Protocol C4541003

A PHASE 1, RANDOMIZED, DOUBLE-BLIND, SPONSOR-OPEN, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACOKINETIC INTERACTION WITH MIDAZOLAM OF MULTIPLE ASCENDING ORAL DOSES OF PF-07258669 IN HEALTHY NON-JAPANESE, JAPANESE, AND OLDER ADULT PARTICIPANTS

Statistical Analysis Plan
(SAP)

Version: **3**

Date: 04 Aug 2023

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 / 30 Nov 2021	Original: 24 Aug 2021 Amendment 1: 12 Oct 2021	N/A	N/A
2 / 07 Feb 2023	Amendment 2: 13 Jan 2022 Amendment 3: 09 Mar 2022 Amendment 4: 09 Nov 2022	Updates based on SAP template update, protocol amendments, A&R plan review and Blinded Data Reviews.	Rationale: the protocol has been amended to 1) add older adult cohort [PA2] 2) add CCI [redacted]; collect urine in PART B for measurement of 6β-hydroxycortisol/cortisol [PA3] 3) allow participants to be allocated to 1of 3 diets (eg, standard, HCHC, or HFHC), with optional additional cohorts of participants allocated to the HFHC diet (with a larger sample size). [PA4] The following sections were revised as part of this rationale: • Updated protocol title • Section 2.2: updated endpoints to match protocol amendments.

			• Section 2.3: added text verbatim from the protocol amendments to reflect the changes to the protocol amendments; updated Schema to reflect protocol amendments • Sections 3 and 6: updated endpoints to match protocol amendments. • Section 5.2; added reporting by population and dietary allocation • Section 6: updated description of 'populations' to include older cohort and dietary allocation • Appendix 5: added abbreviations Rationale: Changes to reflect current SAP template: • Section 2.2: added "Type" and "Estimand" columns to objective/endpoint/estimand table • Updated links throughout Rationale: Changes to reflect updates based on ongoing review of tables: • Section 3.3.1.4: removed relative changes wording • Section 3.3.1.5; corrected 24 hour baseline timepoint • Section 5.2.4: updated statistical model diagnostics wording; • Section 5.2.5: Added clarification for placebo dose • Section 6: clarified study and period day definitions

			• Section 6.3.1.2: added additional flexibility to do exploratory Emax modelling • Section 6.3.1.4: clarified MMRM analysis method • Appendix 4: updated dTarget and predicted ED50 (i.e. to use the Q8H dose); updated the Hill SD
3 / 04 Aug 2023	N/A	Updates based on A&R plan review and Blinded Data Reviews.	• Sections 3.2.1, 6.2.1 and 6.3.1.1: added also for plasma M4 metabolite (PF-07275428) where possible. Results may not be included in the CSR. • Sections 5.2.4 and 6: added clarification for records collected after discontinuation of study drug for selected tertiary endpoints • Section 6.1.1.2 - change from baseline in ALT and AST will be summarized and plotted. • Appendix 5 – added abreviations

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C4541003. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

Text in *italics* is taken directly from the protocol.

2.1. Modifications to the Analysis Plan Described in the Protocol

Not applicable.

2.2. Study Objectives, Endpoints, and Estimands

Type	Objective	Endpoint	Estimand
Part A			
Primary:			
Safety	• *To evaluate the safety and tolerability of multiple, ascending, oral doses of PF-07258669 in healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled).*	• *Assessment of adverse events, clinical safety laboratory tests, vital signs, continuous cardiac monitoring, 12-lead ECGs, respiratory rate, oral body temperature, physical examinations, neurological examination findings, C-SSRS,* CCI [redacted]	N/A
Secondary:			
PK	• *To evaluate the plasma and urine PK of single and multiple oral doses of PF-07258669 in healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled).*	• *PF-07258669 plasma PK on Days 1 and 14: C_{max}, AUC_{tau}, T_{max}, dose-normalized C_{max}, and dose-normalized AUC_{tau}* • *PF-07258669 urine PK on Day 14: Ae_{tau}, Ae_{tau}%, and CL_r (if data permit)*	N/A
Tertiary:			
PK	• *To evaluate additional PK parameters of PF-07258669 following single and multiple oral doses to healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled).*	• *PF-07258669 plasma PK on Day 14: CL/F, C_{min}, C_{av}, PTR, R_{ac}, $R_{ac,Cmax}$, $t_{½}$, and V_z/F (if data permit)*	N/A
Other	• *To evaluate the effect of multiple, ascending doses of PF-07258669 administered for 14 days in healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled) on body weight.*	• *Change from baseline in body weight on Days 7 and 14*	N/A
CCI [redacted]			
CCI [redacted]			
CCI [redacted]			

	CCI [redacted]		
Biomarkers	• *To evaluate the potential for induction of CYP3A with administration of multiple, ascending doses of PF-07258669 administered for 14 days in healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled).*	• *Change from baseline on Day 14:* o *Urinary 6β-hydroxycortisol/cortisol ratio;* o *Plasma 4β-hydroxycholesterol/cholesterol ratio;* o *Serum extracellular vesicles (if analyzed).*	N/A
Biomarkers	• *To conduct exploratory profiling for plasma metabolites of PF-07258669 in steady-state plasma samples in healthy non-Japanese, Japanese (if enrolled), and older adult participants (if enrolled).*	• *Qualitative plasma levels of potential metabolites of PF-07258669 at steady-state.:*	N/A
Part B			
Primary:			
PK	• *To evaluate the effects of PF-07258669 on midazolam PK in healthy adult participants*	• *Midazolam plasma PK parameters alone and in combination with PF-07258669 on Period 1/Day 1, Period 2/Day 2, and Period 2/Day10: Cmax, AUClast, and AUCinf (if data permit).*	N/A
Secondary:			
Safety	• *To evaluate the safety and tolerability of midazolam alone and in combination with PF-07258669 when administered to healthy adult participants*	• *Adverse events, vital signs measurements, continuous pulse oximetry, 12-lead ECGs, physical examination findings, and clinical safety laboratory measurements*	N/A
Tertiary:			
PK	• *To evaluate the effects of PF-07258669 on additional PK parameters of midazolam in healthy adult participants*	• *Midazolam plasma PK parameters alone and in combination with PF-07258669 on Period 1/Day 1, Period 2/Day 2, and Period 2/Day 10: Tmax, CL/F, Vz/F, and t½ (if data permit).*	N/A
Biomarkers	• *To evaluate the potential for induction of CYP3A with administration of multiple, ascending doses of PF-*	• *Change from baseline:* o *Urinary 6β-hydroxycortisol/cortisol ratio on Day 7(if analyzed);*	N/A

	07258669 in healthy adult participants	○ *Plasma 4β-hydroxycholesterol/cholesterol ratio;* ○ *Serum extracellular vesicles (if analyzed)*	
CCI [redacted]			

2.3. Study Design

This study consists of 2 parts. Part A will evaluate the safety, tolerability, and PK of multiple ascending doses of PF-07258669. Part B will assess the effects of PF-07258669 on midazolam PK. A total of up to approximately 150 participants are planned to be enrolled in this study. Participants who discontinue prior to completion of the study for reasons unrelated to safety may be replaced at the discretion of the investigator and sponsor. Participants withdrawn for safety reasons will not be replaced.

