Protocol C4671019

A PHASE 1, OPEN-LABEL, RANDOMIZED, SINGLE DOSE, 2-SEQUENCE, 2-PERIOD CROSSOVER STUDY TO EVALUATE THE EFFECT OF HIGH-FAT MEAL ON THE RELATIVE BIOAVAILABILITY OF PF-07321332 BOOSTED WITH RITONAVIR IN HEALTHY ADULT PARTICIPANTS

Statistical Analysis Plan (SAP)

Version: 1.0

Date: 07 Dec 2021

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APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1.0 07 Dec 2021	Original 18 Oct 2021	N/A	N/A

2. INTRODUCTION

This is a Phase 1, open-label, single dose, randomized, 2-treatment, 2-sequence, 2-period crossover study to evaluate the effect of a high-fat meal on the relative bioavailability of PF-07321332 boosted with ritonavir following single dose oral administration of PF-07321332 in combination with ritonavir using 150 mg tablet formulation of PF-07321332 in healthy adult participants.

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C4671019. 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.

2.1. Study Objectives and Endpoints

Objectives	***Endpoints***
Primary:	***Primary:***
• *To evaluate the effect of a high-fat meal on the exposure of PF-07321332 boosted with ritonavir following a single oral dose of PF-07321332 in combination with ritonavir using 150 mg tablet formulation of PF-07321332.*	• *The ratio of AUC_{last}, AUC_{inf} (if data permit) and C_{max} of PF-07321332.*
Secondary:	***Secondary:***
• *To characterize the pharmacokinetic parameters of PF-07321332 boosted with ritonavir following a single oral dose of PF-07321332 in combination with ritonavir.*	• *T_{max}, and $t_{½}$, CL/F and V_z/F (if data permit).*
• *To evaluate the safety and tolerability of PF-07321332/ritonavir in healthy participants.*	• *Assessment of TEAEs, clinical laboratory abnormalities, vital signs, and 12-lead ECGs.*

2.2. Study Design

This is a Phase 1, open-label, single dose, randomized, 2-treatment, 2-sequence, 2-period crossover study to evaluate the effect of a high-fat meal on the relative bioavailability of PF-07321332 boosted with ritonavir following single dose oral administration of PF-07321332 in combination with ritonavir using 150 mg tablet formulation of PF-07321332 in healthy adult participants. The study will consist of 2 treatments: a single oral dose of 300 mg PF-07321332 (2 × 150 mg tablets) under fasted conditions and 3 doses of 100 mg ritonavir at -12 hour, 0 hour and 12 hour related to PF 07321332 dosing (Treatment A), and a single oral dose of 300 mg PF 07321332 (2 × 150 mg tablets) under fed conditions and 3 doses of 100 mg ritonavir at -12 hour, 0 hour and 12 hour related to PF-07321332 dosing (Treatment B).

*There will be a total of 2 treatment sequences shown in **Table 2** with the assigned Treatments A or B in each treatment period.*

Table 2. Treatment Sequences

	Period 1	*Washout (4 days between PF-07321332 dosing)*	***Period 2***
Sequence 1 (n=6)	*Treatment A*		*Treatment B*
Sequence 2 (n=6)	*Treatment B*		*Treatment A*

Each enrolled participant will participate in 2 study periods to receive 2 different treatments according to the sequence determined by randomization:

- *Treatment A: Single oral dose of 300 mg PF-07321332 (2 × 150 mg tablets) under fasted conditions and 3 doses of 100 mg ritonavir at -12 hour, 0 hour and 12 hour related to PF-07321332 dosing. (Reference)*
- *Treatment B: Single oral dose of 300 mg PF 07321332 (2 × 150 mg tablets) under fed conditions and 3 doses of 100 mg ritonavir at -12 hour, 0 hour and 12 hour related to PF-07321332 dosing. (Test)*

A total of approximately 12 healthy male and/or female participants will be randomly assigned to investigational product such that approximately 6 participants will be enrolled to each sequence. Participants who withdraw from the study may be replaced at the sponsor's discretion in collaboration with the Investigator.

The total planned duration of participation, from the screening visit to the last Follow-up phone call, is approximately 10 weeks. Participants will be screened within 28 days prior to the first dose of investigational products and if all eligibility criteria are fulfilled, the participants will report to the CRU on Day -1 and will be required to stay in the CRU for 8 days and 7 nights.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

The primary endpoints are the plasma PK endpoints which are described in Section 3.3.

