Protocol C4671052

A Phase 1, Randomized, Fixed Sequence, Multiple-Dose, Open-Label Study to Estimate the Effect of Nirmatrelvir (PF-07321332)/Ritonavir on Rosuvastatin Pharmacokinetics in Healthy Adult Participants

Statistical Analysis Plan
(SAP)

Version: 1

Date: 26 May 2023

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

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 / 26 May 2023	Original 03 Apr 2023	N/A	N/A

2. INTRODUCTION

Nirmatrelvir is a potent and selective inhibitor of the SARS-CoV-2 M^{pro} that was developed for the treatment of COVID-19. Ritonavir is a strong CYP3A4 inhibitor being used to inhibit the metabolism of nirmatrelvir in order to increase plasma concentrations of nirmatrelvir to values that are efficacious.

The purpose of the study is to assess the effect of nirmatrelvir/ritonavir on the plasma PK of rosuvastatin, a sensitive OATP1B1 substrate. Both nirmatrelvir and ritonavir have the potential to inhibit OATP1B1. Rosuvastatin is considered a well-known sensitive substrate of OATP1B1 and is likely to be a concomitant medication in patients who have COVID-19 and are prescribed nirmatrelvir/ritonavir. Therefore, this study will assess the impact of nirmatrelvir/ritonavir on the pharmacokinetics of rosuvastatin.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C4671052.

2.1. Modifications to the Analysis Plan Described in the Protocol

None.

2.2. Study Objectives and Endpoints

Objectives	***Endpoints***
Primary:	***Primary:***
• *To assess the effect of multiple doses of nirmatrelvir/ritonavir on the plasma pharmacokinetics of a single, oral dose of rosuvastatin in healthy participants.*	• *AUC_{inf}, C_{max} of rosuvastatin.*
Secondary:	***Secondary:***
• *To further characterize the plasma pharmacokinetics of a single, oral dose of rosuvastatin administered with nirmatrelvir/ritonavir.*	• *AUC_{last}, T_{max}, $t_{½}$, CL/F, and V_z/F of rosuvastatin.*

Objectives	*Endpoints*
• *To evaluate the safety and tolerability of a single oral dose of rosuvastatin when co-administered with nirmatrelvir/ritonavir.*	• *Vital signs, laboratory tests and adverse events.*

2.3. Study Design

This is a Phase 1, randomized, fixed sequence, multiple-dose, open-label study of the effect of nirmatrelvir/ritonavir on rosuvastatin PK in healthy adult participants.

A total of approximately 12 healthy male and/or female participants will be enrolled in the study. Participants who discontinue from the study may be replaced at the sponsor's discretion. Participants will be screened within 28 days prior to the first dose of investigational product. Participants will report to the CRU the day prior to Day 1 dosing (i.e., Day -1) in Period 1. Participants will remain in the CRU for a total of 11 days and 10 nights (including Period 1 and Period 2). To adequately remove any drug effects of rosuvastatin from Period 1 to Period 2, there will be a minimum 5-day washout period between the 2 rosuvastatin dosing events. The total rosuvastatin PK will then be assessed over 72 hours.

Participants will receive 2 treatments across 2 periods, as outlined in Table 2 below:

Table 2. Treatment Sequence

Sequence	*Period 1*	*Washout*	*Period 2*
Sequence 1 (N = 12)	*Treatment A*	*At least 5 days between the 2 rosuvastatin dosing events*	*Treatment B*

N = number of enrolled participants.

