Protocol *C3601006*

AN OPEN-LABEL, PARALLEL-GROUP, PHARMACOKINETIC STUDY OF MULTIPLE INTRAVENOUS DOSES OF AZTREONAM AND AVIBACTAM IN SUBJECTS WITH SEVERE RENAL IMPAIRMENT AND NORMAL RENAL FUNCTION

Statistical Analysis Plan (SAP)

Version: **2.0**

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Revision History

Version	Date	Author(s)	Summary of Changes/Comments
1	July 29, 2018	PPD	Not Applicable
2	August 27, 2021	PPD	• **Section 2.2:** Addition of an exploratory objective: To determine concordance/comparability of capillary microsample plasma concentrations with venous plasma concentrations for aztreonam and avibactam in healthy subjects with normal renal function (Cohort 1). • **Section 8.2.2:** Addition of statistical analysis methodology about Capillary Plasma Microsamples.

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

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

This Statistical Analysis Plan for Study C3601006 is based on the Protocol Amendment 1, dated 14 February 2021 as follows:

- Added a tertiary/exploratory objective to Section 2.2, to determine concordance/comparability of capillary microsample plasma concentrations with venous plasma concentrations for aztreonam and avibactam.
- New Section 8.2.2 added to describe additional scatter plots.

2. INTRODUCTION

Aztreonam-avibactam (ATM-AVI) is a combination product that contains the β-lactam antibiotic aztreonam (ATM) and the non-β-lactam β-lactamase inhibitor avibactam (AVI). ATM-AVI is being developed to treat infections caused by resistant Gram-negative pathogens, including those with metallo-β lactamase (MBL)-mediated drug resistance.

Dose adjustment for aztreonam and avibactam are warranted in renal impairment given that both are eliminated primarily as unchanged substances by the kidney. This study is therefore being conducted to evaluate the effect of severe renal impairment on the PK, safety and tolerability of ATM-AVI. Results from this study along with previous renal impairment PK data from each of the ATM-AVI components will be used to confirm proposed ATM-AVI dosing in severe renal impairment which was based on modelling/simulation.

2.1. Study Design

This is a Phase 1, open-label, parallel-group study where an IV loading dose (30 min infusion) followed by multiple IV doses (3 hr infusion) of ATM-AVI will be administered to subjects with severe renal impairment (not on dialysis) and to healthy subjects with normal renal function. Subjects with the following levels of renal function will be enrolled:

Table 1. Group Assignment Based on eGFR (estimated glomerular filtration rate) Estimates

Cohort (n)	*Description*	*Estimated eGFRa (mL/min)*
1 (n=6 subjects)	*None (Normal)*	*≥80*
2 (n=5 to 6 subjects)	*Severe (Not on dialysis)*	*>15 - ≤30*

Abbreviations: BSA = body surface area; eGFR = estimated glomerular filtration rate; MDRD = Modification of Diet in Renal Disease.

a. Estimate of eGFR based on the MDRD formula adjusting for BSA. The Day -2 eGFR values will be used for group placement.

- *Step 1: eGFR (mL/min/1.73 m^2) = 175 × $(S_{cr,\ std})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American) where $S_{cr,\ std}$ denotes serum creatinine measured with a standardized assay.*

- *Step 2: Convert the MDRD-derived, BSA-adjusted eGFR obtained above to absolute eGFR (mL/min) for eligibility assessment using the following equation:*

    - eGFR (mL/min) = eGFR (mL/min/1.73 m^2) × subject's BSA where BSA is calculated as BSA = (Weight$^{0.425}$ × Height$^{0.725}$) × 0.007184.

Approximately 6 subjects will be enrolled into Cohorts 1 and 2. Due to the potential difficulty in recruiting subjects with eGFR ≤30 mL/min, the number of subjects to be enrolled in this group will be approximately 5-6 in order to have at least 5 evaluable subjects.

CL_{CR} will also be estimated from a spot serum creatinine measurement using the following Cockcroft-Gault (C-G) equation:

1. CL_{cr} (mL/min) = $\frac{[140 - \text{Age (years)}] \text{ x total body weight (kg) x (0.85 for females)}}{72 \text{ x serum creatinine (mg/dL)}}$

Note that eGFR calculated by the MDRD equation will be used for group placement. Nevertheless, renal function will be estimated using both C-G and MDRD equations in this study.

