Protocol *C4611003*

A PHASE 1, OPEN-LABEL, SINGLE-DOSE STUDY TO INVESTIGATE THE MASS BALANCE, METABOLISM AND EXCRETION OF [^{14}C]-PF-07304814 IN HEALTHY PARTICIPANTS USING A ^{14}C-MICROTRACER APPROACH

Statistical Analysis Plan (SAP)

Version: 1.0

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Revision History

Version	Date	Author(s)	Summary of Changes/Comments
1.0	October 12, 2021	PPD	Not Applicable

NOTE: *Italicized* text within this document has been taken verbatim from the Protocol.

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

None.

2. INTRODUCTION

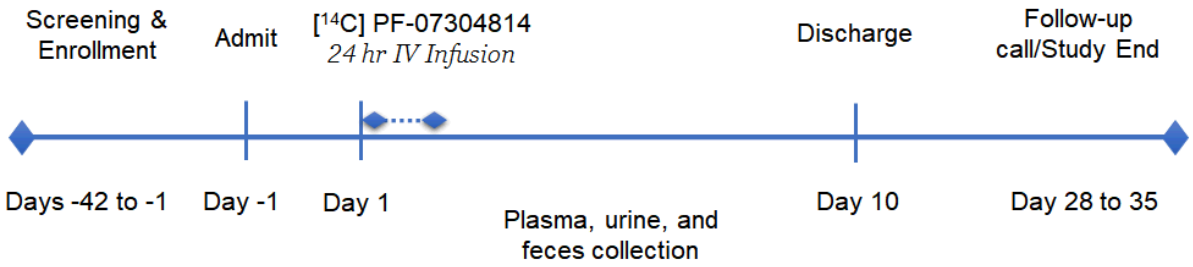
PF-07304814 is a phosphate prodrug of PF-00835231, a potent and selective inhibitor of the SARS-CoV-2 3CL protease, that is being developed as a constant-rate continuous IV infusion for the treatment of patients hospitalized with COVID-19.

The purpose of this study is to characterize mass balance and further the understanding of PK, metabolism, and elimination of PF-07304814 and PF-00835231 (active moiety). The knowledge of routes of elimination and metabolites is useful for evaluating the likelihood of effects of renal or hepatic impairment on the disposition of PF-07304814 and PF-00835231 (active moiety). The biotransformation analysis will be used to identify metabolites of PF-07304814, if possible. The knowledge gained in this study may guide study designs to address potential drug-drug interactions (DDI) and special population studies.

2.1. Study Design

This is a Phase 1, open-label, single-dose, single-center study to characterize mass balance and evaluate the pharmacokinetics, metabolism, and route and extent of elimination of [^{14}C]-PF-07304814 in healthy male and female (of non-childbearing potential) participants. The study will enroll approximately 5 healthy participants. Each participant will receive a single, 24-hour constant-rate, continuous IV infusion of 500 mg PF-07304814 containing approximately 420 nCi [^{14}C] PF-07304814. The total planned duration of participation, from the Screening visit to the last Follow-up phone call, is approximately up to 11 weeks.

Study Schema:



2.2. Study Objectives

2.2.1. Primary Objectives

- *To confirm mass balance and characterize the routes of elimination of [^{14}C]-PF-07304814 and drug related materials following 420 nCi/500 mg dose of [^{14}C]-PF-07304814.*

- *To evaluate the PK of PF-07304814 and PF-00835231 in plasma and characterize the PK of total [^{14}C] in plasma.*

2.2.2. Secondary Objectives

- *To identify metabolites of PF-07304814 in plasma, urine and feces, if possible.*

- *To evaluate the safety and tolerability of a single, 24-hour constant-rate, continuous infusion of 500 mg PF-07304814 containing 420 nCi [^{14}C]-PF-07304814 in healthy participants.*

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

No formal interim analysis will be conducted for this study. As this is an open-label study, the sponsor may conduct unblinded reviews of the data during the course of the study for the purpose of safety assessment, facilitating PK/PD modeling, and/or supporting clinical development.

