Protocol *C4671014*

A PHASE 1, OPEN-LABEL, FIXED SEQUENCE, 2-PERIOD CROSSOVER STUDY TO ESTIMATE THE EFFECT OF CARBAMAZEPINE ON THE PHARMACOKINETICS OF PF-07321332 BOOSTED WITH RITONAVIR IN HEALTHY PARTICIPANTS

Statistical Analysis Plan
(SAP)

Version: 1.0

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1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1.0/ 21 SEP 2021	N/A	N/A	N/A

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C4671014. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

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 this study is to estimate the effect of carbamazepine, a strong inducer of CYP3A4 on the PK of PF-07321332/ritonavir in healthy participants.

2.1. Study Objectives, Endpoints

2.1.1. Primary Objective

- *To estimate the effect of carbamazepine on the PK of PF-07321332 after a single dose of PF-07321332/ritonavir (reference) compared to the PK of PF-07321332 after a single dose of PF-07321332/ritonavir, following multiple dosing with carbamazepine (test).*

2.1.2. Secondary Objectives

- *To estimate the effect of carbamazepine on the PK of ritonavir after a single dose of PF-07321332/ritonavir (reference) compared to the PK of ritonavir after a single dose of PF-07321332/ritonavir, following multiple dosing with carbamazepine (test).*

- *To evaluate the safety and tolerability of PF-07321332/ritonavir alone and, following multiple dosing with carbamazepine.*

- *To characterize additional PK parameters of both PF-07321332 and ritonavir after a single dose of PF-07321332/ritonavir (reference) compared to the PK of both PF-07321332 and ritonavir, following multiple dosing with carbamazepine.*

2.2. Study Design

This is a Phase 1, fixed sequence, 2-period study to estimate the effect of a strong CYP3A4 inducer, carbamazepine, on the PK of PF-07321332 and ritonavir in healthy participants. A total of approximately 12 healthy male and/or female participants will be enrolled into the study. The purpose of this study is to characterize the effects of carbamazepine on the single dose PK of PF-07321332 300 mg/ritonavir 100 mg. Safety and tolerability will also be assessed throughout the duration of the study. This study will consist of 2 periods. Period 1 (P1) will include PF-07321332 300 mg/ritonavir 100 mg as a single oral dose, and Period 2 (P2) will include a 15-day dosing of carbamazepine administered orally. Carbamazepine will start from 100 mg BID on Days 1, 2, and 3, and then, will titrate up to 200 mg BID on Days 4, 5, 6, and 7 in P2. Carbamazepine will eventually titrate up to and maintain at 300 mg BID stably in the rest of P2 from Days 8 to 15 (Figure 2 below). On Day 14 in the P2, a single dose of PF-07321332 300 mg/ritonavir 100 mg will be administered after multiple-dosed carbamazepine. PK sampling will be included in both Periods 1 and 2 to determine PK parameters.

Safety assessments will be performed during Screening, on Day -1 prior to dosing, and at specified time points. PEs, vital sign measurements, and clinical laboratory tests will be conducted, and AEs will be monitored to assess safety. The total participation time (eg, CRU confinement time for study procedures) for each participant in this study is approximately 18 days/17 nights (excluding screening and follow-up contact). A safety follow-up call will be made to participants 28 to 35 days from administration of the dose of study intervention.

The total planned duration of participation, from the Screening visit to the last Follow-up phone call, is approximately 11 to 12 weeks.

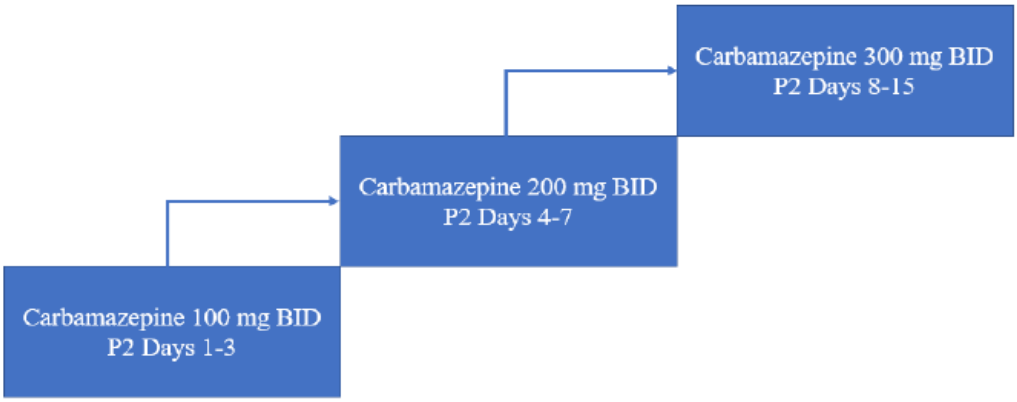
Participants who withdraw from the study may be replaced at the discretion of the investigator upon consultation with the sponsor.

Figure 1. The Schematic Represents the Study Design

Screening Days -28 to -2	Period 1 (P1) (Days 1 and 2)*	Period 2 (P2) (Days 1-16)	Follow-up Days 28-35
	(Reference) PF-07321332 300 mg/ritonavir 100 mg single dose	(Test) Carbamazepine+: 100 mg BID on Days 1-3, 200 mg BID on Days 4-7, and 300 mg BID on Days 8-15 in P2 + PF-07321332 300 mg/ritonavir 100 mg single dose on Day 14 in P2	
	PK sampling 0-48 hrs	PK sampling 0-48 hrs	

*P1 includes Day -1.
+.Carbamazepine will start from 100 mg BID on Days 1, 2, and 3, and then, will titrate up to 200 mg BID on Days 4, 5, 6, and 7 in P2. Carbamazepine will eventually titrate up to and maintain at 300 mg BID stably in the rest of P2 from Days 8 to 15.