2.3.1. Part A

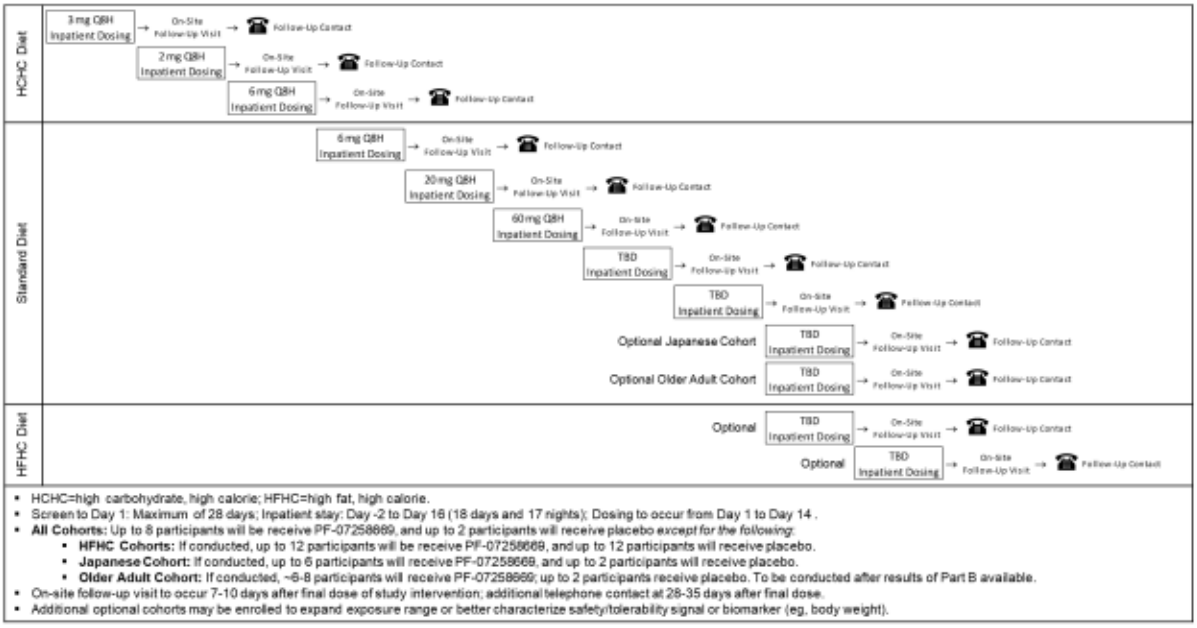
Part A of this study is a randomized, investigator- and participant-blind, sponsor-open, placebo-controlled evaluation of the safety, tolerability, and PK of PF-07258669 after administration of multiple ascending oral doses to healthy adult participants. An optional cohort of healthy adult Japanese participants may also be evaluated if results in non-Japanese participants support further evaluation. Up to 3 different dietary allocations (ie, standard, high carbohydrate-high calorie, and high fat-high calorie diets) may be employed. An optional cohort of healthy adult Japanese participants may also be evaluated if results in non-Japanese participants support further evaluation. In addition, an optional cohort of older adult participants may also be evaluated if results in younger participants support further evaluation. The cohort of older participants will only be evaluated after the results of Part B of this study are available.

Approximately 138 healthy adult participants may be enrolled in Part A, of which approximately 8 may be Japanese and 8-10 may be older participants.

Precautionary sentinel dosing will be used in each cohort of Part A in which PF-07258669 exposures (C_{max} and/or AUC_{24}) are projected to be higher than the exposures evaluated in the single ascending dose clinical study C4541001 or at the discretion of the investigator in response to safety signals observed in a previous cohort. If the projected exposures have been evaluated in a previous cohort of the current study, sentinel dosing may be omitted. When sentinel dosing is employed, 2 participants (1 receiving PF-07258669 and 1 receiving placebo) will be dosed before the remaining participants of that cohort are dosed. Safety and tolerability data through at least 48 hours after the first dose of study intervention for the sentinel participants will be reviewed prior to dosing the remaining participants of that cohort.

Additional optional cohorts may be enrolled to repeat a dose or to expand the dose/exposure range or better characterize a particular safety or tolerability signal or clinical biomarker of interest (eg, body weight).

Figure 1. Study Design Part A



2.3.2. Part B

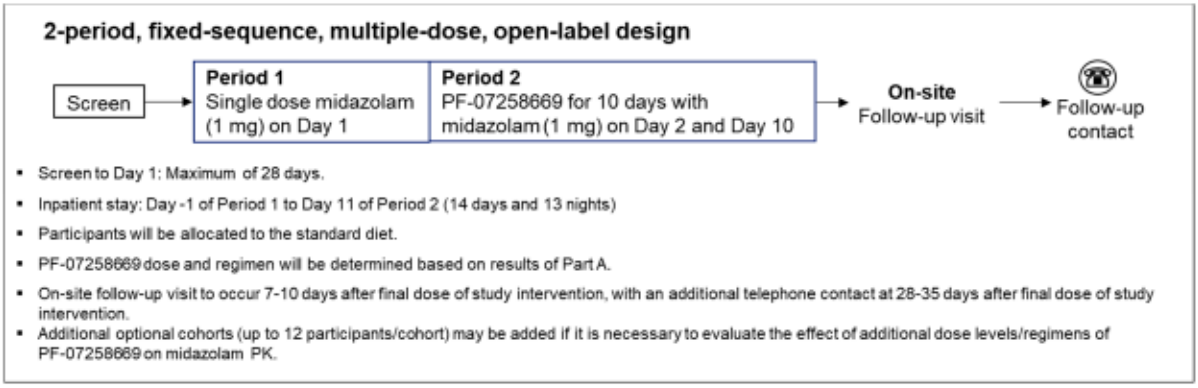
Part B will be conducted if the results of Part A support further evaluation of PF-07258669. Part B is a 2-period, fixed-sequence, multiple-dose, open-label design to evaluate the effect of PF-07258669 on midazolam PK in healthy adult participants. A total of approximately 12 healthy adult participants per cohort will be enrolled in Part B. All participants in Part B will be allocated to standard diet.

Precautionary sentinel dosing may be used in Part B if autoinhibition of PF-07258669 PK is observed in Part A of the study, or at the discretion of the investigator. Based on potential time-dependent inhibition of CYP3A4/5 at high doses of PF 07258669, near maximal inhibition of CYP3A4/5 is predicted to occur on Period 2/Day 2. Therefore, if sentinel dosing is employed, 2 participants will be dosed, and if no untoward effects are observed through at least 24 hours after midazolam dosing in Period 2/Day 2 for the sentinel participants, the remaining participants of that cohort will then be dosed. If untoward effects are observed, a lower dose of PF-07258669 may be evaluated in another cohort.

Initially, a PF-07258669 dose/exposure level similar to the MTD determined in Part A is planned to be used to maximize the possibility of detecting a PK interaction with midazolam in Part B. However, a lower dose level may be evaluated based on the results of Part A. Additional optional cohorts may be added if it is necessary to evaluate the effect of additional dose levels of PF-07258669 on midazolam PK. If the PF-07258669/midazolam combination used in the first cohort of Part B is well tolerated, sentinel dosing may not be

used in subsequent cohorts if the PF-07258669 dose used in any additional cohort of Part B is less than the PF-07258669 dose evaluated in the first cohort of Part B.

Figure 2. Study Design Part B



3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

3.1.1. Part A: Safety and Tolerability

Assessment of adverse events, clinical safety laboratory tests, vital signs, continuous cardiac monitoring, 12-lead ECGs, respiratory rate, oral body temperature, physical examinations, neurological examination findings, C-SSRS, CCI [redacted].

3.1.1.1. Adverse Events

Any events occurring following the start of treatment, or increasing in severity, will be counted as treatment emergent. Events that occur in a non-treatment period (for example Follow-up) will be counted as treatment emergent and attributed to the most recent treatment taken.

A 3-tier approach for summarizing adverse events (AEs) will not be used due to the low number of participants planned to be recruited.

3.1.1.2. Clinical Safety Laboratory Tests

Safety laboratory tests will be performed as described in the protocol.
Baseline will be defined as the assessments on Day -2. However, *at the discretion of the investigator, non-sentinel participants may begin their stay at the CRU on the same day as the sentinel participants in order to standardize conditions for all participants of a given cohort. In that case, admission and baseline activities can be started either on their first day in the CRU or on Day -2 of their stay*, in which case baseline will be the last pre-dose measurement.

