Protocol *B1871063*

A PHASE 1, OPEN-LABEL, RANDOMIZED, 3-PERIOD, 6-SEQUENCE, CROSSOVER STUDY TO EVALUATE THE BIOAVAILABILITY OF BOSUTINIB ADMINISTERED AS CAPSULE CONTENTS MIXED WITH APPLESAUCE OR YOGURT RELATIVE TO INTACT CAPSULES IN HEALTHY PARTICIPANTS UNDER FED CONDITION

Statistical Analysis Plan (SAP)

Version: 1.0

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Revision History

Version	Date	Author(s)	Summary of Changes/Comments
1.0	May 26, 2021	PPD	Not Applicable

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

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

None.

2. INTRODUCTION

The purpose of this study is to compare the bioavailability of bosutinib when orally administered as capsule contents mixed with applesauce or yogurt to the oral administration of intact capsules in healthy participants under fed condition. Bosutinib (PF-05208763) is an orally active Src/Abl kinase inhibitor with potent antiproliferative and proapoptotic activity in cultured chronic myelogenous leukemia cells, and antiproliferative activity in primitive progenitor chronic myelogenous leukemia cells from patients.

2.1. Study Design

This will be a Phase 1, open-label, randomized, single dose, 3-period, 6-sequence, crossover study in approximately 18 healthy participants. Participants will be randomized to 1 of the 6 sequences of treatment described below.

Table 1. Study Schematics

Sequence	***Period 1***	***Washout Period***	***Period 2***	***Washout Period***	***Period 3***
1 (n=6)	*C*	*At least 14 days between successive bosutinib doses*	*A*	*At least 14 days between successive bosutinib doses*	*B*
2 (n=6)	*B*		*C*		*A*
3 (n=6)	*A*		*B*		*C*
4 (n=6)	*B*		*A*		*C*
5 (n=6)	*A*		*C*		*B*
6 (n=6)	*C*		*B*		*A*

n = number of participants.

Treatment A (Test): Contents of 500 mg (100 mg x 5) bosutinib capsules mixed with 45 mL of applesauce under fed condition.

Treatment B (Test): Contents of 500 mg (100 mg x 5) bosutinib capsules mixed with 45 mL of full fat yogurt under fed condition.

Treatment C (Reference): 500 mg (100 mg x 5) bosutinib intact capsules under fed condition.

Note: "Enrolled" means a participant's agreement to participate in a clinical study following completion of the informed consent process and screening. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity after screening.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.

Since the mean plasma elimination t½ of bosutinib is approximately 34 hours (range 18 to 55 hours), there will be a minimum 14-day washout period between successive doses of bosutinib.

Blood samples for PK analysis will be collected predose and at 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, and 144 hours post the bosutinib dose.

Participants will remain in the study for up to approximately 13 weeks, including the screening and follow-up periods. Participants will be screened within 28 days of the first dose of IP and if all study entry criteria are fulfilled, the participants will report to the CRU on the day prior to Day 1 dosing (Day -1) of Period 1. Following an overnight fast of at least 10 hours, on Day 1 of each period, participants will receive a high-fat and high-calorie breakfast (see study protocol' Section 5.3.1) prior to dosing which will need to be completely consumed within 20 minutes. For reference Treatment C, bosutinib intact capsules will be administered with approximately 240 mL of ambient temperature water. For Test Treatments A and B, bosutinib capsule contents will be administered as granules mixed with 45 mL of applesauce or yogurt, respectively, followed by approximately 240 mL of ambient temperature water. Intact capsules (Treatment C) and capsule contents mixed with applesauce or yogurt (Treatments A and B, respectively) will be swallowed and not chewed.

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Participants will be confined in the CRU for a minimum of 5 days until completion of the 72 hour PK sample collection on Day 4 in each period. Participants will be discharged at the discretion of the investigator. The investigator could choose to confine participants in the CRU and discharge them following the 96 hour PK sample on Day 5. Participants discharged on Day 5 will be required to return to the CRU for outpatient visit on Day 7 of each period. Alternatively, participants may be discharged from the CRU on Day 4, provided that they are able to return for outpatient visits on Days 5 and 7. A follow-up phone call will be made at least 28 calendar days and up to 35 calendar days after the last administration of the IP to capture any potential AE, prior/concomitant treatments and confirm appropriate contraceptive usage.

Tolerability and safety will be assessed for all treatments by monitoring AEs, ECGs, BP, pulse rate and safety laboratory data.

If there are participants who withdraw or discontinue treatment and are considered to be non-evaluable with respect to the primary PK objective, additional participants can be enrolled at the discretion of the investigator upon consultation with the sponsor.

2.2. *Study Objectives*

Primary:

- *To estimate the relative bioavailability of a single 500 mg dose of bosutinib when administered as capsule contents mixed with applesauce to intact capsules under fed condition in adult healthy participants.*

- *To estimate the relative bioavailability of a single 500 mg dose of bosutinib when administered as capsule contents mixed with yogurt to intact capsules under fed condition in adult healthy participants.*

Secondary:

- *To evaluate the PK of bosutinib when administered as capsule contents mixed with applesauce or yogurt and as intact capsules to healthy participants under fed condition.*

- *To evaluate the safety and tolerability of bosutinib when administered as capsule contents mixed with applesauce or yogurt and as intact capsules to healthy participants under fed condition.*

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3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

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.

