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

Study Intervention Number:	PF-07258669
Study Intervention Name:	N/A
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Phase:	1

Brief Title: A Phase 1 Study of Multiple Ascending Doses of PF-07258669 in Healthy Adult Participants

Document History

Document	Version Date
Amendment 4	09 November 2022
Amendment 3	09 March 2022
Amendment 2	13 January 2022
Amendment 1	12 October 2021
Original protocol	24 August 2021

This amendment incorporates all revisions to date, including amendments made at the request of country health authorities and IRBs/ECs and any global protocol administrative clarification letter.

Protocol Amendment Summary of Changes Table

Amendment 4 (09 November 2022)

Overall Rationale for the Amendment: The protocol is being amended to allow participants to be allocated to 1 of 3 diets (eg, standard, HCHC, or HFHC), with optional additional cohorts of participants allocated to the HFHC diet (with a larger sample size). These additional optional cohorts may allow for better characterization of safety, tolerability, or clinical biomarker of interest (eg, body weight). The following changes to C4541003 protocol are now proposed:

Section # and Name	Description of Change	Brief Rationale	Substantial or Nonsubstantial
1.1 (Synopsis), 1.2 (Schema, Figure 1), 4.1.1 (Part A), 4.2.1 (Rationale for Part A), 9.3.4 (Tertiary/Exploratory Endpoints), and 9.5.1 (Sample Size Determination)	Optional cohorts of participants allocated to the HFHC diet were added (with a larger sample size).	These cohorts may allow for better characterization of safety, tolerability, or clinical biomarker of interest (eg, body weight).	Substantial
1.1 (Synopsis), 1.2 (Schema), 2.3.1 (Risk Assessment), 4.1 (Overall Design), 4.1.1 (Part A), 4.1.2 (Part B), 4.2.1 (Rationale for Part A), 4.2.2 (Rationale for Part B), 5.3.1 (Meals and Dietary Restrictions), 9.3.3.3 [Statistical Methods for PF-07258669 PK Data (Part A only), 9.3.4 (Tertiary/Exploratory Endpoints), and 9.5.1 (Sample Size Determination)	Cohorts will be allocated to 1 of 3 diets (ie, standard, HCHC, or HFHC). Participants in Part B will only be allocated to standard diet.	The influence of diet on safety and tolerability (eg, liver function tests, triglycerides) has been reported in published literature in healthy participants resident in a CRU. Allocation to 1 of 3 diets will facilitate a more comprehensive assessment of safety, tolerability, PK, and exploratory endpoints.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial or Nonsubstantial
1.3.1 (Part A – Overall Schedule of Activities) and text throughout document	Updated numbering of Appendices 8-15.	Addition of Kidney Safety Monitoring Guidelines (Appendix 7) required subsequent appendices to be re-numbered.	Nonsubstantial
2.2.6 (Clinical Overview)	Added summary of safety, tolerability, and PK data observed to date in the current study.	Provides the most up to date summary of clinical safety, tolerability, and PK data for PF-07258669.	Nonsubstantial
4.3.3.1 (Part A PF-07258669 Dose Selection	Added rationale for dose selection for participants allocated to HFHC diet.	Doses administered to allocated to the HFHC diet (if enrolled) will be less than or equal to the highest safe and well tolerated dose evaluated in healthy adult non-Japanese participants allocated to standard diet in Part A.	Nonsubstantial
10.2 (Appendix 2: Clinical Laboratory Tests)	Added cystatin C to chemistry laboratory assessments.	Aligns with current best practice for clinical safety assessments in multiple ascending dose studies.	Nonsubstantial
10.7 (Appendix 7: Kidney Safety: Monitoring Guidelines)	Added kidney safety monitoring guidelines as an appendix.	Aligns with current best practice for clinical safety assessments in multiple ascending dose studies.	Nonsubstantial
10.14 (Appendix 14: Protocol Amendment History)	Moved Summary of Changes table for Amendment 3 from beginning of protocol to the appendix.	Moved to highlight changes for Amendment 4 at the beginning of the protocol.	Nonsubstantial
10.15 (Appendix 15: Abbreviations)	Added new abbreviations.	New abbreviations were added to protocol text.	Nonsubstantial
11 (References)	Added new references.	To support rationale for dietary allocations, cystatin C, and kidney safety monitoring.	Nonsubstantial

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1. PROTOCOL SUMMARY

1.1. Synopsis

Brief Title: A Phase 1 Study of Multiple Ascending Doses of PF-07258669 in Healthy Adult Participants

Rationale: This study will be the first time multiple ascending doses of PF-07258669 are administered to humans. The safety, tolerability, and PK of multiple ascending doses of PF-07258669 will be evaluated in Part A. The effect of PF-07258669 on midazolam PK will be evaluated in Part B if results from Part A support further development of PF-07258669.

Objectives and Endpoints:

Objectives	Endpoints
Part A	
Primary:	**Primary:**
• 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].
Secondary:	**Secondary:**
• 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).
Tertiary/Exploratory:	**Tertiary/Exploratory:**
• 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). • 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.	• 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). • Change from baseline in body weight on Days 7 and 14.
CCI [redacted]	

• CCI [redacted]
• CCI [redacted]
• 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).
• 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).
	CCI [redacted]
	CCI [redacted]
	• CCI [redacted]
	• Change from baseline on Day 14:
	  • Urinary 6β-hydroxycortisol/cortisol ratio;
	  • Plasma 4β-hydroxycholesterol/cholesterol ratio;
	  • Serum extracellular vesicles (if analyzed).
	• Qualitative plasma levels of potential metabolites of PF-07258669 at steady-state.

Part B	
Primary:	**Primary:**
• 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/Day 10: C_{max}, AUC_{last}, and AUC_{inf} (if data permit).
Secondary:	**Secondary:**
• 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.
Tertiary/Exploratory:	**Tertiary/Exploratory:**

• To evaluate the effects of PF-07258669 on additional PK parameters of midazolam in healthy adult participants.
• To evaluate the potential for induction of CYP3A with administration of multiple doses of PF-07258669 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: T_{max}, CL/F, V_z/F, and $t_{½}$ (if data permit).
	• Change from baseline:
	  • Urinary 6β-hydroxycortisol/cortisol ratio on Day 7 (if analyzed);
	  • Plasma 4β-hydroxycholesterol/cholesterol ratio on Day 10;
	  • Serum extracellular vesicles on Day 10 (if analyzed).

CCI

[redacted]

Brief Summary: 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. Up to 3 different dietary allocations (ie, standard, HCHC, and HFHC diets) may be employed in this study. Optional cohorts of healthy adult Japanese participants and/or older adult participants may also be evaluated if results in other cohorts support further evaluation. 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.

Part B of this study 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. Part B will be conducted if the results of Part A support further evaluation of PF-07258669. 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. 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.

Number of Participants: Approximately 138 healthy adult participants may be enrolled in Part A of this study, of whom approximately 8 may be Japanese and approximately 8-10 may be older adult participants. Approximately 12 healthy adult participants will be enrolled in Part B. Additional optional cohorts may be enrolled in Part A 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). Similarly, additional optional cohorts may

be enrolled in Part B to evaluate the effect of additional dose levels of PF-07258669 on midazolam PK.

The optional cohort of older adult participants, if enrolled, will be conducted after the results of Part B are available.

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.

Intervention Groups and Duration: Participants of Part A of this study will receive multiple doses of PF-07258669 or placebo for 14 days. In Period 1 of Part B, participants will receive a single dose of midazolam. In Period 2 of Part B, participants will receive multiple doses of PF-07258669 for 10 days. Midazolam will be co-administered with PF-07258669 on Days 2 and 10 of Part B. PF-07258669 and placebo will be administered as tablets. Midazolam will be administered as an oral solution.

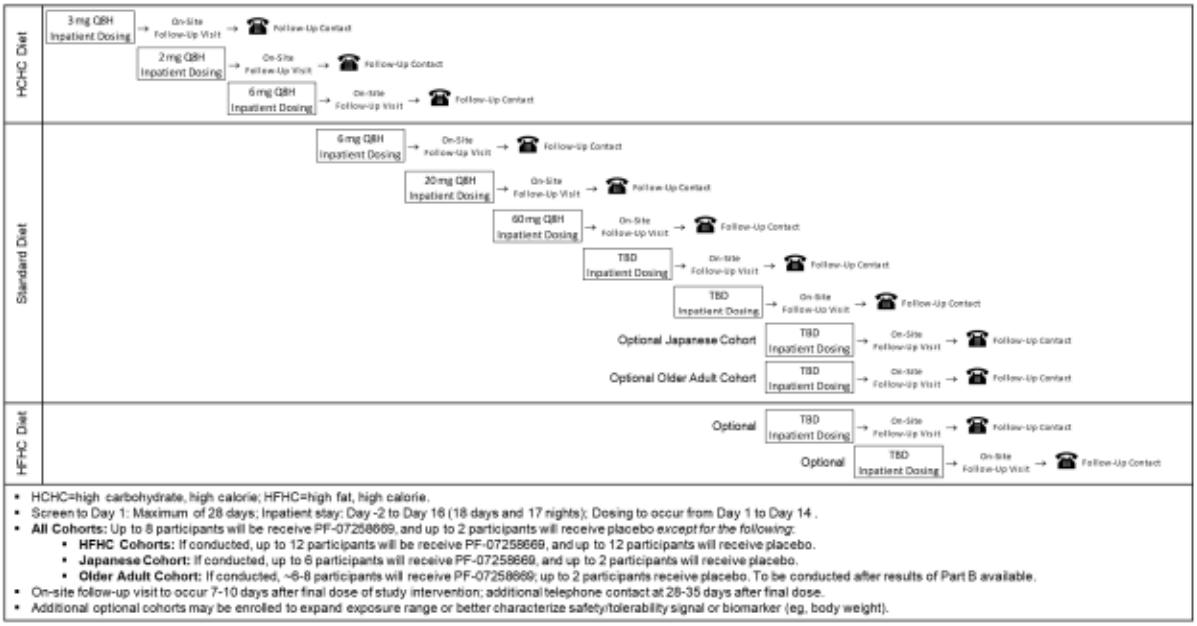
Data Monitoring Committee or Other Independent Oversight Committee: No

Statistical Methods: The sample size has been chosen based on the need to minimize exposure to humans of a new chemical entity while allowing adequate characterization of safety, tolerability, and PK data at each dose level. For the optional HFHC cohorts, the sample size was selected to provide acceptable operating characteristics to assess change from baseline in body weight. All safety analyses will be performed on the Safety Analysis Set, which is defined as all participants randomly assigned to study intervention and who receive at least 1 dose of study intervention. Participants will be analyzed according to the study intervention they actually received. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate. The PK parameters for PF-07258669 and midazolam will be derived from the plasma concentration-time profiles.

PK parameters and concentrations of PF-07258669 and midazolam will be descriptively summarized by dose, dietary allocation, and nominal time, as appropriate.

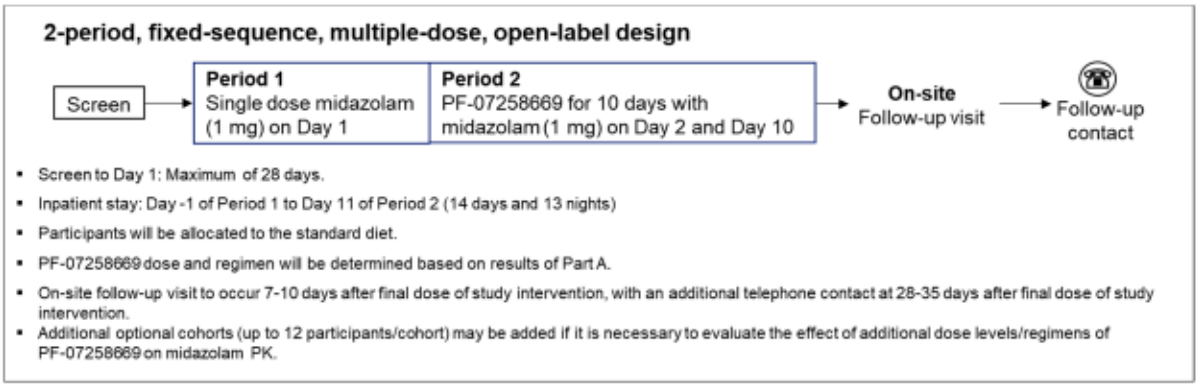
1.2. Schema

Figure 1. Part A: Multiple Ascending Dose Evaluation



- HCHC=high carbohydrate, high calorie; HFHC=high fat, high calorie.
- Screen to Day 1: Maximum of 28 days; Inpatient stay: Day -2 to Day 16 (18 days and 17 nights); Dosing to occur from Day 1 to Day 14 .
- **All Cohorts:** Up to 8 participants will be receive PF-07258669, and up to 2 participants will receive placebo *except for the following*:
  - **HFHC Cohorts:** If conducted, up to 12 participants will be receive PF-07258669, and up to 12 participants will receive placebo.
  - **Japanese Cohort:** If conducted, up to 8 participants will receive PF-07258669, and up to 2 participants will receive placebo.
  - **Older Adult Cohort:** If conducted, ~6-8 participants will receive PF-07258669; up to 2 participants receive placebo. To be conducted after results of Part B available.
- On-site follow-up visit to occur 7-10 days after final dose of study intervention; additional telephone contact at 28-35 days after final dose.
- Additional optional cohorts may be enrolled to expand exposure range or better characterize safety/tolerability signal or biomarker (eg, body weight).

Figure 2. Part B: DDI Assessment



- Screen to Day 1: Maximum of 28 days.
- Inpatient stay: Day -1 of Period 1 to Day 11 of Period 2 (14 days and 13 nights)
- Participants will be allocated to the standard diet.
- PF-07258669 dose and regimen will be determined based on results of Part A.
- On-site follow-up visit to occur 7-10 days after final dose of study intervention, with an additional telephone contact at 28-35 days after final dose of study intervention.
- Additional optional cohorts (up to 12 participants/cohort) may be added if it is necessary to evaluate the effect of additional dose levels/regimens of PF-07258669 on midazolam PK.

1.3. Schedule of Activities

The SoA table provides an overview of the protocol visits and procedures. Refer to the STUDY ASSESSMENTS AND PROCEDURES section of the protocol for detailed information on each procedure and assessment required for compliance with the protocol. The investigator may schedule visits (unplanned visits) in addition to those listed in the SoA table, in order to conduct evaluations or assessments required to protect the well-being of the participant.

1.3.1. Part A – Overall Schedule of Activities

Visit Identifier See Appendix 15 for abbreviations in this table	Screening	Study Day [all activities at 0 h (prior to dosing) unless otherwise specified]																	Follow-Up		ET/ DC
Days Relative to Day 1[a]	Day -28 to Day -3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Visit: 21-24[b]	Contact: 42-49[b]	
Informed consent and demography	X																					
Outpatient visit	X																		X			
Inpatient stay at CRU		X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	X[c]			
Review inclusion/exclusion criteria	X	X																				
Medical/medication history	X	X																				
History of alcohol, tobacco, and illegal drug use	X	X																				
Contraception check	X	X																	X	X	X	X
COVID-19 related measures[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Physical examination[e] (body weight and height at Screening only)	X	X																				X
Neurological examination		X					X[f]						X[f]									X
Review concomitant treatments	X	X																		X	X	X
Serious/non-serious AE monitoring	X	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	X	X	X	X
CCI (including body weight)			X	X						X							X		X	X		X
CCI																						
C-SSRS[g]	X	X		Refer to Section 1.3.2						X[f]							Refer to Section 1.3.2			X		X
Respiratory rate					X[h]		X[i]			X[i]			X[i]					X[h]				X
Oral body temperature					X[h]		X[i]			X[i]			X[i]					X[h]				X
Supine 12-Lead ECG[j]	X				X[h]		X[i]			X[i]			X[i]					X[h]	X	X		X
Triplicate supine BP and PR																						
Orthostatic (supine and standing) BP and PR	X				X[h]		X[i]			X[i]			X[i]					X[h]	X	X		X
Continuous cardiac telemetry[k]			X							X												
CCI																						

Visit Identifier See Appendix 15 for abbreviations in this table	Screening	Study Day [all activities at 0 h (prior to dosing) unless otherwise specified]																		Follow-Up		ET/ DC
Days Relative to Day 1[a]	**Day -28 to Day -3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Visit: 21-24[b]**	**Contact: 42-49[b]**	
CCI																						
Blinded study intervention administration[p]					X	X	X	X	X	X	X	X	X	X	X	X						
Blood sampling for:																						
Serum FSH[q]	X																					
HbcAb, HbsAg, HbsAb, HCVAb, HIV testing	X																					
Clinical safety laboratory tests, including comprehensive lipid panel[r]	X	X		Refer to Section 1.3.2	X		X			X			X				Refer to Section 1.3.2		X	X		X
Retained research sample: biomarkers (Prep B1)																						
Retained research sample: biomarkers (Prep B2)																						
Retained research sample: genetics (Prep D1.5)																						
Serum extracellular vesicles																						
PF-07258669 plasma PK					X[h]		X			X			X					X[h]	X[s]			X
4β-hydroxycholesterol/cholesterol																						
Metabolite profiling																		X[h]				
CCI																						
Urine sampling for:																						
Urine drug test	X	X																				
Clinical safety laboratory tests[r]	X	X		Section 1.3.2	X		X			X			X				Section 1.3.2		X	X		X
PF-07258669 PK																						
6β-hydroxycortisol/cortisol ratio[t]			X															X[h]				
CCI																						

a. Day relative to start of dosing of blinded study intervention (Day 1).

b. On-site follow-up visit to occur 7-10 days after administration of the final dose of study intervention. Follow-up contact may occur via telephone contact and must occur 28-35 days after administration of the final dose of study intervention.

c. Discharge from CRU. Japanese participants may remain in-house until the follow-up visit.

d. Per CRU procedures.

e. Complete physical examination must either be conducted at Screening or Day -2. Brief physical examination may be performed on Day -2 if a complete physical examination is performed at Screening. Brief physical examinations may be performed as appropriate at other times at the investigator's discretion if there are findings during the previous examination or new/open adverse events.

f. Conducted at approximately 2 hours post morning dose.

g. Baseline/Lifetime assessment to be performed at Screening; "Since last visit" assessments should be performed at other timepoints.

h. These assessments on Day 2 and Day 15 are collected 24 hours after the previous day's morning dose and are designated as 24H samples for Days 1 and 14 in Section 1.3.2.

i. Conducted prior to morning dose and at approximately 1 hour (T_{max}) post morning dose.

j. Single 12-lead ECG at Screening, discharge, ET/DC, and follow-up visit; all other times must collect triplicate ECGs per nominal time point.

k. Baseline continuous cardiac telemetry will be recorded for at least 2 hours on Day -1. On Days 1, 7, and 14, continuous cardiac telemetry will begin at least 30 minutes prior to morning dose and continue for 8 hours after the morning dose.

l. Day -1: To be conducted as time-matched baseline [ie, 0H (approximately 15 min before breakfast), 1.5H, 4H (approximately 15 min before lunch), and 10H (approximately 15 min before dinner). 0H measurement on Day 1 also serves as 24H Day -1 baseline measurement. 0H on Day -1 is approximately 24 hours prior to administration of the first dose of study intervention.

m. Days 4, 7, and 10: To be conducted at 0H (approximately 15 min before breakfast), 1.5H, 4H (approximately 15 min before lunch), 10H (approximately 15 min before dinner), and 24H (approximately 15 min before breakfast on Days 5, 8, and 11, respectively).

n. Day -1: To be conducted as time-matched baseline [ie, 0H (approximately 15 min after breakfast; serves as 24H baseline measurement), 4H (approximately 15 min after lunch), and 10H (approximately 15 min after dinner). 0H on Day -1 is approximately 24 hours prior to administration of the first dose of study intervention.

o. Days 4, 7, and 10: To be conducted at 4H (approximately 15 min after lunch), 10H (approximately 15 min after dinner), and 24H (approximately 15 min after breakfast on Days 5, 8, and 11, respectively).

p. Study intervention to be administered Q8H or Q12H (as outlined in Section 1.3.2) with the exception of Day 14 in which only a morning dose will be administered.

q. Females who have been amenorrheic for at least 12 months.

r. Participants should fast for at least 12 hours prior to sample collection.

s. Day 16 PK sample to be collected at 48 hour after the final dose of study intervention on Day 14.

t. On Day -1, urine collection for 6β-hydroxycortisol/cortisol to occur over 0-24 hours. The end of the 24-hour collection time will occur prior to dosing on the morning of Day 1.

CCI

[redacted]

1.3.2. Part A – Schedule and Procedures for Days 1 and 14 Only (unless otherwise noted)

Hours Relative to Dosing at 0H	0	0.25	0.5	1	1.5	2	4	6	8	10	12	16	18	24
Continued inpatient stay at CRU	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Serious/non-serious AE monitoring	→	→	→	→	→	→	→	→	→	→	→	→	→	→
C-SSRS (Day 14 only)						X								
Respiratory rate	X			X				X			X			X[a]
Oral body temperature	X			X				X			X			X[a]
Triplicate supine 12-lead ECG	X[b]		X	X	X	X	X	X	X		X			X[a]
Triplicate supine BP and PR	X	X			X	X	X		X		X			
Orthostatic (supine and standing) BP and PR[c]	X			X				X			X			X[a]
Continuous cardiac telemetry[d]	X	→	→	→	→	→	→	→	X					
CCI														
Blood sampling for:														
Clinical safety laboratory tests, including comprehensive lipid panel (Day 14 only)[e]	X													
Retained research sample for biomarkers (Prep B1) [Day 1 only][f]	X													
Retained research sample for biomarkers (Prep B2) [Day 1 only][f]	X													
Retained research sample for genetics (Prep D1.5) [Day 1 only][f]	X													
Serum extracellular vesicles	X													
PF-07258669 PK[g]	X	X	X	X	X	X	X	X	X		X			X[a]
4β-hydroxycholesterol/cholesterol	X													
Metabolite profiling (0H on Day 1 and all indicated time points on Day 14)	X			X		X	X		X					X[a]
CCI														
Urine sampling for:														
Clinical safety laboratory tests (Day 14 only)[e]	X													
PK predose spot urine blank (Day 1 only)	X													
PF-07258669 PK (Day 14 only)	X	→	→	→	→	→	→	→	X[h]	→	X[h]			
6β-hydroxycortisol/cortisol ratio (Day 14 only)[i]	X	→	→	→	→	→	→	→	→	→	→	→	→	X[a]
CCI														
Blinded study intervention administration														
If Q8H regimen[j]	X								X			X		
If Q12H regimen[j]	X										X			

a. The 24H assessments for Days 1 and 14 occur prior to morning dosing on Day 2 and 24 hours after Day 14 morning dosing, respectively.

b. Day 1 only: At -1H, -0.5H, and 0H prior to the morning dose, triplicate 12 lead ECGs will be obtained at each time point. The average of the triplicate ECG measurements over the 3 pre-dose measurement times (total of 9 ECG measurements) collected before morning dose administration on Day 1 will serve as each participant's baseline QTc value.

c. 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.

d. Continuous cardiac telemetry to commence at least 30 minutes prior to morning dose and continue for 8 hours after morning dose.

e. Participants should fast for at least 12 hours prior to sample collection.

f. Prep B1, B2, and D1.5 Retained Research Samples: If not collected on the designated collection day, collect at the next available time point when biospecimens are being collected in conjunction with a participant visit.

g. The PK sampling scheme is subject to change pending observed data in previous cohorts. Sampling times and/or duration of sampling may be modified and/or extended if data generated in previous cohorts indicate that the $t_{½}$ is longer than expected.

h. On Day 14, urine collection for PK to occur over 0-τ, according to dosing frequency (ie, 0-8H for Q8H; 0-12H for Q12H).

i. On Day 14, urine collection for 6β-hydroxycortisol/cortisol [redacted] to occur over 0-24 hours post morning dose. The end of the 24-hour collection time for Day -1 will occur the morning on Day 1 prior to dosing.

j. Study intervention to be administered Q8H or Q12H with the exception of Day 14 in which only a morning dose will be administered.