All subjects must have stable renal function to enter the study. Stable renal function is defined as ≤25% difference between 2 measurements of eGFR obtained, from the same laboratory, on 2 separate occasions during the screening period that are at least 72 hours but no more than 14 days apart. Additionally, difference between eGFR Screening 1 value and Day -2 value should be ≤25%. The Day -2 eGFR value will be used for group placement. The CrCL value will be recorded at the same time eGFR is determined.

The total participation time for each subject in this study will be approximately 6 days (excluding Screening).

2.2. Study Objectives

Primary Objective

- *To evaluate the pharmacokinetics of the co-administration of aztreonam and avibactam following multiple doses via IV infusion in subjects with severe renal impairment and in healthy subjects with normal renal function.*

Secondary Objective

- *To evaluate the safety and tolerability of multiple doses of aztreonam and avibactam in subjects with severe renal impairment and in healthy subjects with normal renal function.*

Tertiary/Exploratory Objective

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- *To determine concordance/comparability of capillary microsample plasma concentrations with venous plasma concentrations for aztreonam and avibactam in healthy subjects with normal renal function.*

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

No formal interim analysis will be conducted for this study. However, 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 dose-escalation decisions, facilitating PK/PD modeling, and/or supporting clinical development.

Final analysis will follow the official database release. As this will be an open-label study, there is no formal unblinding of the randomization code.

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

No hypotheses are required.

4.2. Statistical Decision Rules

No decision rules are required.

5. ANALYSIS SETS

5.1. Pharmacokinetic (PK) Analysis Set

5.1.1. Concentration Analysis Set

The PK concentration population is defined as all subjects treated with study drug who have at least 1 ATM and AVI concentration measurement.

5.1.2. Parameter Analysis Set

The PK parameter analysis population is defined as all subjects treated with study drug who have at least 1 of the PK parameters of interest.

5.2. Pharmacodynamic Analysis Set

None.

5.3. Safety Analysis Set

All subjects who receive at least 1 dose of study medication will be included in the safety analyses and listings.

5.4. Other Analysis Sets

None.

5.5. Treatment Misallocations

All analyses will be performed on an "as-treated" basis and will not include data from subjects who are allocated to a cohort but not treated.

If a subject takes a treatment that is not consistent with the cohort they are allocated to, for example takes a treatment out of sequence or takes the same treatment twice, then they will be reported under the treatment that they actually receive for all safety, PK and pharmacodynamic analyses, where applicable.

5.6. Protocol Deviations

Subjects who experience events that may affect their PK profile (eg, lack of compliance with dosing) may be excluded from the PK analysis. At the discretion of the pharmacokineticist a concentration value may also be excluded if the deviation in sampling time is of sufficient concern or if the concentration is anomalous for any other reason.

A full list of protocol deviations will be compiled and reviewed to identify major and minor deviations prior to database closure.

5.6.1. Deviations Assessed Prior to Randomization

At Screening, the investigator will assess subjects against the inclusion and exclusion criteria as set out in Sections 4.1 and 4.2 of the protocol.

5.6.2. Deviations Assessed Post-Randomization

A full list of protocol deviations for the study report will be compiled prior to database closure. Any significant deviation from the protocol will be reviewed prior to database closure and a decision taken regarding evaluation for each analysis population.

6. ENDPOINTS AND COVARIATES

6.1. Efficacy Endpoint(s)

None.

6.2. Safety Endpoints

Any events occurring following start of treatment or increasing in severity will be counted as treatment emergent.

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

- *adverse events,*

- *laboratory data,*
- *vital signs data,*
- *ECG results.*

6.3. Other Endpoints

6.3.1. PK Endpoints

Blood and urine samples for PK analysis of aztreonam and avibactam will be taken according to the Schedule of Activities given in the protocol.

The following PK parameters will be calculated for aztreonam and avibactam (if possible) from the concentration-time data using standard noncompartmental methods:

Table 2. Noncompartmental PK Parameters

Matrix	PK Parameter	Analysis Scale	Aztreonam and Avibactam
Plasma	$AUC_{0\text{-}24,ss}$	ln	A, D
	$AUC_{0\text{-}\tau}$	ln	D
	C_{max}	ln	A, D
	T_{max}	R	D
	$t_{1/2}^{*}$	R	D
	C_{τ}	ln	D
	CL^{*}	ln	D
	V_Z^{*}	ln	D
	V_{ss}	ln	D
Urine	CL_R	ln	D
	$Ae_{0\text{-}\tau}$	R	D
	$Ae_{0\text{-}\tau}$ (%)	R	D

Key: A=analyzed using statistical model, D=displayed with descriptive statistics, ln=natural-log transformed, R=raw (untransformed), *=if data permits, ss=steady state, τ = 6 hours for normal renal function (Cohort 1) and 8 hours for severe renal impairment (Cohort 2).