- *Treatment A (rosuvastatin, Reference): Single oral administration of rosuvastatin 10 mg tablet on Day 1 in the morning (AM dose).*

- *Treatment B (rosuvastatin + nirmatrelvir/ritonavir, Test): Nirmatrelvir/ritonavir 300 mg (2 × 150 mg)/100 mg tablets q12h (BID) for 2 days (Day 1 morning [AM] and evening [PM] and Day 2 morning [AM], 3 doses total) with a single oral administration of rosuvastatin 10 mg tablet on Day 2 in the morning (AM dose). Nirmatrelvir and ritonavir will be dosed within no more than 5 minutes of each other. Rosuvastatin single dose will be dosed within 5 minutes after the Day 2 morning (AM) nirmatrelvir/ritonavir dose.*

The expected duration of participants from Screening to the follow-up telephone contact will be approximately 11 weeks.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

The primary endpoints are the plasma AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} of rosuvastatin. The test/reference ratios for AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} will be derived with Treatment B (rosuvastatin + nirmatrelvir/ritonavir) as the test treatment and Treatment A (rosuvastatin) as the reference treatment.

Plasma PK parameters of rosuvastatin will be derived (as data permit) from the concentration-time data using standard noncompartmental methods as outlined in the Table 3. Actual PK sampling times will be used in the derivation of rosuvastatin PK parameters when available. 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. Definitions of PK Parameters

Parameter	***Definition***	***Method of Determination***
*AUC_{inf}**	*Area under the concentration-time curve from time 0 extrapolated to infinity.*	*$AUC_{last} + (C_{last}*/k_{el})$, where C_{last}* is the predicted plasma concentration at the last quantifiable time point from the log-linear regression analysis.*
*AUC_{last}***	*Area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last})*	*Linear/Log trapezoidal method.*
C_{max}	*Maximum observed concentration*	*Observed directly from data*
T_{max}	*Time for C_{max}*	*Observed directly from data as time of first occurrence*
*$t_{½}$**	*Terminal half-life*	*$Log_e(2)/k_{el}$, where k_{el} is the terminal phase rate constant calculated by a linear regression of the loglinear concentration-time curve. Only those data points judged to describe the terminal loglinear decline will be used in the regression.*
*CL/F**	*Apparent clearance*	*$Dose/AUC_{inf}$*
*V_z/F**	*Apparent volume of distribution*	*$Dose/(AUC_{inf} \bullet k_{el})$*

**If data permit.*
***T_{last} will also be included in order to provide additional context for AUC_{last}. Tlast will only be listed, not summarized.*

3.2. Secondary Endpoints

3.2.1. Other PK parameters of rosuvastatin

The secondary endpoints include other plasma PK parameters of rosuvastatin such as AUC_{last}, T_{max}, $t_{1/2}$, CL/F and V_z/F.

3.2.2. Safety data

Other secondary endpoints are the safety and tolerability data, discussed in Section 3.5.

3.3. Baseline Variables

Baseline characteristics will be collected according to the schedule of activities (SoA) as specified in the protocol.

3.4. Other Endpoint

One predose blood sample for PK analysis of nirmatrelvir/ritonavir will be collected on Period 2 Day 2.

3.5. Safety Endpoints

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

- adverse events (AE)
- laboratory data
- vital signs data

3.5.1. Adverse Events

Any adverse events occurring following start of treatment will be considered as treatment emergent adverse event (TEAE). Events that occur during follow-up within the lag time of up to 35 days after the last dose of study intervention will be counted as treatment emergent and attributed to the last treatment taken. The time period for collecting AEs ("active collection period") for each participant begins from the time the participant provides informed consent.

3.5.2. 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 not take into account whether each participant's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

For both periods, the baseline measurement is the predose measurement on Period 1 Day -1. Changes from baseline will be defined as the change between the postdose and baseline measurements.

3.5.3. Vital Signs

Supine blood pressure (BP), pulse rate (PR) and temperature will be measured at times specified in the SoA given in the protocol.

For both periods, the baseline measurement is the predose measurement on Day 1 in each period. Changes from baseline will be defined as the change between the postdose and baseline measurements.

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 releasing the database and classifications will be documented per standard operating procedures.