1.3.3. Part B – Overall Schedule of Activities

Visit Identifier	Screening	Period 1			Period 2										Follow-Up		ET/DC
Day Relative to Day 1[a]	Day -28 to Day -2	-1	1	2	1	2	3	4	5	6	7	8	9	10	11	Visit: 17-20[b]	Contact: 38-45[b]	
Informed consent and demography	X																	
Outpatient visit	X															X		
Inpatient stay at CRU		X	→	→	→	→	→	→	→	→	→	→	→	→	X[c]			
Review inclusion/exclusion criteria	X	X																
Medical/medication history	X	X																
History of alcohol, tobacco, and illegal drug use	X	X																
Contraception check	X	X													X	X	X	X
COVID-19 related measures[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Physical examination[e] (body weight and height at screen only)	X	X																X
Review concomitant treatments	X	X														X	X	X
Serious/non-serious AE monitoring	X	X	→	→	→	→	→	→	→	→	→	→	→	→	X	X	X	X
CCI (including body weight)			X[f]		X[f]						X[f]				X[f]	X		X
CCI																		
CCI																		
Respiratory rate			X[f]		X[g]			X[g]			X[g]			X[g]				X
Oral body temperature			X[f]		X[g]			X[g]			X[g]			X[g]				X
Supine 12-Lead ECG	X[h]		X[i]		X[j]			X[j]			X[j]			X[j]	X[h]	X[h]		X[h]
Supine vital signs (BP and PR)			X[i]		X[j]			X[j]			X[j]			X[j]				
Orthostatic (supine and standing) BP and PR	X[h]		X[i]		X[j]			X[j]			X[j]			X[j]	X[h]	X[h]		X[h]
Continuous pulse oximetry			X[k]			X[k]								X[k]				
Midazolam dosing			X			X[l]								X[l]				
PF-07258669 dosing[m]					X	X	X	X	X	X	X	X	X	X				
Blood sampling for:																		
Clinical safety laboratory tests, including comprehensive lipid panel[n]	X	X		X		X		X			X			X	X	X		X
Serum FSH[o]	X																	
HBcAb, HBsAg, HBsAb, HCVAb, HIV testing	X																	
Retained research sample for biomarkers (Prep B1)[p]			X[f]															
Retained research sample for biomarkers (Prep B2)[p]			X[f]															
Retained research sample for genetics (Prep D1.5)[p]			X[f]															
Midazolam plasma PK			X[q]	X[r]		X[q]	X[r]							X[q]	X[r]			X

Visit Identifier	Screening	Period 1			Period 2											Follow-Up		ET/DC
Day Relative to Day 1[a]	Day -28 to Day -2	-1	1	2	1	2	3	4	5	6	7	8	9	10	11	Visit: 17-20[b]	Contact: 38-45[b]	
Serum extracellular vesicles			X[f]											X[f]				
4β-hydroxycholesterol/cholesterol			X[f]											X[f]				
Urine sampling for:																		
Urine drug test	X	X																
Clinical safety laboratory tests[n]	X	X		X		X		X			X			X	X	X		X
6β-hydroxycortisol/cortisol ratio		X	X[s]								X	X[s]						
CCI																		

a. Day relative to start of dosing of blinded study intervention (Day 1).
b. On-site follow-up visit to occur 7-10 days after administration of the final dose of study intervention. Follow-up contact may occur via telephone contact and must occur 28-35 days after administration of the final dose of study intervention.
c. Discharge from CRU.
d. Per CRU procedures.
e. Complete physical examination must either be conducted at Screening or Day -1. Brief physical examination may be performed on Day -1 if a complete physical examination is performed at Screening. Brief physical examinations may be performed as appropriate at other times at the investigator's discretion if there are findings during the previous examination or new/open adverse events.
f. Prior to morning dosing. Day 11 CCI to occur before breakfast.
g. Conducted prior to morning dose and at approximately 1 hour post morning dose of PF-07258669.
h. Single 12-lead ECG and vital signs at Screening, discharge, follow-up visit, and ET/DC.
i. Triplicate 12-lead ECG and vital signs on Day 1 of Period 1 prior to dosing and approximately 1 hour post midazolam dose. 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.
j. Triplicate 12-lead ECG and vital signs on Days 1, 4, 7, and 10 of Period 2 prior to dosing and approximately 1 hour post morning dose of PF-07258669. 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.
k. Continuous pulse oximetry to begin 30 minutes prior to midazolam dosing and to continue until at least 6 hours after midazolam dosing.
l. On days of co-administration of midazolam and PF-07258669 (Day 2 and Day 10 of Period 2), midazolam will be administered within 5 minutes after the morning dose of PF-07258669.
m. The dose and dose regimen of PF-07258669 will be determined based on the results of Part A of this study.
n. Participants should fast for at least 12 hours prior to sample collection.
o. Females who have been amenorrheic for at least 12 months.
p. Prep B1, B2, and D1.5 Retained Research Samples: If not collected on the designated collection day, collect at the next available time point when biospecimens are being collected in conjunction with a participant visit.
q. Midazolam PK blood samples to be collected prior to dosing, and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 16 hours post-dose.
r. Midazolam PK blood sample to be collected at 24 hours after midazolam dose that was administered on Period 1/Day 1, Period 2/Day 2, or Period 2/Day 10.
s. These assessments are to occur over 0-24 hours on Day -1 and Day 7. The end of the 24-hour measurement time will occur on the mornings of Day 1 and Day 8, respectively.

2. INTRODUCTION

PF-07258669 is a small molecule MC4R antagonist that is being developed for the treatment of metabolic diseases with catabolic imbalances associated with unintended weight loss, such as geriatric anorexia. Based on preclinical data, antagonism of MC4R may increase appetite, food intake, and body weight in humans.

2.1. Study Rationale

This study will be the first time multiple ascending doses of PF-07258669 are administered to humans. The purpose of Part A of this study is to evaluate the safety, tolerability, and PK of multiple ascending doses of PF-07258669 in healthy adult participants. Healthy adult Japanese and/or older adult participants may also be enrolled in Part A. Safety, tolerability, and PK results obtained from Japanese and/or older adult participants will be used to support participation of Japanese and/or older adult participants in future clinical studies. The purpose of Part B of this study is to evaluate the effect of PF-07258669 on midazolam PK in healthy adult participants.

2.2. Background

2.2.1. MC4R and Geriatric Anorexia

Geriatric anorexia is the loss of appetite or decreased food intake in later life[1] and is associated with adverse outcomes including malnutrition, sarcopenia, frailty, and increased mortality.[2-5] MC4R is a stimulatory GPCR primarily expressed in the brain and is considered the master regulator of energy balance and thus plays a key role in the regulation of appetite and body weight.[6,7] Downstream MC4R signaling has been reported to be dysregulated in both elderly adults and aged rodents[1,8,9] where an imbalance in MC4R signaling has been reported in aged rats with increased activity or hypersensitivity of the receptor, resulting in reduced food intake and weight loss.[10-12] Inhibition of MC4R signaling has also consistently been shown to increase food intake and body weight in multiple preclinical models of chronic illness such as induced cachexia; lipopolysaccharide-induced anorexia,[13] heart failure,[14] chronic kidney disease,[15] and cancer.[16,17] Taken together, these data suggest that antagonism of MC4R may increase appetite, food intake, CCI [redacted].

2.2.2. Nonclinical Pharmacology

PF-07258669 was shown to be a specific antagonist of MC4R in rats, dogs, and humans CCI [redacted]. The functional antagonistic potency of PF-07258669 showed specificity for human MC4R (K_d of CCI [redacted] nM) over the other human receptor isoforms, where K_d values were CCI [redacted] nM for human MC1R, MC2R, and MC5R and CCI [redacted] nM for human MC3R. CCI [redacted]

CCI

[redacted]

2.2.3. Nonclinical Pharmacokinetics and Metabolism

CCI

[redacted]

2.2.4. Biopharmaceutics

CCI

[redacted]

CCI [redacted]

2.2.5. Summary of Nonclinical Toxicology Studies

PF-07258669 was evaluated in a series of safety pharmacology and toxicity studies in rats and dogs up to 1 month in duration, as well as in a battery of genetic toxicity studies.

CCI [redacted] Details of the nonclinical safety program are provided in the investigator's brochure. The nonclinical safety profile of PF-07258669 has been adequately characterized to support progression into clinical trials of up to 4 weeks.

2.2.6. Clinical Overview

The clinical study C4541001 was the first study to administer PF-07258669 to humans and investigated the safety, tolerability, and PK of single ascending doses of PF-07258669 and/or placebo to 29 healthy adult participants. The study was a randomized, placebo-controlled, investigator- and participant-blind, sponsor-open, sequential design. Safety, tolerability, and PK data have been generated at single doses of placebo and PF-07258669 ranging from 0.1 mg to 300 mg. PF-07258669 was well tolerated with an acceptable safety profile, and there were no deaths, SAEs, or serious AEs reported. A total of 19 treatment-emergent adverse events were reported across 14 of 29 participants. All adverse events were graded as mild in severity. There were no reported discontinuations from study or from study drug due to AEs. Five AEs were considered treatment-related by the investigator (4 AEs in PF-07258669 groups and 1 AE in placebo group). Incidence rates of AEs were similar across treatment groups: 7 of the 19 AEs were reported in 6 participants receiving placebo, and 12 AEs were reported in 10 participants receiving PF-07258669 at 1 mg to 300 mg. There was no evidence of any increased incidence of AEs with increasing dose of PF-07258669.

There were 4 AEs related to asymptomatic elevations in serum triglycerides reported across 2 participants (2 AEs per participant). Three of these 4 AEs followed PF-07258669 (30 mg, 100 mg, 200 mg) and the remaining AE occurred following placebo. Given the crossover study design, the fluctuations in serum triglycerides observed in these participants before dosing, after placebo, and fluctuations in triglycerides during the follow-up after completion of dosing, a convincing association of PF-07258669 dosing with triglyceride elevation was not apparent.

Other than the AEs related to serum triglycerides above, there were no clinically significant adverse trends noted in safety laboratory data. With the exception of an AE of asymptomatic orthostatic hypotension, no clinically significant adverse changes in vital signs were observed. There were no clinically significant adverse trends observed in ECG parameters.

Following administration of single oral doses of PF-07258669 under fasted conditions, median T_{max} ranged from 0.75 to 1.25 hours across the dose groups. The mean terminal $t_{½}$ ranged from 2.5 to 13 hours. In general, both C_{max} and AUC_{inf} increased in an approximate dose-proportional manner across the dose range evaluated within each cohort (0.1-3 mg in Cohort 1 and 10-200 mg in Cohort 2). However, less than dose proportional increases of C_{max} and AUC_{inf} were observed when comparing Cohort 1 and Cohort 2. A summary of single-dose PF-07258669 PK parameters from C4541001 is presented in Table 1.

Table 1. Single-Dose PF-07258669 PK Parameters in C4541001

Cohort	Dose (mg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{inf} (ng•h/mL)	Terminal $t_{½}$ (h)
1	0.1	0.8439 (13%)	1 (1-1.5)	2.659 (14%)	2.508 ± 0.924
	0.3	3.053 (35%)	1 (0.5-1)	8.252 (28%)	3.828 ± 0.923
	1	10.72 (40%)	0.75 (0.5-1)	28.55 (28%)	4.572 ± 0.934
	3	26.48 (35%)	0.75 (0.5-1)	76.63 (28%)	5.013 ± 0.528
2	10	53.30 (24%)	1 (0.5-1)	183.0 (15%)	5.558 ± 0.790
	30	188.1 (3%)	1 (1-1)	586.9 (11%)	6.698 ± 1.651
	100	573.4 (45%)	1 (0.5-1.5)	2156 (12%)	6.415 ± 1.591
	200	1173 (41%)	1.25 (1-2)	4033 (21%)	6.880 ± 1.970
3	300	1604 (64%)	1 (0.5-1.5)	6036 (32%)	12.99 ± 10.045

Data are presented as geometric mean (%CV) for C_{max} and AUC_{inf}, median (range) for T_{max}, and arithmetic mean ± standard deviation for terminal $t_{½}$.

CCI

As of October 2022, PF-07258669 doses have been escalated from 2 mg Q8H to 20 mg Q8H. A total of 50 participants have been enrolled in the current study, receiving either PF-07258669 or placebo. Preliminary draft C_{max} and AUC_{tau} have increased in an approximate dose-proportional manner. Median T_{max} ranged from 0.5 to 1 hour post-dose. The mean t½ ranged from 4.8 to 7.7 hours across the dose range evaluated. All reported adverse events have been mild or moderate, CCI

2.3. Benefit/Risk Assessment

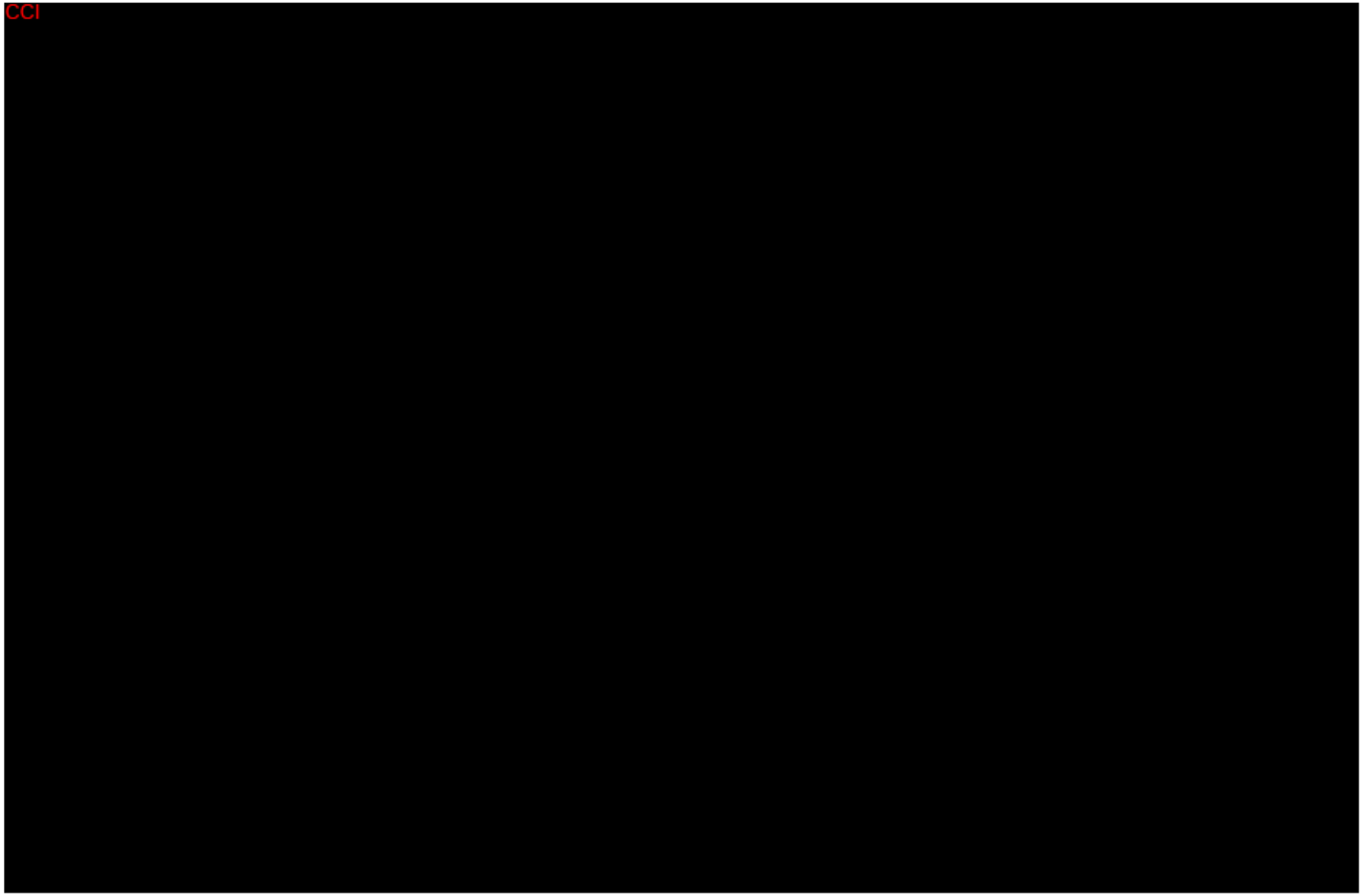
Study C4541003 is the first time that PF-07258669 will be administered as repeated doses to humans. For the healthy participants participating in this multiple, oral dose study, no clinical benefit is expected. The study is designed to provide the basis for further clinical development of PF-07258669 as a potential new, pharmacological agent for the treatment of geriatric anorexia. Postulated risks based on nonclinical studies and clinical data to date are summarized in Section 2.2.5 and Section 2.2.6. The clinical impact of these potential risks will be minimized through the proposed cautious dose escalation process, wherein higher doses of PF-07258669 will be administered only after lower doses have been found to be safe and well tolerated with an acceptable safety profile. Predefined human exposure stopping limits will not be exceeded. In addition, this study may use precautionary sentinel dosing (as outlined in Section 4.1.1 and Section 4.1.2) and includes standard, intensive, inpatient monitoring of the participants during the dosing period.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of PF-07258669 may be found in the IB, which is the SRSD for this study. The SRSD for midazolam may be found in the approved SmPC[28] in the European Union.

2.3.1. Risk Assessment

All study intervention risks are communicated through the IB.

CCI



CCI

[redacted]

• **Study Intervention – Midazolam**		
Decreased respiratory rate	Respiratory depression is a recognized potential side effect of midazolam. Other potential side effects include hypotension and somnolence.	• All participants in Part B will be monitored closely for any side effects on days of midazolam dosing as outlined in Section 1.3.3. • Continuous pulse oximetry will be conducted in Part B starting 30 minutes prior to each midazolam dose and continuing until at least 6 hours after each midazolam dose. • Precautionary sentinel dosing may be employed as described in Section 4.1.2.
Other		
Risk COVID-19 contamination during study	During the pandemic, healthy participants could be infected with the SARS-CoV-2 virus through study participation, which could lead to increased health risks for this participant and others in the study. Adverse events could be confounded.	• COVID-19 specific assessments according to the SoA.

Potential laboratory changes, including elevations in liver function tests and lipid parameters, in response to unrestricted caloric intake	The influence of diet on safety and tolerability (eg, liver function tests, triglycerides) has been reported in published literature of healthy participants resident in a CRU.	• Up to 3 different dietary allocations (ie, standard, high carbohydrate-high calorie, and high fat-high calorie diets) may be employed in this study to permit assessment of safety and tolerability in the setting of different diets.

2.3.2. Benefit Assessment

The participants in this study are not expected to obtain any specific benefit beyond contributing to the process of developing new therapies in an area of unmet need. They will receive close monitoring of their safety via study procedures undertaken (eg, physical examinations, 12-lead ECGs, vital signs) which will occur as outlined in this protocol.

2.3.3. Overall Benefit/Risk Conclusion

Based on the totality of available clinical and nonclinical data, and taking into account the measures to minimize risk to study participants, the overall benefit/risk profile supports clinical testing of multiple doses of PF-07258669 in this study as part of the clinical development for an indication of geriatric anorexia.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Part A	
Primary:	**Primary:**
• 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].
Secondary:	**Secondary:**
• 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).
Tertiary/Exploratory:	**Tertiary/Exploratory:**
• 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). • 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.	• 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). • Change from baseline in body weight on Days 7 and 14.
CCI [redacted]	

	CCI [redacted]
CCI [redacted]	
CCI [redacted]	CCI [redacted]

• 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).
• 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).
	• Change from baseline on Day 14:
	  • Urinary 6β-hydroxycortisol/cortisol ratio;
	  • Plasma 4β-hydroxycholesterol/cholesterol ratio;
	  • Serum extracellular vesicles (if analyzed).
	• Qualitative plasma levels of potential metabolites of PF-07258669 at steady-state.

Part B	
Primary:	**Primary:**
• 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: C_{max}, AUC_{last}, and AUC_{inf} (if data permit).
Secondary:	**Secondary:**
• 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.
Tertiary/Exploratory:	**Tertiary/Exploratory:**

• To evaluate the effects of PF-07258669 on additional PK parameters of midazolam in healthy adult participants
• To evaluate the potential for induction of CYP3A with administration of multiple doses of PF-07258669 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: T_{max}, CL/F, V_z/F, and $t_{½}$ (if data permit).
	• Change from baseline:
	  • Urinary 6β-hydroxycortisol/cortisol ratio on Day 7 (if analyzed);
	  • Plasma 4β-hydroxycholesterol/cholesterol ratio on Day 10;

CCI [redacted]	• Serum extracellular vesicles on Day 10 (if analyzed). CCI [redacted]

4. STUDY DESIGN

4.1. Overall 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.

4.1.1. 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. 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).

For a given participant, the total study duration from screening to the follow-up phone call will be up to approximately 11 weeks. Screening will occur within 28 days prior to the first dose of blinded study intervention. Eligible participants who meet the entry criteria will progress to admission to the CRU for a 18 day (and 17 night) inpatient stay. While inpatient, study intervention will be administered either Q8H or Q12H for 14 consecutive days with the exception of Day 14 in which only a single morning dose will be administered. Participants will remain inpatient for an additional 48 hours after the morning dose on Day 14. Following discharge, participants will return for an on-site follow-up visit 7 to 10 days after administration of the final dose of study intervention. Japanese participants, if enrolled, may remain in-house until the follow-up visit. The follow-up contact may occur via a telephone call and will occur 28 to 35 days after administration of the final dose of study intervention. Dose levels will be escalated across cohorts to bracket the expected clinical dose range, but projected exposures will not exceed the pre-defined human exposure limits. Dose escalation increments between cohorts will be selected such that up to semi-logarithmic exposures will be expected.

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, and these participants would remain in the CRU for up to a 20 day (and 19 night) inpatient stay.

4.1.2. 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 [redacted] 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.

For a given participant, the total study duration from screening to the follow-up phone call will be up to approximately 11 weeks. Screening will occur within 28 days prior to the first dose of study intervention. Eligible participants who meet the entry criteria will progress to admission to the CRU for a 14 day (and 13 night) inpatient stay. While inpatient, participants will receive a single dose of midazolam in Period 1. Period 2 will follow 2 days after administration of midazolam in Period 1. In Period 2, participants will receive PF-07258669 (dose and dose regimen to be determined based on results of Part A) for 10 consecutive days.

Single doses of midazolam will be co-administered within 5 minutes after the morning dose of PF-07258669 on Days 2 and 10. Participants will remain inpatient for 24 hours after the final dose of midazolam on Day 10. Following discharge, participants will return for an on-site follow-up visit 7 to 10 days after administration of the final dose of study intervention. The follow-up contact may occur via a telephone call and will occur 28 to 35 days after administration of the final dose of study intervention.

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.

4.2. Scientific Rationale for Study Design

The population planned for this study will be healthy adult males and females of nonchildbearing potential. Female participants will be confirmed not to be of childbearing potential because embryo-fetal developmental toxicity studies with PF-07258669 have not been conducted. In males, appropriate measures are expected to be followed to minimize potential transfer of PF-07258669 via semen.

4.2.1. Rationale for Part A

Because this is the first time multiple doses of PF-07258669 will be administered to humans, a sequential cohort, escalating oral dose design is planned with careful assessment and ongoing review of safety, tolerability, and PK data of PF-07258669. Precautionary sentinel dosing may also be employed as described in Section 4.1.1.

At each dose level proposed for dose-escalation cohorts of healthy adult non-Japanese participants, approximately 8 participants are planned to receive PF-07258669 and approximately 2 participants are planned to receive placebo. The planned doses in the escalation sequence (Table 2) may be modified or repeated, as guided by emerging safety, tolerability, and PK data but will follow the dose-escalation rules defined in Section 6.5.1. Additional optional cohorts of healthy adult non-Japanese participants may be enrolled to repeat a dose or to expand the dose/exposure range or better characterize the safety and tolerability profile or clinical biomarker of interest (eg, body weight).

The influence of diet on safety and tolerability (eg, liver function tests, triglycerides) has been reported in published literature in healthy participants resident in a CRU.[32] Cohorts of healthy non-Japanese participants will be allocated to 1 of 3 different dietary allocations (ie, standard, HCHC, or HFHC diets) described in Section 5.3.1 to facilitate a more comprehensive assessment of safety, tolerability, PK, and exploratory endpoints. Optional cohorts of Japanese and/or older adult participants will be allocated to standard diet only.

A larger sample size will be used for the additional optional cohorts of healthy adult non-Japanese participants receiving the HFHC diet. If conducted, the first cohort allocated to the HFHC diet will likely evaluate safety, tolerability, PK, and PD at the maximum tolerated dose of PF-07258669 identified in earlier dose-escalation cohorts allocated to standard diet. This cohort will consist of approximately 24 participants with approximately 12 participants planned to receive PF-07258669 and approximately 12 participants planned to receive placebo. An optional second cohort allocated to the HFHC diet may be enrolled to evaluate safety, tolerability, PK, and PD at a lower dose/exposure to further characterize the dose/exposure-response relationship and inform dose selection for later clinical studies. This second cohort may consist of approximately 16 participants with approximately 12 participants planned to receive PF-07258669 and approximately 4 participants planned to receive placebo. Refer to Section 9.5.1 for additional details on the sample size determination.

An optional cohort of approximately 8 healthy adult Japanese participants (approximately 6 receiving PF-07258669 and approximately 2 placebo) may be enrolled in this study to collect safety, tolerability, and PK data of PF-07258669 to support enrollment of Japanese participants in future clinical studies with PF-07258669 if results from previous cohorts support further clinical development of PF-07258669.

An optional cohort of approximately 8-10 older adult participants (approximately 6-8 receiving PF-07258669 and approximately placebo) may be enrolled in this study to collect safety, tolerability, and PK data of PF-07258669 to support enrollment of older adults in future clinical studies with PF-07258669.

To permit an unbiased assessment of safety, the administration of both PF-07258669 and placebo will be blinded to both site staff (except those involved in preparation of doses) as well as all participants in Part A.