6.3.2. PD Endpoints

None.

6.4. Covariates

None.

7. HANDLING OF MISSING VALUES

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

7.1. Concentrations Below the Limit of Quantification

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. (In listings BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification).

7.2. Deviations, Missing Concentrations and Anomalous Values

In summary tables and plots of median profiles, statistics will be calculated having set concentrations to missing if 1 of the following cases is true:

1. A concentration has been collected as ND (ie not done) or NS (ie no sample).

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

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

7.3. Pharmacokinetic Parameters

Actual PK sampling times will be used in the derivation of PK parameters.

If a PK parameter cannot be derived from a subject'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 subject discontinues).

In summary tables, statistics will be calculated by setting NC values to missing; and statistics will be presented for a particular renal function group with ≥3 evaluable measurements. For statistical analyses (ie, analysis of variance), PK parameters coded as NC will also be set to missing; and analyses will not be performed for a particular parameter if more than 50% of the data are NC.

If an individual subject has a known biased estimate of a PK parameter (due for example to an unexpected event such as vomiting before all the compound is adequately absorbed in the body), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methods

The effect of the renal impairment on PK parameters will be assessed by constructing 90% confidence intervals around the estimated difference between each of the Test (severe renal impairment group) and the Reference (normal renal function group) using a one-way ANOVA model based on natural log transformed data.

8.2. Statistical Analyses

8.2.1. Pharmacokinetic Analysis

Analysis of variance (ANOVA) will be used to compare the natural log transformed $AUC_{0-24,ss}$ and C_{max} between the normal renal function group and the severe renal impairment group. The geometric least squares mean point estimate and the associated 90% confidence intervals (CIs) for the difference of each comparison will be estimated.

Aztreonam and avibactam PK parameters $AUC_{0-24,ss}$, C_{max}, $AUC_{0-\tau}$, T_{max}, C_{τ}, $t_{½}$, CL, CL_r, V_Z, V_{ss}, $Ae_{0-\tau}$, $Ae_{0-\tau}$% will be summarized descriptively by group.

Box plots of mean, median and individual subject parameters will be made across both groups for $AUC_{0-24,ss}$, C_{max} and C_{τ}. Concentrations will be listed and summarized descriptively by PK sampling time and group. Summary profiles (means and medians) of the concentration-time data will be plotted across different groups. Individual subject concentration-time profiles will be also presented. For summary statistics and summary plots by sampling time, the nominal PK sampling time will be used; for individual subject plots by time, the actual PK sampling time will be used.

The following PK parameters will be summarized by renal function group:

Table 3. Aztreonam and avibactam PK Parameters to be Summarized Descriptively by Group

Parameter	Summary Statistics
$AUC_{0-24,ss}$, C_{max}, $AUC_{0-\tau}$, C_{τ}, CL, CL_r, V_Z, V_{ss}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
$t_{1/2}$, $Ae_{0-\tau}$, $Ae_{0-\tau}$%	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

Box and whisker plots for individual subject aztreonam and avibactam parameters ($AUC_{0-24,ss}$, $AUC_{0-\tau}$, and C_{max}) will be presented by renal function group and overlaid with geometric means.

Presentations for aztreonam and avibactam concentrations will include:

- A listing of all concentrations sorted by renal function group (present in heading), subject id 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 renal function group and nominal time postdose, where the set of statistics will include n, mean, median, standard deviation, coefficient of variation (cv), minimum, maximum and the number of concentrations above the lower limit of quantification.

- Day 3 median concentrations time plots (on both linear and semi-log scales) against nominal time postdose by renal function group (all renal function groups on the same plot per scale, based on the summary of concentrations by renal function group and time postdose).

- Day 3 mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose by renal function group (all renal function groups on the same plot per scale, based on the summary of concentrations by renal function group and time postdose).

- A listing of all urine concentration interval sorted by renal function group (present in heading), subject ID and nominal collection duration postdose.