Final analysis will follow the official database release. As this will be an open-label study, there is no formal unblinding of the randomization code.

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

No hypotheses are required.

4.2. Statistical Decision Rules

No decision rules are required.

5. ANALYSIS SETS

5.1. Pharmacokinetic (PK) Analysis Set

5.1.1. Concentration Analysis Set

- *The PK concentration analysis set for PF-07304814 is defined as all participants who receive study intervention and have at least one measurable PF-07304814 and PF-00835231 concentration(s).*

- *The PK concentration analysis set for [^{14}C] is defined as all participants who receive study intervention and have at least one [^{14}C] measurement.*

5.1.2. Mass Balance Analysis Set

All participants who receive study intervention and who have evaluable total [^{14}C] concentration (urinary and fecal) data and who had no protocol deviations or AEs (such as diarrhea or severe constipation) that may have affected the mass balance analysis.

5.1.3. Parameter Analysis Set

The parameter analysis population is defined as all participants randomized and treated who have at least 1 of the parameters of primary interest in plasma, urine or feces.

5.2. Pharmacodynamic Analysis Set

None.

5.3. Safety Analysis Set

All participants who receive at least 1 dose of study medication will be included in the safety analyses and listings.

5.4. Other Analysis Sets

None.

5.5. Treatment Misallocations

This is a nonrandomized study. All participants will receive the same treatment.

5.6. Protocol Deviations

Participants who experience events that may affect their PK profile (eg lack of compliance with dosing) may be excluded from the PK analysis. At the discretion of the pharmacokineticist a concentration value may also be excluded if the deviation in sampling time is of sufficient concern or if the concentration is anomalous for any other reason.

A full list of protocol deviations will be compiled and reviewed to identify major and minor deviations prior to database closure.

5.6.1. Deviations Assessed Prior to Randomization

At Screening, the investigator will assess participants against the inclusion and exclusion criteria as set out in Sections 5.1 and 5.2 of the protocol.

5.6.2. Deviations Assessed Post-Randomization

A full list of protocol deviations for the study report will be compiled prior to database closure. Any significant deviation from the protocol will be reviewed prior to database closure and a decision taken regarding evaluation for each analysis population.

6. ENDPOINTS AND COVARIATES

6.1. Efficacy Endpoint(s)

None.

6.2. Safety Endpoints

An adverse event will be considered a Treatment-Emergent Adverse Event (TEAE) if the event started during the effective duration of treatment. All events that start on or after the first dosing day and time/ start time, if collected, but before the last dose plus the lag time (28 days) will be flagged as TEAEs. The algorithm will not consider any events that started prior to the first dose date. Any events occurring following start of treatment or increasing in severity will be counted as treatment emergent.

Events that occur in a non-treatment period (for example, Follow-up) will be counted as treatment emergent and attributed to the previous treatment taken.

The following data are considered in standard safety summaries (see protocol for collection days and list of parameters):

- *adverse events,*
- *laboratory data,*
- *vital signs data,*
- *ECG results.*

6.3. PK Endpoints

6.3.1. Mass Balance

Carbon-14 in urine will be measured using accelerator mass spectrometry and reported as the percentage of the administered dose of [^{14}C] excreted at each time interval and as the total percent of dose excreted in urine.

Carbon-14 in feces will be measured using accelerator mass spectrometry and reported as the percentage of the administered dose of [^{14}C] excreted at each time interval and as the total percent of dose excreted in feces.

Percent recovery of total [^{14}C] in urine and feces will be determined based on total administered dose.

Individual participant and median data profiles will be graphically presented for the cumulative recovery of [^{14}C] in urine, feces and their combination. The total recovery of [^{14}C] in urine, feces and their combination will be listed and summarized. Where possible the rate of excretion of [^{14}C] will be estimated.