Serial plasma PK samples will be collected on Days 1 and 14 to characterize single-dose and multiple-dose PK of PF-07258669. Trough PK samples will also be collected on Days 4, 7, and 10 to aid in the determination of time to steady-state. Urine samples will be collected to evaluate renal clearance of PF-07258669. Sampling times and/or duration of sampling may be modified and/or extended if data generated in previous cohorts indicate that the $t_{½}$ is longer than expected based on preliminary data generated in C4541001.

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[redacted]

CCI [redacted]

A comprehensive lipid panel, consisting of total cholesterol, triglycerides, HDL, and LDL, will be collected after at least 12 hours of fasting and monitored with clinical safety lab tests due to the observation of increased triglycerides and cholesterol noted in the 1-month rat toxicity study. Elevated plasma triglycerides have also been reported in 2 participants in C4541001 single ascending dose clinical study.

Respiratory rate and body temperature will also be measured and monitored due to the observation of transient lower respiratory rate and body temperature in rats after administration of PF-07258669 in toxicology studies.

Body weight will be measured in this study because MC4R antagonism is expected to increase food consumption and body weight. CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted] Similar observations have been made in a mouse model with MC4R deficiency but not in a MC4R knockout mouse model.[21,22]

In vitro data indicate that PF-07258669 is primarily metabolized by CYP3A, as such the use of moderate or strong CYP3A inhibitors or inducers is not permitted during the study and within 28 days or 5 half lives (whichever is longer) prior to first dose of study intervention. Refer to Section 6.8 for additional details. In addition, in vitro data indicate that PF-07258669 may be a potential TDI of CYP3A4/5, as such concomitant medications that are CYP3A substrates may be restricted. Refer to Section 6.8.1 for additional details.

Based on nonclinical data, the potential exists that PF-07258669 may cause weak induction of CYP3A at high doses, representing a regulatory risk. Evaluation of the urinary 6β-hydroxycortisol/cortisol ratio, plasma 4β-hydroxycholesterol/cholesterol ratio, and serum extracellular vesicles will allow for exploratory evaluation of induction potential. However, serum extracellular vesicles may not be analyzed if urinary 6β-hydroxycortisol/cortisol, plasma 4β-hydroxycholesterol/cholesterol, and/or midazolam (Part B) data suggest no induction.

Plasma samples will be collected for exploratory qualitative metabolite profiling to identify potential major metabolites.

Although there is no suspicion of human teratogenicity based on the expected pharmacology of PF-07258669, human reproductive safety data are not available for PF-07258669. Therefore, the use of a highly effective method of contraception is required for male study participants and is recommended for partners of male study participants who are WOCBP (see Appendix 4).

4.2.2. Rationale for Part B

PF-07258669 is a potential TDI of CYP3A4/5 at projected therapeutic doses and a potential inducer of CYP3A4/5 at doses greater than the projected therapeutic dose. Evaluating the potential PK interactions between PF-07258669 and midazolam may provide insights into PK or safety issues that may arise in future clinical studies or inform decisions related to the inclusion of patients in future studies who may be taking concomitant medications that are CYP3A substrates.

CCI [redacted]

CCI [redacted] CCI [redacted] at the projected efficacious dose.

At projected therapeutic exposures, PF-07258669 is not expected to induce CYP3A. Although induction was not observed at the enzyme activity level in vitro at those exposures, PF-07258669 demonstrated CCI [redacted] mRNA induction of CYP3A4. However, at higher exposures associated with the highest doses planned to be evaluated in this study, PF-07258669 has the potential to induce CYP3A.

Given the preponderance of CYP3A4/5 substrates in the intended patient population of elderly patients with anorexia, a clinical DDI study assessing the effect of PF-07258669 on the PK of a sensitive CYP3A4/5 substrate needs to be conducted to determine the effect of PF-07258669 on CYP3A4/5. Midazolam is a sensitive CYP3A4/5 substrate which undergoes extensive metabolism by CYP3A4/5. The PK and PD properties of midazolam make it widely accepted as a probe in vitro, and in clinical studies for assessing CYP3A activity and is recommended by the FDA as a probe substrate for CYP3A DDI studies. The current study is designed to determine the effect of PF-07258669 on the PK of midazolam.

The onset of inhibition effect on CYP3A by potential inhibitors is relatively rapid. Although TDI does not occur as quickly as competitive inhibition, near maximal TDI of CYP3A with clarithromycin was shown to be achieved at approximately 1 day and 4 days in the gut and liver, respectively.[23] Given the short terminal $t_{½}$ (<6.3 hours) observed in C4541001, accumulation of PF-07258669 is expected to be minimal after multiple dosing. Therefore, midazolam will also be co-administered with PF-07258669 on Day 2 of Period 2 to assess the potential effect of CYP3A inhibition by PF-07258669 on midazolam PK, which may inform deconvolution of CYP3A inhibition from potential mixed induction/inhibition that may be observed on Day 10.

Both induction and TDI can take several days to exert their effects on CYP3A activity. Thus, multiple doses of PF-07258669 will be administered for 10 days to ensure that CYP3A TDI and induction can be observed and evaluated, if there is potential for PF-07258669 to induce CYP3A. Midazolam will be co-administered with PF-07258669 on Day 10 of Period 2 to evaluate the potential effect of mixed CYP3A induction and inhibition by PF-07258669 on midazolam PK.

Respiratory depression, hypotension, and somnolence are among the recognized potential side effects of higher doses of midazolam. Although such adverse events are not considered likely at midazolam exposures anticipated in Part B, all participants will be closely monitored for these side effects on days of midazolam dosing. This will include continuous pulse oximetry for all participants from 30 minutes before through at least 6 hours after midazolam dosing.

The plasma 4β-hydroxycholesterol/cholesterol ratio will also be evaluated in Part B to allow for exploratory evaluation of these CYP3A induction biomarkers in the same participants in which the potential midazolam PK DDI was evaluated. Serum extracellular vesicle samples and the urine samples for measurement of the 6β-hydroxycortisol/cortisol ratio will be also collected but may not be analyzed if urinary 6β-hydroxycortisol/cortisol (Part A), plasma 4β-hydroxycholesterol/cholesterol (Parts A and B), and/or midazolam (Part B) data suggest no induction.

CCI [redacted]

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. Optional cohorts may be added if it is necessary to evaluate the effect of additional dose levels of PF-07258669 on midazolam PK.

Precautionary sentinel dosing may be employed as described in Section 4.1.2. Part B participants will be allocated to standard diet only.

4.2.3. Choice of Contraception/Barrier Requirements

Human reproductive safety data are not yet available for PF-07258669, but there is no suspicion of human teratogenicity based on the intended pharmacology of the compound. Therefore, the use of a highly effective method of contraception is required (see Appendix 4).

4.2.4. Collection of Retained Research Samples

Retained Research Samples will be collected and stored for further analyses which may, for example, provide greater understanding of the study intervention.

4.3. Justification for Dose

The proposed dose levels of PF-07258669 in this study were derived based on cumulative clinical and nonclinical data with PF-07258669, including in vitro and in vivo pharmacology and PK data, nonclinical toxicity studies, and the single-dose human safety, tolerability, and PK results from Study C4541001 (Section 2.2.6). Dose levels have been selected to bracket the expected clinical dose and exposure range, while considering uncertainty in the projected clinically efficacious dose. The dose levels and dosing regimens beyond the Cohort 1 starting dose level/regimen may be modified based on emerging human safety, tolerability, and PK data in the current study.

CCI [redacted]

CCI

4.3.2. Human Exposure Stopping Limits

The human exposure stopping limits are based on exposures observed in dogs in nonclinical toxicology studies and are corrected for species-dependent plasma protein binding between dog and human CCI

CCI

4.3.3. Rationale for Dose Selection

4.3.3.1. Part A PF-07258669 Dose Selection

The planned PF-07258669 dose range to be evaluated in Part A of this study is shown in Table 2.

Table 2. Predicted PF-07258669 Steady-State Exposures

Cohort	CCI [redacted]
1	
2	
3	
4	
5	

CCI [redacted]

The starting dose level of PF-07258669 proposed for Part A of this study is CCI [redacted] This starting dose level is projected to be safe given that PF-07258669 has been well tolerated with an acceptable safety profile at single oral doses up to CCI [redacted] Based on safety and tolerability observed in C4541001, no clinically significant adverse effects on ECGs, vital signs, or clinical safety laboratories are expected at this dose.

CCI [redacted]

The dose/exposure range to be studied was selected to account for uncertainties in the projected C_{eff} and the projected therapeutic dose, while also bracketing the expected clinically effective dose range in humans for clinically relevant pharmacological activity of geriatric anorexia and providing safety coverage for a wide range of PF-07258669 doses.
CCI [redacted]

In addition to MC4R antagonism being a novel approach to treating geriatric anorexia, uncertainties in C_{eff} are also due to the unknown quantitative translation of aged-rat food intake and body weight data to humans. Doses higher than the projected human therapeutic dose may be evaluated in future clinical studies to maximize the potential to observe

efficacy. Q8H dosing in this study allows for evaluation of higher AUCs than have been evaluated in the single ascending dose study (C4541001) to date. In addition, Q12H dosing may also be explored to evaluate higher AUCs than have been evaluated in C4541001 or if multiple dose PK is not as expected (eg, if the observed t½ is longer than expected due to auto-inhibition of CYP3A).

Exposures at the provisional doses outlined in Table 2 were projected using a preliminary population PK model that was developed using preliminary draft single-dose PK data generated in C4541001. CCI [redacted]

Dose escalation increments are planned to be up to approximately semi-logarithmic increases in exposure from the previous highest dose. If auto-inhibition is observed, resulting in greater than dose proportional exposure increases, the dose escalation scheme in Table 2 will not be used. Dose levels will be adjusted such that predicted exposures will not exceed 3.3-fold increments between dose levels. Actual doses, target exposures, dose regimens, and dose increments may be repeated or adjusted (higher or lower) during the study based on emerging human safety, tolerability, and PK data, but projected exposures will not exceed the pre-defined human exposure limits. Other dose regimens (eg, Q6H or Q12H) or titration schemes may also be administered. Any decision to alter the planned dosing schemes will be made jointly by the investigator and the sponsor after careful evaluation of all available data.

The PK data from both C4541001 and the present study will be used to predict exposures for subsequent dose levels. The subsequent dose levels will be selected based on review of observed safety, tolerability, and PK up to Day 14 from previous dose levels.

Doses administered to non-Japanese participants allocated to the HFHC diet (if enrolled), Japanese participants (if enrolled) and/or older adult participants (if enrolled) will be less than or equal to the highest safe and well-tolerated dose evaluated in healthy adult non-Japanese participants allocated to standard diet in Part A. If enrolled, the first cohort allocated to the HFHC diet will likely evaluate safety, tolerability, PK, and PD at the maximum tolerated dose of PF-07258669 identified in earlier dose-escalation cohorts allocated to standard diet. An optional second cohort allocated to the HFHC diet may be enrolled to evaluate safety, tolerability, PK, and PD at a lower dose/exposure to further characterize the dose/exposure-response relationship and inform dose selection for later clinical studies. Based on the projected clearance pathways of PF-07258669 (Section 2.2.3), significant differences in PF-07258669 exposures between non-Japanese, Japanese, and older adults are not anticipated.

The maximum dose of PF-07258669 to be administered in Part A will not exceed a total daily dose of 2000 mg.

4.3.3.2. Part B Dose Selection

4.3.3.2.1. PF-07258669 in Part B

The PF-07258669 dose level/regimen to be used in Part B will be determined based on the results of Part A. Dose level(s)/regimen(s) projected to achieve exposures less than or equal to those associated with the MTD determined in Part A will be used to evaluate potential PK interactions between PF-07258669 and midazolam in Part B. The results of Part B will be used to inform PF-07258669 dose selection and decisions related to the inclusion of patients who may be taking concomitant medications in future clinical studies.

The MTD is planned to be evaluated to maximize the interactions between PF-07258669 and midazolam. However, lower doses of PF-07258669 may be evaluated for reasons such as, but not limited to, unexpected exposures, higher than expected variability, or if unexpected safety or tolerability signals are observed. If nonlinear PF-07258669 PK resulting in higher than expected exposures is observed in Part A due to potential auto-inhibition of CYP3A, a PF-07258669 dose level less than the MTD may be evaluated. Additional optional cohorts may be added if it is necessary to evaluate the effect of additional dose levels of PF-07258669 on midazolam PK.

Although TDI does not occur as quickly as competitive inhibition, near maximal TDI of CYP3A with clarithromycin was shown to be achieved at approximately 1 day and 4 days in the gut and liver, respectively. Both inducers and time-dependent inhibitors can take several days to exert their effects on enzyme activity. Thus, multiple doses of PF-07258669 will be administered for 10 days. If there is a potential for CYP3A4/5 TDI and/or induction due to PF-07258669, dosing for this duration will ensure that it can be observed and evaluated.

The maximum dose of PF-07258669 to be administered in Part B will not exceed a total daily dose of 2000 mg.

4.3.3.2.2. Midazolam in Part B

The recommended dose of midazolam oral solution for premedication prior to surgery and sedation for brief diagnostic and therapeutic procedures and for the short-term treatment of sleep disorders is 7.5 to 15 mg.[28] Midazolam has also been administered at doses of 2 to 15 mg to assess CYP3A DDIs[25,26] and has been generally well tolerated. CCI [redacted]
Therefore, a 1 mg oral dose of midazolam is planned to be administered. This dose is expected to provide sufficient exposure to characterize midazolam clearance in the absence of PF-07258669 while avoiding saturation of first-pass extraction and minimizing AEs in the presence of PF-07258669.

However, based on the results of Part A, the predicted PF-07258669 exposure at the planned dose, and whether nonlinear PF-07258669 PK is observed due to potential auto-induction or auto-inhibition of CYP3A, the midazolam dose may be adjusted higher or lower, respectively, such that midazolam concentrations can be more adequately assessed (ie, higher

concentrations further above the LLOQ) and predicted concentrations remain within a range that will not have undue pharmacological effects. A midazolam dose >1 mg will only be used if net auto-induction is observed in Part A. The maximum dose of midazolam to be administered in Part B will not exceed 8 mg.

While the predicted exposure of midazolam when co-administered with PF-07258669 is not expected to exceed that of an 8 mg midazolam dose, the proposed exposure limit of midazolam (or max exposure expected) will not exceed that of a 15 mg single oral dose of midazolam which is known to be safe and generally well-tolerated. Across 23 studies (identified using the University of Washington drug interaction database) using 15 mg oral midazolam, mean C_{max} and AUC were approximately 78 ± 28 ng/mL (range 43-171) and 249 ± 98 ng•h/mL (range 138-578), respectively.[29]

4.4. End of Study Definition

The end of the study is defined as the date of the last scheduled procedure shown in the SoA for the last participant in the trial.

A participant is considered to have completed the study if he/she has completed all periods of the study, including the last scheduled procedure shown in the SoA.

5. STUDY POPULATION

This study can fulfill its objectives only if appropriate participants are enrolled. The following eligibility criteria are designed to select participants for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular participant is suitable for this protocol.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age and Sex:

1. Healthy male participants and females of nonchildbearing potential must be 18 to 60 years of age, inclusive, at the time of signing the ICD.

   For optional cohort of older adult participants only: Male participants and female participants of non-childbearing potential must be 65 to 90 years of age, inclusive, at the time of signing the ICD. Attempts will be made to ensure that the age composition of this cohort (eg, approximately 70% of participants ≥70 years of age) is comparable to that of the anticipated patient population in later clinical studies.

- Refer to Appendix 4 for reproductive criteria for male (Section 10.4.1) and female (Section 10.4.2) participants. Female participants must meet criteria for nonchildbearing potential specified in Section 10.4.3.

Type of Participant and Disease Characteristics:

2. Female participants of nonchildbearing potential and male participants who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests, and cardiac monitoring.

   For optional cohort of older adult participants only: Participants must be in a stable condition at admission. These participants must be in reasonably good health as determined by the investigator based on a detailed medical history, full physical examination, vital signs assessments, 12-lead ECG, and clinical laboratory tests. Participants with mild, chronic, stable disease (eg, controlled hypertension, non-insulin dependent diabetes, osteoarthritis) may be enrolled if deemed medically prudent by the investigator.

3. **Japanese participants only:** Participants enrolling as Japanese must have 4 biological Japanese grandparents who were born in Japan.

4. Participants who are willing to avoid direct sunlight exposure or any high intensity ultraviolet light exposure from admission to the follow-up contact and to apply sunscreen/lotion with a high sun protection factor and to wear eye protection, as appropriate.

5. Participants who are willing and able to comply with all scheduled visits, treatment plan, laboratory tests, lifestyle considerations, and other study procedures.

Weight:

6. BMI of 17.5 to 28.5 kg/m^2; and a total body weight >50 kg (110 lb).

   For optional cohort of older adult participants only: BMI of 17.5 to 32.4 kg/m^2; and a total body weight >50 kg (110 lbs). Efforts will be made to enroll at least 3 older adult participants with BMI <25 kg/m^2, if feasible.

Informed Consent:

7. Capable of giving signed informed consent as described in Appendix 1, which includes compliance with the requirements and restrictions listed in the ICD and in this protocol.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions:

1. Evidence or history of clinically significant hematological, renal, endocrine (including, but not limited to, thyroid disease, diabetes insipidus), pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric (including, but not limited to, primary polydipsia, obsessive compulsive disorder, anxiety disorder, schizophrenia), neurological (including, but not limited to, seizure disorder, traumatic brain injury), immunodeficiency (including, but not limited to, severe infection that required ICU admission, prolonged hospitalization, or prolonged treatment) or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at the time of dosing), as well as presence of clinical laboratory abnormalities defined in Exclusion Criterion #19 below.

   For optional cohort of older adult participants only: Participants with chronic conditions (eg, hypertension) that are controlled by either diet or stable doses of medications may be included. See Section 6.8 for further information on concomitant medications. Recent evidence (ie, within previous 6 months) or history of unstable disease or moderate to severe conditions which would, in the investigator's opinion, interfere with the study evaluations or have an impact on the safety of participants.

2. History of symptomatic orthostatic hypotension.

3. History of symptomatic bradycardia.

4. Any condition possibly affecting drug absorption (eg, bariatric surgery, gastrectomy, cholecystectomy).

5. History of phototoxicity or photosensitivity.

6. History of HIV infection, hepatitis B, or hepatitis C; positive testing for HIV, hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb) or hepatitis C antibody (HCVAb). Hepatitis B vaccination is allowed. As an exception a positive HBsAb test due to hepatitis B vaccination is permissible.

7. History of eating disorders (eg, anorexia nervosa, bulimia nervosa, binge-eating disorder, avoidant/restrictive food intake disorder).

8. Part A only: For participants who answer "Yes" the C-SSRS questions 4 or 5, a risk assessment should be done by a qualified MHP (ie, a psychiatrist or licensed PhD level clinical psychologist) to assess whether it is safe for the participant to participate in the study. In addition, participants deemed by the investigator to be at significant risk of suicidal or violent behavior should be excluded.

9. Other medical or psychiatric condition including recent (within the past year) or active suicidal ideation/behavior or laboratory abnormality, or other conditions or situations related to the COVID-19 pandemic (eg, contact with positive case, residence, or travel to an area with high incidence) that may increase the risk of study participation or, in the investigator's judgment, make the participant inappropriate for the study.

Prior/Concomitant Therapy:

10. Use of prescription or nonprescription drugs and dietary and herbal supplements within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study intervention. Refer to Section 6.8 for additional details.

    For optional cohort of older adult participants only:

    - Participants taking daily prescription or non-prescription medications (that are not moderate or strong CYP3A inducers or inhibitors) for management of acceptable chronic medical conditions are to be on a stable dose, as defined by no change in dose for the 28 days or 5 half-lives (whichever is longer) before the screening visit.
        - Use of stable concomitant mediations noted above that are CYP3A substrates may be restricted. Refer to Section 6.8.1 for restrictions regarding use of CYP3A substrates.
    - All medications must be reviewed on a case-by-case basis by the investigator and approved by the sponsor during the screening period for eligibility purposes.

11. Use of moderate or strong CYP3A inhibitors or inducers within 28 days or 5 half-lives (whichever is longer) prior to Screening. Refer to Section 6.8 for additional details.

Prior/Concurrent Clinical Study Experience:

12. Previous administration with an investigational drug within 30 days (or as determined by the local requirement) or 5 halflives preceding the first dose of study intervention used in this study (whichever is longer).

Diagnostic Assessments:

13. A positive urine drug test at Screening (Parts A and B), Day -2 (Part A), or Day -1 (Part B).

14. Screening supine BP ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), following at least 5 minutes of supine rest. If BP is ≥140 mm Hg (systolic) or ≥90 mm Hg

(diastolic), the BP should be repeated 2 more times and the average of the 3 BP values should be used to determine the participant's eligibility.

15. Screening supine blood pressure values <90 mmHg (systolic) or <50 mmHg (diastolic) following at least 5 minutes of rest. If BP is <90 mmHg (systolic) or <50 mmHg (diastolic), the BP should be repeated 2 more times and the average of the 3 BP values should be used to determine the participant's eligibility.

16. Evidence of orthostatic hypotension at screening visit, defined as a decrease of ≥20 mmHg in systolic blood pressure and/or ≥10 mmHg in diastolic blood pressure 2 minutes after standing from a supine position.

17. A heart rate of <45 bpm on baseline standard 12-lead ECG performed at screening visit.

18. Baseline standard 12-lead ECG that demonstrates clinically relevant abnormalities that may affect participant safety or interpretation of study results (eg, baseline QTcF interval >450 msec, complete LBBB, signs of an acute or indeterminate-age myocardial infarction, ST-T interval changes suggestive of myocardial ischemia, second- or third-degree AV block, or serious bradyarrhythmias or tachyarrhythmias). If the baseline uncorrected QT interval is >450 msec, this interval should be rate-corrected using the Fridericia method and the resulting QTcF should be used for decision making and reporting. If QTcF exceeds 450 msec, or QRS exceeds 120 msec, the ECG should be repeated 2 more times and the average of the 3 QTcF or QRS values should be used to determine the participant's eligibility. Computer-interpreted ECGs should be overread by a physician experienced in reading ECGs before excluding a participant.

19. Participants with **ANY** of the following abnormalities in clinical laboratory tests at screening, as assessed by the studyspecific laboratory and confirmed by a single repeat test, if deemed necessary:

    - AST **or** ALT level ≥1.25× ULN;
    - Total bilirubin level ≥1.5× ULN; participants with a history of Gilbert's syndrome may have direct bilirubin measured and would be eligible for this study provided the direct bilirubin level is ≤ ULN;
    - eGFR <81 mL/min/1.73m^2 (<60 mL/min/1.73m^2 for optional cohort of older adult participants only);
    - Fasting serum triglycerides >2× ULN.

20. Positive test result by PCR for SARS-CoV-2 infection at the time of Screening (Parts A and B), Day -2 (Part A), or Day -1 (Part B).

Other Exclusions:

21. History of alcohol abuse or binge drinking and/or any other illicit drug use or dependence within 6 months of Screening. Binge drinking is defined as a pattern of 5 (male) and 4 (female) or more alcoholic drinks in about 2 hours. As a general rule, alcohol intake should not exceed 14 units per week (1 unit = 8 ounces (240 mL) beer, 1 ounce (30 mL) of 40% spirit or 3 ounces (90 mL) of wine).

22. Use of tobacco or nicotine containing products in excess of the equivalent of 5 cigarettes/day or 2 chews of tobacco/day.

23. Blood donation (excluding plasma donations) of approximately 1 pint (500 mL) or more within 60 days prior to dosing.

24. History of sensitivity to heparin or heparin-induced thrombocytopenia.

25. Part B only: Participants who have a history of sensitivity reactions to midazolam, or who according to the product label for midazolam would be at increased risk if dosed with midazolam (ie, including but not limited to participants with a history of myasthenia gravis or tendinitis/tendon rupture).

26. Unwilling or unable to comply with the criteria in the Lifestyle Considerations section of this protocol.

27. Actively following a calorie-restricted diet for purposes of intentional weight loss.

28. Investigator site staff or Pfizer employees directly involved in the conduct of the study, site staff otherwise supervised by the investigator, and their respective family members.

5.3. Lifestyle Considerations

The following guidelines are provided:

5.3.1. Meals and Dietary Restrictions

- Participants must abstain from all food and drink (except water or study intervention administration) at least 12 hours prior to any safety laboratory evaluations.

- Part A: On Days 1 and 14 (serial PK sampling days), participants will abstain from all food and drink (except water or study intervention administration) for at least 12 hours overnight prior to morning dosing. Water is permitted until 1 hour prior to morning study intervention administration. Water may be consumed without restriction beginning 1 hour after morning dosing. Food may be consumed beginning 4 hours after morning dosing.

- There are no food or drink restrictions (other than those described below) for afternoon/evening dosing or for dosing on other days in Part A, with the exception of 12 hour fasting (except water or study intervention administration) on Days 2, 4, 7, and 10 in which safety laboratory evaluations are conducted.

