Protocol *B7451034*

A PHASE 1, RANDOMIZED, 2-WAY CROSSOVER, OPEN LABEL STUDY TO ESTIMATE THE EFFECT OF PF-04965842 ON MATE1/2K ACTIVITY, USING METFORMIN AS A PROBE, IN HEALTHY PARTICIPANTS

Statistical Analysis Plan (SAP)

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SAP Author: PPD

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Table Revision History

Version	Date	Author(s)	Summary of Changes/Comments
Final	December 14, 2018	PPD	Not Applicable

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

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None.

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

None.

2. INTRODUCTION

PF-04965842 is a Janus kinase (JAK) 1 inhibitor that is currently being developed for the treatment of moderate to severe atopic dermatitis (AD).

Within vitro data indicating PF-04965842 may have the potential to inhibit multidrug and toxic compound extrusion transporter (MATE)1 and MATE2K, a clinical interaction study is needed to assess the effect of PF-04965842 on the activity of these transporters.

The primary purpose of this study is to assess the effect of PF-04965842 on the in vivo pharmacokinetics (PK) of metformin, a specific substrate for MATE1 and MATE2K.

2.1. Study Design

This is a Phase 1, randomized, 2-way crossover, open label study of the effect of PF-04965842 on metformin PK in healthy adult participants. The effect of PF-04965842 on N1-methylnicotinamide (NMN) PK and its correlation to the effect on metformin PK will also be assessed.

A total of approximately 12 healthy male and/or female participants will be enrolled in the study so that approximately 6 participants will be enrolled in each treatment sequence. Each treatment sequence will consist of 2 periods. Participants who discontinue from the study may be replaced at the sponsor's discretion. The replacement participant will receive the same treatment sequence as the participant who discontinued.

Participants will be screened within 28 days of the first dose of investigational product.

Participants will report to the clinical research unit (CRU) the day prior to Day 1 (ie, Day -1) dosing in Period 1 for both treatment sequences. In both sequences, participants will remain in the CRU for a total of 8 days and 7 nights (including Period 1 and Period 2). To adequately remove any drug effects of metformin (plasma half-life ~ 6 hours/whole blood half-life ~ 18 hours) from Period 1 to Period 2, there will be a minimum 4-day washout period between 2 dosing events. The minimum 3-day washout for PF-04965842 in Sequence 2 is sufficient as the half-life of PF-04965842 is ~3-5 hours. The metformin PK will be assessed over 48 hours. Participants will be assigned to one of the following 2 treatment sequences.

Table 1. Study Schematics

Sequence	*Period 1*	***Washout Period***	***Period 2***
1 *(6 subjects)*	*Treatment A*	At least 4 days from metformin administration	*Treatment B*
2 *(6 subjects)*	*Treatment B*	At least 4 days from metformin + PF-04965842 administrationa	*Treatment A*

Abbreviation: QD = once daily.
a. At least a 3 day washout is required from the last dose of PF-04965842.

- *Treatment A (Reference): Single oral administration of metformin 500 mg on Day 1.*
- *Treatment B (Test): Concomitantly single oral administration of metformin 500 mg on Day 1 and oral administration of PF-04965842 200 mg QD for 2 days on Days 1-2.*

All treatments administered on Day 1 will be administered following a moderate-fat meal. PF-04965842 can be dosed with or without food on Day 2 in Treatment B.

2.2. Study Objectives

Primary Objective

- *To estimate the effect of PF-04965842 on MATE1/2K activity via the pharmacokinetic assessment of a single, oral dose of metformin in healthy participants.*

Secondary Objective

- *To evaluate the PK, safety and tolerability of a single oral dose of metformin when co-administered with PF-04965842.*

Exploratory/Tertiary Objectives

- *To evaluate the effects of PF-04965842 on N^1-methylnicotinamide.*
- *To correlate the effects of PF-04965842 on N^1-methylnicotinamide versus metformin.*
- *To enable exploratory research through the collection of banked biospecimens, unless prohibited by local regulations or ethics committee decision.*

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

No formal interim analysis will be conducted for this study. However, as this is an sponsor-open 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

There are no statistical hypotheses.