Any clinical laboratory abnormalities of potential clinical concern will be described. To determine if there are any clinically significant laboratory abnormalities, the haematological,

clinical chemistry, urinalysis and other safety tests will be assessed against the criteria specified in the sponsor reporting standards. The assessment will take into account whether each participant's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

3.1.1.3. Vital Signs

Triplicate supine and single orthostatic blood pressure (BP) and pulse rate measurements will be taken as described in the protocol. Note that some of the supine measurements are collected as part of the orthostatic assessment. When timing of orthostatic assessments coincides with timing of triplicate supine assessments, the last of the triplicate supine readings will be used as the supine reading for the orthostatic assessment. The average of the triplicate measurements (when appropriate) will be calculated for each vital signs parameter.

Baseline for supine BP and pulse rate will be defined as the average of the triplicate measurements collected at the pre-dose (0 hour) assessment on Day 1. Baseline for standing BP and pulse rate (as part of the orthostatic vital signs) will be defined as the pre-dose (0 hour) assessment on Day 1.

The following endpoints will be determined:

- Postural differences (supine - standing) for systolic and diastolic BP and for pulse rate (at each orthostatic assessment timepoint)
- Change from baseline (CFB) in supine, standing and postural differences for systolic and diastolic BP and for pulse rate
- The minimum and maximum post-dose supine and standing for systolic and diastolic BP and for pulse rate
- The maximum post-dose postural differences for systolic and diastolic BP
- The minimum post-dose postural differences for pulse rate
- The maximum decrease and increase from baseline over all measurements taken post-dose for supine, standing and postural differences for systolic and diastolic BP and pulse rate.

The maximum increase from baseline will be calculated by selecting the maximum change from baseline over the respective period, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken. Similarly, the maximum decrease from baseline will be determined by selecting the minimum value of the changes from baseline. In cases where a participant does not show a decrease, the minimum increase should be taken.

3.1.1.4. Continuous Cardiac Monitoring

Continuous cardiac monitoring will be performed using telemetry as outlined in the protocol.

All abnormal rhythms will be recorded and reviewed by the investigator for the presence of rhythms of potential clinical concern. The time, duration, and description of the clinically significant event will be recorded in the CRF. Events deemed of clinical concern will be recorded as AEs and will be summarized as part of the standard AE outputs.

3.1.1.5. 12-lead Electrocardiograms

A single supine 12-lead ECG will be obtained on all participants at screening, discharge and follow-up. 12-lead ECGs will be recorded in triplicate on all participants at all other times detailed in the protocol. The average of the triplicate readings (when appropriate) will be calculated for each ECG parameter.

The QT, QTcF, PR, RR, QRS interval and heart rate will be recorded at each assessment time. If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

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

The average of the triplicate ECG measurements over the 3 pre-dose measurement times (-1H, -0.5H, and predose 0H; total of 9 ECG measurements) collected before morning dose administration on Day 1 will serve as each participant's baseline value.

The following endpoints will be determined: -

- change from baseline in QT, QTcF, PR, QRS interval and heart rate
- maximum post-dose QTcF, PR and QRS interval
- maximum increase from baseline over all measurements taken post-dose for QTcF, PR and QRS values

The maximum increase from baseline will be calculated by selecting the maximum change from baseline over the respective period, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

3.1.1.6. Respiratory Rate

Respiratory rate will be measured at times specified in the protocol.

Baseline will be the pre-dose (0 hour) assessment on Day 1.

Change from baseline in respiratory rate will be determined, as well the maximum increase and decrease from baseline over all measurements taken post-dose.

3.1.1.7. Oral Body Temperature

Oral body temperature will be measured at times specified in the protocol.

Baseline will be the pre-dose (0 hour) assessment on Day 1.

Change from baseline in body temperature will be determined, as well the maximum increase and decrease from baseline over all measurements taken post-dose.

3.1.1.8. Physical and Neurological Examinations

Physical and neurological examinations will be performed as described in the protocol. P*hysical examination, and neurological examination information, as applicable, collected during the course of the study will be considered source data and will not be required to be*

reported, unless otherwise noted. However, any untoward findings identified on physical and/or neurological examinations conducted during the active collection period will be captured as AEs, if those findings meet the definition of an AE.

3.1.1.9. C-SSRS

The C-SSRS is a validated tool to evaluate suicidal ideation and behaviour. The 'Baseline/Lifetime' assessment is to be performed at Screening. All other assessments should use the 'Since Last Visit' assessment. Data relevant to the assessment of suicidality will be mapped to the Columbia-Classification Algorithm of Suicide Assessment (C-CASA) codes as given in Appendix 2.

CCI

[redacted]

3.1.2. Part B: Midazolam PK Parameters

- *Midazolam plasma PK parameters alone and in combination with PF-07258669 on Period 1/Day 1, Period 2/Day 2, and Period 2/Day10: C_{max}, AUC_{last}, and AUC_{inf} (if data permit).*

Blood samples for PK analysis of midazolam will be collected according to the protocol.

Plasma PK parameters (on Period 1/Day 1, Period 2/Day 2 and Period 2/Day 10) for midazolam will be derived from the plasma concentration-time profiles using standard non-compartmental methods, as data permit.

Table 2 shows the analysis scale and method for each parameter.

Table 2. Midazolam PK Parameters

Parameter	Analysis Scale	Period 1/Day 1 (Midazolam)	Period 2/Day 2 (Midazolam + PF-07258669)	Period 2/Day 10 (Midazolam + PF-07258669)
C_{max}	ln	A, D	A, D	A, D
AUC_{last}	ln	A, D	A, D	A, D
AUC_{inf}*	ln	A, D	A, D	A, D

*=if data permit. Abbreviations: A=analyzed using a statistical model; D=displayed with descriptive statistics as outlined in Table 6 in Section 6.1.2; ln=natural-log transformed.

3.2. Secondary Endpoint(s)

3.2.1. Part A: PF-07258669 Plasma and Urine PK Parameters

- *PF-07258669 plasma PK on Days 1 and 14: Cmax, AUCtau, Tmax, dose-normalized Cmax, and dose-normalized AUCtau*

- *PF-07258669 urine PK on Day 14: Ae_{tau}, Ae_{tau}%, and CLr (if data permit)*

Plasma PK parameters will also be calculated for the M4 metabolite (PF-07275428) where possible and as applicable. Blood and urine samples for PK analysis of PF-07258669 will be collected according to the protocol.

Plasma PK parameters (on Days 1 and 14) and urine PK parameters (on Day 14) for PF-07258669 will be derived from the plasma and urine concentration-time profiles using standard non-compartmental methods, as data permit.

Table 3 shows the analysis scale and method for each parameter.

Table 3. PF-07258669 PK parameters

Parameter	Analysis Scale	PF-07258669
Plasma Parameters		
C_{max}	ln	D
AUC_{tau}	ln	D
T_{max}	R	D
C_{max} (dn)	ln	D
AUC_{tau} (dn)	ln	D
Urine Parameters		
Ae_{tau}	ln	D
Ae_{tau}%	ln	D
CLr	ln	D

Abbreviations: (dn)=Dose normalized; D=displayed with descriptive statistics as outlined in Table 7 in Section 6.2.1; ln=natural-log transformed; R=raw (untransformed).

3.2.2. Part B: Safety and Tolerability

- *Adverse events, vital signs measurements, continuous pulse oximetry, 12-lead ECGs, physical examination findings, and clinical safety laboratory measurements*

See sections 3.1.1.1, 3.1.1.2, 3.1.1.3, 3.1.1.5 and 3.1.1.8 for details on the safety endpoints.

See sections 3.1.1.6 and 3.1.1.7 for details on additional safety endpoints (respiratory rate and oral body temperature).

Continuous pulse oximetry will be performed as outlined in the protocol. Events deemed of clinical concern will be recorded as AEs and will be summarized as part of the standard AE outputs.

For Part B vital signs, ECG, clinical safety laboratory measurements, respiratory rate and oral body temperature data, baseline will be defined as the last pre-dose assessment on Day 1,

Period 1. Where appropriate, as outlined in the protocol, the average of triplicate measures will be used for this baseline.