Figure 2. Titration Scheme for Carbamazepine



The end of the study is defined as the date of last scheduled procedure shown in the Schedule of Activities for the last participant in the trial.

A participant is considered to have completed the study if he/she has completed all parts of the study, including the last scheduled procedure shown in the Schedule of Activities.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

- *Plasma PF-07321332 PK parameters: C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated).*

3.2. Secondary Endpoint(s)

- *Plasma ritonavir PK parameters: C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated).*
- *Safety: treatment-emergent adverse events, clinical laboratory tests, vital signs, PE, and ECGs.*
- *Plasma PF-07321332 and ritonavir PK parameters: T_{max}, AUC_{last}, CL/F, V_z/F, and t½, as data permit.*

3.3. 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.

3.3.1. 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 (28-35 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.

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.

3.3.2. Laboratory Data

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 pre-dose measurement taken in each study period.

3.3.3. Vital Signs Data

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

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

3.3.4. ECG Data

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:

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

3.3.5. Other Safety Data

If any additional safety data is collected and databased it will be listed only.

3.4. Pharmacokinetic(PK) Endpoints

The plasma PK parameters for PF-07321332 will be derived from the concentration time profiles. Actual PK sampling times will be used in the derivation of PKparameters. In the case that actual PK sampling times are not available, nominal PK sampling time will be used in the derivation of PK parameters.

Table 2. Plasma PK Parameters

PK Parameter	Analysis Scale	PF-07321332 and Ritonavir
AUC_{last}	ln	A, D
AUC_{tau} (if provided)	ln	A, D
AUC_{inf}^{*}	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

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

4.1. For Purposes of Analysis, the Following Analysis Sets are Defined

Population	**Description**
Enrolled	*"Enrolled" means a participant, or his or her legally authorized representative's, agreement to participate in a clinical study following completion of the informed consent process and screening. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity after 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.*
PK Concentration	*The PK concentration population is defined as all participants assigned to investigational product and treated who have at least 1 concentration measured.*
PK Parameter	*The PK parameter analysis population is defined as all participants assigned to investigational product and treated who have at least 1 of the PK parameters of primary interest measured.*
Safety	*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.*

4.2. 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.

4.3. 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.

4.3.1. 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.

4.3.2. 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.

5. GENERAL METHODOLOGY AND CONVENTIONS

The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

5.1. Hypotheses and Decision Rules

No statistical hypothesis will be tested in this study. There are no statistical decision rules.

5.2. General 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 algori.

5.2.1. Statistical Analyses

The plasma concentrations of PF-07321332 and ritonavir will be listed and descriptively summarized by nominal PK sampling time and treatment. Individual participants, as well as mean and median profiles of the plasma concentration time data will be plotted by treatment for each analyte using actual (for individual) and nominal (for mean and median) times respectively. Mean and median profiles will be presented on both linear and semi-log scales. For comparison of AUC_{inf} and C_{max} with and without carbamazepine, box and whisker plots of these parameters will be plotted by treatment for each analyte.

Natural log transformed parameters (AUC_{inf}, AUC_{last}, and C_{max}) of PF-07321332 and ritonavir 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 coadministered with carbamazepine will be the Test treatment.

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 3. Plasma PK Parameters to be Summarized Descriptively by Analyte and Treatment

Parameter	Summary Statistics
AUC_{inf}*, AUC_{last}, AUC_{tau} (if provided), 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_{inf}, AUC_{last} and C_{max}) will be presented by analyte and treatment and overlaid with geometric means.

Supporting data from the estimation of t½ and AUC_{last} 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 and ritonavir 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.

5.3. Methods to Manage Missing Data

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

5.3.1. 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).

5.3.2. 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.

5.3.3. 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.

6. ANALYSES AND SUMMARIES

6.1. Study Conduct and Participant Disposition

Participant evaluation groups will show end of study participant disposition and will show which participants were analyzed 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.

6.1.1. 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.

6.1.2. 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.

6.2. Safety Summaries and Analyses

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.

6.2.1. Adverse Events

Adverse events will be reported in accordance with the sponsor reporting standards by treatment (PF-07321332 300 mg/ritonavir 100 mg alone (Period 1) and multiple dose carbamazepine coadministered with single dose PF-07321332 300 mg/ritonavir 100 mg Period 2 until end of study).

6.2.2. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the sponsor reporting standards. Baseline is as defined in Section 3.3.2.

6.2.3. Vital Signs Data

For each planned time-point, baseline values and change from baseline values within each treatment will be summarized with descriptive statistics and categorical incident counts (using sponsor default standards). Baseline is as defined in Section 3.3.3.

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

6.2.4. 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 3.3.4.

ECG endpoints and changes from baseline (QTcF, PR, QRS), over all planned 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 4. 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.

6.2.5. Concomitant Medications and Nondrug Treatments

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

6.2.6. 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.

6.2.7. Suicidal Ideation and Behavior Risk Data

If available, this data will be provided in listings.

6.2.8. 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, HLA-A*3101, HLA-B*1502 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 16.

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.

7. INTERIM ANALYSES

None.

8. REFERENCES

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

9. APPENDICES

Appendix 1. SAS CODE FOR ANALYSES

Programming Note:

- The statistical analysis of ritonavir should be stored in a statistics exploratory folder within the program for regulatory purpose.

An example of the PROC MIXED code is provided below:

```
proc mixed data=tab.pk;
        class trt participant;
        model l&var=trt/ ddfm=KR;
        random participant /subject=participant;
        lsmeans trt;
        estimate 'Test vs Reference' trt  -1  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 = PF-07321332/ritonavir alone (Reference);
B = PF-07321332/ritonavir + carbamazepine (Test)

*/
```