3.2. Secondary Endpoint(s)

The secondary endpoints related to PK are described in Section 3.3. The secondary endpoints related to safety/tolerability are described in Section 3.5.

3.3. Other Endpoint(s)

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

Plasma PK parameters of PF-07321332 will be derived (as data permit) from the concentration-time data using standard noncompartmental methods. Actual PK sampling times will be used in the derivation of PK parameters. 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 3. Noncompartmental PK Parameters of PF-07321332 to be calculated

Parameter	Analysis Scale	PF-07321332
AUC_{inf}*	ln	A, D
AUC_{last}	ln	A, D
C_{max}	ln	A, D
T_{max}	R	D
$t_{½}$*	R	D
CL/F*	ln	D
V_z/F*	ln	D

*=if data permits.
Abbreviations: A = analyzed using a statistical model; D=displayed with descriptive statistics as outlined in Table 4 in Section 6.1; ln=natural-log transformed; R=raw (untransformed)

T_{last} will also be provided as a support parameter for AUC_{last}. T_{last} values will only be listed and not summarized.

3.4. Baseline Variables

Baseline variables are those collected on Day 1 prior to dosing or before Day 1. Baseline for laboratory data, vital signs and ECG are defined in Sections 3.5.2, 3.5.3 and 3.5.4 respectively.

3.5. Safety Endpoints

3.5.1. Adverse Events

An adverse event is 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 end of the study will be flagged as TEAEs. The algorithm will not consider any events that started prior to the first dose date of PF-07321332.

A 3-tier approach for summarizing AEs will not be used due to the low number of participants planned to be recruited.

3.5.2. Laboratory Data

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

Baseline will be the last pre-dose measurement prior to administration of PF-07321332 in period 1.

To determine if there are any clinically significant laboratory abnormalities, the hematological, 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.

3.5.3. Vital Signs

Single supine blood pressure, respiratory rate, temperature and pulse measurements will be taken at times detailed in the SoA given in the protocol.

Baseline will be the last pre-dose measurement prior to administration of PF-07321332 in period 1.

The following vital signs endpoints will be determined:

- Change from baseline in supine systolic and diastolic blood pressure, pulse rate, temperature and respiratory rate.

3.5.4. ECG

12-lead ECGs will be recorded on all participants at times detailed in the SoA given in the protocol.

Baseline will be the last pre-dose measurement prior to administration of PF-07321332 in period 1.

The following ECG endpoints will be determined:

- Change from baseline in QT interval, heart rate, QTcF interval, PR interval, and QRS complex.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to unblinding and releasing the database and classifications will be documented per standard operating procedures.

Population	Description
Enrolled	"Enrolled" means a participant'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.
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.
PK Concentration Set	All participants randomly assigned to study intervention and who take at least 1 dose of study intervention and in whom at least 1 concentration value is reported.
PK Parameter Set	All participants randomly assigned to study intervention and who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of interest are reported.

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

There is no statistical hypothesis testing planned for this study and no statistical decision rules will be applied.

5.2. General Methods

Unless otherwise stated, all summaries and plots will be presented by treatment.

5.2.1. Analyses for Continuous Endpoints

Continuous variables will be presented using summary statistics: number of observations, arithmetic mean, standard deviation, cv%, median, minimum and maximum values.

Log transformed continuous variables will be presented using summary statistics: number of observations, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.

5.2.2. Analyses for Categorical Endpoints

Categorical variables will be presented using summary statistics: number of observations and percentages.

5.3. Methods to Manage Missing Data

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

In all PK concentration 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 (LLQ).

For PK summary tables and plots of mean/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/statistician.

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 dose with ≥3 evaluable measurements. For statistical analyses, 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

For all outputs produced, the following treatment labels and ordering will be used:

PF-07321332 300 mg/ritonavir 100 mg Fed
PF-07321332 300 mg/ritonavir 100 mg Fasted

6.1. Primary Endpoint(s)

The plasma PK parameters detailed in Section 3.3 will be listed and summarized for participants in the PK Parameter Set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3. Each PK parameter will be summarized by treatment. Each summary will include the set of summary statistics as specified in Table 4.

Table 4. PK Parameters to be Summarized Descriptively

Parameter	Summary Statistics
AUC_{last}, AUC_{inf}, C_{max}, CL/F and 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, standard deviation, minimum, maximum.

For AUC_{inf}, AUC_{last} and C_{max} a listing of the individual participant ratios (Treatment B (fed)/Treatment A (fasted)) will be provided. 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 $t_{½}$ will be listed by treatment where applicable: 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}. These data may be included in the CSR.