3.3. Other Endpoint(s)

3.3.1. Part A

3.3.1.1. Additional PF-07258669 PK Parameters

- *PF-07258669 plasma PK on Day 14: CL/F, Cmin, Cav, PTR, Rac, Rac,Cmax, t½, and Vz/F (if data permits).*

These PK parameters will also be calculated for M4 metabolite (PF-07275428) where possible.

Additional plasma PK parameters (on Day 14) for PF-07258669 will be derived from the plasma concentration-time profiles using standard non-compartmental methods, as data permit.

Table 4 shows the analysis scale and method for each of these additional parameters.

Table 4. Additional PF-07258669 PK parameters

Parameter	Analysis Scale	PF-07258669
CL/F	ln	D
C_{min}	ln	D
C_{av}	ln	D
PTR	ln	D
R_{ac}	ln	D
$R_{ac,Cmax}$	ln	D
t½*	R	D
Vz/F*	ln	D

*=if data permits. Abbreviations: D=displayed with descriptive statistics as outlined in Table 8 in Section 6.3.1.1; ln=natural-log transformed; R=raw (untransformed).

3.3.1.2. Body Weight

- *Change from baseline in body weight on Days 7 and 14.*

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

Baseline will be defined as the pre-dose (0 hour) assessment on Day 1.

Change from baseline and percent change from baseline will be calculated for each post-baseline timepoint.

CCI

CCI

CCI

CCI [redacted]

3.3.1.7. Potential for Induction of CYP3A

- *Change from baseline on Day 14:*
  - *Urinary 6β-hydroxycortisol/cortisol ratio;*
  - *Plasma 4β-hydroxycholesterol/cholesterol ratio;*
  - *Serum extracellular vesicles (if analyzed).*

Blood samples for the measurement of 4β-hydroxycholesterol, cholesterol and extracellular vesicles, and urine samples for the measurement of 6β-hydroxycortisol and cortisol will be collected at times specified in the protocol.

Baseline for urinary 6β-hydroxycortisol/cortisol ratio will be the sample on Day -1. Baseline for plasma 4β-hydroxycholesterol/cholesterol ratio and serum extracellular vesicles (if analysed) will be the pre-dose (0 hour) sample on Day 1.

Change from baseline and percent change from baseline will be calculated at each post-baseline timepoint.

3.3.1.8. Qualitative Metabolite Profiling

- *Qualitative plasma levels of potential metabolites of PF-07258669 at steady-state.*

Metabolite profiling may be conducted as part of this study and used for internal exploratory purposes. These data will not be included in the CSR.

3.3.1.9. Other Endpoints

CCI [redacted]

Retained Research Samples for biomarkers and genetics will be collected as part of this study and may be used for internal exploratory purposes. These data will not be included in the CSR.

3.3.2. Part B

3.3.2.1. Additional Midazolam PK Parameters

- *Midazolam plasma PK parameters alone and in combination with PF-07258669 on Period 1/Day 1, Period 2/Day 2, and Period 2/Day 10: Tmax, CL/F, Vz/F, and t½ (if data permit).*

Additional plasma PK parameters (on Period 1/Day 1, Period 2/Day 2 and Period 2/Day 10) for midazolam will be derived from the plasma concentration-time profiles using standard non-compartmental methods, as data permit.

Table 5 shows the analysis scale and method for each of these additional parameters.

Table 5. Additional Midazolam PK Parameters

Parameter	Analysis Scale	Period 1/Day 1 (Midazolam)	Period 2/Day 2 (Midazolam + PF-07258669)	Period 2/Day 10 (Midazolam + PF-07258669)
T_{max}	R	D	D	D
CL/F*	ln	D	D	D
Vz/F*	ln	D	D	D
t½*	R	D	D	D

*=if data permits. Abbreviations:D=displayed with descriptive statistics as outlined in Table 9 in Section 6.3.2.1; ln=natural-log transformed; R=raw (untransformed)

3.3.2.2. Potential for Induction of CYP3A

- *Change from baseline on Day 10:*
  - *Urinary 6β-hydroxycortisol/cortisol ratio on Day 7(if analyzed);*
  - *Plasma 4β-hydroxycholesterol/cholesterol ratio*
  - *Serum extracellular vesicles (if analyzed)*

Blood samples for the measurement of urinary 6β-hydroxycortisol, cortisol, plasma 4β-hydroxycholesterol, cholesterol and extracellular vesicles will be collected at times specified in the protocol.

Baseline will be the sample on Day 1 Period 1, prior to morning dosing.

Change from baseline and percent change from baseline will be calculated for Day 10 Period 2.

CCI [redacted]

CCI

[redacted]

3.4. Baseline Variables

Not applicable.

3.5. Safety Endpoints

See Section 3.2.1 for safety endpoints for Part A.

See Section 3.2.2 for safety endpoints for Part B.

3.5.1. COVID-19

Participants will undergo COVID-19 related measures per CRU procedures.

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 unblinding and releasing the database and classifications will be documented per standard operating procedures.

Participant Analysis Set	***Description***
Enrolled/ Randomly assigned to study intervention	*"Enrolled" means a participant's agreement to participate in a clinical study following completion of the informed consent process and 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. A participant will be considered enrolled if the informed consent is not withdrawn prior to participating in any study activity after screening.*
Full Analysis Set (FAS)	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention, for the given part of the study (Part A or B).*
Safety Analysis Set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention, for the given part of the study (Part A or B). Participants will be analyzed according to the product they actually received.*
PK Concentration Set	*All participants randomly assigned to study intervention and who take at least 1 dose of midazolam and/or study intervention and in whom at least 1 plasma or urine concentration value is reported, for the given part of the study (Part A or B).*

Participant Analysis Set	*Description*
PK Parameter Set	*All participants randomly assigned to study intervention and who take at least 1 dose of midazolam and/or study intervention and have at least 1 of the PK parameters of interest calculated, for the given part of the study (Part A or B).*

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

There is no statistical hypothesis testing planned for this study and no statistical decision rules will be applied.

5.2. General Methods

Part A and Part B of the study will be presented separately.

Unless otherwise stated, all summaries and plots will be presented by treatment group, with data from different populations (e.g. non-Japanese, Japanese and older adult participants), if appropriate, reported separately. Each dietary allocation (ie, standard, high carbohydrate-high calorie, and high fat-high calorie diets) will also be reported separately.

If a dose level is repeated across 2 or more cohorts (within a diet), the data may be combined and/or reported separately.

5.2.1. Analyses for Continuous Endpoints

Unless otherwise stated, continuous variables will be presented using summary statistics: number of observations, arithmetic mean, standard deviation, median, minimum and maximum values.

Log transformed continuous variables will be presented using summary statistics: number of observations, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.

5.2.2. Analyses for Categorical Endpoints

Categorical variables will be presented using summary statistics: number of observations and percentages.

5.2.3. Mixed Effects Model

A *mixed effects model with treatment as a fixed effect and participant as a random effect* will be used. *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.*

Example SAS code is provided in Appendix 3.

5.2.4. Mixed Model Repeated Measures

An MMRM model will be used with treatment, time (as a factor), treatment-by-time interaction, baseline and baseline-by-time interaction as fixed effects. Participant will be fitted as a random effect in the model with time as a repeated effect within each participant. An unstructured covariance matrix will be fitted to the repeated times within participant (other covariance matrices will be considered if necessary, e.g. if the model does not converge). Records collected after discontinuation of study drug will be excluded.

The Least Squares (LS) means (and standard errors, 90% confidence intervals and 2-sided p-values) will be obtained for each treatment at each timepoint. Differences (and standard errors, 90% confidence intervals and 2-sided p-values) between LS means will also be obtained, comparing each dose of PF-07258669 versus placebo. If data have been logged, LS means and differences (including CI's) will be back transformed to give percent changes from baseline ([relative change from baseline -1]*100) and percent change from placebo ([ratio of relative change from baseline -1]*100), as appropriate.

Standard SAS output will be provided to support the statistical summary table for the analysis model, but will not be included in the CSR.

Example SAS code is provided in Appendix 3.

