Statistical Analysis Plan

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Study Number: TAK-227-1001

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STATISTICAL ANALYSIS PLAN

Study Number: TAK-227-1001

A Randomized, Open-Label, Single-Dose, Three-Way Crossover Evaluation of the Effect of Food on the Pharmacokinetics, Safety, and Tolerability of TAK-227 in Healthy Adult Participants

Phase: 1

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Study Title: A Randomized, Open-Label, Single-Dose, Three-Way Crossover Evaluation of the Effect of Food on the Pharmacokinetics, Safety, and Tolerability of TAK-227 in Healthy Adult Participants

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TABLE OF CONTENTS

1.0 OBJECTIVES, ENDPOINTS AND ESTIMANDS 8
1.1 Objectives 8
1.1.1 Primary Objective 8
1.1.2 Secondary Objective 8
1.1.3 Exploratory Objectives 8
1.2 Endpoints 8
1.2.1 Primary Endpoints 8
1.2.2 Secondary Endpoints 9
1.2.3 Exploratory Endpoints 9
1.2.4 Additional Exploratory Endpoints 9
1.3 Estimands 10
2.0 STUDY DESIGN 10
3.0 STATISTICAL HYPOTHESES AND DECISION RULES 12
3.1 Statistical Hypotheses 12
3.2 Statistical Decision Rules 12
3.3 Multiplicity Adjustment 12
4.0 SAMPLE-SIZE DETERMINATION 13
5.0 ANALYSIS SETS 13
5.1 Safety Set 13
5.2 PK Set 13
6.0 STATISTICAL ANALYSIS 13
6.1 General Considerations 13
6.1.1 Handling of Treatment Misallocations 15
6.2 Study Information 15
6.3 Disposition of Participants 15
6.4 Demographic and Other Baseline Characteristics 15
6.4.1 Demographics 15
6.4.2 Medical History and Concurrent Medical Conditions 16
6.5 Medication History and Concomitant Medications 16
6.6 Efficacy Analysis 16
6.7 Safety Analysis 16
6.7.1 Adverse Events 17
6.7.2 Adverse Events of Special Interest 17
6.7.3 Clinical Laboratory Evaluation 18

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6.7.4 Vital Signs 19
6.7.5 12-Lead ECG 19
6.7.6 Physical Examinations 20
6.7.7 Overdose 20
6.7.8 Extent of Exposure and Compliance 20
6.8 Pharmacokinetic Analysis 20
6.9 Patient Reported Outcomes and Health Care Utilization Endpoints Analysis 21
6.10 Interim Analysis 21
6.11 Preliminary Analysis 21
6.12 Data Monitoring Committee/Internal Review Committee/ [Other Data Review Committees] 22
7.0 REFERENCES 22
8.0 CHANGES TO PROTOCOL PLANNED ANALYSES 22
9.0 APPENDIX 22
9.1 Changes From the Previous Version of the SAP 22
9.2 Data Handling Conventions 22
9.3 Analysis Software 22

LIST OF IN-TEXT TABLES

Table 2.a Study Schematic 10
Table 2.b Randomization Sequence 11
Table 2.c Study Treatments for Study Drug 12
Table 6.a Collection of Laboratory Samples 18
Table 6.b Collection of Vital Signs 19
Table 6.c Collection of ECGs 19
Table 6.d Collection of Blood Samples for Pharmacokinetic Analysis 20