- Part B: Participants must abstain from all food and drink (except water or study intervention administration) for at least 12 hours prior to morning dosing with midazolam on Day 1 of Period 1 and the days in which midazolam is co-administered with PF-07258669 (Day 2 and Day 10 of Period 2). Water is permitted until 1 hour prior to midazolam administration. Water may be consumed without restriction beginning 1 hour after midazolam dosing. Food may be consumed beginning 4 hours after morning dosing on Day 1 of Period 1 and the morning dosing on days in which midazolam is co-administered with PF-07258669 (Day 2 and Day 10 of Period 2). There are no food or drink restrictions (other than those described below) for all doses and other days of Part B, with the exception of 12 hour fasting (except water or study intervention administration) on Day 2 of Period 1 and Days 2, 4, 7, 10, and 11 in which safety laboratory evaluations are conducted, provided other restrictions are followed.

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- Noncaffeinated drinks (except grapefruit or grapefruit-related citrus fruit juices; see below) may be consumed with meals and snacks.

- Participants will refrain from consuming red wine, grapefruit, or grapefruit-related citrus fruits (eg, Seville oranges, pomelos, fruit juices) from 7 days prior to the first dose of study intervention until collection of the final PK blood sample.

- Lunch will be provided approximately 4 hours after morning dosing.

- Dinner will be provided approximately 9 to 10 hours after morning dosing.

- Cohorts will be allocated to 1 of 3 diets as follows:

Diet	Daily kcal Limit	Fat	Protein	Carbohydrate
Standard	<3200	30%	15%	55%
HCHC	No limit	30%	15%	55%
HFHC	<4500	55%	15%	30%

Refer to Section 1.2 for dietary allocation to planned cohorts. Additional cohorts may be added to any dietary allocation as needed.

- All participants enrolled in a given cohort will have the same dietary allocation. The decision regarding dietary allocation will be communicated to CRU prior to admission of the cohort, and CRU staff will inform participants of dietary allocation.

- For **standard diet**, main meals will be constructed to yield a total daily nutritional composition of approximately 55% carbohydrate, 30% fat, and 15% protein. The daily caloric intake per participant should not exceed approximately 3200 kcal, as per standard CRU procedure for Phase 1 studies in healthy participants. Second helpings at meal times will not be offered, and snacks will be limited in keeping with daily calorie limit. The nutritional composition of snacks will be flexible to cater to participant preferences.

- For **HCHC diet**, main meals will be constructed to yield a total daily nutritional composition of approximately 55% carbohydrate, 30% fat, and 15% protein. Participants will be provided with a minimum of approximately 3200 kcal per day, without any maximum daily calorie limit. Participants will be offered second helpings at meal times and supplemental snacks between meals and after dinner. The nutritional composition of snacks will be flexible to cater to participant preferences.

- For **HFHC diet**, main meals will be constructed to yield a total daily nutritional composition of approximately 30% carbohydrate, 55% fat, and 15% protein. Daily calorie intake per participant should not exceed approximately 4500 kcal. This macronutrient breakdown and daily calorie limit is informed by published data from healthy male participants resident in a CRU that demonstrate tolerability without precipitating abnormalities in key laboratory parameters (eg, LFTs).[32] Second helpings at meal times and supplemental snacks between meals and after dinner will be available for participants upon request. The nutritional composition of snacks available to participants following HFHC diet will offer a higher fat than carbohydrate content.

- Participants are not required to accept, consume, or complete food offerings.

- Menu choices, portion sizes, and access to second helpings and supplement snacks will be consistent across cohorts allocated to the same diet as much as feasible.

- Participants will eat separately from participants of other simultaneous studies as much as is feasible.

5.3.2. Caffeine, Alcohol, and Tobacco

- Participants will abstain from caffeine-containing products for 24 hours prior to the start of dosing until collection of the final PK sample of each study period.

- Participants will abstain from alcohol for 24 hours prior (or as specified above for red wine) to admission to the CRU and continue abstaining from alcohol until collection of the final PK sample of each study period. Participants may undergo an alcohol breath test or blood alcohol test at the discretion of the investigator.

- Participants will abstain from the use of tobacco- or nicotine-containing products for 24 hours prior to dosing and during confinement in the CRU.

5.3.3. Activity

- Participants will abstain from strenuous exercise (eg, heavy lifting, weight training, calisthenics, aerobics) for at least 48 hours prior to each blood collection for clinical laboratory tests. Walking at a normal pace will be permitted.

- In order to standardize the conditions on serial PK sampling days (Part A – Days 1 and 14; Part B – Period 1/Day 1 and Period 2/Days 2 and 10), participants will be required to refrain from lying down (except when required for BP, PR, RR, and ECG measurements) for first 4 hours after morning dose, and may be required to follow meals and dietary restrictions.

- In Part A only, participants will be confined to the procedure room for the first 4 hours after dosing on Days 1, 7, and 14 during continuous cardiac monitoring, except to use the bathroom. After this, if the equipment setup allows, participants may be ambulatory during the ECG monitoring period, but should not engage in strenuous activities.

- Participants will be advised to avoid direct sunlight exposure or any high intensity ultraviolet light exposure, from admission through the onsite follow-up visit. In addition, participants will be instructed to apply sun cream/lotion with a high sun protection factor of ≥50 and to use eye protection, as appropriate.

5.3.4. Daily Medication (Optional Cohort of Older Participants Only)

Older participants should continue to take their permitted daily prescription or non-prescription medications at approximately the same time each day during the study. These participants must be established on a stable dose of all permitted medications, as defined by no change in dose or dose regimen for the 28 days or 5 half-lives (whichever is longer) before the screening visit.

5.3.5. Contraception

The investigator or his or her designee, in consultation with the participant, will confirm that the participant has selected an appropriate method of contraception for the individual participant and his or her partner(s) from the permitted list of contraception methods (see Section 10.4.4) and will confirm that the participant has been instructed in its consistent and correct use. At time points indicated in the schedule of activities (SoA), the investigator or designee will inform the participant of the need to use highly effective contraception consistently and correctly and document the conversation and the participant's affirmation in the participant's chart (participants need to affirm their consistent and correct use of at least 1 of the selected methods of contraception) considering that their risk for pregnancy may have changed since the last visit. In addition, the investigator or designee will instruct the participant to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the participant or partner.

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention/enrolled in the study. Screen failure data are collected and remain as source and are not reported on the CRF.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened if the prior reason(s) for not meeting the eligibility criteria have been resolved.

6. STUDY INTERVENTIONS(S) AND CONCOMITANT THERAPY

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, medical device(s), or study procedure(s) intended to be administered to a study participant according to the study protocol.

For the purposes of this protocol, study intervention refers to PF-07258669, placebo for PF-07258669, or midazolam.

6.1. Study Intervention(s) Administered

PF-07258669 and matching placebo will be supplied as tablets to the CRU in bulk along with individual dosing containers for unit dosing. Tablets will be provided in strengths of 1 mg, 5 mg, 25 mg, and 100 mg.

PF-07258669 and matching placebo will be presented to the participants in individual dosing containers.

For Part B, commercially-available midazolam oral solution (2 mg/mL, 30 mL) will be provided to the CRU.

6.1.1. Administration

Administration of study intervention will occur under the conditions described in the Meals and Dietary Restrictions in Section 5.3.1.

In order to standardize the conditions on serial PK sampling days (Part A – Days 1 and 14; Part B – Period 1/Day 1 and Period 2/Days 2 and 10), all participants will be required to refrain from lying down (except when required for BP, PR, RR, and ECG measurements), eating, and drinking beverages other than water during the first 4 hours after the morning dosing.

- Part A: Participants will receive study intervention at approximately 08:00 hours (±2 hours) from Day 1 to the morning of Day 14. On days of serial PK sampling (ie, Days 1 and 14), participants will abstain from all food and drink (except water or study intervention administration) for at least 12 hours overnight prior to morning dosing. Investigator site personnel will administer study intervention during each period with ambient temperature water to a total volume of approximately 240 mL. Participants will swallow the study intervention whole, and will not manipulate or chew the study intervention prior to swallowing. Additional water (up to 100 mL) may be consumed by the participants (eg, extra rinse) in an attempt to ensure that the full dose is consumed.

  For optional cohort of older participants only: Healthy older participants should continue to take their permitted daily prescription or non-prescription medications at approximately the same time each day during the study. On days of dense PK sampling (morning dosing on Day 1 and Day 14), administration of concomitant medications should occur at least 1-2 hours prior to morning dosing of PF-07258669 or withheld until 4 hours after morning dosing of PF-07258669. There are no restrictions on other days of the study.

- Part B: In Period 1, following an overnight fast of at least 12 hours, participants will receive midazolam at approximately 08:00 hours (±2 hours), followed by intake of 50 mL of ambient temperature water. Additional water (up to 100 mL) may be consumed by the participants (eg, extra rinse) in an attempt to ensure that the full dose is consumed.

  The dosing regimen for PF-07258669 to be used in Period 2 will be based on the results of Part A of this study. In Period 2, the first dose of PF-07258669 will be administered at 08:00 hours (±2 hours) on Day 1. PF-07258669 dosing will continue from Day 1 through Day 10. Investigator site personnel will provide PF-07258669 to the participant with ambient temperature water to a total volume of approximately 240 mL. Participants will swallow the tablets whole and will not manipulate or chew the tablets prior to swallowing. Additional water (up to 100 mL) may be consumed by the participants (eg, extra rinse) in an attempt to ensure that the full dose is consumed.

  On the days of co-administration of midazolam and PF-07258669 in Part B (Day 2 and Day 10): Following an overnight fast of at least 12 hours (except water or study intervention administration), PF-07258669 will be administered to participants at approximately 08:00 hours (±2 hours) with ambient temperature water to a total volume of approximately 240 mL and will be followed by the administration of

midazolam within 5 minutes which will be followed by intake of 50 mL of ambient temperature water. Additional water (up to 100 mL) may be consumed by the participants (eg, extra rinse) in an attempt to ensure that the full doses of PF-07258669 and midazolam are consumed on days of co-administration.

6.2. Preparation, Handling, Storage, and Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study interventions received and any discrepancies are reported and resolved before use of the study intervention.

2. Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated recording) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff. At a minimum, daily minimum and maximum temperatures for all site storage locations must be documented and available upon request. Data for nonworking days must indicate the minimum and maximum temperatures since previously documented for all site storage locations upon return to business.

3. Any excursions from the study intervention label storage conditions should be reported to Pfizer upon discovery along with any actions taken. The site should actively pursue options for returning the study intervention to the storage conditions described in the labeling, as soon as possible. Once an excursion is identified, the study intervention must be quarantined and not used until Pfizer provides permission to use the study intervention. Specific details regarding the definition of an excursion and information the site should report for each excursion will be provided to the site in the IP manual and CRU site procedures.

4. Any storage conditions stated in the SRSD will be superseded by the storage conditions stated on the label.

5. Study interventions should be stored in their original containers.

6. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records), such as the IPAL or sponsor-approved equivalent. All study interventions will be accounted for using a study intervention accountability form/record.

7. Further guidance and information for the final disposition of unused study interventions are provided in the CRU's site procedures and IP manual. All destruction must be adequately documented. If destruction is authorized to take place at the investigator site, the investigator must ensure that the materials are destroyed in

compliance with applicable environmental regulations, institutional policy, and any special instructions provided by Pfizer.

Upon identification of a product complaint, notify the sponsor within 1 business day of discovery, as described in the IP manual.

6.2.1. Preparation and Dispensing

Within this protocol, preparation refers to the investigator site activities performed to make the study intervention ready for administration or dispensing to the participant by qualified staff. Dispensing is defined as the provision of study intervention, concomitant treatments, and accompanying information by qualified staff member(s) to a healthcare provider, participant in accordance with this protocol. Local health authority regulations or investigator site guidelines may use alternative terms for these activities.

PF-07258669 and matching placebo tablets will be prepared at the CRU in the individual dosing containers by 2 operators, 1 of whom is an appropriately qualified and experienced member of the study staff (eg, physician, nurse, physician's assistant, nurse practitioner, pharmacy assistant/technician, or pharmacist). The tablets will be provided in unit dose containers and labeled in accordance with Pfizer regulations and the clinical site's labeling requirements. See the IP Manual and/or CRU's site procedure for instructions on how to prepare the study intervention for administration.

Midazolam (2 mg/mL oral solution) will be prepared and dispensed at the CRU in the individual oral syringes by 2 operators, 1 of whom is an appropriately qualified and experienced member of the study staff (eg, physician, nurse, physician's assistant, nurse practitioner, pharmacy assistant/technician, or pharmacist) as allowed by local, state and institutional guidance. The oral solution will be provided in unit dose oral syringes and labeled in accordance with Pfizer regulations and the clinical site's labeling requirements. CRU staff should refer to the SmPC on how to prepare midazolam for administration to participants.

6.3. Measures to Minimize Bias: Randomization and Blinding

6.3.1. Allocation to Study Intervention

The investigator will assign participant numbers to the participants as they are screened for the study. Pfizer will provide a randomization schedule to the investigator and, in accordance with the randomization numbers, the participant will receive the study treatment regimen assigned to the corresponding randomization number.

Participants will be randomly assigned to receive study intervention from a central randomization scheme.

In Part A of this study, investigators and participants will remain blinded to each participant's assigned study intervention throughout the course of the study. In order to maintain this blind, an otherwise uninvolved third party (for example, pharmacist) will be responsible for the preparation and dispensing of all study intervention according to the randomization schedule and assigned treatment for the individual participant.

In Part B of this study, the investigator's knowledge of the treatment should not influence the decision to enroll a particular participant or affect the order in which participants are enrolled.

6.3.2. Breaking the Blind

The method for breaking the blind in this study will be manual. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a participant's treatment assignment unless this could delay further management of the participant. If a participant's treatment assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. When the blinding code is broken, the reason must be fully documented in the CRF.

Blood specimens will be obtained from all participants for PK analysis to maintain the study blind at the investigator site. Only the investigator site staff and blinded study monitor, if assigned, will be blinded to study treatment. A limited number of Pfizer study team personnel will be unblinded to participant treatments in order to permit real-time interpretation of the safety and PK data and provide information necessary to potentially alter the dose-escalation sequence. The blinded study monitor, if assigned, will remain blinded to treatment until all monitoring for the study has been completed. Specimens from participants randomized to placebo will not be routinely analyzed. To minimize the potential for bias, treatment randomization information will be kept confidential by Pfizer unblinded personnel and will not be released to the blinded investigator or blinded investigator site personnel until the study database has been locked or the investigator requests unblinding for safety reasons.

6.4. Study Intervention Compliance

When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second qualified member of the study site staff.

When participants are dosed at the site, they will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the CRF. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention. Study site personnel will examine each participant's mouth to ensure that the study intervention was ingested.

6.5. Dose Modification

The decision to proceed to the next dose level and/or dose regimen of PF-07258669 (eg, an increase, decrease, or repeat of a previous dose level) will be made by the study team and the investigator based on safety, tolerability, and preliminary PK data obtained at the prior dose level. With the exceptions of the optional Japanese and older adult cohorts, at least 8 participants (including at least 1 placebo participant) must complete the prior dose level in Part A. Safety, tolerability, and PK data through at least 14 days will be reviewed.

The midazolam dose in Part B may also be modified as outlined in Section 4.3.3.2.2.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety, tolerability, and/or PK findings at a given dose level or to add cohorts to evaluate additional dose levels. The study procedures for these additional participant(s)/cohort(s) will be the same as that described for other study participants/cohorts.

6.5.1. Dose Escalation and Stopping Rules

Precautionary sentinel dosing may be employed in Part A and Part B as described in Section 4.1.1 and Section 4.1.2, respectively.

Dose escalation stopping rules will be used to determine whether the maximal tolerated dose has been attained. Dose escalation may be stopped if it is determined that the limits of safety and/or tolerability have been reached. This decision will be made after a discussion takes place between the sponsor study team and the investigator. The sponsor study team may not overrule the investigator's decision to stop dose escalation. If dose escalation is stopped because of any of these criteria, additional cohorts may receive the same or lower doses of the study intervention.

The dose escalation will be terminated based on the following criteria:

- If 50% or more of the participants receiving active drug at a given dose level (but not participants receiving placebo) develop similar clinically significant laboratory, ECG, or vital sign abnormalities, in the same organ class, indicating dose-limiting intolerance.

- Severe nonserious AEs, considered as, at least, possibly related to study intervention administration, in 2 participants at a given dose level (but not participants receiving placebo), independent of within or not within the same system organ class, indicating dose-limiting intolerance.

- Dosing will be paused for any SAE that occurs in a participant receiving active treatment until causality is fully assessed by the PI and sponsor. Dosing may resume if the SAE is determined to be not drug-related by the PI and sponsor. If the SAE is determined to be either drug-related or unknown, either dosing will cease or the SAE will be evaluated by the sponsor's protocol review committee (or similar review group), which is independent of the study team and investigators. If the protocol

review committee determines that dosing may resume, a plan that mitigates risks to participants with the resumption of dosing will be implemented. Such a plan could include a revision of inclusion/exclusion criteria, repeating or reducing the dose, or adding appropriate safety monitoring.

- CCI [redacted] Dosing may resume if the AE is determined to not be drug-related by the investigator and sponsor. If the AE is determined to be either drug related or unknown, either dosing will cease or the AE will be evaluated by the sponsor's protocol review committee (or similar review group), which is independent of the study team and investigators, to provide further direction.

- It is determined that the limit of safety and/or tolerability has been reached. This decision will be made following discussions between the study team and the investigator.

- Other findings that, at the discretion of the study team and investigator, indicate that dose escalation should be halted.

- CCI [redacted]

- If, based on the observed data, the group mean C_{max} or AUC (based on total plasma concentration) of the next planned dose is projected to exceed the escalation limits, that dose will not be explored. Modified doses may be explored if they are not expected to exceed PK stopping criteria.

Progression to the next dose will occur if the last dose was well tolerated and after satisfactory review of the available safety and PK data.

6.6. Continued Access to Study Intervention After the End of the Study

No intervention will be provided to study participants at the end of their study participation.

6.7. Treatment of Overdose

6.7.1. Overdose of PF-07258669

For this study, any dose of PF-07258669 greater than 2000 mg within a 24-hour time period will be considered an overdose.

There is no specific treatment for an overdose.

In the event of an overdose, the investigator or treating physician should:

1. Contact the medical monitor within 24 hours.

2. Closely monitor the participant for any AEs/SAEs and laboratory abnormalities for at least 5 half-lives or 28 calendar days after the overdose of PF-07258669 (whichever is longer).

3. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

4. Overdose is reportable to Pfizer Safety **only when associated with an SAE**.

5. Obtain a blood sample for PK analysis within 5 days from the date of the last dose of study intervention if requested by the medical monitor (determined on a case-by-case basis).

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the medical monitor based on the clinical evaluation of the participant.

6.7.2. Overdose of Midazolam

For this study, any dose of midazolam that exceeds 15 mg, or any clinical signs/symptoms indicative of exaggerated midazolam pharmacology (including but not limited to excessive sedation, hypotension, or respiratory depression) that requires medical intervention will be considered an overdose.

In the event of an overdose, the investigator or treating physician should:

1. Immediately institute cardiorespiratory support in keeping with ILS guidelines. The competitive benzodiazepine antagonist, flumazenil, may be used at the discretion of the investigator in accordance with local protocols. The participant may be transferred to an affiliated hospital facility if deemed necessary at the discretion of the investigator. The participant must be closely monitored through recovery to baseline.

2. Obtain a blood sample for both midazolam PK and PF-07258669 analysis, if possible, as soon as the participant has been medically stabilized or at the earliest possible time.

3. Contact the medical monitor within 24 hours.

4. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

5. Overdose is reportable to Pfizer Safety **only when associated with an SAE**.

Decisions regarding continued participation in Part B, dose interruptions or modifications will be made by the investigator in consultation with the medical monitor based on the clinical evaluation of the participant.

6.8. Concomitant Therapy

Use of prescription or nonprescription drugs and dietary and herbal supplements are prohibited within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study intervention and for the duration of the study. See Section 6.8.1 for exceptions for the optional cohort of older adult participants.

Treatments taken within 28 days before the first dose of study intervention will be documented as a prior treatment. Treatments taken after the first dose of study intervention will be documented as concomitant treatments.

For all participants, all concomitant treatments taken during the study must be recorded with indication, daily dose, and start and stop dates of administration. All participants will be questioned about concomitant treatment at each clinic visit.

Females taking hormone replacement therapy may be eligible to participate in this study if they are willing to discontinue therapy at least 28 days prior to the first dose of study intervention and remain off hormonal therapy for the duration of the study.

As PF-07258669 is primarily metabolized by CYP3A, use of moderate or strong CYP3A inhibitors or inducers are prohibited for all participants within 28 days or 5 half-lives (whichever is longer) prior to Screening and for the duration of the study. A non-exhaustive list of excluded concomitant medications is in Appendix 13. If a medication is not listed, it should not automatically be assumed it is permitted to co-administer. Therefore, all medications must be reviewed on a case-by-case basis by the investigator and approved by the sponsor during the screening period for eligibility purposes.

Limited use of nonprescription medications that are not believed to affect participant safety or the overall results of the study may be permitted on a case-by-case basis following approval by the sponsor. Acetaminophen/paracetamol may be used at doses of ≤1 g/day.

6.8.1. Optional Cohort of Older Adult Participants Only

Participants in the optional cohort of older adult participants only may take certain daily prescription or non-prescription medications for management of acceptable chronic medical conditions. They are to be on a stable dose, as defined by no change in dose for the 28 days or 5 half-lives (whichever is longer) before the screening visit. However, concomitant administration of moderate or strong CYP3A inhibitors or inducers are not allowed. Additionally, as described below, depending on results of Part B of the study, concomitant administration of certain CYP3A substrates may also not be allowed.

In vitro, PF-07258669 is a potential time-dependent inhibitor and/or inducer of CYP3A. The effect of PF-07258669 on a probe CYP3A substrate, midazolam, will be assessed in Part B of this study. The results of Part B of this study will inform the ability to co-adminster PF-07258669 with CYP3A substrates in this study.

Based on the overall interpretation of the results of Part B, and taking into account the dose level/regimen to be evaluated in the older adult cohort, the following restrictions will apply regarding use of CYP3A substrates in older adult participants:

- If no interaction (ie, PF-07258669 results in <1.25-fold increase in midazolam AUC) – no restrictions;
- If a mild interaction (ie, PF-07258669 results in ≥1.25- to <2-fold increase in midazolam AUC) – use of sensitive CYP3A substrates with narrow therapeutic index will be restricted;
- If a moderate interaction (ie, PF-07258669 results in ≥2- to <5-fold increase in midazolam AUC) or a strong interaction (ie, PF-07258669 results in ≥5-fold increase in midazolam AUC) – use of sensitive CYP3A substrates and CYP3A substrates with narrow therapeutic index will be restricted.

A non-exhaustive list of prohibited and precautionary medications is provided in Appendix 13. If a medication is not listed, it should not automatically be assumed it is permitted to co-administer. Therefore, all medications must be reviewed on a case-by-case basis by the investigator and approved by the sponsor during the screening period for eligibility purposes.

Healthy older participants should continue to take their permitted daily prescription or non-prescription medications at approximately the same time each day during the study. On days of dense PK sampling (morning dosing on Day 1 and Day 14), administration of concomitant medications should occur at least 1-2 hours prior to morning dosing of PF-07258669 or withheld until 4 hours after morning dosing of PF-07258669. There are no restrictions on other days of the study.

6.8.2. Rescue Medicine

There is no rescue therapy to reverse the AEs observed with PF-07258669; standard medical supportive care must be provided to manage the AEs.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue study intervention. Reasons for permanent discontinuation of study intervention include the following: adverse event or some other (administrative) reason.

If study intervention is permanently discontinued, the participant will not remain in the study for further evaluation. See the SoA for data to be collected at the time of discontinuation of study intervention.

In the event of discontinuation of study intervention, it must be documented on the appropriate CRF/in the medical records whether the participant is discontinuing further receipt of study intervention or also from study procedures, posttreatment study follow-up, and/or future collection of additional information.

7.1.1. ECG Changes

A participant who meets either of the following bulleted criteria based on the average of triplicate ECG readings will be withdrawn from the study intervention.

- QTcF >500 msec.
- Change from baseline: QTcF >60 msec.

If a clinically significant finding is identified (including, but not limited to, changes from baseline in QTcF after enrollment), the investigator or qualified designee will determine if the participant can continue in the study and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

7.1.2. Potential Cases of Acute Kidney Injury

Abnormal values in SCr concurrent with presence or absence of increase in BUN that meet the criteria below, in the absence of other causes of kidney injury, are considered potential cases of acute kidney injury and should be considered important medical events.

An increase of ≥0.3 mg/dL (or ≥26.5 µmol/L) in SCr level relative to the participant's own baseline measurement should trigger another assessment of SCr as soon as practically feasible, preferably within 48 hours from awareness.

If the second assessment (after the first observations of ≥0.3 mg/dL [or ≥26.5 µmol/L] in SCr relative to the participant's own baseline measurement) is ≥0.4 mg/dL (or ≥35.4 µmol/L), the participant should be discontinued from the study and adequate, immediate, supportive measures taken to correct apparent acute kidney injury.

