Protocol *C4671015*

COVID-19: A PHASE 1, OPEN-LABEL, FIXED SEQUENCE, 2-PERIOD CROSSOVER STUDY TO ESTIMATE THE EFFECT OF ITRACONAZOLE ON THE PHARMACOKINETICS OF PF-07321332/RITONAVIR IN HEALTHY PARTICIPANTS

Statistical Analysis Plan (SAP)

Version: 1.0

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Revision History

Version	Date	Author(s)	Summary of Changes/Comments
1.0	July 20, 2021	PPD	Not Applicable

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

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NONE

APPENDICES

CCI [redacted]

[redacted]

[redacted]

1. AMENDMENTS FROM PREVIOUS VERSION(S)

None.

2. INTRODUCTION

PF-07321332 is a potent and selective inhibitor of the SARS-CoV-2 3CL protease that is currently being developed as an oral treatment of COVID-19. Ritonavir is a strong CYP3A4 inhibitor being used to inhibit the metabolism of PF-07321332 in order to increase plasma concentrations of PF-07321332 to values that are anticipated to be efficacious.

The purpose of the study is to evaluate the effect of the strong CYP3A4 inhibitor, itraconazole, on the PK of PF-07321332 in healthy participants. Results from this study will provide guidance for dosing recommendations with concomitant medications during Phase 3 development and further establish the safety margins of PF-07321332/ritonavir.

2.1. Study Design

This is a Phase 1, open-label, 2-period, fixed sequence crossover study to estimate the effect of the strong CYP3A4 inhibitor, itraconazole, on the PK of PF-07321332 and ritonavir in healthy participants. The study will consist of 2 treatments: multiple oral doses of 300 mg PF-07321332/100 mg ritonavir alone and multiple oral doses of 300 mg PF-07321332/100 mg ritonavir in combination with itraconazole. A total of approximately 12 healthy male and/or female participants will be enrolled into the study to ensure at least 9 participants will complete the study. The treatment will consist of a single fixed sequence. Participants who discontinue from the study for non-safety reasons may be replaced at the sponsor's discretion in collaboration with the Investigator.

Healthy participants will be screened to determine eligibility within 28 days prior to study treatment. Medical history and results of physical examination, physical measurements, vital signs, 12-lead ECGs, and clinical laboratory evaluations will determine eligibility.

In Period 1, each enrolled participant will receive 300 mg PF-07321332/100 mg ritonavir administered orally, q12h for 5 doses, from Day 1 morning to Day 3 morning. Serial PK samples will be collected up to 48 hours post the 5th dose on Day 3 to establish baseline exposure of PF-07321332. PK samples will also be collected predose the morning and evening doses of PF-07321332/ritonavir on Day 2 to confirm steady state.

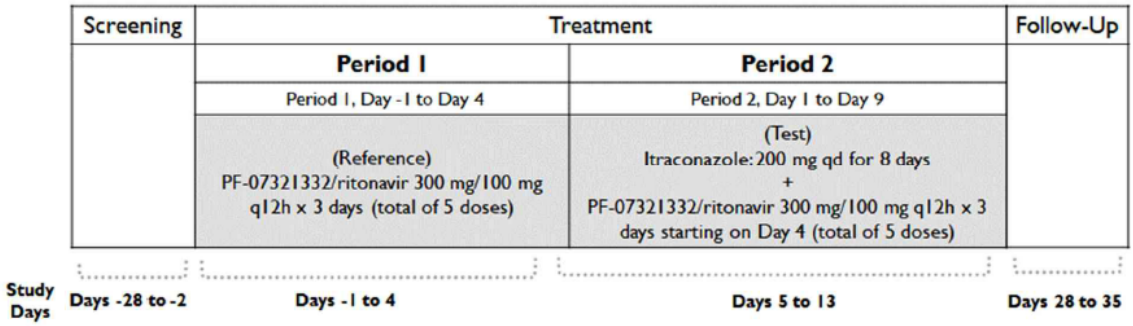
Period 2 will begin on Study Day 5 (referred to as Period 2, Day 1). Participants will receive 200 mg itraconazole, administered orally, QD, on Period 2, Days 1 through 8, inclusive. On Days 4, 5, and 6, participants will receive 300 mg PF-07321332/100 mg ritonavir administered orally, q12h for 5 doses, from Period 2, Day 4 morning to Day 6 morning.

Following the final administration of PF-07321332/ritonavir on the morning of Day 6, participants will undergo serial PK sampling up to 72 hours post the last dose. PK samples will also be collected predose the morning and evening doses of PF-07321332/ritonavir on Day 5 to confirm steady state. Participants will be discharged from the CRU on Period 2, Day 9 following completion of all assessments.

If a participant has any clinically significant, study related abnormalities at the conclusion of a scheduled inpatient portion of the study, the Pfizer medical monitor (or designated representative) should be notified and the participant may be asked to remain in the CRU until such abnormalities are deemed not clinically significant, or it is safe for outpatient follow-up.