Statistical Model Diagnostics

The presence of outliers will be investigated for this model. An outlier will be defined as any response data value with a studentized (conditional) residual greater than 3, or less than -3. A listing will be presented of any participants meeting these criteria and will be included with standard SAS output. The assumptions of normality will be verified graphically using residual plots. For each fitted model, a set of conditional studentized residual plots will be produced, including residual plot, histogram of normality, quartile-quartile (QQ) plot and summary of fit statistics. The residual plots will not be included in the CSR.

If there are outliers or major deviations from normality, then the effect of these on the conclusions may 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.

5.2.5. Bayesian Emax Model

A four-parameter Emax model will be used, with dose as a continuous variable. The model structure will take the form: -

$$CFB = E_0 + \frac{E_{max} * dose^{Hill}}{ED_{50}{}^{Hill} + dose^{Hill}}$$

E_0 is the placebo effect, *dose* is the target randomized dose (placebo dose = 0), E_{max} is the maximum effect, ED_{50} is the dose producing 50% of the maximum effect and *Hill* is the slope parameter. The model will utilize a Bayesian methodology approach with weakly informative priors as described in Appendix 4.

Estimates of the model parameters of E_0, E_{max}, ED_{50} and *Hill* and their 95% credible intervals will be produced.

The posterior medians and 90% credible intervals (5th and 95th percentiles of the relevant posterior distribution) will be reported for each target randomized dose (including placebo) and their differences relative to placebo.

If convergence cannot be obtained, a posterior predictive check for non-monotone dose response produces a predictive fit probability <0.05, or visual inspection of the data does not support a dose-response Emax relationship, the model may be simplified or the analysis may not be performed.

5.3. Methods to Manage Missing Data

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

5.3.1. Concentrations Below the Limit of Quantification

In all PK data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero.

In all exploratory biomarker data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to the lower limit of quantification (LLQ).

In listings, BLQ values will be reported as "<LLQ" where LLQ will be replace with the value for the LLQ.

5.3.2. Deviations, Missing Concentrations and Anomalous Values

In PK and exploratory biomarker 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 (i.e. not done) or NS (i.e. no sample),
2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist/statistician.

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

Participants who experience events that may affect their PK profile (e.g. lack of compliance with dosing or vomiting) 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.

5.3.3. Pharmacokinetic Parameters

Actual PK sampling times will be used in the derivation of PK parameters. For PK parameter calculations, the sponsor's standard rules will be applied.

If a PK parameter cannot be derived from a participant's concentration data, the parameter will be coded as NC (i.e. 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 group/analyte with ≥3 evaluable measurements. For statistical analyses (i.e. mixed effects model), 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 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.

6. ANALYSES AND SUMMARIES

For all presentations, study day is relative to start of study treatment (Day 1); period day is relative to the start of treatment period, unless otherwise specified.

For endpoints Body Weight, CCI , CCI , CCI [redacted] the records collected after discontinuation of study drug will be excluded from all summaries, figures and analysis tables. Data will be listed only.

6.1. Primary Endpoint(s)

6.1.1. Part A: Safety and Tolerability

6.1.1.1. Adverse Events

Adverse events will be listed and summarized by treatment and overall, in accordance with sponsor reporting standards using the safety population defined in Section 4.

6.1.1.2. Clinical Safety Laboratory Tests

Laboratory data will be listed and summarized by treatment, in accordance with the sponsor reporting standards using the safety population defined in Section 4. Baseline is as defined in Section 3.1.1.2.

Change from baseline in ALT and AST will be summarized descriptively by treatment and timepoint, as described in Section 5.2.1. The safety analysis set (as defined in Section 4) will

be used. Mean change from baseline will be plotted against time post-dose. On each plot there will be one line for each treatment and 90% CIs.

6.1.1.3. Vital Signs

Absolute values and changes from baseline in supine, standing and postural changes for systolic and diastolic blood pressure (BP) and pulse rate will be listed and summarised by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.1.1.3.

Mean changes from baseline for supine, standing and postural changes for systolic and diastolic BP and pulse rate will be plotted against time post-dose. On each plot there will be one line for each treatment. Corresponding individual plots of changes from baseline will also be produced for each treatment.

Maximum decrease from baseline for supine and standing systolic and diastolic BP, maximum increase from baseline for supine and standing pulse rate, maximum increase from baseline for postural differences in systolic and diastolic BP, and maximum decrease from basleine for postural difference in pulse rate will be summarized by treatment, according to sponsor reporting standards.

Maximum/minimum absolute values and changes from baseline for vital signs (for supine, standing and postural) will also be summarised descriptively by treatment using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post-dose time points will be counted in these categorical summaries.

6.1.1.4. 12-lead Electrocardiograms

Absolute values and *changes from baseline for the ECG parameters (ie, QT interval, heart rate, QTcF interval, PR interval and QRS complex) will be summarized by treatment and time*point according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.1.1.5.

Mean changes from baseline for QT interval, heart rate and QTcF interval will be plotted against time post-dose. On each plot there will be one line for each treatment. Corresponding individual plots of changes from baseline will also be produced for each treatment.

Maximum increase from baseline for QTcF will be summarized by treatment, according to sponsor reporting standards.

Maximum/minimum absolute values and changes from baseline for QTcF, PR and QRS intervals will also be summarized descriptively by treatment using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post dose timepoints will be counted in these categorical summaries.

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

If more than 1 ECG is collected at a nominal time after dose administration (for example, triplicate ECGs), the mean of the replicate measurements will be used to represent a single observation at that time point. If any of the 3 individual ECG tracings has a QTcF value >500 msec, but the mean of the triplicates is not >500 msec, the data from the participant's individual tracing will be described in a safety section of the CSR in order to place the >500 msec value in appropriate clinical context. However, values from individual tracings within triplicate measurements that are >500 msec will not be included in the categorical analysis unless the average from the triplicate measurements is also >500 msec. Changes from baseline will be defined as the change between the postdose QTcF value and the average of the predose triplicate values on Day 1.

Changes from baseline in QTcF will be plotted separately against drug concentrations. This will be a scatter plot for all observations where QTcF and drug concentration are recorded. Placebo data will also be included (with drug concentration set to zero). Different symbols will be used for each treatment. *In addition, an attempt will be made to explore and characterize the relationship between plasma concentration and QT interval length using a PK/PD modeling approach. If a PK/PD relationship is found, the impact of participant factors (covariates) on the relationship will be examined. The results of such analyses may not be included in the CSR.*

6.1.1.5. Respiratory Rate

Absolute values and changes from baseline in respiratory rate will be listed and summarized by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.1.1.6.

Mean change from baseline will be plotted against time post-dose. On each plot there will be one line for each treatment. Corresponding individual plots of changes from baseline will also be produced for each treatment.

Maximum increase and decrease from baseline for respiratory rate will be summarized by treatment, according to sponsor reporting standards.

6.1.1.6. Oral Body Temperature

Absolute values and changes from baseline in oral body temperature will be listed and summarized by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.1.1.7.

Mean change from baseline will be plotted against time post-dose. On each plot there will be one line for each treatment. Corresponding individual plots of changes from baseline will also be produced for each treatment.

Maximum increase and decrease from baseline for oral body temperature will be summarized by treatment, according to sponsor reporting standards.

6.1.1.7. C-SSRS

Screening ("Lifetime") and post-screening ("Since Last Visit") C-SSRS data (mapped to C-CASA scores, as described in Section 3.1.1.9) will be listed and summarized categorically, as outlined in Section 5.2.2, by treatment and timepoint, using the safety population defined in Section 4.

CCI

[redacted]

6.1.2. Part B: Midazolam PK Parameters

Plasma concentrations of midazolam will be listed and summarized descriptively by nominal PK sampling time and treatment (midazolam alone on Period 1/Day 1, co-administration on Period 2/Day 2, and co-administration on Period 2/Day 10) using the PK Concentration Set (as defined in Section 4). Presentations will include:

- a listing of all concentrations sorted by participant ID, treatment and nominal time post-dose. 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 post-dose, 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.
- individual concentration-time plots by treatment (on both linear and semi-log scales) against actual time post-dose (there will be separate spaghetti plots for each treatment, with a line for each participant per scale).
- individual concentration-time plots by participant (on both linear and semi-log scales) against actual time post-dose (there will be separate spaghetti plots for each participant, with a line for each treatment per scale).
- median concentration-time plots (on both linear and semi-log scales) against nominal time post-dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time post-dose).
- mean concentration-time plots (on both linear and semi-log scales) against nominal time post-dose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time post-dose).