4.2. Statistical Decision Rules

There are no statistical decision rules.

5. ANALYSIS SETS

5.1. Pharmacokinetic (PK) Analysis Set

5.1.1. Concentration Analysis Set

The pharmacokinetic (PK) concentration population is defined as all enrolled subjects who received at least 1 dose of metformin and in whom at least 1 plasma concentration value is reported.

5.1.2. Parameter Analysis Set

The PK parameter analysis population is defined as all participants randomized and treated who have at least 1 of the PK parameters of primary interest in at least 1 treatment period.

5.2. Pharmacodynamic Analysis Set

None.

5.3. Safety Analysis Set

All participants randomly assigned to investigational product and who take at least 1 dose of investigational product. Participants will be analyzed according to the product they actually received.

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 randomized but not treated.

If a subject takes a treatment that is not consistent with the treatment they are randomized 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.

Events that occur in a non-treatment period (for example, Washout or Follow-up) will be counted as treatment emergent and attributed to the previous treatment taken.

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.*

6.2.1. Adverse Events

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

Events that occur in a non-treatment period (for example, Washout or Follow-up) will be counted as treatment emergent and attributed to the previous treatment taken.

6.2.2. Laboratory Safety Tests

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 take into account whether each subject's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

Baseline is defined as the last predose measurement taken in each study period.

6.2.3. Vital Signs Data

Supine measurements will be taken at times detailed in the Schedule of Activities given in the protocol.

Baseline is the last predose recording in each study period.

6.2.4. ECG Results

QT interval, QTc, PR, RR, QRS and heart rate will be recorded at each assessment time indicated in the Schedule of Activities given in the protocol.

If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

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

Baseline is the last predose recording in each study period.

The average of the triplicate measurements will be calculated prior to analyzing the data. Baseline will be defined as the average of the triplicate predose recordings in each study period.

6.2.5. Other Safety Data

Additional safety data will be collected as described in the protocol and will be listed if collected in the sponsor's database.

6.3. Other Endpoints

6.3.1. PK Endpoints

PK analysis of metformin and NMN (N1-methylnicotinamide) will be taken according to the Schedule of Activities given in the protocol.

The following PK parameters will be calculated by treatment and by analyte (if applicable) from the concentration-time data and urine concentrations using standard noncompartmental methods:

Table 2. Noncompartmental PK Parameters

PK Parameter	Analysis Scale	Metformin	NMN (N1-methylnicotinamide)
AUC_{inf}*	ln	A, D	
AUC_{last}	ln	A, D	D
AUC_{24}	ln	D	A, D
C_{max}	ln	A, D	A, D
T_{max}	R	D	D
$t_{1/2}$*	R	D	
CL/F*	ln	D	
V/F*	ln	D	
Ae*	R	D	D
Ae%*	R	D	
CL_r*	ln	A, D	A, D

Key: A=analyzed using statistical model, D=displayed with descriptive statistics, ln=natural-log transformed, R=raw (untransformed), *=if data permits

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 treatment 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 interactive effect on PK parameters will be determined by constructing 90% confidence intervals around the estimated difference between the Test and Reference treatments using a mixed effects model based on natural log transformed data. The mixed effects model will be implemented using SAS Proc Mixed, with REML estimation method and Kenward-Roger degrees of freedom algorithm.

8.2. Statistical Analyses

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

Natural log transformed AUCinf, AUClast, Cmax and CLr of metformin will be analyzed using a mixed effect model with sequence, period and treatment as fixed effects and participant within sequence as a random effect.

Additionally, AUC24, Cmax and CLr of NMN will also be analyzed using a mixed effect model with sequence, period and treatment as fixed effects and participant within sequence as a random effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% confidence intervals will be obtained from the model. The adjusted mean differences and 90% confidence intervals for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% confidence intervals for the ratios. Metformin alone will be the Reference treatment, while the metformin co-administered with PF-04965842 will be the Test treatment.

