Protocol C3991041

A PHASE 1, OPEN-LABEL, FIXED-SEQUENCE STUDY TO EVALUATE THE EFFECT OF ITRACONAZOLE AND CYCLOSPORINE ON THE SINGLE-DOSE PHARMACOKINETICS OF PF-07081532 IN OVERWEIGHT OR OBESE ADULT PARTICIPANTS

Statistical Analysis Plan
(SAP)

Version: 1.0

Date: 22 Mar 2023

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APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1.0 / 22 Mar 2023	Original 31 Jan 2023	N/A	N/A

2. INTRODUCTION

GLP-1 is a neuroendocrine hormone that is predominantly released from the small intestine in response to food intake. GLP-1 activation of the GLP-1R stimulates insulin release, inhibits glucagon secretion in a glucose-dependent manner, and delays gastric emptying. In addition, GLP-1 has been shown to increase satiety and suppress food intake.

PF-07081532 is an orally administered, potent and selective GLP-1R agonist in development as adjunct to diet and exercise, to improve glycemic control in T2DM, and for chronic weight management in a population that is overweight with co-morbidities or who have obesity.

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C3991041.

2.1. Modifications to the Analysis Plan Described in the Protocol

None.

2.2. Study Objectives and Endpoints

Objectives	*Endpoints*
Primary:	***Primary:***
• *To estimate the effect of MD itraconazole on the single-dose PK of PF-07081532 in otherwise healthy, overweight or obese participants.*	• *PF-07081532 PK parameter: AUC_{inf} (if data permit[a] otherwise AUC_{last})*
• *To estimate the effect of SD cyclosporine on the single-dose PK of PF-07081532 in otherwise healthy, overweight or obese participants.*	• *PF-07081532 PK parameter: AUC_{inf} (if data permit[a] otherwise AUC_{last})*
Secondary:	***Secondary:***
• *To evaluate the safety and tolerability of PF-07081532 administered separately and in combination with itraconazole or cyclosporine in otherwise healthy, overweight or obese participants.*	• *Assessment of TEAEs, clinical laboratory abnormalities, vital signs, body weight, and ECG parameters.* • *Assessment of mental health as determined by C-SSRS and PHQ-9.*
• *To evaluate the effects of itraconazole or cyclosporine on additional PK parameters for PF-07081532.*	• *Additional plasma PK parameters for PF-07081532: C_{max} and T_{max}; and CL/F, V_z/F, $t_{½}$, as data permit.*

a. Should it be deemed that too few AUC_{inf} estimates (eg, less than 12 for a single treatment) are obtained from the evaluable participants, AUC_{last} may be selected as the primary endpoint for CSR reporting.

2.3. Study Design

This is a Phase 1, open-label, fixed-sequence, 3-period study to evaluate the effect of multiple doses of itraconazole and a single dose of cyclosporine on the single-dose PK of PF-07081532 in otherwise healthy, overweight or obese adult participants. The 3 study periods will be conducted consecutively without a break.

All participants will provide informed consent and undergo Screening evaluations to determine their eligibility. Screening will occur within 28 days of the first dose of study intervention on Period 1 Day 1. Participants who discontinue from the study before completing all assessments may be replaced at the discretion of the investigator and sponsor.

Approximately 16 participants will be enrolled in the study such that approximately 12 evaluable participants complete the study.

The total duration of participation from the Screening Visit to the F/U telephone contact will be approximately 82 days or 12 weeks, approximately 3 weeks of which will be conducted on an inpatient basis. The 21-day inpatient portion of the study will be conducted as follows:

***Period 1**: Days -1 to 5 (Study Days -1 to 5; participants are admitted to the CRU on Day -1), 40 mg SD PF-07081532 on Day 1;*

***Period 2**: Days 1 to 5 (Study Days 6 to 10), 40 mg SD PF-07081532 plus 600 mg SD cyclosporine on Day 1;*

Period 3*: Days 1 to 10 (Study Days 11 to 20; participants are discharged from the CRU on Study Day 20), 200 mg itraconazole QD ×9 days plus 40 mg SD PF-07081532 on Day 4.*

A telephone F/U contact will occur 28-35 days from the last dose of study intervention (Period 3, Day 9).