Participant Analysis Set	***Description***
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.*
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.*
PK Concentration Set	*All participants who take at least 1 dose of study intervention and in whom at least 1 concentration value is reported.*
PK Parameter Set	*All participants who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of primary interest are reported.*

5. GENERAL METHODOLOGY AND CONVENTIONS

Final analysis will be performed after study participant data set release following last participant last visit.

5.1. Hypotheses and Decision Rules

No statistical hypothesis will be tested in this study.

5.2. General Methods

5.2.1. Analyses for Binary/Categorical Endpoints

For binary or categorical variables, number of participants, numbers and percentages of participants meeting the categorical criteria will be presented in accordance with the Clinical Data Interchange Standards Consortium and Pfizer Standards (CaPS).

5.2.2. Analyses for Continuous Endpoints

For continuous variables, the data will be summarized using the number of participants, mean, median, standard deviation (SD), minimum, and maximum in accordance with the CaPS. For appropriate PK parameters, geometric mean and geometric coefficient of variation (%CV) will also be summarized.

5.3. Methods to Manage Missing Data

5.3.1. Pharmacokinetic Data

Methods to handle missing PK data are described below.

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.

Deviations, Missing Concentrations and Anomalous Values:

In summary tables and plots of median profiles, statistics will be calculated having set concentrations to missing if one of the following cases is true:

1. A concentration has been collected as ND (i.e., not done) or NS (i.e., 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.

An anomalous concentration value is one that, after verification of bioanalytical validity, is grossly inconsistent with other concentration data from the same individual or from other participants. For example, a BLQ concentration that is between quantifiable values from the same dose is considered as anomalous. Anomalous concentration values may be excluded from PK analysis at the discretion of the PK analyst.

PK 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 (i.e., not calculated). (Note that NC values will not be generated beyond the day that a participant discontinues).

In summary tables, statistics will not be presented for a particular treatment group if more than 50% of the data are NC. For statistical analyses, PK parameters coded as NC will also be set to missing.

If an individual participant has a known biased estimate of a PK parameter (due for example to a dosing error or an unexpected event such as vomiting before all the compound is adequately absorbed from the gastrointestinal tract), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses. For instance, if a participant has a vomiting event post dose that is within a duration of time that is 2-times the derived median T_{max} for the population for the administered treatment, then the

pharmacokineticist should consider the exclusion of the PK concentration data collected following the initial vomiting event in that treatment period and the PK parameter data reported for that treatment period from the datasets used to calculate summary statistics or statistical analyses.

5.3.2. Safety Data

Missing values in standard summaries of safety data will be imputed according to CaPS.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

Plasma AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} of rosuvastatin will be summarized by treatment group and will include the set of summary statistics as specified in Table 4.

For the evaluation of the effect of multiple doses of nirmatrelvir/ritonavir on the PK of rosuvastatin, *natural log transformed AUC_{inf} (if data permit), AUC_{last} and Cmax will be analyzed using a mixed effect model with treatment as a 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. Treatment A (rosuvastatin) will be the Reference treatment while Treatment B (rosuvastatin + nirmatrelvir/ritonavir) will be the Test treatment.*

For AUC_{inf}, AUC_{last} and C_{max}, a listing of the individual participant ratios (Test/Reference) will be provided. Box and whisker plots for AUC_{inf}, AUC_{last} and C_{max} will be plotted by treatment and overlaid with geometric means.

Residuals from the models 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 CSR. 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.

6.2. Secondary Endpoints

6.2.1. Other PK parameters of rosuvastatin

Secondary endpoints include other PK parameters of rosuvastatin such as AUC_{last}, T_{max}, $t_{1/2}$, CL/F and V_z/F. The PK parameters will be listed and summarized descriptively by treatment group in accordance with Pfizer data standards on the PK Parameter Set, as data permit. Missing values will be handled as detailed in Section 5.3.1. Each PK parameter will be summarized by treatment group and will include the set of summary statistics as specified in Table 4.

