Protocol *C4891009*

An Interventional, Phase 1, Open-Label, Fixed Sequence, 2-Period Study to Estimate the Effect of Multiple Doses of Itraconazole on the Pharmacokinetics Of Single Dose ARV-471 in the Fed Condition in Healthy Adult Males, and Females of Nonchildbearing Potential

Statistical Analysis Plan (SAP)

Version: 1

Date: 12 Sep 2022

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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 12 Sep 2022	Original 20 Jul 2022	N/A	N/A

2. INTRODUCTION

ARV-471 (also known as PF-07850327) is a potent, selective, orally bioavailable PROteolysis TArgeting Chimeric (PROTAC®) small molecule that induces degradation of the ER. ARV-471 is a hetero-bifunctional PROTAC molecule that simultaneously binds the estrogen receptor (ER) and the cereblon E3 ligase complex, enabling protein-protein interactions between ER and the ligase complex. As a result, the ER becomes poly-ubiquitinated on accessible lysine residues and subsequently undergoes targeted degradation by the proteasome to affect its elimination from cells.

Itraconazole and its primary metabolite (hydroxy-itraconazole) are specific strong inhibitors of CYP3A. Since ARV-471 is a substrate for CYP3A, concomitant administration of multiple doses of itraconazole along with ARV-471 may lead to increased systemic exposure of ARV-471. The objective of this study is to estimate the effect of multiple doses of itraconazole on the PK of ARV-471.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C4891009.

2.1. Modifications to the Analysis Plan Described in the Protocol

None.

2.2. Study Objectives, Endpoints, and Estimands

The following are the objectives and endpoints in this study. Estimand framework will not be applied to this Phase 1 study in healthy participants.

Objectives	*Endpoints*
Primary:	***Primary:***
• *To estimate the effect of multiple doses of itraconazole on the pharmacokinetics of a single oral dose of ARV-471.*	• *Plasma AUC_{inf} and C_{max} of ARV-471 and ARV-473, as data permits.*
Secondary:	***Secondary:***
• *To estimate the effect of multiple doses of itraconazole on the pharmacokinetics of a single oral dose of ARV-471.* • *To evaluate the safety and tolerability of a single dose of ARV-471 administered to healthy participants in the absence and presence of multiple doses of itraconazole under fed conditions.*	• *Plasma AUC_{last}, T_{max}, $t_{1/2}$, CL/F and V_z/F of ARV-471 and ARV-473, as data permits, and as appropriate.* • *Safety laboratory tests, physical examination, vital signs, electrocardiograms (ECG), concomitant medication and adverse event monitoring.*

2.3. Study Design

This is a Phase 1, open-label, 2-period fixed-sequence crossover study to estimate the effect of multiple doses of the strong CYP3A inhibitor, itraconazole, on ARV-471 and ARV-473 pharmacokinetics in healthy males, and females of nonchildbearing potential. A total of approximately 12 participants will be enrolled in the study. The study will consist of 2 periods; Period 1 = single dose of ARV-471 alone and Period 2 = multiple doses of itraconazole + single dose of ARV-471. Following Period 1, a washout period of at least 10 days must occur between the 2 single doses of ARV-471. Following administration of ARV-471 in each period, participants will undergo serial PK sampling. Participants who withdraw may be replaced at the discretion of the sponsor.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

The primary endpoints of the study are plasma AUC_{inf} (if data permit) and C_{max} of ARV-471 and ARV-473 following a single dose administration of ARV-471 (alone) and coadministered with multiple doses of itraconazole. Adjusted geometric mean ratios of AUC_{inf} and C_{max} will be derived. If data do not permit derivation of plasma AUC_{inf}, plasma AUC_{last} will be used in place of AUC_{inf}.

ARV-471 and ARV-473 PK parameters, as appropriate, following a single dose administration of ARV-471 will be derived from the ARV-471 and ARV-473 plasma concentration versus time profiles using non-compartmental methods as data permit. The PK

parameters to be assessed in this study, their definition, and method of determination are outlined in Table 2. Actual PK sampling times will be used in the derivation of PK parameters whenever possible. In the case that actual PK sampling times are not available, nominal PK sampling time may be used in the derivation of PK parameters.

Table 2. Definitions of PK Parameters

Parameter[a]	***Definition***	***Method of Determination***
AUC_{inf}	*Area under the plasma concentration-time profile from time zero extrapolated to infinite time*	$AUC_{last} + (C_{last}/k_{el})$, *where* C_{last} *is the predicted plasma concentration at the last quantifiable time point and* k_{el} *is the elimination rate constant estimated from the log-linear regression analysis.*
C_{max}	*Maximum plasma concentration*	*Observed directly from the data.*
AUC_{last}	*Area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration* (C_{last})	*Linear-log trapezoidal method.*
T_{max}	*Time for* C_{max}	*Observed directly from the data as time of first occurrence.*
$t_{½}$	*Terminal plasma elimination half-life*	$Log_e(2)/k_{el}$, *Only those data points judged to describe the terminal log-linear decline will be used in the regression.*
CL/F	*Apparent clearance after oral dose*	$Dose/AUC_{inf}$ *after oral dose.*
V_z/F	*Apparent volume of distribution after oral dose*	$Dose/(AUC_{inf} * k_{el})$ *after oral dose.*
C_{last}	*Last measurable observed concentration*	*Observed directly from the data.*
T_{last}	*The time for* C_{last}	*Observed directly from the data.*

a. As data permit.