The nominal PK sampling time will be used for summary statistics and relevant median plots, whereas for individual participant plots by time, the actual PK sampling time will be used.

The midazolam PK parameters, detailed in Section 3.1.2, will be listed, summarized descriptively and analyzed by treatment for participants in the PK Parameter set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3.

Each PK parameter will be summarized for each treatment using the summary statistics as specified in the table below: -

Table 6. Midazolam PK Parameters to be Summarized Descriptively

Parameter	Summary Statisticis
C_{max}, AUC_{last}, AUC_{inf}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.

Supporting data from the estimation of AUCinf will be listed by treatment: 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. These data may be included in the clinical study report.

Box and whisker plots for individual PK parameters (Cmax, AUCinf and AUClast) will be presented by treatment and overlaid with geometric means and individual datapoints.

Natural log transformed AUC_{inf}, AUC_{last} and C_{max} of midazolam *will be analyzed using a mixed effects model* as described in Section 5.2.3. *Midazolam alone (Period 1) will be the Reference treatment, while the midazolam co-administered with PF-07258669 (on Day 2 or 10 in Period 2) will be the Test treatments.*

Additional PK analyses may be performed if deemed appropriate and may not be included in the CSR.

If additional cohorts are included in the study, all summaries and analyses will also include cohort/dose.

6.2. Secondary Endpoint(s)

6.2.1. Part A: PF-07258669 Plasma and Urine PK Parameters

Plasma and urine *concentrations of PF-07258669* and plasma concentrations of M4 metabolite (PF-07275428) where possible *will be listed and summarized descriptively by dose, population* (non-Japanese, Japanese [if enrolled], and older adult participants [if enrolled]), *dietary allocation, day and nominal PK sampling time* using the PK Concentration Set defined in Section 4. Presentations will include:

- a listing of all concentrations sorted by participant ID and nominal time post-dose. 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 dose, population, dietary allocation, day and nominal time post-dose, 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.
- individual concentration-time plots by dose, population (on both linear and semi-log scales) and dietary allocation, against actual time post-dose (there will be separate spaghetti plots for each dose per scale), paged by day and coloured by dose.

- median concentrations-time plots (on both linear and semi-log scales) against nominal time post-dose by dose, population and dietary allocation (all doses on the same plot per scale, based on the summary of concentrations by dose, population, dietary allocation and time post-dose), paged by day and coloured by dose.
- mean concentrations-time plots (on both linear and semi-log scales) against nominal time post-dose by dose, population and dietary allocation (all doses on the same plot per scale, based on the summary of concentrations by dose and time post-dose), paged by day and coloured by dose.
- Median and mean pre-dose concentration-time plots (on both linear and semi-log scales) against day by dose, population and dietary allocation (all doses on the same plot per scale), based on the summary of concentrations by dose and time post-dose), coloured by dose.

The nominal PK sampling time will be used for summary statistics and relevant median plots, whereas for individual participant plots by time, the actual PK sampling time will be used.

The plasma and urine PF-07258669 PK parameters and plasma M4 metabolite (PF-07275428) where possible and as applicable, detailed in Section 3.2.1, *will be listed and summarized descriptively by dose, population, dietary allocation and day, as applicable,* for participants in the PK Parameter set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3.

Each PK parameter will be summarized by dose, population, dietary allocation and day (Days 1 or 14, as appropriate) using the summary statistics as specified in the table below: -

Table 7. PF-07258669 PK Parameters to be Summarized Descriptively

Parameter	Statistical Summaries
C_{max}, AUC_{tau}, C_{max}(dn), AUC_{tau} (dn)	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
Ae_{tau}, Ae_{tau}%, CLr	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%

To assess the relationship between the PK parameters and dose, *dose normalized AUC_{tau} and C_{max} may be plotted against dose (using a logarithmic scale) for each day* separately, *and will include individual participant values and the geometric means for each dose. The data from the Japanese participants (if enrolled) and older adult participants (if enrolled)* and diet, *will be identified by different symbols/colors.* Geometric means will have a different symbol than the individual values. The values will be dose normalized (to a 1 mg dose) by dividing the individual values and raw geometric means by dose.

Additional PK analyses may be performed if deemed appropriate and may not be included in the CSR. Results from M4 metabolite (PF-07275428) may not be included in the CSR.

6.2.2. Part B: Safety and Tolerability

6.2.2.1. Adverse events

See section 6.1.1.1.

6.2.2.2. Vital signs

Absolute values and changes from baseline in supine, standing and postural changes for systolic and diastolic blood pressure (BP) and pulse rate will be listed and summarised by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.2.2.

Mean changes from baseline for supine, standing and postural changes for systolic and diastolic BP and pulse rate will be plotted against time post-dose. Corresponding individual plots of changes from baseline may also be produced, or combined with the mean plot.

Maximum/minimum absolute values and changes from baseline for (for supine, standing and postural) vital signs will also be summarised descriptively by treatment using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post dose time points will be counted in these categorical summaries.

6.2.2.3. ECGs

Absolute values and changes from baseline for the ECG parameters (ie, QT interval, heart rate, QTcF interval, PR interval and QRS complex) will be listed and summarized by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.2.2.

Mean changes from baseline for QT interval, heart rate and QTcF interval will be plotted against time post-dose. Corresponding individual plots of changes from baseline may also be produced, or combined with the mean plot.

Maximum absolute values and changes from baseline for QTcF, PR and QRS intervals will also be summarized descriptively by treatment using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post dose timepoints will be counted in these categorical summaries.

6.2.2.4. Clinical Safety Laboratory Measurements

Laboratory data will be listed and summarized by treatment, in accordance with the sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.2.2.

6.2.2.5. Respiratory Rate

Absolute values and changes from baseline in respiratory rate will be listed and summarized by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.2.2.

Mean change from baseline will be plotted against time post-dose. Corresponding individual plots of changes from baseline may also be produced or combined with the mean plot.

6.2.2.6. Oral Body Temperature

Absolute values and changes from baseline in oral body temperature will be listed and summarized by treatment and timepoint, according to sponsor reporting standards, using the safety population defined in Section 4. Baseline is as defined in Section 3.2.2.

Mean change from baseline will be plotted against time post-dose. Corresponding individual plots of changes from baseline may also be produced or combined with the mean plot.

6.3. Other Endpoint(s)

6.3.1. Part A

6.3.1.1. Additional PF-07258669 PK Parameters

Additional plasma PK parameters for PF-07258669 and M4 plasma metabolite (PF-07275428) where possible and as applicable, as described in Section 3.3.1.1, will be listed and summarized descriptively by dose, population, day, and dietary allocation, as applicable, for participants in the PK Parameter set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3.

Each PK parameter (if data permit) will be summarized by dose, population and dietary allocation on Day 14 using the summary statistics as specified in the table below: -

Table 8. Additional PF-07258669 PK Parameters to be Summarized Descriptively

Parameter	Summary Statisitics
CL/F, Vz/F, C_{min}, C_{av}, PTR, R_{ac}, R_{ac} C_{max}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
t½	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

Supporting data from the estimation of t½ will be listed by treatment: 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. These data may be included in the clinical study report.

Additional PK analyses may be performed if deemed appropriate and may not be included in the CSR. Results from plasma M4 metabolite (PF-07275428) may not be included in the CSR.

6.3.1.2. Body Weight

Change from baseline and percent change from baseline body weight will be listed and summarized descriptively by treatment and timepoint, as described in Section 5.2.1. The FAS (as defined in Section 4) will be used.

Mean change from baseline and percent change from baseline will be plotted against time post-dose. On each plot there will be one line for each treatment. Corresponding individual plots of changes from baseline will also be produced for each treatment.