Participants should return to the investigator site and be evaluated as soon as possible, preferably within 48 hours from awareness of the second assessment confirming abnormal SCr result. This evaluation should include laboratory tests, detailed history, and physical assessment. In addition to repeating SCr, laboratory tests should include serum BUN, serum creatine kinase, and serum electrolytes (including at a minimum potassium, sodium, phosphate/phosphorus, and calcium), in addition to urinary dipstick, urine microscopic examination, and urinary indices. All cases confirmed on repeat testing as meeting the laboratory criteria for acute kidney injury, with no other cause(s) of laboratory abnormalities identified, should be considered potential cases of drug-induced kidney injury irrespective of availability of all the results of the investigations performed to determine etiology of the abnormal SCr. If ≥2 healthy participants in a given cohort are noted to have 2 consecutive

SCr results of ≥0.3 mg/dL (or ≥26.5 µmol/L), an assessment of whether the finding may be considered an adverse drug reaction should be undertaken.

7.2. Participant Discontinuation/Withdrawal From the Study

A participant may withdraw from the study at any time at his/her own request. Reasons for discontinuation from the study include the following:

- Refused further study procedures;
- Lost to follow-up;
- Death;
- Study terminated by sponsor;
- Discretion of the investigator or sponsor for safety or behavioral reasons, or the inability of the participant to comply with the protocol required schedule of study visits or procedures.

At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted. See the SoA for assessments to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The early discontinuation visit applies only to participants who are enrolled/randomized and then are prematurely withdrawn from the study. Participants should be questioned regarding their reason for withdrawal.

The participant will be permanently discontinued from the study intervention and the study at that time.

If a participant withdraws from the study, he/she may request destruction of any remaining samples taken and not tested, and the investigator must document any such requests in the site study records and notify the sponsor accordingly.

If the participant withdraws from the study and also withdraws consent (see Section 7.2.1) for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

7.2.1. Withdrawal of Consent

Participants who request to discontinue receipt of study intervention will remain in the study and must continue to be followed for protocol-specified follow-up procedures. The only exception to this is when a participant specifically withdraws consent for any further contact with him or her or persons previously authorized by the participant to provide this information. Participants should notify the investigator in writing of the decision to withdraw

consent from future follow-up, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is only from further receipt of study intervention or also from study procedures and/or posttreatment study follow-up, and entered on the appropriate CRF page. In the event that vital status (whether the participant is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

7.3. Lost to Follow up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for/attend a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible. Counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study;
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record;
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

The investigator (or an appropriate delegate at the investigator site) must obtain a signed and dated ICD before performing any study-specific procedures.

Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.

Safety issues should be discussed with the sponsor immediately upon occurrence or awareness to determine whether the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Participants will be screened within 28 days prior to administration of the study intervention to confirm that they meet the study population criteria for the study. If the time between screening and dosing exceeds 28 days as a result of unexpected delays (eg, delayed drug shipment), then participants do not require rescreening if the laboratory results obtained prior to first dose administration meet eligibility criteria.

A participant who qualified for this protocol but did not enroll from an earlier cohort/group may be used in a subsequent cohort/group without rescreening, provided laboratory results obtained prior to the first dose administration meet eligibility criteria for this study. In addition, other clinical assessments or specimen collections, eg, banked biospecimens, may be used without repeat collection, as appropriate.

Every effort should be made to ensure that protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator must take all steps necessary to ensure the safety and well-being of the participant. When a protocol-required test cannot be performed, the investigator will document the reason for the missed test and any corrective and preventive actions that he or she has taken to ensure that required processes are adhered to as soon as possible. The study team must be informed of these incidents in a timely manner.

Any safety, laboratory, or analyte results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

If an IV catheter is utilized for blood sample collections, ECGs and vital sign assessments (pulse rate and BP) should be collected prior to the insertion of the catheter.

For samples being collected and shipped, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site prior to initiation of the study.

The total blood sampling volume for individual participants in this study is approximately 260 mL for Part A and approximately 250 mL for Part B. The actual collection times of blood sampling may change. Additional blood samples may be taken for safety assessments at times specified by Pfizer, provided the total volume taken during the study does not exceed 550 mL during any period of 56 consecutive days.

To prepare for study participation, participants will be instructed on the information in the Lifestyle Considerations and Concomitant Therapy sections of the protocol.

8.1. Efficacy Assessments

No efficacy assessments are being conducted in this study.

8.2. Safety Assessments

Planned time points for all safety assessments are provided in the SoA. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

8.2.1. Physical Examinations

Physical examinations are to be performed at the nominal timepoints specified in the SoA. Additional physical examinations will be permitted, as necessary, to ensure appropriate collection of safety data.

A complete physical examination will include, at a minimum, head, ears, eyes, nose, mouth, skin, heart and lung examinations, lymph nodes, and gastrointestinal, musculoskeletal, and neurological systems.

A brief physical examination will include, at a minimum, assessments of general appearance, the respiratory and cardiovascular systems, and participant-reported symptoms.

Physical examinations may be conducted by a physician, trained physician's assistant, or nurse practitioner as acceptable according to local regulation.

Height and weight will also be measured and recorded as per the SoA. For measuring weight, a scale with appropriate range and resolution is used and must be placed on a stable, flat surface. Participants must remove shoes, bulky layers of clothing, and jackets so that only light clothing remains. They must also remove the contents of their pockets and remain still during measurement of weight. Please refer to Section 8.5.1 for a more complete description of body weight assessment.

Physical examination findings collected during the study will be considered source data and will not be required to be reported, unless otherwise noted. Any untoward physical examination findings that are identified during the active collection period and meet the definition of an AE or SAE (Appendix 3) must be reported according to the processes in Sections 8.3.1 to 8.3.3.

8.2.2. Neurological Examinations (Part A only)

Neurological examinations may be conducted by a physician, trained physician's assistant, or nurse practitioner as acceptable according to local regulation at the nominal timepoints specified in the SoA. The neurological exam will consist of assessment of higher cortical function, the cranial nerves, motor function, deep tendon reflexes, sensory exam, and coordination and gait. The exam should be done to the extent needed to assess the participant for any potential changes in neurological status, as determined by the investigator (or designee).

8.2.3. Vital Signs

BP and pulse rate will be measured at the nominal timepoints specified in the SoA. Additional collection times, or changes to collection times, of BP and pulse rate will be permitted, as necessary, to ensure appropriate collection of safety data. Supine BP will be measured with the participant's arm supported at the level of the heart, and recorded to the nearest mm Hg after approximately 5 minutes of rest. The same arm (preferably the dominant arm) will be used throughout the study. Participants should be instructed not to speak during measurements.

The same properly sized and calibrated BP cuff will be used to measure BP each time. The use of an automated device for measuring BP and pulse rate is acceptable; however, when done manually, pulse rate will be measured in the brachial/radial artery for at least 30 seconds. When the timing of these measurements coincides with a blood collection, BP and pulse rate should be obtained prior to the nominal time of the blood collection. When triplicate measurements of supine BP or pulse rate are required per SoA, measurements should be collected 2-4 minutes apart.

The procedure for collecting postural or orthostatic data will be:

- Assess BP after the participant is in the supine position for a minimum of 5 minutes; when timing of orthostatic BP assessment coincides with timing of triplicate supine BP assessment, the last of the triplicate supine readings will be used as the supine reading for the orthostatic assessment.
- Have the participant stand up for 2 minutes.
- Assess BP after the participant is in the standing position for approximately 2 minutes.

Orthostatic hypotension is defined as a decrease of ≥20 mm Hg for systolic BP or ≥10 mm Hg for diastolic BP 2 minutes after standing from a supine position. Orthostatic hypotension may be symptomatic or asymptomatic. Symptoms of orthostatic hypotension are those that develop upon assuming the erect posture from a supine position and may include: lightheadedness, dizziness, blurred vision, weakness, fatigue, cognitive impairment, nausea, palpitations, tremulousness, headache, and/or neck ache. Repeated measurements of supine/standing BP should be obtained in the event of either asymptomatic or symptomatic orthostatic hypotension at the discretion of the investigator.

If a participant manifests an increase in pulse rate of ≥30 beats per minute [bpm] with a decrease of <20 mm Hg for systolic BP or <10 mm Hg for diastolic BP 2 minutes after standing from a supine position, repeated measurements of supine/standing BP should be obtained. Similarly, if a participant has symptoms suggestive of orthostasis, but not documented orthostatic hypotension, repeated measurements of supine/standing BP should be obtained. Lesser degrees of BP reduction may still be considered clinically significant if

the participant becomes symptomatic upon standing, especially in the presence of a significant increase in pulse rate (≥30 bpm).

8.2.3.1. Respiratory Rate

Respiratory rate will be measured at times specified in the SoA. After approximately 5 minutes rest in supine position, respiratory rate will be measured by observing and counting the respirations of the participant for 30 seconds and multiplied by 2. When BP is to be taken at the same time, respiration measurement will be done during the 5 minutes of rest and before BP measurement.

8.2.3.2. Oral Body Temperature

Body temperature will be measured orally.

8.2.4. Electrocardiograms

Standard 12-lead ECGs utilizing limb leads (with a 10 second rhythm strip) should be collected at times specified in the SoA section of this protocol using an ECG machine that automatically calculates the heart rate and measures PR, QT, and QTc intervals and QRS complex. Alternative lead placement methodology using torso leads (eg, Mason-Likar) should not be used given the potential risk of discrepancies with ECGs acquired using standard limb lead placement. All scheduled ECGs should be performed after the participant has rested quietly for at least 5 minutes in a supine position.

On Day 1 at -1H, -0.5H, and 0H prior to the morning dose, triplicate 12-lead ECGs will be obtained approximately 2 to 4 minutes apart at each time point. The average of the triplicate ECG measurements over the 3 pre-dose measurement times (total of 9 ECG measurements) collected before morning dose administration on Day 1 will serve as each participant's baseline QTc value.

To ensure safety of the participants, a qualified individual at the investigator site will make comparisons to baseline measurements. Additional ECG monitoring will occur if a) the mean value from the triplicate measurements for any postdose QTcF interval is increased by ≥60 msec from the baseline **and** is >450 msec; or b) an absolute QT value is ≥500 msec for any scheduled ECG. If either of these conditions occurs, then a single ECG measurement must be repeated at least hourly until QTc values from 2 successive ECGs fall below the threshold value that triggered the repeat measurement.

If a) a postdose QTcF interval remains ≥60 msec from the baseline **and** is >450 msec; or b) an absolute QT value is ≥500 msec for any scheduled ECG for greater than 4 hours (or sooner, at the discretion of the investigator); or c) QTcF intervals get progressively longer, the participant should undergo continuous ECG monitoring. A cardiologist should be consulted if QTcF intervals do not return to less than the criteria listed above after 8 hours of monitoring (or sooner, at the discretion of the investigator).

In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads be placed in the

same positions each time in order to achieve precise ECG recordings. If a machine-read QTc value is prolonged, as defined above, repeat measurements may not be necessary if a qualified medical provider's interpretation determines that the QTcF values are in the acceptable range.

ECG values of potential clinical concern are listed in Appendix 8.

8.2.4.1. Continuous Cardiac Monitoring by Telemetry (Part A only)

All abnormal rhythms will be recorded and reviewed by the study physician 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. In addition, a printed record of the tracing(s) of the clinically significant rhythm(s) will be made and retained with other source documents.

Telemetry should be collected using a centralized system that also allows for the storage and advanced analysis of all recorded data in order to preserve important events for future evaluations. Holter monitoring should not be used in parallel with continuous telemetry, unless it is the only means of data storage available at the investigator site, or verifiable arrhythmia quantification is required. To establish a baseline, telemetry should be recorded for at least 2 hours on Day -1. This may be done immediately prior to dosing or at some 2-hour continuous interval in the 24 hours prior to dosing, as long as the recording is performed when the participant is awake. Telemetry may be stopped within a reasonably short period of time prior to dosing, in order to avoid interference with study operations conducted immediately before dosing. However, it is expected that the telemetry leads will be in place and the system connected prior to dosing.

8.2.5. Continuous Pulse Oximetry (Part B Only)

Continuous pulse oximetry will be performed on midazolam dosing days in Part B as outlined in the SoA. Monitoring will be initiated at least 30 minutes prior to midazolam dosing and will continue through at least 6 hours after midazolam dosing. The duration of monitoring may be extended at discretion of the investigator. All abnormal oxygen saturation alerts will be recorded and reviewed by the study physician for potential clinical concern. Clinical correlation through review of the participant will be sought for any alerts considered of potential clinical concern. Guidance with respect to overdose of midazolam outlined in Section 6.7.2 should be considered in the setting of abnormal oxygen saturation alerts deemed concerning. The time, duration, and description of the clinically significant event will be recorded in the CRF. Continuous pulse oximetry should be collected using a centralized system that also allows for the storage and advanced analysis of all recorded data in order to preserve important events for future evaluations.

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CCI [redacted]

8.2.8. Clinical Safety Laboratory Assessments

See Appendix 2 for the list of clinical safety laboratory tests to be performed and the SoA for the timing and frequency. All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 14 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

See Appendix 6 for suggested actions and follow-up assessments in the event of potential drug-induced liver injury.

Participants may undergo random urine drug testing at the discretion of the investigator. Drug testing conducted prior to dosing must be negative for participants to receive study intervention.

8.2.9. COVID-19 Related Measures

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

CCI

[redacted]

8.2.11. Suicidal Ideation and Behavior Risk Monitoring

8.2.11.1. Columbia Suicide Severity Rating Scale (Part A Only)

The C-SSRS is an interview based rating scale to systematically assess suicidal ideation and suicidal behavior. Versions are available for Screening/Baseline (Lifetime assessment) assessments and subsequent assessments (Since Last Visit assessment) during the study.

Baseline/Lifetime assessment to be performed at Screening. All other assessments should use the Since Last Visit assessment.

The C-SSRS should be administered by an appropriately trained staff member.

At each suicidality assessment, participants felt to have significant suicidal ideation with actual plan and intent or suicidal behavior, must be evaluated by a clinician/MHP skilled in the evaluation of suicidal ideation and behavior in the participants by virtue of training or experience (eg, psychiatrist, licensed clinical psychologist) who will determine if it is safe for the participant to participate/continue in the trial. Specific criteria that indicate a need for such an assessment are:

- Suicide ideation associated with actual intent and/or plan in the past year; ("YES" answer to C-SSRS questions 4 "some intent to act without specific plan" or 5 "specific plan and intent");
- Previous history of suicide behaviors in the past 5 years (a "YES" answer to any of the suicidal behavior items of the C-SSRS with the behavior occurring in the past 5 years);
- In the investigator's judgment, a risk assessment or exclusion is warranted.

Other possible suicidal ideation and behavior AEs or other clinical observations may, based on the judgment of the investigator, also trigger a risk assessment and require a narrative.

Suicidality AEs or other clinical observations may, based on the judgment of the investigator and clinician/MHP, also trigger a risk assessment and a narrative using information from the C-SSRS, and available information, prior to screening and baseline information, and the

clinician/MHP assessment. When there is a positive response to any question on the C-SSRS, the investigator should determine whether an AE has occurred.

At the Screening/Baseline assessment, a risk assessment will be done by qualified staff to determine whether it is safe for the participant to be enrolled or to continue to participate in the trial.

Participants who respond "YES" to items 4, 5 or to any behavioral question of the C-SSRS at any time after the baseline visit will be assessed by clinician/MHP to determine whether it is safe for the participant to continue in the trial.

Participants who respond "YES" to items 4, 5 or to any behavioral question of the C-SSRS on more than one occasion during a trial must have their suicidality managed appropriately by the investigator together with clinician/MHP (or the investigator alone if the investigator is a qualified mental health professional). Depending on the specifics of the participant as assessed by the investigator and/or clinician/MHP, the participant may be discontinued from the trial.

8.2.11.2. Rater Qualifications

For specific rating assessments, only qualified raters will be allowed to evaluate and/or rate participants in this study. The minimum qualifications a rater must meet for each study rating assessment will be outlined in the C-SSRS Resource Guide provided to each participating site. The level of experience with the target population (or equivalent), specific scale experience (or equivalent), and certification required (if applicable) will be listed and used to determine whether a rater is approved for a given assessment. The rater must become certified to perform selected study assessments before he or she can participate in the conduct of the study. For specifically defined assessments, rater training and standardization exercises may be conducted, and written documentation will be provided by the site for each rater's certification. In return, each site will be provided written documentation outlining each rater's certification for specific study assessments. Recertification may be required at periodic intervals during the study. The raters who administer specific study assessments will be documented in a centralized location and all site staff who administer ratings will be verified in the site study documentation during the conduct of the study.

8.3. Adverse Events, Serious Adverse Events, and Other Safety Reporting

The definitions of an AE and an SAE can be found in Appendix 3.

AEs may arise from symptoms or other complaints reported to the investigator by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative), or they may arise from clinical findings of the Investigator or other healthcare providers (clinical signs, test results, etc.).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible to pursue and obtain adequate information both to determine the outcome and to assess whether the

event meets the criteria for classification as an SAE or caused the participant to discontinue the study intervention (see Section 7.1).

During the active collection period as described in Section 8.3.1, each participant will be questioned about the occurrence of AEs in a nonleading manner.

In addition, the investigator may be requested by Pfizer Safety to obtain specific follow-up information in an expedited fashion.

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each participant begins from the time the participant provides informed consent, which is obtained before the participant's participation in the study (ie, before undergoing any study-related procedure and/or receiving study intervention), through and including a minimum of 28 calendar days, except as indicated below, after the last administration of the study intervention.

Follow-up by the investigator continues throughout and after the active collection period and until the AE or SAE or its sequelae resolve or stabilize at a level acceptable to the investigator.

For participants who are screen failures, the active collection period ends when screen failure status is determined.

If the participant withdraws from the study and also withdraws consent for the collection of future information, the active collection period ends when consent is withdrawn.

If a participant permanently discontinues or temporarily discontinues study intervention because of an AE or SAE, the AE or SAE must be recorded on the CRF and the SAE reported using the CT SAE Report Form.

Investigators are not obligated to actively seek information on AEs or SAEs after the participant has concluded study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has completed the study, and he/she considers the event to be reasonably related to the study intervention, the investigator must promptly report the SAE to Pfizer using the CT SAE Report Form.

8.3.1.1. Reporting SAEs to Pfizer Safety

All SAEs occurring in a participant during the active collection period as described in Section 8.3.1 are reported to Pfizer Safety on the CT SAE Report Form immediately upon awareness and under no circumstance should this exceed 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

8.3.1.2. Recording Nonserious AEs and SAEs on the CRF

All nonserious AEs and SAEs occurring in a participant during the active collection period, which begins after obtaining informed consent as described in Section 8.3.1, will be recorded on the AE section of the CRF.

The investigator is to record on the CRF all directly observed and all spontaneously reported AEs and SAEs reported by the participant.

Reporting of AEs and SAEs for participants who fail screening are subject to the CRF requirements as described in Section 5.4.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3. Follow-Up of AEs and SAEs

After the initial AE or SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. For each event, the investigator must pursue and obtain adequate information until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3).

In general, follow-up information will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Any information relevant to the event, such as concomitant medications and illnesses, must be provided. In the case of a participant death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer Safety.

Further information on follow-up procedures is given in Appendix 3.

8.3.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities toward the safety of participants and the safety of a study intervention under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/ECs, and investigators.

Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives SUSARs or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the SRSD(s) for the study and will notify the IRB/EC, if appropriate according to local requirements.

8.3.5. Environmental Exposure, Exposure During Pregnancy or Breastfeeding, and Occupational Exposure

Environmental exposure, occurs when a person not enrolled in the study as a participant receives unplanned direct contact with or exposure to the study intervention. Such exposure may or may not lead to the occurrence of an AE or SAE. Persons at risk for environmental exposure include healthcare providers, family members, and others who may be exposed. An environmental exposure may include exposure during pregnancy, exposure during breastfeeding, and occupational exposure.

Any such exposure to the study intervention under study are reportable to Pfizer Safety within 24 hours of investigator awareness.

8.3.5.1. Exposure During Pregnancy

An EDP occurs if:

- A female participant is found to be pregnant while receiving or after discontinuing study intervention.
- A male participant who is receiving or has discontinued study intervention exposes a female partner prior to or around the time of conception.
- A female is found to be pregnant while being exposed or having been exposed to study intervention due to environmental exposure. Below are examples of environmental EDP:
    - A female family member or healthcare provider reports that she is pregnant after having been exposed to the study intervention by ingestion, inhalation, or skin contact.
    - A male family member or healthcare provider who has been exposed to the study intervention by ingestion, inhalation, or skin contact then exposes his female partner prior to or around the time of conception.

The investigator must report EDP to Pfizer Safety within 24 hours of the investigator's awareness, irrespective of whether an SAE has occurred. The initial information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

- If EDP occurs in a participant or a participant's partner, the investigator must report this information to Pfizer Safety on the CT SAE Report Form and an EDP Supplemental Form, regardless of whether an SAE has occurred. Details of the pregnancy will be collected after the start of study intervention and until at least 14 days after the last dose of study intervention.

- If EDP occurs in the setting of environmental exposure, the investigator must report information to Pfizer Safety using the CT SAE Report Form and EDP Supplemental Form. Since the exposure information does not pertain to the participant enrolled in the study, the information is not recorded on a CRF; however, a copy of the completed CT SAE Report Form is maintained in the investigator site file.

Follow-up is conducted to obtain general information on the pregnancy and its outcome for all EDP reports with an unknown outcome. The investigator will follow the pregnancy until completion (or until pregnancy termination) and notify Pfizer Safety of the outcome as a follow-up to the initial EDP Supplemental Form. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless preprocedure test findings are conclusive for a congenital anomaly and the findings are reported).

Abnormal pregnancy outcomes are considered SAEs. If the outcome of the pregnancy meets the criteria for an SAE (ie, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly in a live-born baby, a terminated fetus, an intrauterine fetal demise, or a neonatal death), the investigator should follow the procedures for reporting SAEs. Additional information about pregnancy outcomes that are reported to Pfizer Safety as SAEs follows:

- Spontaneous abortion including miscarriage and missed abortion;

- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the investigator assesses the infant death as related or possibly related to exposure to the study intervention.

Additional information regarding the EDP may be requested by the sponsor. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg, follow-up on preterm infants to identify developmental delays). In the case of paternal exposure, the investigator will provide the participant with the Pregnant Partner Release of Information Form to deliver to his partner. The investigator must document in the source documents that the participant was given the Pregnant Partner Release of Information Form to provide to his partner.

8.3.5.2. Exposure During Breastfeeding

An exposure during breastfeeding occurs if:

- A female is found to be breastfeeding while being exposed or having been exposed to study intervention (ie, environmental exposure). An example of environmental exposure during breastfeeding is a female family member or healthcare provider who reports that she is breastfeeding after having been exposed to the study intervention by ingestion, inhalation, or skin contact.

The investigator must report exposure during breastfeeding to Pfizer Safety within 24 hours of the investigator's awareness, irrespective of whether an SAE has occurred. The information must be reported using the CT SAE Report Form. When exposure during breastfeeding occurs in the setting of environmental exposure, the exposure information does not pertain to the participant enrolled in the study, so the information is not recorded on a CRF. However, a copy of the completed CT SAE Report Form is maintained in the investigator site file.

An exposure during breastfeeding report is not created when a Pfizer drug specifically approved for use in breastfeeding women (eg, vitamins) is administered in accord with authorized use. However, if the infant experiences an SAE associated with such a drug, the SAE is reported together with the exposure during breastfeeding.

8.3.5.3. Occupational Exposure

The investigator must report any instance of occupational exposure to Pfizer Safety within 24 hours of the investigator's awareness using the CT SAE Report Form regardless of whether there is an associated SAE. Since the information about the occupational exposure does not pertain to a participant enrolled in the study, the information is not recorded on a CRF; however, a copy of the completed CT SAE Report Form must be maintained in the investigator site file.

8.3.6. Cardiovascular and Death Events

Not applicable.

8.3.7. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

Not applicable.

8.3.8. Adverse Events of Special Interest

CCI [redacted]

CCI [redacted]

8.3.8.1. Lack of Efficacy

This section is not applicable because efficacy is not expected in the study population.

8.3.9. Medical Device Deficiencies

Not applicable.

8.3.10. Medication Errors

Medication errors may result from the administration or consumption of the study intervention by the wrong participant, or at the wrong time, or at the wrong dosage strength.

Exposures to the study intervention under study may occur in clinical trial settings, such as medication errors.

Safety Event	Recorded on the CRF	Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

Medication errors include:

- Medication errors involving participant exposure to the study intervention;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the study participant.

Such medication errors occurring to a study participant are to be captured on the medication error page of the CRF, which is a specific version of the AE page.

In the event of a medication dosing error, the sponsor should be notified within 24 hours.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is recorded on the medication error page of the CRF and, if applicable, any associated AE(s), serious and nonserious, are recorded on the AE page of the CRF.