A safety follow-up call will be made to participants approximately 28 to 35 days from administration of the final dose of study intervention. The total planned duration of participation, from the Screening visit to the last Follow-up phone call, is approximately 10 weeks.

Study Schema:



2.2. Study Objectives

2.2.1. Primary Objective

- *To estimate the effect of multiple doses of itraconazole on the PK of PF-07321332 following multiple doses of PF-07321332/ritonavir.*

2.2.2. Secondary Objectives

- *To evaluate the safety and tolerability of PF-07321332/ritonavir in healthy participants in the absence and presence of multiple doses of itraconazole.*

- *To characterize the PK of PF-07321332 following multiple doses when PF-07321332/ritonavir is administered alone or with itraconazole in healthy participants.*

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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.

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

There are no statistical hypotheses for this study.

4.2. Statistical Decision Rules

There are no statistical decision rules.

5. ANALYSIS SETS

5.1. Enrolled/Randomly Assigned to Study Intervention

"Enrolled" means a participant's, or their legally authorized representative's, agreement to participate in a clinical study following completion of the informed consent process and screening. Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled, unless otherwise specified by the protocol. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity after screening.

5.2. Pharmacokinetic (PK) Analysis Set

5.2.1. Concentration Analysis Set

All participants who take at least 1 dose of study intervention and in whom at least 1 concentration value is reported.

5.2.2. Parameter Analysis Set

All participants who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of interest are reported.

5.3. Pharmacodynamic Analysis Set

None.

5.4. Safety Analysis Set

All participants who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually received.

5.5. Other Analysis Sets

None.

5.6. Treatment Misallocations

All analyses will be performed on an "as-treated" basis and will not include data from participants who are randomized but not treated.

If a participant takes a treatment that is not consistent with the treatment they are randomized to, for example takes a treatment out of sequence or takes the same treatment twice, then they will be reported under the treatment that they actually receive for all safety, PK and pharmacodynamic analyses, where applicable.

5.7. 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.

5.7.1. 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.

5.7.2. 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

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.*

6.2.1. Adverse Events

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.

6.2.2. Laboratory Safety Tests

Safety laboratory tests will be performed as described in the protocol.

To determine if there are any clinically significant laboratory abnormalities, the haematological, clinical chemistry (serum) and urinalysis safety tests will be assessed against the criteria specified in the sponsor reporting standards. The assessment will take into account whether each participant's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

Baseline is defined as the last pre-dose measurement taken in each study period.

6.2.3. Vital Signs Data

Supine measurements will be taken at times detailed in the Schedule of Activities given in the protocol.

Baseline is the last pre-dose recording in each study period.

The following vital signs endpoints will be determined:

- The minimum systolic and diastolic blood pressures and the minimum and maximum pulse rates over all measurements taken post-dose.
- The maximum increase and maximum decrease from baseline over all measurements taken post-dose for systolic and diastolic blood pressures.

The maximum increase from baseline will be calculated by firstly subtracting the baseline value from each post-dose measurement to give the change from baseline. The maximum of these values will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

Similarly, the maximum decrease from baseline will be determined by selecting the minimum value of the changes from baseline. In cases where a participant does not show a decrease, the minimum increase should be taken.

6.2.4. ECG Results

Triplicate QT interval, QTcF, PR, RR, QRS and heart rate will be recorded at each assessment time indicated in the Schedule of Activities given in the protocol. The average of the triplicate readings collected at each assessment time will be calculated for each ECG parameter. Baseline will be defined as the average of the triplicate predose recordings in each study period.

If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

$$QTcF = QT / (RR)^{1/3} \qquad \text{where } RR = 60/HR \text{ (if not provided)}$$

The maximum absolute value (post-dose) and the maximum increase from baseline for QTcF, PR and QRS, over all measurements taken post-dose, will be determined.

The maximum increase from baseline will be calculated by first subtracting the baseline value from each post-dose measurement to give the change from baseline. The maximum of these values will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

6.2.5. Other Safety Data

Additional safety data will be collected as described in the protocol and will be listed if collected in the sponsor's database.

6.3. Other Endpoints

6.3.1. PK Endpoints

Blood samples for PK analysis of PF-07321332 CCI will be taken according to the Schedule of Activities given in the protocol.

The following PK parameters will be calculated for PF-07321332 CCI (if possible) from the concentration-time data using standard non-compartmental methods:

PK Parameter	Analysis Scale	PF-07321332 [redacted]
AUC_{last}	ln	D
AUC_{tau}	ln	A, D
C_{max}	ln	A, D
T_{max}	R	D
$t_{1/2}$*	R	D
CL/F*	ln	D
V_Z/F*	ln	D

Key: A=analyzed using statistical model, D=displayed with descriptive statistics, ln=natural-log transformed, R=raw (untransformed), *=if data permits

6.3.2. 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 treatment with ≥3 evaluable measurements. For statistical analyses (ie, analysis of variance), PK parameters coded as NC will also be set to missing; and analyses will not be performed for a particular parameter if more than 50% of the data are NC.