3.2. Secondary Endpoints

The secondary endpoints are additional plasma PK parameters (including AUC_{last}, T_{max}, $t_{1/2}$, CL/F and V_z/F, defined in Table 2) of ARV-471 and ARV-473, as appropriate, (following a single dose administration of ARV-471 alone and with multiple doses of itraconazole).

Other secondary endpoints of the study include the overall safety profile of a single dose of ARV-471 alone and with multiple doses of itraconazole, as characterized by laboratory tests, vital signs, ECG, concomitant medication and adverse events (discussed in Section 3.5).

3.3. Other Endpoint(s)

None.

3.4. Baseline Variables

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

3.5. Safety Endpoints

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

- Adverse events (AE)
- Laboratory data
- Vital signs data
- ECG results
- Concomitant medication

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 28 days after the last ARV-471 dose will be counted as treatment emergent and attributed to the last treatment taken. Events that occur during the washout period (up to 28 days from the last treatment) between study periods will be counted as treatment emergent and attributed to the previous 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 participants's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

For Period 1, the baseline measurement is the predose measurement on Day -1. For Period 2, the baseline measurement for itraconazole alone is the predose measurement on Day -1 and for ARV-471 with itraconazole the baseline measurement is the predose measurement on Day 4. Changes from baseline will be defined as the change between the postdose and baseline measurements.

3.5.3. Vital Signs

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

For Period 1, the baseline measurement is the predose measurement on Day 1. For Period 2, the baseline measurement is the predose measurement on Day 5. Changes from baseline will be defined as the change between the postdose and baseline measurements.

3.5.4. Electrocardiograms

QT interval, QTcF, PR, QRS and heart rate (HR) will be recorded at each assessment time indicated in the SoA given in the protocol. If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

QTcF = QT / (RR)^(1/3) where RR = 60/HR (if not provided)

For Period 1, the baseline value is the average of the triplicate ECG measurements collected before dose administration on Day 1. For Period 2, the baseline value is the average of the triplicate ECG measurements collected before dose administration on Day 5. Changes from baseline will be defined as the change between the postdose ECG measurement and the derived baseline ECG.

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 over the respective period 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.

3.5.5. Concomitant Medication

Concomitant medications are documented in the case report form (CRF) as treatments taken after the first dose of study intervention.

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	*"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 assignment to study intervention. 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.*
Safety Analysis Set	*All participants enrolled and who take at least 1 dose of study intervention.*

Participant Analysis Set	***Description***
PK Concentration Analysis Set	*All participants who received at least 1 dose of ARV-471 and/or itraconazoleand have at least 1 measurable ARV-471 or ARV-473 concentration.*
PK Parameter Analysis Set	*All participants who received at least 1 dose of ARV-471 and/or itraconazole and have at least 1 of the ARV-471 or ARV-473 PK parameters of primary interest.*

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 (ie, not done) or NS (ie, no sample).

2. A deviation in sampling time is of sufficient concern or a concentration has been flagged as 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 or pharmacokineticist.

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 (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. PK parameter 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 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.

5.3.2. Safety Data

Missing values in standard summaries of AEs, concomitant medications, laboratory data, vital signs, and ECGs will be imputed according to CaPS.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

The primary endpoints AUC_{inf} and C_{max} will be summarized descriptively by treatment and will include the set of summary statistics as specified in Table 3. AUC_{last} will also be included in case AUC_{inf} cannot be derived. Individual participant parameters for AUC_{inf} (if data permit, otherwise AUC_{last}) and C_{max} will be plotted by treatment and overlaid with geometric mean. Summary profiles (means and medians) of concentration time data will be plotted by treatment on linear and semi log scale. Individual participant concentration time profiles will also be presented. For summary statistics and summary 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 ARV-471 and ARV-473 AUC_{inf} (if data permit), AUC_{last} and C_{max} 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% CIs for the ratios. Treatment A (ARV-471 given alone) is the Reference Treatment while Treatments B (ARV-471 given after multiple doses of itraconazole) is 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 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 provided in the clinical study report.

6.2. Secondary Endpoints

6.2.1. Other PK parameters

AUC_{last}, T_{max}, $t_{1/2}$, CL/F, V_z/F, C_{last} and T_{last} *will be summarized descriptively by treatment* and will include the set of summary statistics as specified in Table 3. AUC_{last} will be analyzed using the same mixed effect model used in the primary endpoints.

