Protocol *C4671013*

COVID-19: A PHASE 1, OPEN-LABEL, 3-TREATMENT, 6-SEQUENCE, 3-PERIOD CROSSOVER STUDY TO ESTIMATE THE EFFECT OF PF-07321332/RITONAVIR AND RITONAVIR ON THE PHARMACOKINETICS OF MIDAZOLAM IN HEALTHY PARTICIPANTS

Statistical Analysis Plan (SAP)

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SAP Author: PPD
PPD

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Revision History

Version	Date	Author(s)	Summary of Changes/Comments
Version 1.0	August 30, 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-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 PF-07321332/ritonavir and ritonavir on the PK of midazolam, a CYP3A4 substrate, 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, 3-treatment, 6-sequence, 3-period crossover study to estimate the effect of steady-state PF-07321332/ritonavir and ritonavir on the PK of midazolam in healthy adult participants. The study will consist of 3 treatments: single oral dose of 2 mg midazolam alone (Treatment A), multiple oral doses of 300 mg PF-07321332/100 mg ritonavir in combination with a single oral dose of 2 mg midazolam (Treatment B), and multiple oral doses of 100 mg ritonavir in combination with a single oral dose of 2 mg midazolam (Treatment C). The treatment will consist of 6 treatment sequences shown in the table below, with each participant receiving all 3 treatments, according to the assigned sequence over 3 periods.

A total of approximately 12 healthy male and/or female participants will be enrolled into the study to ensure at least 10 participants will complete the study. Participants who discontinued from the study for non-safety reasons may be replaced at the sponsor's discretion in collaboration with the investigator.

Each enrolled participant will participate in 3 study periods to receive 3 different treatments:

- *Treatment A: Single oral dose of 2 mg midazolam, followed by serial PK sampling and 2-day washout (Treatment A period duration: Day 1 to Day 3)*
- *Treatment B: 300 mg PF-07321332/100 mg ritonavir administered orally, q12h for 9 doses: Day 1 morning to Day 5 morning. On Day 5, participants will receive a single oral dose of 2 mg midazolam administered with the Day 5 morning dose of PF-07321332/ritonavir, followed by serial PK sampling and a 7-day washout (Treatment B period duration: Day 1 to Day 12).*

- *Treatment C: 100 mg ritonavir administered orally, q12h for 9 doses: Day 1 morning to Day 5 morning. On Day 5, participants will receive a single oral dose of 2 mg midazolam administered with the Day 5 morning dose of ritonavir, followed by serial PK sampling and a 7-day washout (Treatment C period duration: Day 1 to Day 12).*

Participants will be randomly assigned to 1 of 6 sequences as in Table 1.

Table 1. Treatment Sequences

Sequence	***Period 1***	***Period 2***	***Period 3***
1 (2 participants)	*Treatment A*	*Treatment B*	*Treatment C*
2 (2 participants)	*Treatment C*	*Treatment A*	*Treatment B*
3 (2 participants)	*Treatment B*	*Treatment C*	*Treatment A*
4 (2 participants)	*Treatment C*	*Treatment B*	*Treatment A*
5 (2 participants)	*Treatment B*	*Treatment A*	*Treatment C*
6 (2 participants)	*Treatment A*	*Treatment C*	*Treatment B*

Participants will be discharged on Period 3, following completion of all assessments.

The total planned duration of participation, from the Screening visit to the last Follow-up phone call, is approximately 13 weeks.

2.2. Study Objectives

2.2.1. Primary Objective

- *To estimate the effect of PF-07321332/ritonavir on the PK of midazolam.*

2.2.2. Secondary Objectives

- *To evaluate the safety and tolerability of PF-07321332/ritonavir in healthy participants in the absence and presence of midazolam.*
- *To estimate the effect of ritonavir on the PK of midazolam.*
- *To evaluate the effects of PF-07321332/ritonavir and ritonavir on additional PK parameters of midazolam in healthy participants.*

CCI [redacted]

- CCI [redacted]
- CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

There are no statistical hypotheses.

4.2. Statistical Decision Rules

There are no statistical decision rules.

5. ANALYSIS SETS

5.1. Pharmacokinetic (PK) Analysis Set

5.1.1. Concentration Analysis Set

The PK concentration population is defined as all participants randomized and treated who have at least 1 concentration in at least 1 treatment period.

5.1.2. Parameter Analysis Set

The PK parameter analysis population is defined as all participants randomized and treated who have at least 1 of the PK parameters of primary interest in at least 1 treatment period.