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

The primary endpoints are plasma PF-07081532 AUC_{inf} (if data permit, otherwise AUC_{last}) when administered with MD itraconazole and with SD cyclosporine.

3.2. Secondary Endpoint(s)

The secondary endpoints are the following:

- Safety and tolerability data, discussed in Section 0.
- Assessment of mental health as determined by C-SSRS and PHQ-9.
- Additional plasma PK parameters (C_{max} and T_{max}; and CL/F, V_z/F, $t_{½}$, as data permit) for PF-07081532.

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

- adverse events (AE)
- laboratory data
- body weight
- mental health
- vital signs data
- electrocardiogram (ECG) results

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 hematological, clinical chemistry (serum) and urinalysis safety tests will be assessed against the criteria specified in the sponsor's 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.

The baseline measurement is the latest predose measurement on Day 1 of Period 1.

3.5.3. Body Weight

Body weight will be measured at times specified in the SoA given in the protocol.

The baseline measurement is the predose measurement on Day 1 of Period 1.

3.5.4. Vital Signs

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

The baseline measurement is the latest predose measurement on Day 1 of Period 1.

3.5.5. 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. QTcF will be derived using Fridericia's heart rate correction formula:

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

The baseline measurement is the latest measurement on Day 1 of Period 1.

3.5.6. Mental health

3.5.6.1. Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is an interview-based rating scale to systematically assess suicidal ideation and suicidal behavior. The "baseline/screening" version of the C-SSRS will be administered at Screening and Day -1 in study. Participants who respond "yes" to Question 4 or 5 (indicating suicidal ideation), or to any suicidal behavioral question on the C-SSRS at

screening or Day -1 will not be permitted in the study (see CCI *The "since last visit" version of the C-SSRS will be administered at the time points specified in the SoA. The C-SSRS will be administered by study site staff who have completed training in its administration. Participants who have recurrent suicidal ideation or behavior during the study should be discontinued from the study and treated appropriately. If a study participant endorses a 4 or 5 on the ideation subscale or any behavioral item of the C-SSRS on 2 or more occasions and is confirmed to have active suicidal ideation or behavior on both occasions by a risk assessment conducted by a qualified MHP, then the participant should be discontinued from the study and treated appropriately.*

3.5.6.2. Patient Health Questionnaire-9 (PHQ-9)

The PHQ9-9 is a 9 item self-report scale for the assessment of depressive symptoms. The PHQ-9 will be completed by participants and reviewed by site staff at the pre-defined time points outlined in the SoA. A PHQ-9 score of ≥15 at Screening or Day -1 indicates clinically significant depression and serves as an exclusion criterion for this study (see section 5.2 of the protocol*).*

The PHQ-9 is a 9 item self-report scale for the assessment of depressive symptoms. The PHQ-9 will be completed by participants and reviewed by site staff at the pre-defined time points outlined in the SoA. The total score will be derived by adding the corresponding values of responses to each item. The total score ranges from 0 to 27, with the following interpretation:

Total Score	Depression Severity
1-4	Minimal depression
5-9	Mild depression
10-14	Moderate depression
15-19	Moderately severe depression
20-27	Severe depression

A participant with a postbaseline assessment with a score of ≥ 15 should be referred to a MHP.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

For purposes of analysis, the following analysis sets are defined:

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*

Participant Analysis Set	*Description*
	the study, are not considered enrolled, unless otherwise specified by the protocol.
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 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, and 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 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.

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 (ie, 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 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.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

Natural log_e transformed AUC_{inf} (if data permit, otherwise AUC_{last}) *of PF-07081532 administered without cyclosporine or coadministered with cyclosporine 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 models. 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. The test treatment will be 'PF-07081532 40 mg singe dose and cyclosporine' (Period 2), which will be reported separately in comparison to the reference treatment of 'PF-07081532 40 mg singe dose without cyclosporine' (Period 1).