6.3.2. PK Parameter Endpoints

- **Plasma PK Parameters**

Plasma PF-07304814 and PF-00835231 (active moiety) concentrations will be reported in units of mass per volume, while total [^{14}C] in plasma will be reported in units of unchanged drug concentration equivalents per volume (eg, ngEq/mL). PK parameters (Table 1) *will be derived from PF-07304814 and PF-00835231 concentrations in plasma and from total [^{14}C] concentration equivalents in plasma. Actual PK sampling times will be used for the derivation of PK parameters.*

The PF-07304814 and PF-00835231 concentrations and PK parameters and the total [^{14}C] concentration equivalents and PK parameters will be listed and summarized using descriptive statistics. Individual and median PF-07304814 and PF-00835231 concentration-time profiles and total [^{14}C] concentration equivalents-time profiles will be graphically presented.

Table 1. Noncompartmental PK Parameters of Plasma

Parameter	Analysis Scale	PF-07304814	PF-00835231	total [^{14}C]
AUC_{inf}[a]	ln	D	D	D
AUC_{last}[a]	ln	D	D	D
C_{max}	ln	D	D	D
T_{max}	R	D	D	D
C_{24}	R	D	D	D
$t_{1/2}$[a]	R	D	D	D
CL[a,b]	ln	D		
V_{ss}[a,b]	ln	D		

Key: a=if data permits, b=PF-07304814 only, D=displayed with descriptive statistics, ln=natural-log transformed, R=raw (untransformed)

- **Urine PK Parameters**

Total urine [^{14}C] concentrations and percent [^{14}C] will be listed and summarized using descriptive statistics. Individual and summary profiles of urine [^{14}C] will be graphically presented.

Data permitting, following urine parameters will be calculated:

Table 2. Noncompartmental PK Parameters of Urine

Parameter	Analysis Scale	PF-07304814	Total [^{14}C]
Total [^{14}C] Urine	R	D	D
% [^{14}C] Urine	R	D	D

6.3.3. Metabolic Profiling and Metabolite Identification

Plasma, urine, and fecal samples will be analyzed for identification of metabolites of PF-07304814, if possible. Results of the metabolic profiling analysis will be detailed in a separate report and will be summarized in the CSR.

6.3.4. PD Endpoints

None.

6.4. Covariates

None.

7. HANDLING OF MISSING VALUES

For the analysis of safety endpoints, the sponsor data standard rules for imputation will be applied.

7.1. Concentrations Below the Limit of Quantification

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. (In listings BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification).

7.2. Deviations, Missing Concentrations and Anomalous Values

In summary tables and plots of median profiles, statistics will be calculated having set concentrations to missing if 1 of the following cases is true:

1. A concentration has been collected as ND (ie, not done) or NS (ie, no sample).

2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist.

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

7.3. Pharmacokinetic Parameters

Actual PK sampling times will be used in the derivation of PK parameters.

If a PK parameter cannot be derived from a participant's concentration data, the parameter will be coded as NC (ie, not calculated). (Note that NC values will not be generated beyond the day that a participant discontinues).

In summary tables, statistics will be calculated by setting NC values to missing; and statistics will be presented for a particular renal function group with ≥3 evaluable measurements.

If an individual participant has a known biased estimate of a PK parameter (due for example to an unexpected event such as vomiting before all the compound is adequately absorbed in the body), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methods

The PK data will be summarized using descriptive statistics.

8.2. Statistical Analyses

8.2.1. Pharmacokinetic Analysis

The PK parameters detailed in Section 6.3 will be listed and summarized for participants in the appropriate analysis sets (as defined in Section 5.1.2). Missing values will be handled as detailed in Section 7.

PK parameters will be summarized as specified in the table below.

Table 3. PK Parameters to be Summarized Descriptively

Parameter	Summary Statistics
AUC_{last}, AUC_{inf}, C_{max}, CL and V_{ss}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
$t_{1/2}$	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

Box and whisker plots for individual participant parameters (AUC_{inf}, AUC_{last} and C_{max}) be presented and overlaid with geometric means.