Medication errors should be reported to Pfizer Safety within 24 hours on a CT SAE Report Form **only when associated with an SAE.**

8.4. Pharmacokinetics

8.4.1. Plasma for Analysis of PF-07258669 Concentrations (Part A Only)

Blood samples of approximately 3 mL, to provide a minimum of approximately 1 mL of plasma, will be collected for measurement of plasma concentrations of PF-07258669 as specified in the SoA. Instructions for the collection and handling of biological samples will be provided in the laboratory manual or by the sponsor. The actual times may change; the actual date and time (24-hour clock time) of each sample will be recorded.

All efforts will be made to obtain the samples at the exact nominal time relative to dosing. Collection of samples up to and including 10 hours after dose administration that are obtained within 10% of the nominal time relative to dosing (eg, within 6 minutes of a 60-minute sample) will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. Collection of samples more than 10 hours after dose administration that are obtained ≤1 hour from the nominal time relative to dosing will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. This protocol deviation window does not apply to samples to be collected more than 10 hours after dose administration at outpatient/follow-up visits with visit windows.

Samples will be used to evaluate the PK of PF-07258669. Samples collected for analyses of PF-07258669 plasma concentrations may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study, for metabolite identification and/or evaluation of the bioanalytical method, or for other internal exploratory purposes.

Genetic analyses will not be performed on these plasma samples unless consent for this was included in the informed consent. Participant confidentiality will be maintained.

Samples collected for measurement of plasma concentrations of PF-07258669 will be analyzed using a validated analytical method in compliance with applicable SOPs.

The PK samples must be processed and shipped as indicated in the instructions provided to the investigator site to maintain sample integrity. Any deviations from the PK sample handling procedure (eg, sample collection and processing steps, interim storage or shipping conditions), including any actions taken, must be documented and reported to the sponsor. On a case-by-case basis, the sponsor may make a determination as to whether sample integrity has been compromised.

Drug concentration information that may unblind the study will not be reported to investigator sites or blinded personnel until the study has been unblinded.

Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The IRB/EC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICD.

8.4.2. Urine for Analysis of PF-07258669 Concentrations (Part A Only)

Urine will be collected as outlined in the SoA.

A spot urine collection will be completed prior to the morning dose on Day 1. Each participant will complete a forced void. A 10 mL aliquot from this urine ("urine blank") will be collected and stored frozen for measurement of PF-07258669 concentrations.

Each participant will empty his/her bladder just prior to dosing on Day 14, and urine collection will occur over 0-τ, according to the dosing frequency (ie, 0-8 hours for Q8H dosing; 0-12 hours for Q12H dosing). Urine will be stored at approximately 4°C during the collection period. At the end of the collection period, the urine will be mixed thoroughly, and the total volume will be measured and recorded. A 10 mL aliquot will be retained for measurement of PF-07258669 concentrations and will be stored frozen at approximately -20°C within 1 hour of the end of the collection interval.

The preferred tubes for collection and storage will be identified and this information will be provided to the site prior to start of the study. Samples will be analyzed using a validated analytical method in compliance with Pfizer standard operating procedures.

As part of understanding the PK of PF-07258669, samples may be used for metabolite identification and/or evaluation of the bioanalytical method. These data will be used for internal exploratory purposes and will not be included in the clinical report. Samples collected for this purpose will be retained in accordance to local regulations and if not used within this timeframe, will be destroyed.

8.4.3. Plasma for Qualitative Metabolite Profiling (Part A only)

Blood samples (2 mL each) to provide sufficient plasma will be collected for metabolite profiling into appropriately labeled tubes containing anticoagulant at times specified in the SoA. These samples may be used for metabolite identification and/or evaluation of the bioanalytical method. Samples to be used for this purpose will be shipped to either a Pfizer-approved BBS facility or other designated laboratory and retained for up to 1 year following the completion of the study. These data may be used for internal exploratory purposes and will not be included in the CSR for this study.

8.4.4. Plasma for Analysis of Midazolam Concentrations (Part B only)

Blood samples of approximately 3 mL, to provide a minimum of approximately 1 mL of plasma, will be collected into appropriately-labeled tubes containing K_2EDTA for measurement of plasma concentrations of midazolam as specified in the SoA. Instructions for the collection and handling of biological samples will be provided in the laboratory manual or by the sponsor. The actual times may change; the actual date and time (24-hour clock time) of each sample will be recorded.

All efforts will be made to obtain the samples at the exact nominal time relative to dosing. Collection of samples up to and including 10 hours after dose administration that are obtained within 10% of the nominal time relative to dosing (eg, within 6 minutes of a 60-minute sample) will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. Collection of samples more than 10 hours after dose administration that are obtained ≤1 hour from the nominal time relative to dosing will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. This protocol deviation window does not apply to samples to be collected more than 10 hours after dose administration at outpatient/follow-up visits with visit windows.

Plasma samples will be analyzed using a validated analytical method in compliance with Pfizer SOPs. Samples collected for analyses of midazolam plasma concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study, for metabolite identification and/or evaluation of the bioanalytical method, or for other internal exploratory purposes.

Genetic analyses will not be performed on these plasma samples unless consent for this was included in the informed consent. Participant confidentiality will be maintained.

Samples collected for measurement of plasma concentrations of midazolam will be analyzed using a validated analytical method in compliance with applicable SOPs.

Detail procedure regarding the collection, processing, storage and shipping of the plasma samples will be provided in the laboratory manual. The PK samples must be processed and shipped as indicated in the instructions provided to the investigator site to maintain sample integrity. Any deviations from the PK sample handling procedure (eg, sample collection and processing steps, interim storage or shipping conditions), including any actions taken, must be documented and reported to the sponsor. On a case-by-case basis, the sponsor may make a determination as to whether sample integrity has been compromised. Any deviation from the specified sample handling procedure resulting in compromised sample integrity will be considered a protocol deviation.

As part of understanding the PK of the study intervention, samples may be used to evaluate safety aspects related to concerns arising during or after the study, for metabolite identification and/or evaluation of the bioanalytical method, as well as for other internal exploratory purposes. These data will not be included in the CSR.

8.5. Pharmacodynamics

8.5.1. Body Weight

All body weight assessments (except the assessment at the screening visit) should be performed using the calibrated digital body weight smart scale incorporated into the FDA approved multi-frequency segmental body composition analyzer described in Section 8.5.2. The same scale should be used for the duration of the study (except at screening). Body weight will be recorded in either pounds (lb) or kilograms (kg), and accuracy to the nearest

0.2 lb (or 0.1 kg); (ie, the scale must be able to distinguish a difference between 150.4 lb (68.4 kg) versus 150.2 lb (68.3 kg). Body weight must be recorded to one decimal place. The scale must be placed on a stable, flat surface. Participants will be blinded to body weight measurement (except at screening).

Body weight measurements should be taken under the following conditions:

- After an overnight fast;
- After void of urine;
- After removal of shoes, bulky layers of clothing, and jackets so that only light clothing remains;
- While remaining still during the measurement.

CCI

CCI

[redacted]

8.6. Genetics

8.6.1. Specified Genetics

Genetics (specified analyses) are not evaluated in this study.

8.6.2. Retained Research Samples for Genetics

A 2-mL blood sample optimized for DNA isolation Prep D1.5 will be collected according to the SoA, as local regulations and IRBs/ECs allow.

Retained Research Samples may be used for research related to the study intervention(s). Genes and other analytes (eg, proteins, RNA, nondrug metabolites) may be studied using the banked samples.

See Appendix 5 for information regarding genetic research. Details on processes for collection and shipment of these samples can be found in the lab manual and other supporting documentation.

8.7. Biomarkers

Collection of samples for biomarker research is also part of this study.

CCI

[redacted]

8.7.2. Plasma for Determination of 4β-Hydroxycholesterol/Cholesterol Ratio

Blood samples (approximately 3 mL) to provide a minimum of approximately 1 mL of plasma for the analysis of 4β-hydroxycholesterol and cholesterol will be collected into appropriately labeled tubes containing lithium heparin. Samples will be collected according to the times outlined in the SoA. Refer to the lab manual for specific instructions.

8.7.3. Urine for Determination of 6β-Hydroxycortisol/Cortisol Ratio

A pre-dose 24-hour urine collection will be undertaken on Day -1 prior to dosing. In addition, 24-hour urine collection will also be undertaken on Day 7 for Part B and Day 14 for Part A. The Day 14 collection will continue from the 0-τ sample that was collected according to the dosing frequency as outlined in Section 8.4.2. At the end of each of the urine collection

periods (0 to 24 hours), the total urine volume will be measured and recorded, and a 5 mL aliquot will be transferred to an appropriately labeled screw-capped polypropylene tube for analysis of cortisol. Another 5 mL aliquot will be transferred to an appropriately labeled screw-capped polypropylene tube for 6β-hydroxycortisol analysis.

8.7.4. Extracellular Vesicle Analysis of CYP3A Induction

Blood samples (for serum) of approximately 4 mL will be collected as outlined in SoA. These serum samples may be analyzed to assess nanovesicle (exosome)-derived biomarkers for drug metabolism pathways;[27] of interest being assessment of CYP3A activity at baseline and following repeated dosing with PF-07258669. Any remaining serum may be tested in other platforms (transcriptomics, metabolomics, proteomics, etc) to assess for potential CYP3A induction, or in relation to the study intervention or geriatric anorexia. These data, if generated, will be used for internal exploratory purposes and will not be included in the CSR.

8.7.5. Biomarkers Sample Handling

Instructions for the collection and handling of biological samples will be provided in the laboratory manual or by the sponsor. Samples will be analyzed using validated analytical methods in compliance with applicable SOPs. These data will be used for internal exploratory purposes and will not be included in the CSR.

The actual times may change, but the number of samples will remain the same. All efforts will be made to obtain the samples at the exact nominal time relative to dosing. Collection of samples up to and including 10 hours after dose administration that are obtained within 10% of the nominal time relative to dosing (eg, within 6 minutes of a 60-minute sample) will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. Collection of samples more than 10 hours after dose administration that are obtained ≤1 hour away from the nominal time relative to dosing will not be captured as a protocol deviation, as long as the exact time of the collection is noted on the source document and the CRF. This protocol deviation window does not apply to samples to be collected more than 10 hours after dose administration at outpatient/follow-up visits with visit windows.

As part of understanding the PD of the study intervention, samples may be used for evaluation of the bioanalytical method, as well as for other internal exploratory purposes. The exploratory biomarker samples must be processed and shipped as indicated in the instructions provided to the investigator site to maintain sample integrity. Any deviations from the exploratory biomarkers sample handling procedures (eg, sample collection and processing steps, interim storage, or shipping conditions), including any actions taken, must be documented and reported to the sponsor. On a case-by-case basis, the sponsor may make a determination as to whether sample integrity has been compromised.

8.7.6. Specified Gene Expression (RNA) Research

Specified gene expression (RNA) research is not included in this study.

8.7.7. Specified Protein Research

Specified protein research is not included in this study.

8.7.8. Specified Metabolomic Research

Specified metabolomic research is not included in this study.

8.7.9. Retained Research Samples for Biomarkers

These Retained Research Samples will be collected in this study:

- 10 mL blood Prep B1 optimized for plasma;
- 10 mL blood Prep B2 optimized for serum.

Retained Research Samples will be collected as local regulations and IRB/ECs allow according to the SoA.

Retained Research Samples may be used for research related to the study intervention(s). Genes and other analytes (eg, proteins, RNA, nondrug metabolites) may be studied using the retained samples.

See Appendix 5 for information regarding genetic research. Details on processes for collection and shipment of these samples can be found in the lab manual and other supporting documentation.

8.8. Immunogenicity Assessments

Immunogenicity assessments are not included in this study.

8.9. Health Economics

Health economics/medical resource utilization and health economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

Detailed methodology for summary and statistical analyses of the data collected in this study is outlined here and further detailed in a SAP, which will be maintained by the sponsor. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

9.1. Statistical Hypotheses

No formal statistical hypothesis testing will be performed in this study.

9.2. Analysis Sets

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

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).
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).

9.3. Statistical Analyses

The SAP will be developed and finalized before any analyses are performed and will describe the analyses and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

9.3.1. General Considerations

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

9.3.2. Safety Endpoints

All safety analyses will be performed on the safety population, for the given part of the study (Part A or Part B).

AEs, ECGs, BP, pulse rate, continuous cardiac monitoring (for Part A only), and safety laboratory data will be reviewed and summarized on an ongoing basis during the study to evaluate the safety of participants. Any clinical laboratory, ECG, BP, and pulse rate abnormalities of potential clinical concern will be described. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

Medical history, physical 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.

Data collected at screening that are used for inclusion/exclusion criteria, such as laboratory data, ECGs, and vital signs, will be considered source data, and will not be required to be reported, unless otherwise noted. Demographic data collected at screening will be reported.

9.3.2.1. Electrocardiogram Analyses

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.

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

Safety QTcF Assessment

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

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

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.

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.

9.3.3. PK Endpoints

The PK concentration and parameter populations for the given parts of the study (Part A or Part B) are defined as in Section 9.2.

9.3.3.1. Derivation of PF-07258669 PK Parameters (Part A Only)

The PK parameters for PF-07258669, following oral dose administration, will be derived from the plasma and urine concentration-time profiles as detailed in Table 3, as data permit. In all cases, actual PK sampling times will be used in the derivation of PK parameters.

Table 3. Derivation of PF-07258669 PK Parameters

Parameter	Day(s)	Definition	Method of Determination
AUC_{tau}	1, 14	Area under the plasma concentration-time profile from time zero to time tau (τ), the dosing interval, where τ = 8 hours for Q8H dosing, and 12 hours for Q12H dosing.	Linear/Log trapezoidal method
C_{max}	1, 14	Maximum plasma concentration during the dosing interval	Observed directly from data
T_{max}	1, 14	Time for C_{max}	Observed directly from data as time of first occurrence
AUC_{tau} (dn)	1, 14	Dose normalized AUC_{tau}	AUC_{tau}/Dose
C_{max} (dn)	1, 14	Dose normalized C_{max}	C_{max}/Dose
CL/F	14	Apparent clearance for oral dosing	Dose/AUC_{tau}
C_{min}	14	Minimum plasma concentration during the dosing interval	Observed directly from data
C_{av}	14	Average plasma concentration during the dosing interval	AUC_{tau}/tau
R_{ac}	14	Observed accumulation ratio	Day 14 AUC_{tau} / Day 1 AUC_{tau}
$R_{ac,Cmax}$	14	Observed accumulation ratio for C_{max}	Day 14 C_{max}/Day 1 C_{max}
PTR	14	Peak-to-trough ratio	C_{max}/C_{min}
$t_{½}$[a]	14	Terminal half-life	$Log_e(2)/k_{el}$, where k_{el} is the terminal phase elimination rate constant calculated by a linear regression of the

Table 3. Derivation of PF-07258669 PK Parameters

Parameter	Day(s)	Definition	Method of Determination
			log-linear concentration-time curve.
V_z/F[a]	14	Apparent volume of distribution for oral dosing	$Dose/(AUC_{tau} * k_{el})$
Ae_{tau}	14	Amount of unchanged drug recovered in urine during the dosing interval	Sum of [urine concentration * sample volume] for each collection over the dosing interval
Ae_{tau} %	14	Percent of dose recovered in urine as unchanged drug	$100* Ae_{tau}/Dose$
CL_r	14	Renal clearance	Ae_{tau}/AUC_{tau}
a. If data permit.			

9.3.3.2. Derivation of Midazolam PK Parameters (Part B Only)

The PK parameters for midazolam will be derived from the plasma concentration-time profiles as detailed in Table 4, as data permit. In all cases, actual PK sampling times will be used in the derivation of PK parameters.

Table 4. Derivation of Midazolam PK Parameters

Parameter	Definition	Method of Determination
AUC_{last}	Area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration (C_{last})	Linear/Log trapezoidal method
AUC_{inf}[a]	Area under the plasma concentration-time profile from time zero extrapolated to infinite time	$AUC_{last} + (C_{last}*/k_{el})$, where $C_{last}*$ is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis.
C_{max}	Maximum plasma concentration	Observed directly from data
T_{max}	Time for C_{max}	Observed directly from data as time of first occurrence
$t_{½}$[a]	Terminal half-life	$Log_e(2)/k_{el}$, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression.
CL/F[a]	Apparent clearance for oral dosing	$Dose/AUC_{inf}$
V_z/F[a]	Apparent volume of distribution for oral dosing	Apparent volume of distribution estimated from terminal phase $V_z/F= Dose /(AUC_{inf}*k_{el})$
a. If data permit.		

9.3.3.3. Statistical Methods for PF-07258669 PK Data (Part A only)

No formal inferential statistics will be applied to the PF-07258669 PK data.

Plasma concentrations of PF-07258669 will be listed and summarized descriptively by dose, population, dietary allocation, day, and nominal PK sampling time. Individual participant, mean and median profiles of the plasma concentration-time data will be plotted by dose, population, dietary allocation, and day using actual (for individual) and nominal (for mean and median) times respectively. Mean and median profiles will be presented on both linear and log scales.

The plasma and urine PF-07258669 PK parameters will be listed and summarized descriptively by dose, population, dietary allocation, and day, as applicable. Dose-normalized AUC_{tau}, and C_{max} may be plotted against dose (using a logarithmic scale) for each day 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) will be identified by different symbols/colors.

Attainment of steady-state will be assessed by a plot of predose concentrations over time. These plots will be used to understand the relationship between the PK parameters and dose.

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

9.3.3.4. Statistical Methods for Midazolam PK Data (Part B only)

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). Individual participant, mean and median profiles of the concentration-time data will be plotted by treatment using actual (for individual) and nominal (for mean and median) times respectively. Mean and median profiles will be presented on both linear and log scales.

The midazolam PK parameters will be listed and summarized descriptively by treatment. AUC_{inf}, AUC_{last}, and C_{max} will be plotted by treatment, and will include individual participant values and the geometric means for each treatment.

Natural log transformed AUC_{inf}, AUC_{last}, and C_{max} of midazolam will be analyzed using a mixed effect model with treatment as a fixed effect and participant as a random effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% 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. 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.

9.3.4. Tertiary/Exploratory Endpoint(s)

Change from baseline in body weight in the optional additional cohorts allocated to the HFHC diet (if conducted) will be analyzed using an MMRM model with treatment, time (as a factor), treatment-by-time interaction, baseline (as a covariate) 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. Further details will be given in the SAP.

The analysis of other exploratory endpoints will be detailed in the SAP.

9.3.5. Other Analyse(s)

Pharmacogenomic or biomarker data from Retained Research Samples may be collected during or after the trial and retained for future analyses; the results of such analyses are not planned to be included in the CSR.

9.4. Interim Analyses

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.

9.5. Sample Size Determination

Participants in either Part A or Part B, who discontinue prior to completion of the study for reasons unrelated to safety, may be replaced at the discretion of the investigator and sponsor.

9.5.1. Part A Sample Size

A sample size of up to approximately 10 healthy adult non-Japanese participants per cohort (8 active, 2 placebo) has been selected to minimize exposure to humans to a new chemical entity while allowing for adequate characterization of safety, tolerability, and PK at each dose level. This dose escalation portion consists of up to 8 planned cohorts. 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).

An optional cohort of approximately 8 healthy Japanese participants (6 active, 2 placebo) may also be included. This sample size has been judged sufficient to obtain a preliminary evaluation of safety, tolerability, and PK data in this population.

In addition, an optional cohort of approximately 8-10 older adult participants (approximately 6-8 active, 2 placebo) may be included. This sample size has been judged sufficient to obtain a preliminary evaluation of safety, tolerability, and PK data in this population.

If conducted, approximately 24 participants will be randomized in a 1:1 ratio to either PF-07258669 or placebo in the first optional cohort of non-Japanese participants allocated to the HFHC diet. This sample size was selected to provide safety and tolerability data under the HFHC diet restrictions and provide acceptable operating characteristics to assess change from baseline in body weight. This yields 80% power to detect a placebo-adjusted increase in body weight of 1.78 kg (comparing PF-07258669 to placebo) at Day 14, using a 1-sided t-test at a 5% level and assuming a conservative SD of 1.7 for body weight change from baseline at Day 14. Data from 14 previous external studies were utilized to derive this conservative estimate of variability.

A second optional cohort of non-Japanese participants allocated to the HFHC diet may also be enrolled to further characterize the dose/exposure-response relationship with respect to change from baseline in body weight. In this cohort (if conducted), approximately 16 participants will be randomized in a 3:1 ratio to either PF-07258669 (approximately 12 participants) or placebo (approximately 4 participants). This sample size was selected to give similar operating characteristics as for the first cohort allocated to the HFHC diet, such that participants on placebo would be pooled across cohorts allocated to the HFHC diet. Approximately 4 participants receiving placebo was selected to maintain the blind in this second optional cohort allocated to HFHC diet.

9.5.2. Part B Sample Size

A sample size of approximately 12 participants per cohort will be enrolled such that approximately 10 evaluable participants per cohort complete the study. This number of evaluable participants should provide sufficient precision to assess the difference in AUC_{inf} and C_{max} for the co-administration of midazolam and PF-07258669 versus midazolam alone. The expected widths of the 90% confidence intervals, with 80% coverage probability, for these comparisons, are shown in Table 5, for a range of possible effects based on a sample size of 10 participants.

Table 5. Expected Widths of the 90% CIs (with 80% Coverage Probability) for Different Possible Estimated Effects for AUC_{inf} and C_{max}

Parameter	Sample Size	Estimated Effect (Test/Reference)	Probable 90% CI	Probable CI Width
AUC_{inf}	N=10	75%	57% to 98%	41%
		100%	76% to 131%	55%
		150%	114% to 197%	82%
		200%	152% to 262%	110%
		300%	229% to 394%	165%
C_{max}	N=10	75%	49% to 114%	65%
		100%	66% to 152%	86%
		150%	99% to 228%	129%
		200%	132% to 304%	172%
		300%	197% to 456%	259%

These estimates are based on an assumed conservative standard deviation of 0.284 (equivalent to a geometric coefficient of variation of 29%) in $\log_e AUC_{inf}$ for midazolam; and 0.438 (equivalent to a geometric coefficient of variation of 46%) in $\log_e C_{max}$. These estimates of variability are based on data from previous internal DDI studies with midazolam.

A single cohort is currently planned, but additional optional cohorts may be added if it is necessary to evaluate the effect of additional dose levels of PF-07258669 on midazolam PK.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and CIOMS International Ethical Guidelines;
- Applicable ICH GCP guidelines;
- Applicable laws and regulations, including applicable privacy laws.

The protocol, protocol amendments, ICD, SRSD(s), and other relevant documents (eg, advertisements) must be reviewed and approved by the sponsor, submitted to an IRB/EC by the investigator, and reviewed and approved by the IRB/EC before the study is initiated.

Any amendments to the protocol will require IRB/EC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC;
- Notifying the IRB/EC of SAEs or other significant safety findings as required by IRB/EC procedures;
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH GCP guidelines, the IRB/EC, European regulation 536/2014 for clinical studies (if applicable), European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations.

10.1.1.1. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

In the event of any prohibition or restriction imposed (ie, clinical hold) by an applicable regulatory authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the study intervention, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study participants against any immediate hazard, and of any serious breaches of this protocol or of the ICH GCP guidelines that the investigator becomes aware of.

10.1.2. Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

The investigator or his/her representative will explain the nature of the study, including the risks and benefits, to the participant and answer all questions regarding the study. The participant should be given sufficient time and opportunity to ask questions and to decide whether or not to participate in the trial.

Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, privacy and data protection requirements, where applicable, and the IRB/EC or study center.

The investigator must ensure that each study participant is fully informed about the nature and objectives of the study, the sharing of data related to the study, and possible risks associated with participation, including the risks associated with the processing of the participant's personal data.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/EC members, and by inspectors from regulatory authorities.

The investigator further must ensure that each study participant is fully informed about his or her right to access and correct his or her personal data and to withdraw consent for the processing of his or her personal data.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date on which the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICD.

Participants must be reconsented to the most current version of the ICD(s) during their participation in the study.

A copy of the ICD(s) must be provided to the participant.

10.1.4. Data Protection

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of participant data.

Participants' personal data will be stored at the study site in encrypted electronic and/or paper form and will be password-protected or secured in a locked room to ensure that only authorized study staff have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site will be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of participants with regard to the processing of personal data, participants will be assigned a single, participant-specific numerical code. Any participant records or data sets that are transferred to the sponsor will contain the numerical code; participant names will not be transferred. All other identifiable data transferred to the sponsor will be identified by this single, participant-specific code. The study site will maintain a confidential list of participants who participated in the study, linking each participant's numerical code to his or her actual identity and medical record ID. In case of data transfer, the sponsor will protect the confidentiality of participants' personal data consistent with the clinical study agreement and applicable privacy laws.

10.1.5. Committees Structure

10.1.5.1. Data Monitoring Committee

This study will not use a DMC.

10.1.6. Dissemination of Clinical Study Data

Pfizer fulfills its commitment to publicly disclose clinical study results through posting the results of studies on www.clinicaltrials.gov (ClinicalTrials.gov), the EudraCT, and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations. In addition, Pfizer reports study results outside of the requirements of local laws/regulations pursuant to its SOPs.