- Attainment of steady state will be evaluated by a graphical presentation of pre-infusion concentration (trough) values on Days 1-3.

For summary statistics, median and mean plots by sampling time, the nominal PK sampling time will be used, for individual subject plots by time, the actual PK sampling time will be used.

8.2.2. Capillary Plasma Microsamples

To determine concordance/comparability of capillary microsample plasma concentrations with venous plasma concentrations, X-Y and Bland-Altman (B-A) scatter plots with limits of agreement (LoA) for a precision assessment will be used.

The Bland-Altman scatterplot will include the difference of the 2 measurements divided by the mean (% difference) for **each** *sample on the vertical axis and the average of the 2 measurements on the horizontal axis. Three horizontal reference lines will be superimposed on the scatterplot - one line at the average percent difference between the measurements and lines to mark the upper and lower limits of 95% CI. The same plot will be generated for difference of the 2 measurements (vertical axis) and the average of the 2 measurements on the horizontal axis.*

If the 2 methods are comparable, then % differences/differences should be negligible, with the mean of the differences close to 0, and show no systematic variation with the mean of the 2 measurements.

Venous and capillary observed plasma concentrations, individual subject differences and % differences of concentrations will be listed and summarized descriptively by PK sampling time.

8.3. Safety Analysis

A set of summary tables split by renal function group will be produced to evaluate any potential risk associated with the safety and toleration of administering PF-06947387.

8.3.1. Treatment and Disposition of Subjects

Subject evaluation groups will show end of study subject disposition and will show which subjects were analyzed for pharmacokinetics, as well as for safety (adverse events and laboratory data). Frequency counts will be supplied for subject discontinuation(s) by renal function group.

Data will be reported in accordance with the sponsor reporting standards.

8.3.2. Demographic and Clinical Examination Data

A break-down of demographic data will be provided for age, race, weight, body mass index, and height. Each will be summarized by sex at birth and 'All Subjects' in accordance with the sponsor reporting standards.

8.3.3. Discontinuation(s)

Subject discontinuations, temporary discontinuations or dose reductions due to adverse events will be detailed and summarized by renal function group.

Data will be reported in accordance with the sponsor reporting standards.

8.3.4. Adverse Events

Adverse events will be reported in accordance with the sponsor reporting standards by renal function group.

8.3.5. Laboratory Data

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

8.3.6. Vital Signs Data

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

The baseline measurement is the last predose measurement.

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

8.3.7. ECG Data

The baseline measurement is the predose measurement.

Baseline values and *changes from baseline for the ECG parameters QT interval, heart rate, QTc interval, PR interval, and QRS interval will be summarized by treatment and time.*

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

	Borderline(msec)	***Prolonged(msec)***
Absolute value	*≥450- ≤480*	*>480*
Absolute change	*30 -≤60*	*>60*

In addition, the number of subjects with corrected and uncorrected QT values >500 msec will be summarized.

If not provided, QTcF will be derived using the following formula: $QTcF = QT/(RR^{0.33})$.

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

8.3.8. Other Safety Data

Physical examination data and medical history collected during the study will not be reported in the clinical study report.

8.3.9. Concomitant Treatments

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

8.3.10. Screening and Other Special Purpose Data

Prior medication(s) and non-drug treatment(s), serum FSH concentrations, urine drug screen, serum or urine B-hCG for all females of childbearing potential, urine or blood cotinine concentration and HIV, HBsAg, and HCVAb testing will be obtained at Screening.

These data will not be brought in-house, and therefore will not be listed.

9. REFERENCES

1. FDA Guidance for Industry – Pharmacokinetics in Patients with Impaired Renal Function - Study Design, Data Analysis, and Impact on Dosing and Labeling. 05/98.

10. APPENDICES

Appendix 1. SAS CODE FOR ANALYSES

An example of the PROC MIXED code is provided below:

```
proc mixed data = tab.pk covtest alpha=0.1;
        class trt subjid;
        model l&var = trt / S covb CL DDFM=KR;
        repeated/ type=un subject=subjid group=trt R;
        lsmeans trt;
        estimate 'Severe vs Normal'  trt   -1  1;
        ods output lsmeans = lsmeans&var;
        ods output solutionf = solution&var;
run;

/* Letter assignments for trt within the estimate statement above are as follows;

A = Normal Renal Function (Reference)
B = Severe Renal Impairment (Test) */;
```