PK parameter summaries:

The ARV-471 and ARV-473 PK parameters, as appropriate, will be summarized descriptively by treatment in accordance with Pfizer data standards for the PK Parameter Analysis Set, as data permit. A listing of the individual participant ratios (Test-Reference) will be provided. Missing values will be handled as detailed in Section 5.3.1. Each ARV-471 and ARV-473 PK parameter will be summarized by treatment and will include the set of summary statistics as specified in Table 3.

Table 3. PK Parameters to be Summarized Descriptively by Treatment

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

PK concentration summaries:

The plasma concentrations of ARV-471 and ARV-473 will be listed and descriptively summarized by nominal PK sampling time and treatment for the PK Concentration Analysis Set. Individual participant and summary profiles (mean and median plots) of the plasma concentration-time data will be plotted by treatment using actual and nominal times,

respectively. Mean and median ARV-471 and ARV-473 plasma concentration profiles will be presented on both linear and semi-log scales.

Presentations for ARV-471 and ARV-473 plasma 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 provided 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].

6.2.2. Safety data

Safety data will be analyzed in accordance with the CaPS (see Section 6.5).

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

Participants evaluation groups will show end of study participant disposition. Frequency counts will be supplied for participant discontinuation(s) by treatment (ARV-471 alone, ARV-471 with itraconazole, and itraconazole alone). 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 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.

Safety data will be summarized for three (3) separately defined treatments: (a) ARV-471 alone (from Period 1, Day 1 up to just before the first dose of itraconazole administered alone in Period 2, Day 1), (b) itraconazole alone (from first dose of itraconazole administered alone in Period 2, Day 1 up to just before coadministration of ARV-471 with itraconazole), and (c) itraconazole with ARV-471 (from coadministration of ARV-471 with itraconazole in Period 2, Day 5 through the end of the study).

A set of summary tables split by these 3 treatments will be produced to evaluate any potential risk associated with the safety and toleration of administering ARV-471 alone, itraconazole alone, and ARV-471 in the presence of itraconazole.

6.5.1. Adverse Events

Adverse events 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 signs data will be listed and summarized by treatment in accordance with the CaPS.

6.5.4. Electrocardiograms

Changes from baseline for the ECG parameters HR, QTcF , PR interval, and QRS complex will be summarized by treatment and time. The frequency of uncorrected QT values above 500 ms will be tabulated.

The number (%) of participants with maximum postdose QTcF values and maximum increases from baseline in the following categories will be tabulated by treatment:

Safety QTcF Assessment

Degree of Prolongation	***Mild (ms)***	***Moderate (ms)***	***Severe (ms)***
Absolute value	*>450-480*	*>480-500*	*>500*
Increase from baseline		*30-60*	*>60*

In addition, PR and QRS maximum values and maximum increases from baseline will also be tabulated by treatment using the following categories:

Safety PR and QRS Assessment

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

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.

7.1. Introduction

Not applicable.

7.2. Interim Analyses and Summaries

Available safety and PK data may be reviewed.

APPENDICES

Appendix 1. Summary of Analyses

Endpoint	Population	Data Inclusion and Rules for Handling Intercurrent Events and Missing Data	Analysis Method
Ratio of ARV-471 and ARV-473 AUC_{inf}, C_{max}, AUC_{last}	PK Parameter Analysis Set	Observed data	Mixed effect ANOVA model
ARV-471 and ARV-473 PK parameters	PK Parameter Analysis Set	Observed data	Descriptive statistics
ARV-471 and ARV-473 PK concentrations	PK Concentration Analysis Set	Observed and imputed (Section 5.3.1) data	Descriptive statistics
Safety data	Safety Analysis Set	Observed and imputed (Section 5.3.2) data	Descriptive statistics

Appendix 2. SAS Code for Analyses

An example of the PROC MIXED code is provided below:

For primary and secondary objectives:

- **Treatment B (Test) vs Treatment A (Reference)**

```
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
A: single dose of ARV-471 alone
B: multiple doses of itraconazole + single dose of ARV-471
*/

Appendix 3. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AE	adverse event
ANOVA	analysis of variance
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
BLQ	below the limit of quantitation
BP	blood pressure
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CI	confidence interval
CL/F	apparent clearance
C_{last}	predicted plasma concentration at the last quantifiable time point from the log-linear regression analysis
C_{max}	maximum plasma concentration
CRF	case report form
CSR	clinical study report
CYP3A	cytochrome P450, family 3, subfamily A
ECG	electrocardiogram
ER	estrogen receptor
HR	heart rate
k_{el}	elimination rate constant estimated from the log-linear regression analysis
LLQ	lower limit of quantitation
ms	milliseconds
N/A	not applicable
NC	not calculated
ND	not done
NS	no sample
PK	pharmacokinetic(s)
PR	pulse rate
QRS	Combination of Q-, R- and S- wave on an electrocardiogram representing ventricular depolarization
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
rBA	relative bioavailability
RR	respiratory rate
SAP	statistical analysis plan
SD	standard deviation
SoA	schedule of activities

Abbreviation	Term
$t_{1/2}$	terminal plasma elimination half-life
TEAE	treatment emergent adverse event
T_{max}	time for C_{max}
V_z/F	apparent volume of distribution after oral dose