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 participants randomized and treated who have at least 1 concentration in at least 1 treatment period.

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 bosutinib 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 study intervention and who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually received.

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5.5. Other Analysis Sets

None.

5.6. Treatment Misallocations

All analyses will be performed on an "as-treated" basis and will not include data from participants who are randomized but not treated.

If a participant takes a treatment that is not consistent with the treatment they are assigned 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 and PK analyses, where applicable.

5.7. Protocol Deviations

Participants 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.

5.7.1. Deviations Assessed Prior to Randomization

At Screening, the investigator will assess participants against the inclusion and exclusion criteria as set out in Sections 5.1 and 5.2 of the protocol.

5.7.2. 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.*

6.3. Other Endpoints

6.3.1. PK Endpoints

Blood samples for PK analysis of bosutinib (PF-05208763) will be taken according to the Schedule of Activities given in the protocol.

The following PK parameters will be calculated for bosutinib (PF-05208763) (if possible) from the concentration-time data using standard noncompartmental methods:

Table 2. Noncompartmental PK Parameters

PK Parameter	Analysis Scale	Bosutinib (PF-05208763)
AUC_{inf}*	ln	A, D
AUC_{last}	ln	A, D
C_{max}	ln	A, D
T_{max}	R	D
$t_{1/2}$*	R	D
CL/F*	ln	D
Vz/F*	ln	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 participant has a known cause for biased estimate of a PK parameter (due, for example, to an event such as vomiting before all of the drug is absorbed in the body), this will be footnoted in summary tables and will not be included in the calculations of summary statistics or statistical analyses. Participants who vomit at or before 2 times of median T_{max} after drug administration may be excluded from PK analyses.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methods

Precision of the estimate of 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

Natural logarithm-transformed bosutinib AUC_{inf}, AUC_{last} and C_{max} will 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% 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.

The following 2 comparisons will be performed:

- *Treatment A (Test) vs Treatment C (Reference);*
- *Treatment B (Test) vs Treatment C (Reference).*

The plasma PK parameters AUC_{inf}, C_{max}, AUC_{last}, T_{max}, CL/F, Vz/F and $t_{½}$ of bosutinib will be summarized descriptively by treatment. For AUC_{inf}, AUC_{last} and C_{max}, a listing of the individual participant ratios (Test-Reference) will be provided for the 2 comparisons described above. Individual participant PK parameters for AUC_{inf}, AUC_{last} and C_{max} will be plotted by treatment and overlaid with geometric mean. Concentrations will be listed and summarized descriptively by PK sampling time and treatment. Individual participant and median profiles of the concentration-time data will be plotted by treatment.

For summary statistics and median 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.

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}, C_{max}, Vz/F, CL/F	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
$t_{1/2}$	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

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

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

Presentations for *bosutinib (PF-05208763)* concentrations will include:

- A listing of all concentrations sorted by subject 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, 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) 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 subject (on both linear and semi-log scales) against actual time postdose [there will be separate plots for each subject (containing all treatments) per scale].

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

All participants 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.

8.3.1. Treatment and Disposition of Participants

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

8.3.2. Demographic and Clinical Examination Data

A breakdown 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 Participants' in accordance with the sponsor reporting standards.

8.3.3. Discontinuation(s)

Participant discontinuations and temporary discontinuations 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 in accordance with the sponsor reporting standards.

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

8.3.6. Vital Signs Data

Vital Signs data will be databased and available upon request.

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

8.3.7. ECG Data

ECG data will be databased and available upon request.

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

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

Screening data will available upon request.

9. REFERENCES

None.

10. APPENDICES

Appendix 1. SAS CODE FOR ANALYSES

An example of the PROC MIXED code is provided below:

Comparison of Treatment A (100 mg x 5 with applesauce under fed condition) and B (100 mg x 5 with full fat yogurt under fed condition) versus Treatment C (100 mg x 5 under fed condition);

```
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 ‘Treatment A vs Treatment C ' trt   1  0  -1  /cl alpha=0.1;
        estimate ‘Treatment B vs Treatment C ' trt   0  1  -1  /cl alpha=0.1;
        ods 'Estimates' out=est&var;
        ods 'lsmeans' out=ls&var;
        ods 'covparms' out=cov&var;
        ods 'tests3' out=tst&var;
run;

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

Treatment A (Test): Contents of 500 mg (100 mg x 5) bosutinib capsules mixed with 45 mL of applesauce under fed condition.

Treatment B (Test): Contents of 500 mg (100 mg x 5) bosutinib capsules mixed with 45 mL of full fat yogurt under fed condition

Treatment C (Reference): 500 mg (100 mg x 5) bosutinib intact capsules under fed condition.
*/
```