In all cases, study results are reported by Pfizer in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the study or the country in which the study was conducted.

www.clinicaltrials.gov

Pfizer posts clinical trial results on www.clinicaltrials.gov for Pfizer-sponsored interventional studies (conducted in patients) that evaluate the safety and/or efficacy of a product, regardless of the geographical location in which the study is conducted. These results are submitted for posting in accordance with the format and timelines set forth by US law.

EudraCT

Pfizer posts clinical trial results on EudraCT for Pfizer-sponsored interventional studies in accordance with the format and timelines set forth by EU requirements.

www.pfizer.com

Pfizer posts public disclosure synopses (CSR synopses in which any data that could be used to identify individual participants have been removed) on www.pfizer.com for Pfizer-sponsored interventional studies at the same time the corresponding study results are posted to www.clinicaltrials.gov.

Documents within marketing authorization packages/submissions

Pfizer complies with the European Union Policy 0070, the proactive publication of clinical data to the EMA website. Clinical data, under Phase 1 of this policy, includes clinical overviews, clinical summaries, CSRs, and appendices containing the protocol and protocol amendments, sample CRFs, and statistical methods. Clinical data, under Phase 2 of this policy, includes the publishing of individual participant data. Policy 0070 applies to new marketing authorization applications submitted via the centralized procedure since 01 January 2015 and applications for line extensions and for new indications submitted via the centralized procedure since 01 July 2015.

Data sharing

Pfizer provides researchers secure access to patient-level data or full CSRs for the purposes of "bona-fide scientific research" that contributes to the scientific understanding of the disease, target, or compound class. Pfizer will make data from these trials available 24 months after study completion. Patient-level data will be anonymized in accordance with applicable privacy laws and regulations. CSRs will have personally identifiable information redacted.

Data requests are considered from qualified researchers with the appropriate competencies to perform the proposed analyses. Research teams must include a biostatistician. Data will not be provided to applicants with significant conflicts of interest, including individuals requesting access for commercial/competitive or legal purposes.

10.1.7. Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

Guidance on completion of CRFs will be provided in the CRF Completion Requirements document.

The investigator must ensure that the CRFs are securely stored at the study site in encrypted electronic and/or paper form and are password-protected or secured in a locked room to prevent access by unauthorized third parties.

The investigator must permit study-related monitoring, audits, IRB/EC review, and regulatory agency inspections and provide direct access to source data documents. This verification may also occur after study completion. It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

Monitoring details describing strategy, including definition of study critical data items and processes (eg, risk-based initiatives in operations and quality, such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, virtual, or on-site monitoring), are provided in the data management plan and monitoring plan maintained and utilized by the sponsor or designee.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data.

Records and documents, including signed ICDs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor. The investigator must ensure that the records continue to be stored securely for as long as they are maintained.

When participant data are to be deleted, the investigator will ensure that all copies of such data are promptly and irrevocably deleted from all systems.

The investigator(s) will notify the sponsor or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with the sponsor or its agents to prepare the investigator site for the inspection and will allow the sponsor or its agent, whenever feasible, to be present during the inspection. The investigator site and investigator will promptly resolve any discrepancies that are identified between the study data and the participant's medical records. The investigator will promptly

provide copies of the inspection findings to the sponsor or its agent. Before response submission to the regulatory authorities, the investigator will provide the sponsor or its agents with an opportunity to review and comment on responses to any such findings.

10.1.8. Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator site.

Data reported on the CRF or entered in the eCRF that are from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data and its origin can be found in the source document locator, which is maintained by the sponsor.

Description of the use of the computerized system is documented in the source document locator, which is maintained by the sponsor.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP guidelines, and all applicable regulatory requirements.

10.1.9. Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the date of the first participant's first visit and will be the study start date.

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time upon notification to the sponsor if requested to do so by the responsible IRB/EC or if such termination is required to protect the health of study participants.

Reasons for the early closure of a study site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/EC or local health authorities, the sponsor's procedures, or the ICH GCP guidelines;
- Inadequate recruitment of participants by the investigator;
- Discontinuation of further study intervention development.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the ECs/IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

Study termination is also provided for in the clinical study agreement. If there is any conflict between the contract and this protocol, the contract will control as to termination rights.

10.1.10. Publication Policy

The results of this study may be published or presented at scientific meetings by the investigator after publication of the overall study results or 1 year after the end of the study (or study termination), whichever comes first.

The investigator agrees to refer to the primary publication in any subsequent publications, such as secondary manuscripts, and submits all manuscripts or abstracts to the sponsor 30 days before submission. This allows the sponsor to protect proprietary information and to provide comments, and the investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study- or Pfizer-intervention related information necessary for the appropriate scientific presentation or understanding of the study results.

For all publications relating to the study, the investigator will comply with recognized ethical standards concerning publications and authorship, including those established by the International Committee of Medical Journal Editors.

The sponsor will comply with the requirements for publication of the overall study results covering all investigator sites. In accordance with standard editorial and ethical practice, the sponsor will support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship of publications for the overall study results will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

If publication is addressed in the clinical study agreement, the publication policy set out in this section will not apply.

10.1.11. Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical personnel for the study is documented in the study contact list located in the clinical trial management system.

To facilitate access to appropriately qualified medical personnel for study-related medical questions or problems, participants are provided with an ECC at the time of informed consent. The ECC contains, at a minimum, (a) protocol and study intervention identifiers, (b) participant's study identification number, (c) site emergency phone number active 24 hours/day, 7 days per week, and (d) Pfizer Call Center number.

The ECC is intended to augment, not replace, the established communication pathways between the investigator, site staff, and study team. The ECC is to be used by healthcare professionals not involved in the research study only, as a means of reaching the investigator or site staff related to the care of a participant. The Pfizer Call Center number should only be used when the investigator and site staff cannot be reached. The Pfizer Call Center number is not intended for use by the participant directly; if a participant calls that number directly, he or she will be directed back to the investigator site.

10.2. Appendix 2: Clinical Laboratory Tests

The following safety laboratory tests will be performed at times defined in the SoA. Additional laboratory results may be reported on these samples as a result of the method of analysis or the type of analyzer used by the clinical laboratory, or as derived from calculated values. These additional tests would not require additional collection of blood. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

Table 6. Protocol Required Safety Laboratory Assessments

Hematology	Chemistry	Urinalysis	Other
Hemoglobin Hematocrit RBC count MCV MCH MCHC Platelet count WBC count Total neutrophils (Abs) Eosinophils (Abs) Monocytes (Abs) Basophils (Abs) Lymphocytes (Abs)	BUN and creatinine Cystatin C eGFR (CKD-EPI) Glucose (fasting) Calcium Sodium Potassium Chloride Total CO_2 (bicarbonate) AST, ALT Total bilirubin Alkaline phosphatase Uric acid Albumin Total protein CCI CCI Lipid panel: • Total cholesterol • Triglycerides • HDL • LDL (calculated)	Local Dipstick: pH Glucose (qual) Protein (qual) Blood (qual) Ketones Nitrites Leukocyte esterase Laboratory: Microscopy[a]	Urine drug screening[b] COVID-19 testing (per CRU procedures) At screening only: • FSH[c] • Hepatitis B surface antigen • Hepatitis B surface antibody • Hepatitis B core antibody • Hepatitis C antibody • Human immunodeficiency virus

a. Only if urine dipstick is positive for blood, protein, nitrites, or leukocyte esterase.
b. The minimum requirement for drug screening includes cocaine, THC, opiates/opioids, benzodiazepines, and amphetamines (others are site and study specific).
c. For confirmation of postmenopausal status only. (Females who have been amenorrheic for at least 12 months).

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF.

Laboratory/analyte results that could unblind the study will not be reported to investigator sites or other blinded personnel until the study has been unblinded.

Any remaining serum/plasma from samples collected for clinical safety laboratory measurements at baseline and at all times after dose administration may be retained and stored for the duration of the study. Upon completion of the study, these retained safety samples may be used for the assessment of exploratory safety biomarkers or unexpected safety findings. These data will not be included in the CSR. Samples to be used for this purpose will be shipped to either a Pfizer-approved BBS facility or other designated laboratory and retained for up to 1 year following the completion of the study.

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting

10.3.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. • Note: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events Meeting the AE Definition

• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital sign measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator. Any abnormal laboratory test results that meet any of the conditions below must be recorded as an AE:
 	
  • Is associated with accompanying symptoms;
 	
  • Requires additional diagnostic testing or medical/surgical intervention;
 	
  • Leads to a change in study dosing (outside of any protocol-specified dose adjustments) or discontinuation from the study, significant additional concomitant drug treatment, or other therapy.
 	
• Exacerbation of a chronic or intermittent preexisting condition, including either an increase in frequency and/or intensity of the condition.
 	
• New condition detected or diagnosed after study intervention administration, even though it may have been present before the start of the study.
 	
• Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
 	
• Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE or SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of an SAE

An SAE is defined as any untoward medical occurrence that, at any dose, meets one or more of the criteria listed below:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline is not considered an AE.

d. Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle), that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Is a suspected transmission via a Pfizer product of an infectious agent, pathogenic or non-pathogenic, is considered serious.

The event may be suspected from clinical symptoms or laboratory findings indicating an infection in a participant exposed to a Pfizer product. The terms "suspected transmission" and "transmission" are considered synonymous. These cases are considered unexpected and handled as serious expedited cases by pharmacovigilance personnel. Such cases are also considered for reporting as product defects, if appropriate.

g. Other situations:

- Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations, such as significant medical events that may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording/Reporting and Follow-Up of AEs and/or SAEs During the Active Collection Period

AE and SAE Recording/Reporting

The table below summarizes the requirements for recording AEs on the CRF and for reporting SAEs on the CT SAE Report Form to Pfizer Safety throughout the active collection period. These requirements are delineated for 3 types of events: (1) SAEs; (2) nonserious AEs; and (3) exposure to the study intervention under study during pregnancy or breastfeeding, and occupational exposure.

It should be noted that the CT SAE Report Form for reporting of SAE information is not the same as the AE page of the CRF. When the same data are collected, the forms must be completed in a consistent manner. AEs should be recorded using concise medical terminology and the same AE term should be used on both the CRF and the CT SAE Report Form for reporting of SAE information.

Safety Event	**Recorded on the CRF**	**Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness**
SAE	All	All
Nonserious AE	All	None
Exposure to the study intervention under study during pregnancy or breastfeeding	All AEs/SAEs associated with exposure during pregnancy or breastfeeding **Note:** Instances of EDP or EDB not associated with an AE or SAE are not captured in the CRF.	All instances of EDP are reported (whether or not there is an associated SAE)* All instances of EDB are reported (whether or not there is an associated SAE).**
Environmental or occupational exposure to the product under study to a non-participant (not involving EDP or EDB).	None. Exposure to a study non-participant is not collected on the CRF.	The exposure (whether or not there is an associated AE or SAE) must be reported.***

* **EDP** (with or without an associated AE or SAE): any pregnancy information is reported to Pfizer Safety using CT SAE Report Form and EDP Supplemental Form; if the EDP is associated with an SAE, then the SAE is reported to Pfizer Safety using the CT SAE Report Form.

** **EDB** is reported to Pfizer Safety using the CT SAE Report Form which would also include details of any SAE that might be associated with the EDB.

*** **Environmental or Occupational exposure:** AEs or SAEs associated with occupational exposure are reported to Pfizer Safety using the CT SAE Report Form.

- When an AE or SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostic reports) related to the event.
- The investigator will then record all relevant AE or SAE information in the CRF.

- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to Pfizer Safety in lieu of completion of the CT SAE Report Form/AE or SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by Pfizer Safety. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to Pfizer Safety.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE or SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Moderate: Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL. Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- Severe: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling, limiting self care ADL. Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE or SAE. The investigator will use clinical judgment to determine the relationship.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration, will be considered and investigated.

- The investigator will also consult the IB and/or product information, for marketed products, in his/her assessment.
- For each AE or SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE or SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.
- If the investigator does not know whether or not the study intervention caused the event, then the event will be handled as "related to study intervention" for reporting purposes, as defined by the sponsor. In addition, if the investigator determines that an SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, and report such an assessment in the dedicated section of the CT SAE Report Form and in accordance with the SAE reporting requirements.

Follow-Up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations, as medically indicated or as requested by the sponsor, to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other healthcare providers.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide Pfizer Safety with a copy of any postmortem findings, including histopathology.
- New or updated information will be recorded in the originally submitted documents.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4. Reporting of SAEs

SAE Reporting to Pfizer Safety via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to Pfizer Safety will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as the data become available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to Pfizer Safety by telephone.

SAE Reporting to Pfizer Safety via CT SAE Report Form

- Facsimile transmission of the CT SAE Report Form is the preferred method to transmit this information to Pfizer Safety.
- In circumstances when the facsimile is not working, notification by telephone is acceptable with a copy of the CT SAE Report Form sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the CT SAE Report Form pages within the designated reporting time frames.

10.4. Appendix 4: Contraceptive and Barrier Guidance

10.4.1. Male Participant Reproductive Inclusion Criteria

Male participants are eligible to participate if they agree to the following requirements during the intervention period and for at least 28 days after the last dose of study intervention, which corresponds to the time needed to eliminate reproductive safety risk of the study intervention(s):

- Refrain from donating sperm.

PLUS either:

- Be abstinent from heterosexual intercourse with a female of childbearing potential as their preferred and usual lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent;

OR

- Must agree to use contraception/barrier as detailed below:
  - Agree to use a male condom when having sexual intercourse with a woman of childbearing potential who is not currently pregnant.
- In addition to male condom use, a highly effective method of contraception may be considered in WOCBP partners of male participants (refer to the list of highly effective methods below in Section 10.4.4).

10.4.2. Female Participant Reproductive Inclusion Criteria

A female participant is eligible to participate if she is not pregnant or breastfeeding and is not a WOCBP (see definitions below in Section 10.4.3).

The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

10.4.3. Woman of Childbearing Potential

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before the first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

1. Premenopausal female with 1 of the following:

   - Documented hysterectomy;
   - Documented bilateral salpingectomy;
   - Documented bilateral oophorectomy.

   For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

   Note: Documentation for any of the above categories can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview. The method of documentation should be recorded in the participant's medical record for the study.

2. Postmenopausal female.

   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. In addition:
     - A high FSH level in the postmenopausal range must be used to confirm a postmenopausal state in women under 60 years old and not using hormonal contraception or HRT.
     - A female on HRT and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

10.4.4. Contraception Methods

Contraceptive use by men or women should be consistent with local availability/regulations regarding the use of contraceptive methods for those participating in clinical trials.

Highly Effective Methods That Have Low User Dependency (for WOCBP partners of male participants)

1. Implantable progestogen-only hormone contraception associated with inhibition of ovulation.
2. Intrauterine device.

3. Intrauterine hormone-releasing system.

4. Bilateral tubal occlusion (eg, bilateral tubal ligation).

5. Vasectomized partner.

    - A vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. The spermatogenesis cycle is approximately 90 days.

Highly Effective Methods That Are User Dependent (for WOCBP partners of male participants)

6. Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation.

    - Oral;
    - Intravaginal;
    - Transdermal.

7. Progestogen-only hormone contraception associated with inhibition of ovulation.

    - Oral;
    - Injectable.

8. Sexual abstinence.

    - Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

One of the following effective barrier methods must be used in addition to the highly effective methods listed above that are user dependent:

- Male or female condom with or without spermicide;
- Cervical cap, diaphragm, or sponge with spermicide;
- A combination of male condom with either cervical cap, diaphragm, or sponge with spermicide (double-barrier methods).

10.5. Appendix 5: Genetics

Use/Analysis of DNA

- Genetic variation may impact a participant's response to study intervention, susceptibility to, and severity and progression of disease. Therefore, where local regulations and IRBs/ECs allow, a blood sample will be collected for DNA analysis.
- The scope of the genetic research may be narrow (eg, 1 or more candidate genes) or broad (eg, the entire genome), as appropriate to the scientific question under investigation.
- The samples may be analyzed as part of a multistudy assessment of genetic factors involved in the response to PF-07258669/placebo or study interventions of this class to understand treatments for the disease(s) under study or the disease(s) themselves.
- The results of genetic analyses may be reported in the CSR or in a separate study summary, or may be used for internal decision making without being included in a study report.
- The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained as indicated:
    - Retained samples will be stored indefinitely or for another period as per local requirements.
- Participants may withdraw their consent for the storage and/or use of their Retained Research Samples at any time by making a request to the investigator; in this case, any remaining material will be destroyed. Data already generated from the samples will be retained to protect the integrity of existing analyses.
- Samples for genetic research will be labeled with a code. The key between the code and the participant's personally identifying information (eg, name, address) will be held securely at the study site.

10.6. Appendix 6: Liver Safety: Suggested Actions and Follow-Up Assessments

Potential Cases of Drug-Induced Liver Injury

Humans exposed to a drug who show no sign of liver injury (as determined by elevations in transaminases) are termed "tolerators," while those who show transient liver injury but adapt are termed "adaptors." In some participants, transaminase elevations are a harbinger of a more serious potential outcome. These participants fail to adapt and therefore are "susceptible" to progressive and serious liver injury, commonly referred to as DILI. Participants who experience a transaminase elevation above 3 × ULN should be monitored more frequently to determine if they are "adaptors" or are "susceptible."

In the majority of DILI cases, elevations in AST and/or ALT precede TBili elevations (>2 × ULN) by several days or weeks. The increase in TBili typically occurs while AST/ALT is/are still elevated above 3 × ULN (ie, AST/ALT and TBili values will be elevated within the same laboratory sample). In rare instances, by the time TBili elevations are detected, AST/ALT values might have decreased. This occurrence is still regarded as a potential DILI. Therefore, abnormal elevations in either AST OR ALT in addition to TBili that meet the criteria outlined below are considered potential DILI (assessed per Hy's law criteria) cases and should always be considered important medical events, even before all other possible causes of liver injury have been excluded.

The threshold of laboratory abnormalities for a potential DILI case depends on the participant's individual baseline values and underlying conditions. Participants who present with the following laboratory abnormalities should be evaluated further as potential DILI (Hy's law) cases to definitively determine the etiology of the abnormal laboratory values:

- Participants with AST/ALT and TBili baseline values within the normal range who subsequently present with AST OR ALT values >3 × ULN AND a TBili value >2 × ULN with no evidence of hemolysis and an alkaline phosphatase value <2 × ULN or not available.

- For participants with baseline AST **OR** ALT **OR** TBili values above the ULN, the following threshold values are used in the definition mentioned above, as needed, depending on which values are above the ULN at baseline:

  - Preexisting AST or ALT baseline values above the normal range: AST or ALT values >2 times the baseline values AND >3 × ULN; or >8 × ULN (whichever is smaller).

  - Preexisting values of TBili above the normal range: TBili level increased from baseline value by an amount of at least 1 × ULN **or** if the value reaches >3 × ULN (whichever is smaller).

Rises in AST/ALT and TBili separated by more than a few weeks should be assessed individually based on clinical judgment; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor.

The participant should return to the investigator site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history, and physical assessment.

In addition to repeating measurements of AST and ALT and TBili for suspected Hy's law cases, additional laboratory tests should include albumin, CK, direct and indirect bilirubin, GGT, PT/INR, total bile acids, and alkaline phosphatase. Consideration should also be given to drawing a separate tube of clotted blood and an anticoagulated tube of blood for further testing, as needed, for further contemporaneous analyses at the time of the recognized initial abnormalities to determine etiology. A detailed history, including relevant information, such as review of ethanol, acetaminophen/paracetamol (either by itself or as a coformulated product in prescription or over-the-counter medications), recreational drug, or supplement (herbal) use and consumption, family history, sexual history, travel history, history of contact with a jaundiced person, surgery, blood transfusion, history of liver or allergic disease, and potential occupational exposure to chemicals, should be collected. Further testing for acute hepatitis A, B, C, D, and E infection, liver imaging (eg, biliary tract), and collection of serum samples for acetaminophen/paracetamol drug and/or protein adduct levels may be warranted.

All cases demonstrated on repeat testing as meeting the laboratory criteria of AST/ALT and TBili elevation defined above should be considered potential DILI (Hy's law) cases if no other reason for the LFT abnormalities has yet been found. **Such potential DILI (Hy's law) cases are to be reported as SAEs, irrespective of availability of all the results of the investigations performed to determine etiology of the LFT abnormalities.**

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology.

10.7. Appendix 7: Kidney Safety: Monitoring Guidelines

10.7.1. Laboratory Assessment of Change in Kidney Function and Detection of Kidney Injury

Standard kidney safety monitoring requires assessment of baseline and postbaseline serum creatinine (SCr measurement to estimate glomerular filtration rate [SCr-based eGFR] or creatinine clearance [eCrCl]). Baseline and postbaseline serum Scys makes it feasible to distinguish AKI from other causes of Scr increase. If SCr increase is confirmed after baseline, then reflex measurement of Scys is indicated to estimate the combined SCr-Scys eGFR calculation (for adults only).

Regardless of whether kidney function monitoring tests are required as a routine safety monitoring procedure in the study, if the investigator or sponsor deems it necessary to further assess kidney safety and quantify kidney function, then these test results should be managed and followed per standard of care.

10.7.2. Age-Specific Kidney Function Calculation Recommendations

10.7.2.1. Adults (18 Years and Above)—2021 CKD-EPI Equations[33]

2021 CKD-EPI SCr Only	Scr *(mg/dL)*	Scys *(mg/L)*	Recommended eGFR Equation
Female	if ≤0.7	N/A	$eGFR = 143 \times (Scr/0.7)^{-0.241} \times (0.9938)^{Age}$
Female	if >0.7	N/A	$eGFR = 143 \times (Scr/0.7)^{-1.200} \times (0.9938)^{Age}$
Male	if ≤0.9	N/A	$eGFR = 142 \times (Scr/0.9)^{-0.302} \times (0.9938)^{Age}$
Male	if >0.9	N/A	$eGFR = 142 \times (Scr/0.9)^{-1.200} \times (0.9938)^{Age}$
2021 CKD-EPI SCr-Scys Combined	**Scr *(mg/dL)***	**Scys *(mg/L)***	**Recommended eGFR Equation**
Female	if ≤0.7	if ≤0.8	$eGFR = 130 \times (Scr/0.7)^{-0.219} \times (Scys/0.8)^{-0.323} \times (0.9961)^{Age}$
Female	if ≤0.7	if >0.8	$eGFR = 130 \times (Scr/0.7)^{-0.219} \times (Scys/0.8)^{-0.778} \times (0.9961)^{Age}$
Female	if >0.7	if ≤0.8	$eGFR = 130 \times (Scr/0.7)^{-0.544} \times (Scys/0.8)^{-0.323} \times (0.9961)^{Age}$
Female	if >0.7	if >0.8	$eGFR = 130 \times (Scr/0.7)^{-0.544} \times (Scys/0.8)^{-0.778} \times (0.9961)^{Age}$
Male	if ≤0.9	if ≤0.8	$eGFR = 135 \times (Scr/0.9)^{-0.144} \times (Scys/0.8)^{-0.323} \times (0.9961)^{Age}$
Male	if ≤0.9	if >0.8	$eGFR = 135 \times (Scr/0.9)^{-0.144} \times (Scys/0.8)^{-0.778} \times (0.9961)^{Age}$
Male	if >0.9	if ≤0.8	$eGFR = 135 \times (Scr/0.9)^{-0.544} \times (Scys/0.8)^{-0.323} \times (0.9961)^{Age}$
Male	if >0.9	if >0.8	$eGFR = 135 \times (Scr/0.9)^{-0.544} \times (Scys/0.8)^{-0.778} \times (0.9961)^{Age}$

10.7.3. Adverse Event Grading for Kidney Safety Laboratory Abnormalities

AE grading for decline in kidney function (ie, eGFR or eCrCl) will be according to KDIGO criteria.[34]

10.8. Appendix 8: ECG Findings of Potential Clinical Concern

ECG Findings That May Qualify as AEs
• Marked sinus bradycardia (rate <40 bpm) lasting minutes. • New PR interval prolongation >280 msec. • New prolongation of QTcF to >480 msec (absolute) or by ≥60 msec from baseline. • New-onset atrial flutter or fibrillation, with controlled ventricular response rate: ie, rate <120 bpm. • New-onset type I second-degree (Wenckebach) AV block of >30 seconds' duration. • Frequent PVCs, triplets, or short intervals (<30 seconds) of consecutive ventricular complexes.
ECG Findings That May Qualify as SAEs

• QTcF prolongation >500 msec.
• New ST-T changes suggestive of myocardial ischemia.
• New-onset left bundle branch block (QRS >120 msec).
• New-onset right bundle branch block (QRS >120 msec).
• Symptomatic bradycardia.
• Asystole:
  • In awake, symptom-free participants in sinus rhythm, with documented periods of asystole ≥3.0 seconds or any escape rate <40 bpm, or with an escape rhythm that is below the AV node.
  • In awake, symptom-free participants with atrial fibrillation and bradycardia with 1 or more pauses of at least 5 seconds or longer.
  • Atrial flutter or fibrillation, with rapid ventricular response rate: rapid = rate >120 bpm.
• Sustained supraventricular tachycardia (rate >120 bpm) ("sustained" = short duration with relevant symptoms or lasting >1 minute).