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ABBREVIATIONS

λ_z	terminal disposition phase rate constant
AE	adverse event
AUC_{∞}	area under the concentration-time curve from time 0 to infinity, calculated using the observed value of the last quantifiable concentration
AUC_{∞_pred}	area under the concentration-time curve from time 0 to infinity, calculated using the predicted value of the last quantifiable concentration
$AUC_{extrap}\%$	area under the curve from the last quantifiable concentration to infinity, calculated using the observed value of the last quantifiable concentration, expressed as a percentage of AUC_{∞}
$AUC_{extrap}\%_{\_pred}$	area under the curve from the last quantifiable concentration to infinity, calculated using the predicted value of the last quantifiable concentration, expressed as a percentage of AUC_{∞_pred}
AUC_{last}	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
BLQ	below the lower limit of quantitation
CI	confidence interval
CL/F	apparent clearance after extravascular administration, calculated using the observed value of the last quantifiable concentration
$CL/F_{\_pred}$	apparent clearance after extravascular administration, calculated using the predicted value of the last quantifiable concentration
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CPAP	clinical pharmacology analysis plan
CRF	case report form
CRU	clinical research unit
CSR	clinical study report
CV	coefficient of variance
DMP	data management plan
ECG	electrocardiogram
Geom CV	geometric coefficient of variance
Geom Mean	geometric mean
GMR	geometric least-squares mean ratio
ICF	informed consent form
LSM	least-squares mean
Mean	arithmetic mean
MedDRA	Medical Dictionary for Regulatory Activities
MPR AUC_{∞}	metabolite-to parent AUC_{∞} ratio
MPR AUC_{∞_pred}	metabolite-to parent AUC_{∞_pred} ratio
MPR C_{max}	metabolite-to parent C_{max} ratio
n	number of observations
PK	pharmacokinetic
SAE	serious adverse event
SAP	statistical analysis plan

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SD	standard deviation
SEM	standard error of the mean
SOC	System Organ Class
$t_{1/2z}$	terminal disposition phase half-life
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{lag}	lag time to first quantifiable concentration (calculated as lag time to observation prior to the first observation with a measurable (non-zero) concentration)
t_{max}	time to first occurrence of C_{max}
V_z/F	apparent volume of distribution during the terminal disposition phase after extravascular administration, calculated using the observed value of the last quantifiable concentration
$V_z/F_{\_pred}$	apparent volume of distribution during the terminal disposition phase after extravascular administration, calculated using the predicted value of the last quantifiable concentration
WHO	World Health Organization

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1.0 OBJECTIVES, ENDPOINTS AND ESTIMANDS

1.1 Objectives

1.1.1 Primary Objective

To assess the pharmacokinetics (PK) of a single oral dose of 50 mg TAK-227 administered 30 minutes following the start of a high-fat/high-calorie meal or ~30 minutes before the start of a high-fat/high-calorie meal in healthy adult participants relative to administration under fasting conditions in healthy adult participants.

1.1.2 Secondary Objective

To evaluate the safety and tolerability of a single oral dose of 50 mg TAK-227 when administered under fasting conditions, 30 minutes following the start of a high-fat/high-calorie meal, and ~30 minutes before the start of a high-fat/high-calorie meal in healthy adult participants.

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1.1.3 Exploratory Objectives

- *To evaluate other PK parameters for a single oral dose of 50 mg TAK-227 when administered under fasting conditions, 30 minutes following the start of a high-fat/ high-calorie meal, and ~30 minutes before the start of a high-fat/high-calorie meal in healthy adult participants.*
- *To evaluate TAK-227 metabolites PK parameters for a single oral dose of 50 mg TAK-227 when administered under fasting conditions, 30 minutes following the start of a high-fat/high-calorie meal, and ~30 minutes before the start of a high-fat/high-calorie meal in healthy adult participants.*

1.2 Endpoints

1.2.1 Primary Endpoints

The primary endpoints will be assessed through evaluation of the following PK parameters for TAK-227 in plasma:

- *Maximum observed concentration (C_{max})*
- *Area under the concentration-time curve (AUC) from time 0 to the time of the last quantifiable concentration (AUC_{last})*
- *AUC from time 0 to infinity, calculated using the observed value of the last quantifiable concentration (AUC_{∞})*

1.2.2 Secondary Endpoints

The secondary endpoints will be assessed through evaluation of the following safety parameters:

- *Treatment-emergent adverse events (TEAEs) and their frequency, severity, seriousness, and causality assessments*
- *Changes in vital signs, electrocardiograms (ECGs), and clinical laboratory results (hematology, chemistry, and urinalysis) from baseline to post-baseline time points, and findings from physical examinations.*

1.2.3 Exploratory Endpoints

The exploratory endpoints will be assessed through evaluation of the following PK parameters for TAK-227 and its metabolites in plasma (if applicable, but are not limited to):