PK parameters will be summarized descriptively by analyte and by treatment, as applicable, in accordance with Pfizer Data Standards. Concentrations will be listed and summarized descriptively by analyte, nominal PK sampling time and treatment. Individual subject and median profiles of the plasma concentration-time data will be plotted by treatment using actual and nominal times, respectively. Median profiles will be presented on both linear-linear and log-linear scales.

Individual changes from baseline for metformin CLr versus NMN CLr will be plotted and a Pearson correlation coefficient (r) will be presented.

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 clinical study report. 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.

Table 3. PK Parameters to be Summarized Descriptively by Treatment

Parameter	Summary Statistics
AUC_{inf}, AUC_{last}, AUC_{24}, C_{max}, CL/F, CL_r, V/F	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
$t_{1/2}$, AE, Ae%	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

Supporting data from the estimation of t½ and AUCinf will be listed by treatment and analyte: terminal phase rate constant (kel); goodness of fit statistic from the log-linear regression (r2); the percent of AUCinf based on extrapolation (AUCextrap%); and the first, last, and number of time points used in the estimation of kel. This data may be included in the clinical study report.

Presentations for metformin and NMN concentrations will include:

- A listing of all concentrations sorted by analyte, subject ID, period 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 (for each analyte) by treatment 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.

- Median concentrations time plots (on both linear and semi-log scales for each analyte) 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 for each analyte) 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 for each analyte) against actual time postdose (there will be separate spaghetti plots for each treatment per scale).

- Individual concentration time plots by subject (on both linear and semi-log scales for each analyte) against actual time postdose [there will be separate plots for each subject (containing all treatments) per scale].

- Individual changes from baseline for metformin CL_r versus NMN CL_r will be evaluated using a regression line and Pearson correlation coefficient (r).

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.

8.3. Safety Analysis

A set of summary tables split by treatment will be produced to evaluate any potential risk associated with the safety and toleration of administering study treatments.

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 treatment.

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 treatment.

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 treatment.

8.3.5. Laboratory Data

Laboratory data will be listed and summarized by treatment in accordance with the sponsor reporting standards. Baseline is as defined in Section 6.2.2.

8.3.6. Vital Signs Data

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

For each planned time point, baseline values and change from baseline values within each treatment will be summarized with descriptive statistics (using sponsor default standards). Baseline is as defined in Section 6.2.3.

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

8.3.7. ECG Data

Baseline and changes from baseline in PR, QT, QRS, heart rate and QTcF will be summarized by treatment and time postdose. Baseline is as defined in Section 6.2.4.

Changes from baseline for the ECG parameters QT interval, heart rate, QTc interval, PR interval, and QRS complex will be summarized by treatment and time.

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

Safety QTc Assessment

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

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

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

8.3.8. Other Safety Data

None.

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, Serology (HIV, HepBsAg, HepBcAb, HepBsAb, HCVAb testing), QuantiFERON® - TB Gold Test, Medical, Drug, Tobacco and Alcohol History, and urine or blood cotinine concentration will be obtained at Screening.

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

8.3.11. Other Analyses

Pharmacogenomic (PGx) and endogenous biomarker PK data, other than NMN, will be collected and retained for potential future analyses, but will not be analyzed, specifically, for this study.

9. REFERENCES

1. ICH E14 - The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. CHMP/ICH/2/04.

10. APPENDICES

Appendix 1. SAS CODE FOR ANALYSES

- Analyze AUC_{inf}, AUC_{last}, C_{max} and CL_r of metformin.
- Analyze AUC_{24}, C_{max} and CL_r of NMN.

An example of the PROC MIXED code is provided below:

```
proc mixed data=tab.pk;
        class seq period trt subject;
        model l&var=seq period trt/ ddfm=KR;
        random subject(seq) /subject=subject(seq);
        lsmeans trt;
        estimate 'Test vs Reference' 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 = metformin alone (Reference);

B = metformin co-administered with PF-04965842 (Test) */;
```