Change from baseline will be analyzed using an MMRM model as described in Section 5.2.4. Separate models will be fitted to the different populations (e.g. non-Japanese, Japanese and older adult participants), and dietary allocation if appropriate. Each model will include all planned timepoints up to Day 16. Plots of LS means and differences (including CIs for both) will be produced over time (all treatments on the same plot with different colours/symbols for each treatment). Additionally, plots of LS means and differences (including confidence intervals for both), for Day 14 only, will be produced over treatment. These plots of LS means and differences may be combined across the different populations, as appropriate.

A Bayesian Emax model will also be applied to the standard diet, non-Japanese LS means for Day 14 only, as described in Section 5.2.5. Plots of posterior medians and differences (including credible intervals for both), for Day 14 only, will be produced versus dose. This analysis may use total daily dose and/or may only include a subset of treatments (e.g. all doses for a single dosing regimen).

Further exploratory analysis may be performed (e.g. with all populations included), if deemed appropriate and may not be included in the CSR.

CCI

CCI

CCI

6.3.1.7. Potential for Induction of CYP3A

Absolute values, change from baseline, and percent change from baseline in urinary 6β-hydroxycortisol/cortisol ratio and plasma 4β-hydroxycholesterol/cholesterol ratio will be summarized descriptively by treatment and timepoint, as described in Section 5.2.1. The FAS (as defined in Section 4) will be used.

Box and whisker plots for change from baseline in urinary 6β-hydroxycortisol/cortisol ratio and plasma 4β-hydroxycholesterol/cholesterol ratio will be presented by treatment and overlaid with geometric means and individual datapoints.

CCI

[redacted]

6.3.2. Part B

6.3.2.1. Additional Midazolam PK Parameters

Additional plasma PK parameters for Midazolam as described in Section 3.3.2.1 will be listed and summarized descriptively by treatment for participants in the PK Parameter set (as defined in Section 4). Missing values will be handled as detailed in Section 5.3.

Each PK parameter (if data permit) will be summarized for each treatment using the summary statistics as specified in the table below: -

Table 9. Additional Midazolam PK parameters to be Summarized Descriptively

Parameter	Summary Statisticis
CL/F*, Vz/F*	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.
t½*	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.

*=if data permit

Supporting data from the estimation of t½ will be listed by treatment: 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. These data may be included in the clinical study report.

Additional PK analyses may be performed if deemed appropriate and may not be included in the CSR.

If additional cohorts are included in the study, all summaries and analyses will also include cohort/dose.

6.3.2.2. Potential for Induction of CYP3A

Absolute values and change from baseline in plasma 4β-hydroxycholesterol/cholesterol ratio will be summarized descriptively by treatment, as described in Section 5.2.1. The FAS (as defined in Section 4) will be used.

Box and whisker plots for change from baseline in plasma 4β-hydroxycholesterol/cholesterol ratio will be presented by treatment and overlaid with geometric means and individual datapoints.

CCI [redacted]

CCI [redacted]

6.4. Subset Analyses

No subset analyses will be performed.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

Demographic data (age, biological sex, race, ethnicity, body weight, body mass index and height) will be summarized by treatment and overall, for participants in the safety populations for Part A and Part B (as defined in Section 4), separately, as described in Sections 5.2.1 or 5.2.2 (as appropriate).

6.5.2. Study Conduct and Participant Disposition

Participant evaluation groups will show end of study participant disposition by treatment and overall, for Part A and Part B separately, and will show which participants were analyzed for PK and safety. Frequency counts and percentages will be supplied for participant discontinuation(s) by treatment.

6.5.3. Concomitant Medications and Nondrug Treatments

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

6.6. Safety Summaries and Analyses

See Section 6.1.1 for Part A safety summaries.

See Section 6.2.2 for Part B safety summaries.

6.6.1. COVID-19

Positive COVID-19 status will be listed. Additional tables may be produced, in accordance with sponsor reporting standards, using the safety population defined in Section 4.

7. INTERIM ANALYSES

7.1. Introduction

No formal interim analysis will be conducted for this study. As this is a 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. A limited number of the sponsor's team members (excluding site staff) may conduct the unblinded reviews.

7.2. Interim Analyses and Summaries

Not Applicable.

8. REFERENCES

1. clinDR: Simulation and Analysis Tools for Clinical Dose Response Modeling. https://CRAN.R-project.org/package=clinDR

2. Thomas, N., Sweeney, K., and Somayaji, V. (2014). Meta-analysis of clinical dose response in a large drug development portfolio, Statistics in Biopharmaceutical Research, Vol. 6, No.4, 302-317. <doi:10.1080/19466315.2014.924876>

3. Thomas, N., and Roy, D. (2016). Analysis of clinical dose-response in small-molecule drug development: 2009-2014. Statistics in Biopharmaceutical Research, Vol. 6, No.4, 302-317 <doi:10.1080/19466315.2016.1256229>

4. Wu, J., Banerjee, A., Jin, B., Menon, S., Martin, S., and Heatherington, A. (2017). Clinical dose-response for a broad set of biological products: A model-based meta-analysis. Vol. 9, 2694-2721. <doi:10.1177/0962280216684528?>

APPENDICES

Appendix 1. Categorical Classes for ECG and Vital Signs of Potential Clinical Concern

Categories for QTcF

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

Categories for PR and QRS

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	

Categories for Vital Signs

Systolic BP (mm Hg)	min. < 90	max. ≥ 160
Systolic BP (mm Hg) change from baseline	max. decrease ≥ 30	max. increase ≥ 30
Systolic BP (mmHg) postural difference (supine – standing)	max. ≥ 20	
Diastolic BP (mm Hg)	min. < 50	max. ≥ 90
Diastolic BP (mm Hg) change from baseline	max. decrease ≥ 20	max. increase ≥ 20
Diastolic BP (mmHg) postural difference (supine – standing)	max. ≥ 10	
Supine pulse rate (bpm)	min. < 40	max. > 120
Standing pulse rate (bpm)	min. < 40	max. > 140
Pulse rate (bpm) postural difference (supine – standing)	min. ≤ -30	

Measurements that fulfill these criteria are to be listed in the report.

Appendix 2. C-SSRS Mapped to C-CASA - Suicidal Ideation and Behavior Events and Codes

Table 10. C-SSRS Mapped to C-CASA (Suicidality Events and Codes)

Event Code	C-CASA Event	C-SSRS Response
Suicidal Ideation		
1	Passive	"Yes" on "Wish to be dead"
2	Active: Nonspecific (no method, intent, or plan)	"Yes" on "Non-Specific Active Suicidal Thoughts"
3	Active: Method, but no intent or plan	"Yes" on "Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act"
4	Active: Method and intent, but no plan	"Yes" on "Active Suicidal Ideation with Some Intent to Act, without Specific Plan"
5	Active: Method, intent, and plan*	"Yes" on "Active Suicidal Ideation with Specific Plan and Intent"
Suicidal Behavior		
1	Completed suicide	"Yes" on "Completed Suicide"
2	Suicide attempt	"Yes" on "Actual Attempt"
3	Interrupted attempt	"Yes" on "Interrupted attempt"
4	Aborted attempt	"Yes" on "Aborted attempt"
5	Preparatory actions toward imminent suicidal behaviors	"Yes" on "Preparatory Acts or Behavior"
Self-injurious behavior, no suicidal intent		
	Self-injurious behavior, no suicidal intent	"Yes" on "Has subject engaged in Non-suicidal Self-Injurious Behavior?"

*According to C-SSRS, the definition of *plan* includes intent (i.e., intent to complete the suicide is implicit with the concept of plan). Thus, there is no need for the category *method and plan, but no intent*.

