Protocol *C4671012*

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 DABIGATRAN IN HEALTHY PARTICIPANTS

Statistical Analysis Plan (SAP)

Version: 1.0

SAP Author: PPD
(PPD)

Date: 30-AUG-2021

Revision History

Version	Date	Author(s)	Summary of Changes/Comments
1.0	August 30, 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

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 this study is to estimate the effect of PF-07321332/ritonavir and ritonavir on the PK of dabigatran in healthy participants.

2.1. Study Design

This is a Phase 1, open-label, randomized, 3-treatment, 6-sequence, 3-way crossover design to estimate the effect of PF-07321332/ritonavir or ritonavir on the PK of a P-gp substrate, dabigatran in healthy participants.

This study will consist of 3 treatments. In T1, dabigatran will be administered orally as a 75 mg single dose followed by a 3-day washout. In T2, PF-07321332/ritonavir 300 mg/100 mg q12h will be administered as a multiple dose over a period of 2 days. In the morning on Day 2, 75 mg of dabigatran will be administered orally as a single dose. Treatment 2 will be followed by a 3-day washout. In T3, ritonavir 100 mg q12h will be administered as a multiple dose over a period of 2 days. In the morning on Day 2, 75 mg of dabigatran will be administered orally as a single dose. Each washout period will begin after the first dose of study treatment. A total of approximately 24 healthy male and/or female participants will be enrolled into the study. Participants who discontinue from the study may be replaced at the sponsor's discretion in collaboration with the Investigator.

Participants will randomly be assigned to 1 of 6 sequences as follows:

Table 1. Study Schematic

Sequence	***Period 1***	***Period 2***	***Period 3***
Sequence 1	*Treatment 1*	*Treatment 2*	*Treatment 3*
Sequence 2	*Treatment 3*	*Treatment 1*	*Treatment 2*
Sequence 3	*Treatment 2*	*Treatment 3*	*Treatment 1*
Sequence 4	*Treatment 3*	*Treatment 2*	*Treatment 1*
Sequence 5	*Treatment 2*	*Treatment 1*	*Treatment 3*
Sequence 6	*Treatment 1*	*Treatment 3*	*Treatment 2*

The following describes the study design as an example for Sequence 1 (T1-T2-T3):

Participants in T1 (Day 1) will receive dabigatran orally administered as a 75 mg dose followed by a 3-day washout. Serial PK samples will be collected up to 48 hours after single dose administration to determine PK parameters.

T2 will begin on Study Day 5 (referred to as T2 Day 1). Participants in T2 (Day 1) will receive multiple doses of PF-07321332/ritonavir 300 mg/100 mg q12h on Days 1 and 2 followed by a single dose of 75 mg dabigatran administered orally on Day 2. A 3-day washout will ensue the co-administration of PF-07321332/ritonavir and dabigatran. Serial PK samples will be collected up to 48 hours following the administration of PF-07321332/ritonavir co-administered with a single oral dose of 75 mg dabigatran.

T3 will begin on Study Day 10 (referred to as T3 Day 1). Participants in T3 (Day 1) will receive multiple dose administration of ritonavir 100 mg q12h on Days 1 and 2. In the morning on Day 2, participants will be administered a single oral 75 mg dose of dabigatran. Serial PK samples will be collected up to 48 hours following multiple dose administration of ritonavir 100 mg co-administered with a single oral dose of 75 mg dabigatran.

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

2.2. Study Objectives

2.2.1. Primary Objective

- *To estimate the effect of the multiple doses of PF-07321332/ritonavir co-administered with dabigatran orally (test) compared to dabigatran administered orally as a single dose (reference).*

2.2.2. Secondary Objectives

- *To estimate the effect of the multiple doses of ritonavir co-administered with dabigatran orally (test) compared to dabigatran administered orally as a single dose (reference).*

- *To evaluate the safety and tolerability of dabigatran alone and following co-administration with multiple dosing of PF-07321332/ritonavir or ritonavir.*

- *To characterize the PK of dabigatran and PF-07321332 in study treatments.*

CCI [redacted]

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 statistical hypothesis will be tested in this study.

4.2. Statistical Decision Rules

There are no statistical decision rules.

5. ANALYSIS SETS

5.1. Enrolled Set

"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.

5.2. Pharmacokinetic (PK) Analysis Set

5.2.1. Concentration Analysis Set

The PK concentration population is defined as all participants assigned to investigational product and treated who have at least 1 concentration measured.

5.2.2. Parameter Analysis Set

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.

5.3. Pharmacodynamic Analysis Set

None.

5.4. 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.

5.5. 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, and PK 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, 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 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:

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

If not supplied, QTcB will be derived using Bazett's heart rate correction formula:

$$QTcB = QT / (RR)^{1/2} \qquad \text{where RR = 60/HR (if not provided)}$$

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 for PK analysis of PF-07321332, ritonavir, and dabigatran (total) will be taken according to the Schedule of Activities given in the protocol.

The following PK parameters will be calculated for PF-07321332, ritonavir, and dabigatran (total) (if possible) from the concentration-time data using standard noncompartmental methods:

Table 2. Noncompartmental PK Parameters

PK Parameter	Analysis Scale	PF-07321332	Ritonavir	Dabigatran (Total)
AUC_{last}	ln	D	D	A, D
AUC_{inf}*	ln	NA	NA	A, D
AUC_{tau}	ln	D	D	NA
C_{max}	ln	D	D	A, D
T_{max}	R	D	D	D
$t_{1/2}$*	R	D	D	D
CL/F*	ln	D	D	D
Vz/F*	ln	D	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

The plasma concentrations of PF-07321332, ritonavir and dabigatran will be listed and descriptively summarized by nominal PK sampling time and treatment. Individual participant, 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 dabigatran, box and whisker plots of these parameters will be plotted by treatment for each analyte.

Natural log transformed parameters (AUC_{inf} [if data permit)], AUC_{last}, and C_{max}) of dabigatran will be analyzed using a mixed effect model with treatment, period and sequence as fixed effects and participant within sequence 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. Dabigatran administered alone will be the reference treatment and PF-07321332/ritonavir co-administered with dabigatran and ritonavir co-administered with dabigatran will be 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 3. PK Parameters to be Summarized Descriptively by Treatment

Parameter	Summary Statistics
AUC_{inf}, AUC_{last}, AUC_{tau}, 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 PF-07321332, ritonavir and dabigatran 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 PF-07321332, ritonavir and dabigatran.

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

Blood pressure, pulse rate, respiratory rate and temperature will be measured at the time points as mentioned in the schedule of activities in the protocol.

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

8.3.7. ECG Data

ECG 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-COV-2 infection per the Schedule of Activities, 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, urine or blood cotinine concentration, TSH &Free T4, HIV, HBsAg, HBsAb, HBcAb, HCVAb will be obtained at Screening.

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

9. REFERENCES

Not Applicable.

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 'T2 vs T1' trt -1  1 0 /cl alpha=0.1;
        estimate 'T3 vs T1' 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;

T1 = Dabigatran (Reference);
T2 = PF-07321332/ritonavir + dabigatran (Test)
T3 = ritonavir + dabigatran (Test) */;
```