Presentations for PF-07321332 concentrations will be presented using participants in the PK Concentration Set (as defined in Section 4) and will include:

- a listing of all concentrations sorted by participant ID, treatment 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.

- 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 spaghetti plots for each participant per scale).

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

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

The scale used for the x-axis (time) of these plots will be decided on review of the data, and will depend on how long PF-07321332 concentration is quantifiable in the matrix.

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.

Natural log transformed AUC_{inf} (if data permit), AUC_{last} and C_{max} will be analyzed using a mixed effect model with sequence, period and treatment as fixed effects and participant within sequence as a random effect. The Kenward-Roger adjustment for the degrees of freedom will be used. 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. Treatment A (PF-07321332/ritonavir administered under fasted condition) is the Reference treatment and Treatment B (PF-07321332/ritonavir administered under fed condition) is the Test treatment.

6.2. Secondary Endpoint(s)

The secondary endpoints for plasma PK and their analyses are described Section 6.1.

6.3. Other Endpoint(s)

None.

6.4. Subset Analyses

No subset analyses will be performed.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Study Conduct and Participant Disposition

Participant evaluation groups will show participant disposition by treatment. Frequency counts and percentages will be supplied for participant discontinuation(s) by treatment. Data will be reported in accordance with the sponsor reporting standards.

6.5.2. Demographic Data

Demographic data (age, biological sex, race, ethnicity, weight, body mass index and height) will be summarized by treatment sequence and overall (if applicable) in accordance with the sponsor reporting standards.

6.5.3. Concomitant Medications and Nondrug Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be reported according to current sponsor reporting standards.

6.5.4. Other Screening Data

These data will not be recorded in the study database, and therefore will not be listed.

6.6. Safety Summaries and Analyses

All safety analyses will be performed on the Safety Analysis Set.

6.6.1. Adverse Events

Adverse events will be performed on the Safety Analysis Set.

6.6.2. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the sponsor reporting standards. Baseline is as defined in Section 3.5.2.

6.6.3. Vital Signs

Participants meeting the categorical criteria will be listed by treatment. All planned and unplanned post-dose time points will be counted in this listing. Absolute values and changes from baseline vital sign data will be listed.

6.6.4. ECG

ECG endpoints and changes from baseline (QTcF, PR and QRS) will also be listed by treatment using categories as defined in Appendix 1 (for QTcF these correspond to the Pfizer Guidance in Section 8). All planned and unplanned postdose time points will be counted in this listing. Absolute values and changes from baseline ECG data will be listed.

7. INTERIM ANALYSES

7.1. Introduction

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.

7.2. Interim Analyses and Summaries

N/A

8. REFERENCES

Pfizer Guidance for Evaluation of QT / QTc Interval Prolongation and Proarrhythmic Potential for Non-antiarrhythmic Drugs; Members of the Cardiovascular Safety & Advisory Council (CVSAC); January 26, 2018

9. APPENDICES

Appendix 1. Categorical Classes for ECG and Vital Signs of Potential Clinical Concern

Categories for QTcF

Absolute value of QTcF (msec)	>450 and ≤480	>480 and ≤500	>500
Increase from baseline in QTcF (msec)	>30 and ≤60	>60	

Categories for PR and QRS

PR (ms)	max. ≥300	
PR (ms) increase from baseline	Baseline >200 and max. ≥25% increase	Baseline ≤200 and max. ≥50% increase
QRS (ms)	max. ≥140	
QRS (ms) increase from baseline	≥50% increase	

Categories for Vital Signs

Systolic BP (mm Hg)	min. <90	
Systolic BP (mm Hg) change from baseline	max. decrease ≥30	max. increase ≥30
Diastolic BP (mm Hg)	min. <50	
Diastolic BP (mm Hg) change from baseline	max. decrease ≥20	max. increase ≥20
Supine pulse rate (bpm)	min. <40	max. >120

Appendix 2. List of Abbreviations

Abbreviation	Term
AE	adverse event
AUC	area under the curve
BLQ	below the limit of quantitation
BP	blood pressure
CI	confidence interval
CL/F	apparent clearance
C_{max}	maximum observed concentration
CRF	case report form
CSR	clinical study report
ECG	electrocardiogram
LLOQ	lower limit of quantification
LS	least-squares
LSM	least-squares mean
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	mixed-effects model with repeated measures
N/A	not applicable
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
T_{max}	time to reach C_{max}
$t_{½}$	terminal elimination half-life
V_z/F	apparent volume of distribution for extravascular dosing