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 interactive effect on PK parameters will be determined by constructing 90% confidence intervals around the estimated difference between the Test and Reference treatments using a mixed effects model based on natural log transformed data. The mixed effects model will be implemented using SAS Proc Mixed, with REML estimation method and Kenward-Roger degrees of freedom algorithm.

8.2. Statistical Analyses

Natural log transformed parameters (AUC_{tau} and C_{max}) of PF-07321332 will be analyzed using a mixed effect model with treatment as fixed effect and participant as a random effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% CIs will be obtained from the model. The adjusted mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CI for the ratios. PF-07321332/ritonavir administered alone will be the Reference treatment and PF-07321332/ritonavir co-administered with itraconazole will be the Test treatment. Additionally, the aforementioned analysis will be performed for the same PK parameters of [redacted] and is intended to not be included in the clinical study report.

Residuals from the model will be examined for normality and the presence of outliers via visual inspection of plots of residuals vs predicted values and normal probability plots of residuals but these will not be included in the clinical study report. If there are major deviations from normality or outliers then the effect of these on the conclusions will be investigated through alternative transformations and/or analyses excluding outliers.

Justification for any alternative to the planned analysis will be given in the report of the study.

The plasma concentrations of PF-07321332 CCI *will be listed and descriptively summarized by analyte, nominal PK sampling time and treatment.*

Table 1. PK Parameters to be Summarized Descriptively by Analyte and Treatment

Parameter	Summary Statistics
AUC_{tau}, AU_{Clast}, C_{max}, CL/F*, V_Z/F*	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.

*: if data permit

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

Supporting data from the estimation of $t_{½}$ and AUC_{tau} will be listed by analyte and treatment: terminal phase rate constant (k_{el}); goodness of fit statistic from the log-linear regression (r^2); 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 PF-07321332 CCI concentrations will include:

- A listing of all concentrations sorted by participant ID, period and nominal time post-dose. The concentration listing will also include the actual times. Deviations from the nominal time will be given in a separate listing.

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

- Median concentrations time plots (on both linear and semi-log scales) against nominal time post-dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time post-dose).

- Mean concentrations time plots (on both linear and semi-log scales) against nominal time post-dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time post-dose).

- Individual concentration time plots by treatment (on both linear and semi-log scales) against actual time post-dose (there will be separate spaghetti plots for each treatment per scale).

- Individual concentration time plots by participant (on both linear and semi-log scales) against actual time post-dose [there will be separate plots for each participant (containing all treatments) per scale].

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

All safety analyses will be performed on the safety population. A set of summary tables split by treatment 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) by treatment.

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 by treatment.

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 by treatment.

8.3.5. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the sponsor reporting standards. Baseline is as defined in Section 6.2.2.

8.3.6. Vital Signs Data

For each planned time-point, baseline values and change from baseline values within each treatment will be summarized with descriptive statistics (using sponsor default standards). Baseline is as defined in Section 6.2.3.

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

8.3.7. ECG Data

Baseline and changes from baseline in PR, QT, QRS, heart rate and QTcF will be summarized by treatment and time post-dose. Baseline is as defined in Section 6.2.4.

ECG endpoints and changes from baseline (QTcF, PR, QRS), over all measurements taken post-dose, will also be summarized descriptively by treatment using categories as defined in the ECG Findings of Potential Clinical Concern appendix of the protocol and for QTcF values corresponding to ICH E14[1] thresholds below. Numbers and percentages of participants meeting the categorical criteria will be provided and individual values listed in the study report.

Table 2. E14 QTcF Categorical Thresholds

Parameter	Mild (msec)	Moderate (msec)	Severe (msec)
QTcF (msec)	450 < max ≤480	480 < max ≤500	max >500
QTcF (msec) increase from baseline		30 ≤ max ≤60	max >60

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

8.3.8. Concomitant Treatments

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

8.3.9. COVID-19 Test Results

Participants will be tested for SARS-COV-2 infection by PCR prior to being admitted to the CRU for confinement and a subsequent SARS-CoV-2 test will be performed after 4 days (ie, upon completion of 4 × 24 hours in house), or if they develop COVID-19 like symptoms. The test results per visit and for each treatment will be provided in listings.

8.3.10. Screening and Other Special Purpose Data

Prior medication(s) and non-drug treatment(s), serum FSH concentrations, medical history, physical examination, HBcAb, HBsAb, HBsAg, HCVAb, and HIV test will be assessed at Screening.

Urine drug screen will be done at Screening and Period 1 Day -1. Serum or urine pregnancy test for all females of childbearing potential and contraception check will be done at Screening, Period 1 Day -1 and Period 2 Day 9.

Data for prior medication(s) and non-drug treatment(s), urine drug screen, serum or urine pregnancy test for all females of childbearing potential, contraception check and alcohol/tobacco use will be listed. For rest of the parameters data will not be brought in-house, and therefore will not be listed.

9. REFERENCES

1. ICH E14 - The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. CHMP/ICH/2/04.

[redacted]

[redacted]

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[redacted]

[redacted]