- *Time to first occurrence of C_{max} (t_{max})*
- *Lag time to first quantifiable concentration in plasma (t_{lag})*
- *Terminal disposition phase rate constant (λ_z)*
- *Terminal disposition phase half-life ($t_{½z}$)*
- *Apparent volume of distribution during the terminal disposition phase after oral administration, calculated using the observed value of the last quantifiable concentration (V_z/F; parent only)*
- *Apparent clearance after oral administration, calculated using the observed value of the last quantifiable concentration (CL/F; parent only)*
- *AUC from the last quantifiable concentration to infinity, calculated using the observed value of the last quantifiable concentration, expressed as a percentage of AUC_∞ (AUC_{extrap}%)*

The following PK parameters will also be evaluated for TAK-227 metabolites:

- C_{max}
- AUC_{last}
- AUC_∞

1.2.4 Additional Exploratory Endpoints

The following additional endpoints will be calculated for TAK-227 and any metabolites (if applicable):

- *AUC from time 0 to infinity, calculated using the predicted value of the last quantifiable concentration (AUC_{∞_pred})*
- *Apparent volume of distribution during the terminal disposition phase after oral administration, calculated using the predicted value of the last quantifiable concentration (V_z/F_{pred}; parent only)*

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- *Apparent clearance after oral administration, calculated using the predicted value of the last quantifiable concentration (CL/F_{pred}; parent only)*
- *AUC from the last quantifiable concentration to infinity, calculated using the predicted value of the last quantifiable concentration, expressed as a percentage of AUC_{∞_pred} ($AUC_{extrap}\%\_{pred}$)*
- Metabolite-to-parent AUC_{∞} ratio (MPR AUC_{∞})
- Metabolite-to-parent AUC_{∞_pred} ratio (MPR AUC_{∞_pred})
- Metabolite-to-parent C_{max} ratio (MPR C_{max})

1.3 Estimands

Not applicable.

2.0 STUDY DESIGN

This is a single-center, open-label, single-dose, randomized, 3-period, 6-sequence, crossover study in healthy adults. A study schematic is shown in Table 2.a.

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Table 2.a Study Schematic

Pretreatment	**Treatment Periods 1-2-3**				**Study Exit**	**Follow-up (a)**
Screening	**Check-in**	**Dosing and Safety and PK Assessments**	**Safety and PK Assessments**	**Washout (b)**	Day 2 of Treatment Period 3	7 (±3) days after last dose
Day -28 to first dosing	Day -1 of Treatment Period 1	Day 1	Day 2	Not less than 4 days		
	◄---------- Confinement (c) ---------►					

(a) The clinical research unit (CRU) will contact all participants (including participants who terminate the study early) 7 (± 3) days after the last TAK-227 administration by telephone or other methods per CRU standards to determine if any adverse event (AE) has occurred or concomitant medications have been taken since the last study visit. If clinically significant findings are observed upon discharge, participants may return to the CRU for re-evaluation per Investigator's discretion.
(b) There will be a washout period of not less than 4 days between TAK-227 dosing in each period.
(c) Participants will start the confinement on Day -1 of Treatment Period 1 and will remain confined until Day 2 of Treatment Period 3.

On Day 1 of Treatment Period 1, participants will be randomly assigned to one of 6 possible treatment sequences (Table 2.b).

Table 2.b Randomization Sequence

Sequence	Number of participants (N)	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	4	A	B	C
2	4	B	C	A
3	4	C	A	B
4	4	A	C	B
5	4	B	A	C
6	4	C	B	A

Treatment A: Fasting
Treatment B: Fed predose, high-fat/high-calorie meal administered 30 minutes prior to dosing.
Treatment C: Fed postdose, high-fat/ high-calorie meal administered ~30 minutes after dosing.