Appendix 3. Example SAS Code for Statistical Analyses

Mixed Effects Model:

```
proc mixed data = input_dataset;
        class trt subject;
        model var = trt / ddfm = kr;
        random subject / subject = subject;
        lsmeans trt / diff cl alpha=0.1;
run;
```

MMRM:

```
proc mixed data = input_dataset method = reml;
        class subject trt time;
        model var = trt  base time base*time trt*time /ddfm = kr residual;
        repeated time / subject = subject type = un;
        lsmeans trt*time / diff cl alpha=0.1;
run;
```

Appendix 4. Bayesian Emax Model Methodology Details

Data Provided by Programmer

A dataset (either .txt or .csv) of LS means from the MMRM analysis should be produced by programming for use in R by the reporting statistician and QC statistician. The file should be in the following format (Note that column headers should be labelled as specified below (including capitalization), as R is case sensitive): -

dose	mean	se
0	0	0.3
2.5	0.2	0.4
10	0.4	0.5
40	0.3	0.3
80	1.6	0.4
120	1.8	0.5

Note: *dose* may represent the total daily dose if appropriate.

The residual standard deviation on Day 14 from the unstructured covariance matrix from the MMRM will also be provided to the statisticians.

Fitting the Emax Model

The 4-parameter Emax model will be fitted using the latest version (currently 2.3.5) of the clinDR package (1). This analysis will be conducted by the study statistician. A different statistician will conduct QC of the analysis. The outputs of the analysis will be provided as .txt files to the programming team for creation of programming tables and figures.

The default burn-in and number of samples will be utilized along with thinning of 20, which will include 3 chains to assess convergence.

Model diagnostics will be examined, including trace and auto-correlations plots. If these raise concerns over model convergence, additional burn-ins, samples and thinning will be investigated to improve convergence. Changes to the priors below may also be considered (e.g. increase precision of E_0 and *difTarget*) to improve convergence, if deemed necessary. The final diagnostic plots will not be included in the clinical study report.

Priors

Prior distributions will be specified for the placebo response (E_0), the difference in response (*difTarget*) between the highest dose studied (e.g. *dTarget* = 200mg) and placebo, and the residual standard deviation (*sigma*). Note that Emax is derived from other parameters and is thus not explicitly supplied.

Parameter	Prior
E_0	t(Mean = 0, SD = 17, df=5)
difTarget	t(Mean = 0, SD = 17, df=5)
sigma	Uniform(lb=0, ub=3.5)

E_0: There is insufficient historical data to formally calculate a placebo prior. Therefore, a non-informative t-distribution will be used for the placebo change from baseline on Day 14, with a mean of 0 and a standard deviation equal to 10 times the predicted standard deviation (1.7kg).

difTarget: A non-informative t-distribution will be used for the highest dose placebo-corrected change from baseline on Day 14 with a mean of 0 and a standard deviation equal to 10 times the predicted standard deviation (1.7kg).

sigma: A uniform prior will be used, with a range we are confident will include the population value. Based on a meta-analysis of historical data the standard deviation is not expected to be greater than 3.5kg.

ClinDR default settings will be used to specify prior distributions for the *Hill* parameter and the ED_{50}. The default distributions in the current version of clinDR (version 2.3.5) are based on a meta-analysis of dose response data (from references: 2, 3 and 4) and are listed below. These default distributions will be updated if the meta-data, and their analysis, are updated before the completion of the current study.

Parameter	Prior
$log(ED_{50}/P_{50})$	t(Mean=0, SD=1.73, df=5)
log(Hill)	t(Mean = 0, SD =0.85, df=5)

The correlation between these two parameters is currently -0.45 based on the analysis of the meta-data, which also would be updated if the historical analysis is updated.

The predicted ED_{50} for the compound (P_{50}) = CCI [redacted] based on data from the C4541001 study.

R Code

The following R code is included as an example that will be used as a basis for the analysis: -

```
library(clinDR)
mmrmRes <- read.csv("LSmeans.csv", header=T, stringsAsFactors=F)

# Determine 'effective' subject numbers based on MMRM SD at Day 14:
mmrm_sd <- 0.825                                    # Provided by programming
mmrmRes$n <- trunc((mmrm_sd/mmrmRes$se)^2,0)
```

```
# Set-up priors and MCMC options:
prior_mmrm <- emaxPrior.control(epmu=0, epsca=17,
                                difTargetmu=0, difTargetsca=17,
                                dTarget=600,
                                CCI
                                sigmalow=0, sigmaup=3.5)
mcmc_mmrm <- mcmc.control(chains=3, thin=20, seed=169)

# Run Emax model: #
emaxMMRM <- fitEmaxB(mmrmRes$mean, mmrmRes$dose, prior_mmrm, modType=4,
                     count=mmrmRes$n, msSat=mmrm_s d^2, mcmc=mcmc_mmrm)

# Diagnostics and output:
stan_trace(emaxMMRM$estanfit)                    # Look at trace
stan_dens(emaxMMRM$estanfit)                     # Look at densities
stan_ac(emaxMMRM$estanfit)                       # Look at autocorrelation
summary(emaxMMRM)                                # Summary of model parameters
plot(emaxMMRM)                                   # Look at fitted vs. observed data to check model fit
emaxMMRMout <- predict(emaxMMRM, dosevec=mmrmRes$dose, clev=0.90)        # Get dose predictions

# Posterior predictive check for non-monotone dose response
postCheck<-checkMonoEmax(y=mmrmRes$mean,dose=mmrmRes$dose,
        parm=coef(emaxMMRM),sigma2=(sigma(emaxMMRM))^2,nvec=mmrmRes$n)
```

Data Provided by Statistician

Model parameters, posterior medians and credible intervals, as specified in Section 5.2.5, will be output and provided back to programming after QC is complete.

Appendix 5. List of Abbreviations

Abbreviation	Term
AE	adverse event
Ae_{tau}	amount of unchanged drug recovered in urine during the dosing interval
Ae_{tau} %	percent of dose recovered in urine as unchanged drug
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{24}	area under the concentration-time curve from time 0 to 24 hours
AUCextrap%	area under the concentration-time curve from time 0 to infinity based on extrapolation
AUC_{inf}	area under the concentration-time curve from time 0 to infinity
AUC_{last}	area under the concentration-time curve from 0 to time of last measurable concentration
AUC_{tau}	area under the concentration-time curve at steady state over the dosing interval tau
A&R	analysis and reporting
CCI [redacted]	
BLQ	below the limit of quantitation
BP	blood pressure
bpm	beats per minute
C_{av}	average plasma concentration
C-CASA	Columbia-Classification Algorithm of Suicide Assessment
CFB	change from baseline
CI	confidence interval
CL/F	apparent clearance for oral dosing
CL_r	renal clearance
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
COVID-19	coronavirus disease 2019
CRF	case report form
CRU	clinical research unit
C-SSRS	Columbia Suicide Severity Rating Scale
CSR	clinical study report
cv	coefficient of variation
CYP	cytochrome P450
ECG	electrocardiogram
E0	placebo effect
ED_{50}	dose that produces half-maximal effect
Emax	the maximal effect

Abbreviation	Term
FAS	full analysis set
H	hour
HCHC	high carbohydrate, high calorie
HFHC	high fat, high calorie
HR	heart rate
ID	identification
kel	terminal phase elimination rate constant
L	litres
LLQ	lower limit of quantitation
LS	least-squares
MMRM	mixed-effects model with repeated measures
mmHg	millimetre of mercury
ms	millisecond
msec	millisecond
MTD	maximum tolerated dose
N	number of observations
N/A	not applicable
NC	not calculated
ND	not done
CCI [redacted]	
NS	no sample
PA2	protocol amendment 2
PA3	protocol amendment 3
PA4	protocol amendment 4
PK	pharmacokinetic(s)
PK/PD	pharmacokinetics/pharmacodynamics
PTR	peak-to-trough ratio
Q8H	every 8 hours
QC	quality control
QQ	quartile-quartile
QTcF	corrected QT (Fridericia method)
R_{ac}	observed accumulation ratio
$R_{ac,Cmax}$	observed accumulation ratio for Cmax
CCI [redacted]	
SAP	statistical analysis plan
SD	standard deviation
$t_{½}$	terminal half-life
CCI [redacted]	
T_{max}	time for C_{max}
CCI [redacted]	
V_z/F	apparent volume of distribution for oral dosing