Natural $\log_e$ transformed AUC_{inf} (if data permit, otherwise AUC_{last}) *of PF-07081532 administered without itraconazole or coadministered with itraconazole 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 models. 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. The test treatment will be 'PF-07081532 40 mg singe dose and itraconazole' (Period 3), which will be reported separately in comparison to the reference treatment of 'PF-07081532 40 mg singe dose without itraconazole' (Period 1).*

6.2. Secondary Endpoints

Natural $\log_e$ transformed CCI [redacted] of PF-07081532 administered without cyclosporine or coadministered with cyclosporine 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 models. 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. The test treatment will be 'PF-07081532 40 mg singe dose and cyclosporine' (Period 2), which will be reported separately in comparison to the reference treatment of 'PF-07081532 40 mg singe dose without cyclosporine' (Period 1).

Natural $\log_e$ transformed CCI [redacted] of PF-07081532 administered without itraconazole or coadministered with itraconazole 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 models. 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. The test treatment will be 'PF-07081532 40 mg singe dose and itraconazole' (Period 3), which will be reported separately in comparison to the reference treatment of 'PF-07081532 40 mg singe dose without itraconazole' (Period 1).

6.2.1. PK Concentrations

The plasma concentrations of PF-07081532 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].

6.2.2. PK Parameters

The PK parameters will be summarized descriptively by treatment group in accordance with Pfizer data standards on the PK Parameter Analysis Set, as data permit. *PK parameters, including plasma AUC_{inf}, AUC_{last}, C_{max}, T_{max}, and CL/F, V_z/F, $t_{½}$, as data permit, of PF-07081532 will be summarized descriptively by treatment. For AUC_{inf}, a listing of the individual participant ratios (Test/Reference) will be provided. Box and whisker plots for AUC_{inf} will be plotted by treatment.* 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 .

Table 2. 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 t½ 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.

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 the 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 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

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. Body Weight

Observed value and change from baseline in body weight will be summarized descriptively by treatment.

6.5.4. Vital Signs

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

6.5.5. Electrocardiograms

Changes from baseline for the ECG parameters HR, QTcF, PR interval, and QRS complex will be summarized by period 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 period:

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*

Baseline will be pre-dose on Day 1.

6.5.6. Mental Health

C-SSRS and PHQ-9 data will be listed.

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:

Treatment B (Test) vs Treatment A (Reference)

```
proc mixed data=tab.pk;
        where trt in ("A","B");
        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;
```

Treatment C (Test) vs Treatment A (Reference)

```
proc mixed data=tab.pk;
        where trt in ("A","C");
        class trt participant;
        model l&var=trt / ddfm=KR;
        random participant / subject=participant;
        lsmeans trt;
        estimate 'C 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: 40 mg SD PF-07081532
B: 40 mg SD PF-07081532 plus 600 mg SD cyclosporine
C: 200 mg itraconazole QD ×9 days plus 40 mg SD PF-07081532
*/
```

Appendix 2. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AE	adverse event
AUC_{24}	area under the plasma concentration-time profile from time zero to time 24 hours
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 (C_{last})
BLQ	below the limit of quantitation
BMI	body mass index
BP	blood pressure
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CI	confidence interval
C_{last}	predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis
CL/F	apparent oral clearance
C_{max}	maximum plasma concentration
C-SSRS	Columbia Suicide Severity Rating Scale
CSR	clinical study report
ECG	electrocardiogram
EE	ethinyl estradiol
HR	heart rate
k_{el}	the terminal phase rate constant calculated by a linear regression of the loglinear concentration-time curve
LE	levonorgestrel
LLQ	lower limit of quantitation
MD	multiple dose
mg	milligram
MHP	mental health professional
MR	metabolite to parent ratio
$MRAUC_{inf}$	1-hydroxymidazolam AUC_{inf}/midazolam AUC_{inf}; 5-hydroxyomeprazole AUC_{inf}/omeprazole AUC_{inf}
N/A	not applicable
NC	not calculated
ND	not done
NS	no sample
OC	oral contraceptive
PHQ-9	Patient Health Questionnaire-9
PK	pharmacokinetic(s)
PR	pulse rate

Abbreviation	Term
QD	once daily
QRS	Combination of Q-, R- and S- wave on an electrocardiogram representing ventricular depolarization
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
RR	R-R interval from ECG
SAP	statistical analysis plan
SD	single dose; standard deviation
SoA	schedule of activities
$t_{1/2}$	terminal elimination half-life
T2DM	type 2 diabetes mellitus
TEAE	treatment emergent adverse event
T_{max}	time to C_{max}
V_z/F	apparent volume of distribution