On Day 1 of each treatment period, a single dose of 50 mg TAK-227 will be administered orally under one of 3 different feeding conditions as per the randomization schedule:

- *Fasting (Treatment A),*
- *Fed predose, high-fat/high-calorie meal administered 30 minutes prior to dosing (Treatment B), and*
- *Fed postdose, high-fat/high-calorie meal administered ~30 minutes after dosing (Treatment C).*

Drinking water (except water provided with each dosing) will be restricted 1 hour prior to and 1 hour after each dosing but will be allowed ad libitum at all other times. Other fluids may be given as part of meals and snacks but will be restricted at all other times throughout the confinement period.

For Treatment A, participants will be required to fast overnight for at least 10 hours prior to dosing and will continue the fast for at least 4 hours postdose.

For Treatment B, participants will be required to fast overnight for at least 10 hours until 30 minutes prior to their scheduled morning dose, when they will be given a high fat/high-calorie breakfast[*] which will be entirely consumed within 30 minutes. Participants will fast for at least 4 hours postdose.

For Treatment C, participants will be required to fast overnight for at least 10 hours prior to dosing and will continue the fast for ~30 minutes postdose, at which time they will be given a high fat/high calorie breakfast[*] which will be entirely consumed within 30 minutes. Participants will fast for at least 3 hours following the meal.

The dose regimens are shown in Table 2.c.

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[*] *A high fat/high-calorie breakfast will contain 800-1000 calories and approximately 50% fat. An example of high fat would be 2 slices of buttered toast, 2 eggs fried in butter, 2 strips of bacon, 4 oz of hash brown potatoes, and 8 ounces (approximately 240 mL) of whole milk [FDA, 2022].*

Table 2.c Study Treatments for Study Drug

Treatment [(a)]	Study Drug	Dose	Dose Regimen	Days on Study Drug
Treatment A	TAK-227 capsule	50 mg	Fasted, single dose, oral	Day 1
Treatment B	TAK-227 capsule	50 mg	Fed predose: after a high-fat/high-calorie meal [(b)], single dose, oral	Day 1
Treatment C	TAK-227 capsule	50 mg	Fed postdose: before a high-fat/high-calorie meal [(c)], single dose, oral,	Day 1

(a) There will be a washout period of not less than 4 days between TAK-227 dosing in each treatment.
(b) TAK-227 will be administered 30 minutes following the start of a high-fat/high-calorie meal.
(c) A high-fat/high-calorie meal will be administered ~30 minutes following administration of TAK-227.

Following all treatments, each meal and/or snacks served at the CRU will be standardized and will be similar in caloric content and composition (except for the meals served as part of Treatments B and C) and will be taken at approximately the same time in each treatment period. When confined, standard meals and snacks will be provided at appropriate times, except when they are required to fast. When confined in the CRU, participants will be required to fast from all food and drink except water between meals and snacks.

There will be a washout period of not less than 4 days between TAK-227 dosing in each period. PK sample collections will be conducted predose and up to 36 hours postdose in each treatment period.

Safety and tolerability will be assessed throughout the study by TEAEs, vital signs, ECGs, clinical laboratory evaluations, and physical examinations. The CRU will contact all participants (including participants who terminate the study early) 7 (± 3) days after the last TAK-227 administration by telephone or other methods per CRU standards to determine if any AE has occurred or concomitant medications have been taken since the last study visit. If clinically significant findings are observed upon discharge, participants may return to the CRU for re-evaluation per the Investigator's discretion.

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3.0 STATISTICAL HYPOTHESES AND DECISION RULES

3.1 Statistical Hypotheses

Not applicable.

3.2 Statistical Decision Rules

Not applicable.

3.3 Multiplicity Adjustment

Not applicable.

4.0 SAMPLE-SIZE DETERMINATION

The study is designed to have approximately 18 evaluable participants complete all treatment periods of the study.

Sample size calculations was performed using R package PowerTOST based on the following:

- *The intra-participant CV was assumed at ~0.4 for C_{max} and AUC, which were estimated based on data in Study CEC-2 (a completed Phase 1 Multiple Ascending Dose study)*
- *Reference data set is C_{max} or AUC under fasting condition; test data set is C_{max} or AUC under fed conditions*
- *If the observed geometric least-squares mean ratio (GMR) is at 0.90 or 1.10, the estimated 90% confidence interval (CI) of the GMR, with N = 18, is within (0.70, 1.43) for both C_{max} and AUC*

Accounting for possible dropouts, a total of 24 participants may be enrolled.