- Ventricular rhythms >30 seconds' duration, including idioventricular rhythm (heart rate <40 bpm), accelerated idioventricular rhythm (HR >40 bpm to <100 bpm), and monomorphic/polymorphic ventricular tachycardia (HR >100 bpm (such as torsades de pointes)).
- Type II second-degree (Mobitz II) AV block.
- Complete (third-degree) heart block.

ECG Findings That Qualify as SAEs

- Change in pattern suggestive of new myocardial infarction.
- Sustained ventricular tachyarrhythmias (>30 seconds' duration).
- Second- or third-degree AV block requiring pacemaker placement.
- Asystolic pauses requiring pacemaker placement.
- Atrial flutter or fibrillation with rapid ventricular response requiring cardioversion.
- Ventricular fibrillation/flutter.
- At the discretion of the investigator, any arrhythmia classified as an adverse experience.

The enumerated list of major events of potential clinical concern are recommended as "alerts" or notifications from the core ECG laboratory to the investigator and Pfizer study team, and not to be considered as all inclusive of what to be reported as AEs/SAEs.

CCI

[redacted]

CCI

[redacted]

CCI

[redacted]

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CCI

[redacted]

CCI

[redacted]

CCI

[redacted]

10.13. Appendix 13: Prohibited Concomitant Medications That May Result in DDI

The prohibited concomitant medications listed below should not be taken with PF-07258669 during the study. This is not an all-inclusive list. All medications must be reviewed on a case-by-case basis by the investigator and approved by the sponsor during the screening period for eligibility purposes.

The Pfizer study team is to be notified of any prohibited medications taken during the study.

Prohibited Concomitant Medications

CYP3A Inhibitors[a,b]		CYP3A Inducers[a,b]	
Moderate	**Strong**	**Moderate**	**Strong**
Aprepitant	Boceprevir	Bosentan	Apalutamide
Ciprofloxacin	Cobicistat	Efavirenz	Carbamazepine
Conivaptan	Danoprevir	Etravirine	Enzalutamide
Crizotinib	Dasabuvir	Phenobarbital	Mitotane
Cyclosporine	Elvitegravir	Primidone	Phenytoin
Diltiazem	Indinavir		Rifampin
Dronedarone	Itraconazole		St. John's wort
Erythromycin	Ketoconazole		
Fluconazole	Lopinavir		
Fluvoxamine	Paritaprevir		
Imatinib	Ombitasvir		
Tofisopam	Posaconazole		
Verapamil	Ritonavir		
	Saquinavir		
	Telaprevir		
	Tipranavir		
	Telithromycin		
	Troleandomycin		
	Voriconazole		
Sensitive CYP3A Substrates[a,c]		**CYP3A Substrates with Narrow Therapeutic Index[a,d]**	
Alfentanil	Lovastatin	Alfentanil	
Atorvastatin	Lurasidone	Astemizole	
Avanafil	Maraviroc	Cisapride	
Budesonide	Midazolam	Cyclosporine	
Buspirone	Naloxegol	Dihydroergotamine	
Darifenacin	Nisoldipine	Ergotamine	
Darunavir	Quetiapine	Fentanyl	
Dasatinib	Sildenafil	Pimozide	
Dronedarone	Simvastatin	Quinidine	
Ebastine	Sirolimus	Sirolimus	
Eletriptan	Tacrolimus	Tacrolimus	
Eplerenone	Ticagrelor	Terfenadine	
Everolimus	Tolvaptan		
Ibrutinib	Tipranavir		
Indinavir	Triazolam		
Felodipine	Vardenafil		
Lomitapide			

a. Not an all-inclusive list. Source for CYP3A inhibitors, inducers, and sensitive substrates[30]: US FDA, Drug Development and Drug Interactions – Table of Substrates, Inhibitors and Inducers (https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table5-1) accessed 13-January-2022; Source for CYP3A substrates with narrow therapeutic index[31]: Guidance for Industry – Drug Interaction Studies: Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Feb 2012.

b. All prohibited drugs that are moderate or strong CYP3A inhibitors or inducers require at least a 28 day or 5 half-lives (whichever is longer) washout prior to the first dose of study intervention.

c. Will be prohibited if the midazolam AUC ratio is ≥2 in Part B of this study.

d. Will be prohibited if the midazolam AUC ratio is ≥1.25 in Part B of this study.

In a situation where appropriate medical care of a participant requires the use of a prohibited CYP3A inhibitor, inducer, or substrate: These medications are not permitted in the study except in emergency situations requiring no more than one day of administration. Topical application of antimicrobial and antifungal medications is permitted.

10.14. Appendix 14: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the table of contents. The protocol amendment summary of changes tables for past amendments can be found below:

Amendment 3 (09 March 2022)

Overall Rationale for the Amendment: CCI [redacted] The following changes to C4541003 protocol are now proposed:

Section # and Name	Description of Change	Brief Rationale
CCI [redacted]		
1.1 (Synopsis) and 3 (Objectives and Endpoints)	CCI [redacted]	
1.1 (Synopsis), 1.3.3 (Part B – Overall SoA), 3 (Objectives and Endpoints), 4.2.2 (Rationale for Part B), 8.7.3 (Urine for Determination of 6β-Hydroxycortisol/ Cortisol Ratio)	Added measurement of urinary 6β-hydroxycortisol/ cortisol to Part B.	Urine is now being collected in Part B CCI [redacted] urine collection can be used for measurement of 6β-hydroxycortisol/cortisol, which is a marker of potential CYP3A induction.

Section # and Name	Description of Change	Brief Rationale
CCI [redacted]		
2.2.6 (Clinical Overview)	Text updated to reflect C4541001 is final; added preliminary C4541003 safety, tolerability, and PK data.	Provides the most up to date summary of clinical safety, tolerability, and PK data for PF-07258669.
2.3 (Benefit/Risk Assessment)	Added reference to clinical data.	Clinical data for C4541001 and C4541003 preliminary data are now available.
CCI [redacted]		
5.2 (Exclusion Criteria)	Exclusion Criteria #1 and #7: Included additional examples of excluded conditions.	These additions further clarified, and provided additional examples of, medical conditions of participants not permitted in this study.
CCI [redacted]		
CCI [redacted]		
CCI [redacted]		

Section # and Name	Description of Change	Brief Rationale
CCI [redacted]		
10 (Supporting Documentation and Operational Considerations) and text throughout protocol	Adjusted numbering of appendices.	Addition of CCI [redacted] required re-numbering of other appendices.
10.8 CCI [redacted])	Added questionnaire.	Provides example questionnaire with exact questions participants are to answer.
10.13 (Appendix 13: Protocol Amendment History)	Moved Summary of Changes table for Amendment 2 from beginning of protocol to the appendix.	Moved to highlight changes for Amendment 3 at the beginning of the protocol.
10.14 (Appendix 14: Abbreviations)	Added new abbreviations.	New abbreviations were added to protocol text.

Amendment 2 (13 January 2022)

Overall Rationale for the Amendment:

- Safety, tolerability, and PK may be evaluated in an optional cohort of older adults (aged 65 to 90 years old) to inform future clinical development of PF-07258669. Concomitant medications may be allowed, with some restrictions, in older adult participants.

Eligibility criteria pertaining to heart rate, LDL cholesterol, total cholesterol, and triglycerides have been updated following review of preliminary safety data from C4541001.

Section # and Name	Description of Change	Brief Rationale
Title, 1.1 (Synopsis), 1.2 (Schema), 2.1 (Study Rationale), 2.3.1 (Risk Assessment), 3 (Objectives and Endpoints), 4.1 (Overall Design), 4.1.1 (Overall Design of Part A), 4.2.1 (Rationale for Part A), 4.3.3.1 (Part A PF-07258669 Dose Selection), 6.5 (Dose Modification), 6.8 (Concomitant Therapy), 9.3.3.3. (Statistical Methods for PF-07258669 PK Data [Part A only]), 9.5.1 (Part A Sample Size)	Added an optional cohort of older adult participants to the study, updated the total number of planned participants, and clarified that the Japanese cohort and older adult cohort are optional.	Safety, tolerability, and PK may be evaluated in older adults (aged 65 to 90 years old) to inform future clinical development of PF-07258669. The Japanese and older adult cohorts will only be conducted if results in earlier cohorts of this study support additional evaluation. The older adult cohort will only be conducted after results of Part B are available.
1.1 (Synopsis), 2.2.6 (Clinical Overview), 2.3 (Benefit/Risk Assessment), 2.3.1 (Risk Assessment), 4.1.1 (Part A), 4.2.1 (Rationale for Part A), 4.3.3.1 (Part A PF-07258669 Dose Selection, 8.3.8 (Adverse Events of Special Interest)	Updated information about Study C4541001.	All participants have completed Study C4541001. The preliminary safety, tolerability, and PK data presented in this section were updated accordingly.

Section # and Name	Description of Change	Brief Rationale
5.1 (Inclusion Criteria)	Inclusion Criteria #1, #6, and #19: Defined age range, BMI, and eGFR for the optional cohort of older adult participants.	Age range, BMI, and eGFR proposed to be more representative of older population.
5.1 (Inclusion Criteria), 5.2 (Exclusion Criteria)	Inclusion Criterion #2 and Exclusion Criterion #1: Defined characteristics of older adult participants.	Older adult participants in reasonably good health with mild, chronic, stable disease may be enrolled.
5.2 (Exclusion Criteria)	Exclusion Criteria #1, #10, and #11: Prior/Concomitant Therapy.	Allows older adult participants to take daily prescription or non-prescription medications with some restrictions.
5.2 (Exclusion Criteria)	Exclusion Criterion #17: Reduced HR exclusion to <45 bpm.	Review of the safety data from C4541001 does not reveal any AEs of bradycardia or safety concerns with respect to HR. Thus, the lower limit for HR has been adjusted with these data in mind.
5.2 (Exclusion Criteria)	Exclusion Criteria #1 and #19: Removed exclusion criteria for total cholesterol and LDL and increased triglyceride exclusion to >2× ULN.	Review of laboratory data from C4541001 does not identify any clinically-significant, dose-related adverse trends in total cholesterol, LDL, or triglycerides in relation to dosing with PF-07258669.
2.3.1 (Risk Assessment), 4.2.1 (Rationale for Part A), 5.2 (Exclusion Criteria), 5.3.4 (Daily Medication), 6.1.1 (Administration), 6.8 (Concomitant Therapy), 6.8.1 (Optional Cohort of Older Adult Participants Only), 10.11	Added that some background medications are permitted for older adult participants; provided a list of prohibited medications and criteria for when these medications are restricted.	To enable recruitment of participants for this study, older participants are permitted to take prescription or non-prescription medications for management of acceptable chronic medical conditions, as long as those medications are not specifically excluded in Section 5.2, Section 6.8, Section 6.8.1, or Section 10.11.

Section # and Name	Description of Change	Brief Rationale
(Appendix 11: Prohibited Concomitant Medications That May Result in DDI)		
1.3.1 (Part A Overall SoA), 1.3.2 (Part A SoA for Days 1 and 14), 4.2.1 (Rationale for Part A), [redacted] 10.2 (Appendix 2: Clinical Laboratory Tests)	[redacted]	
1.3.1 (Part A Overall SoA), 1.3.2 (Part A SoA for Days 1 and 14), 1.3.3 (Part B Overall SoA), 8 (Study Assessments and Procedures), 8.7.9 (Retained Research Samples for Biomarkers	Prep B1 added to Prep B2 as retained research samples, and updated blood volume in Section 8 accordingly.	To facilitate future exploratory biomarker analysis.
4.1.1 (Part A Overall Design)	Clarified duration of participant stay in CRU	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.
4.1.2 (Part B Overall Design), 4.3 (Justification for Dose), 4.3.3.2.1 (PF-07258669 in Part B), 6.5 (Dose Modification)	Clarified that Part B dose and dose regimen will be determined by results of Part A	Previously only stated that dose regimen would be informed by results of Part A.
4.2.2 (Rationale for Part B)	Clarified midazolam wording.	Removed redundant text.
4.3.3.1 (Part A PF-07258669 Dose Selection)	Added Q8H dose level expected to achieve therapeutic exposures for context.	Projected therapeutic dose is twice daily and outlined in Section 4.3.1. For context, provided a Q8H dose expected to achieve therapeutic

Section # and Name	Description of Change	Brief Rationale
		exposures since Q8H dosing is being used in this study.
9.3.3.1 (Derivation of PF-07258669 PK Parameters [Part A Only])	Removed Day 7 from Rac,Cmax and Rac.	Dense PK samples are not being collected on Day 7, so these parameters cannot be calculated.
10.4.1 (Appendix 4 – Male Participant Reproductive Inclusion Criteria)	Updated to reduce the preclusion of sperm donation and requirement for either abstinence from intercourse or use of male condom from 28 days plus an additional 90 days to cover a spermatogenesis cycle to 28 days only.	PF-07258669 was assessed in a series of genetic toxicity studies and was not genotoxic based on the totality of in vitro and in vivo data.
10.12 (Appendix 12: Protocol Amendment History)	Moved Summary of Changes for Amendment 1 to Appendix 12).	Simplifies current section to Summary of Changes for current amendment only.
11 (Reference)	Added new reference.	Reference supports classification of potential concomitant medications as strong or moderate CYP3A inhibitors or inducers; or sensitive CYP3A substrates; or CYP3A substrates with narrow therapeutic index.

Amendment 1 (12 October 2021)

Overall Rationale for the Amendment: This amendment added precautionary sentinel dosing to Part A and Part B such that safety and tolerability may be evaluated in a subset of participants in each cohort before dosing other participants in those cohorts. Continuous pulse oximetry was also added as a safety assessment on days of midazolam dosing in Part B.

Section # and Name	Description of Change	Brief Rationale
1.1 (Synopsis), 2.3 (Benefit/Risk Assessment), 2.3.1 (Risk Assessment), 4.1.1 (Overall Design Part A), 4.2.1 (Rationale for Part A), 6.5.1 (Dose Escalation and Stopping Rules)	Added precautionary sentinel dosing to Part A.	Precautionary sentinel dosing may be utilized where applicable in Part A to ensure that safety and tolerability data in a subset of 2 participants within a cohort supports dosing additional participants.
1.1 (Synopsis), 2.3 (Benefit/Risk Assessment), 2.3.1 (Risk Assessment), 4.1.2 (Overall Design Part B), 4.2.2 (Rationale for Part B), 6.5.1 (Dose Escalation and Stopping Rules)	Added precautionary sentinel dosing to Part B.	Precautionary sentinel dosing may be utilized where applicable in Part B to ensure that safety and tolerability data in a subset of 2 participants within a cohort supports dosing additional participants.
1.1 (Synopsis) and 3 (Objectives and Endpoints)	Added continuous pulse oximetry as an additional secondary safety endpoint for Part B.	Respiratory depression is a recognized potential side effect of midazolam. Continuous pulse oximetry was added to begin 30 minutes before and to continue for at least 6 hours after midazolam dosing as an additional safety precaution to monitor participants for any respiratory depression.
1.3.1 (Part A – Overall Schedule of Activities)	Updated COVID-19 assessments and footnotes referring to baseline and post-dose measurements of CCI [redacted]	COVID-19 pandemic and vaccination status is evolving; updates reflect evolving CRU procedures. CCI [redacted]

Section # and Name	Description of Change	Brief Rationale
	CCI [redacted]	CCI [redacted] Additional footnotes were added to clarify.
1.3.2 (Part A – Schedule and Procedures for Days 1 and 14 Only)	Clarified timing of assessments outlined in footnote "a".	PF-07258669 dosing does not occur on Day 15.
1.3.3 (Part B – Overall Schedule of Activities)	• Updated COVID-19 assessments. • Added continuous pulse oximetry on days of midazolam dosing. • Removed supine vital signs from screening, discharge, follow-up visit, and ET/DC. • Updated footnotes.	• COVID-19 pandemic and vaccination status is evolving; updates reflect evolving CRU procedures. • Respiratory depression is a recognized side effect of midazolam. As an additional safety precaution to monitor participants for any respiratory depression, continuous pulse oximetry was added to begin 30 minutes before and at least 6 hours after midazolam dosing. • The SoA has assessments of both supine vital signs and orthostatic vital signs at these timepoints. Supine vitals signs are part of orthostatic vital signs measurements, so this redundancy was removed. • Footnotes were updated to reflect changes in the SoA.
2.2.6 (Clinical Overview)	Clarified exposure increases observed across dose range in C4541001.	Within each cohort in C4541001, exposure increases were approximately dose proportional. Between cohorts, exposure increases were less than dose proportional.
2.3.1 (Risk Assessment)	Added risk assessment and mitigation strategies for midazolam.	Respiratory depression is a recognized side effect of midazolam in Part B. Continuous pulse oximetry will be employed

Section # and Name	Description of Change	Brief Rationale
		on days of midazolam dosing in Part B as a safety precaution.
4.1.1 (Overall Design Part A)	Added flexibility to allow non-sentinel participants to begin their stay at the CRU on the same day as the sentinel participants.	This enables the CRU to standardize conditions for all participants of a given cohort.
4.2.2 (Rationale for Part B)	Added rationale for continuous pulse oximetry.	Assessments added as an additional safety precaution on days of midazolam dosing.
CCI [redacted]	Updated CCI [redacted] and associated exposures.	Population PK model was updated with C4541001 data.
4.3.3.2.2 (Midazolam in Part B)	Updated midazolam dose rationale and added midazolam exposure limits.	A concentration range of midazolam that will not have undue pharmacological effects was not defined in the dose selection rationale. Thus, midazolam exposure limits that will not be exceeded in order to have no expected undue pharmacological effects and side effects have been defined and justified in the protocol.
5.2 (Exclusion Criteria)	Removed exclusion for HDL.	HDL was included in error, and the 1.25x ULN criterion is not applicable to this parameter.
5.3.1 (Meals and Dietary Restrictions), 6.1.1 (Administration)	Clarified that study intervention administration is permitted during fasting.	Clarification was needed to clearly define that both water and study intervention administration is permitted during fasting times prior to safety laboratory assessments.
5.4 (Screen Failures)	Screen failures are allowed to be rescreened.	If the prior reason(s) for not meeting the eligibility criteria have been resolved, participants are allowed to be rescreened.

Section # and Name	Description of Change	Brief Rationale
6.2 (Preparation, Handling, Storage, and Accountability), 6.2.1 (Preparation and Dispensing)	Added reference to IP manual.	IP manual provides detailed guidance on dosing of study intervention.
6.7 (Treatment of Overdose)	Added Level 3 sections for PF-07258669 (6.7.1) and midazolam (6.7.2) overdose.	Added information for midazolam overdose; treatment of overdose is different for PF-07258669 and midazolam.
6.8 (Concomitant Therapy)	Clarified that concomitant medications are prohibited for the duration of the study.	In addition to restrictions of prior/concomitants medications prior to participation in this study, the restrictions should be maintained for the duration of the study.
8.2.5 (Continuous Pulse Oximetry)	Added assessment description.	Added information about procedure for continuous pulse oximetry to be employed in Part B, including expected response to abnormal oxygen saturation alerts of potential clinical concern.
8.2.7 (COVID-19 Related Measures)	Updated section title and COVID-19 assessments.	COVID-19 pandemic and vaccination status is evolving; updates reflect evolving CRU procedures.
10.11 (Abbreviations)	Added new abbreviation.	Abbreviation and definition of ICH and ILS added to protocol amendment text.
11 (References)	Added references.	References support midazolam SmPC and midazolam exposure data from University of Washington Drug Interaction Database.

10.15. Appendix 15: Abbreviations

The following is a list of abbreviations that may be used in the protocol.

Abbreviation	Term
Abs	absolute
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
Ae_{tau}	amount of unchanged drug recovered in urine during the dosing interval
Ae_{tau} %	percent of dose recovered in urine as unchanged drug
AKI	acute kidney injury
CCI [redacted]	
ALT	alanine aminotransferase
AO	aldehyde oxidase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_8	area under the concentration-time curve from time 0 to 8 hours
AUC_{24}	area under the concentration-time curve from time 0 to 24 hours
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
AV	Atrioventricular
BBS	Biospecimen Banking System
CCI [redacted]	
BMI	body mass index
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
C_{av}	average plasma concentration
$C_{b,u}$	unbound brain concentration
C_{eff}	efficacious plasma concentration
CFR	Code of Federal Regulations
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CK	creatine kinase
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{last}	predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis
CL/F	apparent clearance for oral dosing

Abbreviation	Term
CL_p	plasma clearance
CL_r	renal clearance
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CCI	[redacted]
CO_2	carbon dioxide (bicarbonate)
COVID-19	coronavirus disease 2019
$C_{p,u}$	unbound plasma concentration
CRF	case report form
CRO	clinical research organization
CRU	clinical research unit
CSF	cerebrospinal fluid
CSR	clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
CT	clinical trial
CV	coefficient of variation
CYP	cytochrome P450
DC	Discontinuation
DDI	drug-drug interaction
DILI	drug-induced liver injury
DMC	data monitoring committee
dn	dose normalized
DNA	deoxyribonucleic acid
CCI	[redacted]
EC	ethics committee
ECC	emergency contact card
ECG	Electrocardiogram
eCrCl	estimated creatinine clearance
eCRF	electronic case report form
EDB	exposure during breastfeeding
EDP	exposure during pregnancy
eGFR	estimated glomerular filtration rate
EMA	European Medicines Agency
ET	early termination
EU	European Union
EudraCT	European Clinical Trials Database
FAS	full analysis set
FDA	Food and Drug Administration
CCI	[redacted]
FOB	functional observational battery
FSH	follicle-stimulating hormone

Abbreviation	Term
f_u	fraction unbound
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GLP	Good Laboratory Practice
GPCR	G-protein coupled receptor
H	Hours
HBcAb	hepatitis B core antibody
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HCHC	high carbohydrate-high calorie
HCVAb	hepatitis C antibody
HDL	high-density lipoprotein
HFHC	high fat-high calorie
HIV	human immunodeficiency virus
HR	heart rate
HRT	hormone replacement therapy
IB	investigator's brochure
IC_{50}	concentration associated with 50% inhibition
ICD	informed consent document
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ID	identification
ILS	immediate life support
IND	investigational new drug
INR	international normalized ratio
IP manual	investigational product manual
IPAL	Investigational Product Accountability Log
IRB	institutional review board
IV	intravenous
K_2EDTA	dipotassium ethylenediaminetetraacetic acid
K_d	dissociation constant
KDIGO	Kidney Disease Improving Global Outcomes
k_{el}	terminal phase elimination rate constant
K_i	inhibition constant
k_{inact}	Enzyme inactivation rate constant
LBBB	left bundle branch block
LDL	low-density lipoprotein
LFT	liver function test
LLOQ	lower limit of quantitation
mBcrp	mouse breast cancer resistance protein
MC1R	melanocortin-1 receptor

Abbreviation	Term
MC2R	melanocortin-2 receptor
MC3R	melanocortin-3 receptor
MC4R	melanocortin-4 receptor
MC5R	melanocortin-5 receptor
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
CCI	
MHP	mental health professional
CCI	
mRNA	messenger ribonucleic acid
msec	millisecond
MTD	maximum tolerated dose
N/A	not applicable
CCI	
CCI	
NOEL	no-observed-effect-level
NTI	narrow therapeutic index
PBPK	physiologically-based pharmacokinetics
PCR	polymerase chain reaction
PD	pharmacodynamics
P-gp	P-glycoprotein
pH	potential of hydrogen
PI	principal investigator
CCI	
PK	pharmacokinetics
pKa	acid dissociation constant
PK/PD	pharmacokinetics/pharmacodynamics
PR	pulse rate
PT	prothrombin time
PTR	peak-to-trough ratio
PVC	premature ventricular contraction/complex
Q6H	every 6 hours
Q8H	every 8 hours
Q12H	every 12 hours
QTc	corrected QT interval
QTcF	QTc corrected using Fridericia's formula
qual	qualitative
R_{ac}	observed accumulation ratio
$R_{ac,Cmax}$	observed accumulation ratio for C_{max}
RBC	red blood cell

Abbreviation	Term
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCr	serum creatinine
Scys	serum cystatin C
SD	standard deviation
SmPC	Summary of Product Characteristics
SoA	schedule of activities
SOP	standard operating procedure
SRSD	single reference safety document
SSC	special safety concern
SUSAR	suspected unexpected serious adverse reaction
τ	tau; time of dosing interval
$t_{½}$	terminal half-life
CCI [redacted]	
TBili	total bilirubin
TDI	time-dependent inhibition
THC	tetrahydrocannabinol
T_{max}	time to reach C_{max}
CCI [redacted]	
CCI [redacted]	
ULN	upper limit of normal
US	United States
USA	United States of America
UV	ultraviolet
V_{ss}	steady-state volume of distribution
V_z/F	apparent volume of distribution for oral dosing
WBC	white blood cells
WOCBP	woman/women of childbearing potential

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