Should vomiting occur after co-administration of midazolam with PF-07321332/ritonavir, the resulting PK parameters from that participant from the corresponding period may be excluded.

5.2. Pharmacodynamic Analysis Set

None.

5.3. Safety Analysis Set

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

5.4. Other Analysis Sets

None.

5.5. 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, and PK analyses, where applicable.

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

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

- *adverse events,*
- *laboratory data,*
- *vital signs,*
- *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, Washout or 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 planned predose 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 predose 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 postdose.
- The maximum increase and maximum decrease from baseline over all measurements taken postdose for systolic and diastolic blood pressures.

The maximum increase from baseline will be calculated by firstly subtracting the baseline value from each postdose 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

QT interval, QTc, PR, RR, QRS and heart rate will be recorded at each assessment time indicated in the Schedule of Activities given in the protocol.

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 average of the triplicate measurements will be calculated prior to analyzing the data. Baseline will be defined as the average of the triplicate predose recordings in each study period.

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

The maximum increase from baseline will be calculated by first subtracting the baseline value from each postdose 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 will be taken according to the Schedule of Activities given in the protocol for PK analysis of midazolam following single dose administration with and without PF-07321332/ritonavir or midazolam+ritonavir (as data permit).

The following PK parameters will be calculated for midazolam following single dose administration with and without PF-07321332/ritonavir or midazolam+ritonavir will be derived (if possible) from the concentration-time data using standard noncompartmental methods:

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

V_z/F^*	ln	D	D

Key: A=analyzed using statistical model, D=displayed with descriptive statistics, ln=natural-log transformed, NA=not applicable, 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 AUC_{inf} (if data permit), AUC_{last} and C_{max} of midazolam will be analyzed using a mixed effect model with sequence, period and treatment as fixed effects and participant within sequence as a random effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% confidence intervals will be obtained from the model. The adjusted mean differences and 90% confidence intervals for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% confidence intervals for the ratios. Midazolam alone is the Reference treatment whilst midazolam + PF-07321332/ritonavir and midazolam + ritonavir are the Test treatments.

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.

Table 2. PK Parameters to be Summarized Descriptively by Treatment

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

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

Supporting data from the estimation of t½ and AUC_{inf} will be listed by analyte and treatment: 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 midazolam and PF-07321332 concentrations will include:

- A listing of all concentrations sorted by participant ID, period and nominal time postdose. 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 postdose, 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 postdose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time postdose).

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

- Individual concentration time plots by treatment (on both linear and semi-log scales) against actual time postdose (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 postdose [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

A set of summary tables split by treatment will be produced to evaluate any potential risk associated with the safety and toleration of administering midazolam following single dose administration with and without PF-07321332/ritonavir or ritonavir.

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 break down 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

Vital Signs data will be summarized and 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 postdose. Baseline is as defined in Section 6.2.4.

ECG endpoints and changes from baseline (QTcF, PR, QRS), over all measurements taken postdose, will also be summarized descriptively by treatment using categories as defined in the Criteria for Safety Values of Potential Clinical Concern appendix of the protocol and for QTc 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 3. E14 QTcF Categorical Thresholds

Parameter	Criterion		
QTcF (msec)	450≤ value <480	480≤ value <500	≥500
QTcF (msec) increase from baseline	30≤ change <60	change ≥60	

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 Assessment Data

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 4 days (ie, upon completion of 4 x 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.

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

8.3.11. Screening and Other Special Purpose Data

Prior medication(s) and non-drug treatment(s), serum FSH concentrations, urine drug screen, serum or urine B-hCG for all females of childbearing potential, TSH and Free T4, Prep D1, HIV, HBsAg, HBsAb, HBcAb, and HCVAb will be obtained at Screening.

If these data are brought in-house, then they will 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.

10. APPENDICES

Appendix 1. SAS CODE FOR ANALYSES

An example of the PROC MIXED code is provided below:

```
proc mixed data=tab.pk;
        class seq period trt participant;
        model l&var=seq period trt/ ddfm=KR;
        random participant(seq) /participant=participant(seq);
        lsmeans trt;
        estimate 'B vs A' trt -1  1  0/cl alpha=0.1;
        estimate 'C vs A' trt -1  0  1/cl alpha=0.1;
        ods 'Estimates' out=est&var;
        ods 'lsmeans' out=ls&var;
        ods 'covparms' out=cov&var;
        ods 'tests3' out=tst&var;
run;


/* Letter assignments for treatments (trt) within the estimate statement above are as follows;

A = Midazolam alone (Reference);
B = Midazolam + PF-07321332/ritonavir (Test)
C = Midazolam + ritonavir (test) */;
```