Table 4. PK Parameters to be Summarized Descriptively by Treatment

Parameter	Summary Statistics
AUC_{inf}, AUC_{last}, C_{max}, CL/F, V_z/F	N, arithmetic mean, median, SD, %CV, minimum, maximum, geometric mean and geometric %CV
T_{max}	N, median, minimum, maximum
$t_{½}$	N, arithmetic mean, median, SD, %CV, minimum, maximum

Supporting data from the estimation of $t_{½}$ and AUC_{inf} will be listed by analyte and group: 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.

PK Concentrations:

The plasma concentrations 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 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.

Presentations of concentrations will include:

- A listing of all concentrations sorted by participant ID, treatment 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, SD, %CV, minimum, maximum and the number of concentrations above the LLQ.

- 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].

CP-1 samples will be collected for exploratory research of CP-1 as an endogenous biomarker for OATP1B1 inhibition, according to the SoA as specified in the protocol. *CP-1 biomarker data will be listed and descriptively summarized by nominal sampling time and by treatment.*

6.2.2. Safety data

Analyses and summaries of safety data are described in Section 6.5.

6.2.3. Other Endpoint

The plasma concentration of nirmatrelvir/ritonavir collected on Period 2 Day 2 will be listed by nominal and actual times.

6.3. Subset Analyses

There are no planned subset analyses.

6.4. Baseline and Other Summaries and Analyses

6.4.1. Demographic Summaries

Demographic characteristics will be summarized for enrolled population in accordance with the CaPS.

6.4.2. Study Conduct and Participant Disposition

Participant evaluation groups will show end of study participant disposition. Frequency counts will be supplied for participant discontinuation(s) by treatment. Data will be reported in accordance with the CaPS.

6.4.3. Study Treatment Exposure

Study treatment exposure will be listed.

6.4.4. Concomitant Medications and Nondrug Treatments

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

6.5. Safety Summaries and Analyses

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

Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

6.5.1. Adverse Events

AEs will be reported in accordance with the CaPS.

Participant discontinuations due to adverse events will be detailed by treatment. Data will be reported in accordance with the CaPS.

6.5.2. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the CaPS.

6.5.3. Vital Signs

Vital sign data will be listed and summarized by treatment in accordance with the CaPS.

7. INTERIM ANALYSES

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 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.

APPENDICES

Appendix 1. SAS Code for Analyses

An example of the PROC MIXED code is provided below:

For the primary objective:

```
proc mixed data=tab.pk;
        class trt participant;
        model l&var=trt/ ddfm=KR;
        random participant /subject=participant;
        lsmeans trt;
        estimate 'B vs A' 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
Treatment A: rosuvastatin (Reference)
Treatment B: rosuvastatin + nirmatrelvir/ritonavir (Test)      */
```

Appendix 2. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AE	adverse event
AUC_{extrap}%	the percent of AUC_{inf} based on extrapolation
AUC_{inf}	area under the plasma concentration-time profile from time zero extrapolated to infinite time
AUC_{last}	area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration
BID	twice daily
BLQ	below the limit of quantitation
BP	blood pressure
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CI	confidence interval
C_{last}	last quantifiable concentration
CL/F	apparent clearance after oral dose
C_{max}	maximum plasma concentration
COVID-19	coronavirus disease 2019
CP-1	coproporphyrin I
CRU	Clinical research unit
CSR	clinical study report
CYP3A4	cytochrome P450 3A4
ECG	electrocardiogram
k_{el}	terminal phase rate constant
LLQ	lower limit of quantitation
mg	milligram
M^{pro}	main protease
N/A	not applicable
NC	not calculated
ND	not done
NS	no sample
OATP1B1	organic anion transporting polypeptide 1B1
PK	pharmacokinetic(s)
PR	pulse rate
r^2	goodness of fit statistic from the log-linear regression
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SoA	schedule of activities
$t_{½}$	terminal elimination half-life
TEAE	treatment emergent adverse event
T_{max}	time for C_{max}
V_z/F	apparent volume of distribution after oral dose