Supporting data from the estimation of t½ and AUC_{inf} will be listed: terminal phase rate constant (k_{el}); goodness of fit statistic from the log-linear regression (r^2); the percent of AUC_{inf} based on extrapolation ($AUC_{extrap\%}$); and the first, last, and number of time points used in the estimation of k_{el}. This data may be included in the clinical study report.

Presentations for PK concentrations will include:

- A listing of all concentrations sorted by participant id and nominal time postdose. The listing of concentrations will include the actual times. Deviations from the nominal time will be given in a separate listing.

- A summary of concentrations by nominal time postdose, where the set of statistics will include n, mean, median, standard deviation, coefficient of variation (cv) and the number of concentrations above the lower limit of quantification.

- A plot of median PK and radioactivity concentrations against nominal time postdose (based on the summary of concentrations by time postdose), where the median concentration-time profiles for plasma PK, and plasma radioactivity, will be presented together on the same plot.

- A log-linear plot of the median concentrations against nominal times postdose, as described above.

- Plots of individual concentrations against actual time postdose, where profiles for plasma PK will be presented together on the same plot.

- Log-linear plots of individual concentrations against nominal times postdose, as described above.

For summary statistics, median and mean plots by sampling time, the nominal PK sampling time will be used, for individual participant plots by time, the actual PK sampling time will be used.

8.3. Safety Analysis

A set of summary tables will be produced to evaluate any potential risk associated with the safety and toleration of administering study treatments.

8.3.1. Treatment and Disposition of Participants

Participant evaluation groups will show end of study participant disposition and will show which participants were analyzed for pharmacokinetics, as well as for safety (adverse events and laboratory data). Frequency counts will be supplied for participant discontinuation(s).

Data will be reported in accordance with the sponsor reporting standards.

8.3.2. Demographic and Clinical Examination Data

A breakdown of demographic data will be provided for age, race, weight, body mass index, and height. Each will be summarized by sex at birth and 'All Participants' in accordance with the sponsor reporting standards.

8.3.3. Discontinuation(s)

Participant discontinuations, temporary discontinuations or dose reductions due to adverse events will be detailed and summarized.

Data will be reported in accordance with the sponsor reporting standards.

8.3.4. Adverse Events

Adverse events will be reported in accordance with the sponsor reporting standards.

8.3.5. Laboratory Data

For each planned timepoint, baseline values and change from baseline values will be summarized with descriptive statistics per treatment (using sponsor default standards). The laboratory data will also be listed.

The baseline will be defined as the last planned pre-dose measurement.

8.3.6. Vital Signs Data

The baseline measurement is the last pre-dose measurement.

Blood pressure, pulse rate and temperature will be measured as per the schedule of activities mentioned in the protocol.

For each planned timepoint, baseline values and change from baseline values per treatment will be summarized with descriptive statistics (using sponsor default standards).

These data will be listed in accordance with the sponsor reporting standards.

8.3.7. ECG Data

The baseline measurement is the pre-dose measurement.

For each planned time-point, baseline values and change from baseline values for the ECG parameters QT interval, heart rate, QTcF interval, PR interval, and QRS complex will be summarized by treatment and time (using sponsor default standards).

These data will be listed in accordance with the sponsor reporting standards.

8.3.8. Other Safety Data

None.

8.3.9. Concomitant Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be provided in the listings.

8.3.10. COVID-19 specific assessments

Participants will be tested for SARS-COVID-19 infection by RT-PCR prior to being admitted to the clinic for confinement and a subsequent COVID-19 test will be performed after approximately 4 days (ie, upon completion of 4 × 24 hours in house), or if they develop COVID-19 like symptoms. Additional testing may be required by local regulations or by the principal investigator. This data will be provided in listings.

8.3.11. Screening and Other Special Purpose Data

If the screening data is brought in-house, then will be listed.

9. REFERENCES

None

10. APPENDICES