5.0 ANALYSIS SETS

5.1 Safety Set

All participants who received at least one dose of the study drug(s) will be included in the safety evaluations.

5.2 PK Set

All participants who comply sufficiently with the protocol and display an evaluable PK profile (eg, exposure to treatment, availability of measurements and absence of major protocol violations) will be included in the PK analyses.

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6.0 STATISTICAL ANALYSIS

6.1 General Considerations

All PK analyses will be conducted using Phoenix® WinNonlin® Version 8.3.4, or higher. All statistical analyses will be conducted using SAS® Version 9.4. All relevant safety data will be listed by participant and treatment. All table, figure, and listing (TFL) shells and numbering list will be included and specified in the TFL Shells document.

The number of observations (n) will be presented as an integer (no decimal places), arithmetic mean (mean), median, and geometric mean (geom mean) values will be presented to 1 more level of precision than the individual values. Standard deviation (SD) and standard error of the mean (SEM) will be presented to 2 more levels of precision than the individual values. Minimum and maximum values will be presented to the same precision as the individual values. Arithmetic percent coefficient of variation (CV%) and geometric percent coefficient of variation (geom CV%) will be presented to 1 decimal place.

Geometric least-squares means (LSMs) will be reported with 1 more level of precision than the individual data. Geometric LSM ratios (GMRs) and 90% confidence intervals (CIs) for the GMRs will be reported to 2 decimal places. Intra-participant CVs will be reported to 1 decimal place.

Noncompartmental analyses will be used in this study. Concentration values below the lower limit of quantitation (BLQ) will be presented as 'BLQ' in the concentration table listings and footnoted accordingly. BLQ values will be treated as zero for the calculation of summary statistics, the generation of concentration plots, and the calculation of PK parameters, unless they are deemed questionable (eg, BLQ value between measurable values), in which case they will be treated as missing and excluded from the concentration summary statistics and the PK analysis. Values of 0 are not included in the calculation of geom mean and geom CV%.

A participant's PK parameter data will be included in the listings but may be excluded from the descriptive and inferential statistics if one or more of the following criteria are met:

- A predose (0 hour) concentration is greater than 5% of that participant's C_{max} value for the same treatment
- A participant did not meet inclusion/exclusion criteria that may have an effect on the PK (as determined by the Takeda Pharmacology Lead and Celerion Pharmacokinetic Scientist)
- A participant deviates substantially from the protocol defined study procedures including but not limited to dosing, dose timing, sample collection, meal timing, etc. (as determined by the Takeda Clinical Pharmacology Lead and Celerion Pharmacokinetic Scientist)
- A participant may be excluded due to vomiting within twice the median t_{max} of TAK-227 (2 × 2 hours = 4 hours)

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The details on PK parameter calculations and TFLs will be outlined in the clinical pharmacology and analysis plan (CPAP) and TFL Shells document including specifics on the following:

- Insufficient data to determine a reliable $t_{1/2z}$ value and other λ_z-dependent parameters
- PK parameters presented by treatment, including the units, precision, and summary statistics that will be presented in in-text and end-of-text tables
- Concentration data presented by treatment, including the units, precision, and summary statistics that will be presented in end-of-text tables
- Concentration data file used for PK analysis
- PK parameter Phoenix® WinNonlin® output file used to generate the TFLs
- Linear mixed-effect model and non-parametric statistical analysis results presented in in-text and end-of-text tables
- Arithmetic mean concentration-time figures presented as in-text and end-of-text figures
- Listings of concentration data for individual participants in Appendix 16.2.5.

- Individual concentration-time figures presented in Appendix 16.2.6.

Continuous demographic and safety data will be summarized descriptively. For categorical variables, the count and percentages of each possible value will be tabulated, where applicable. The denominator for the percent calculation will be the number of participants in the safety set for overall summaries, and the number of participants dosed with each treatment in by-treatment summaries. For continuous variables, n, mean, SD, minimum, median, and maximum values will be tabulated. The level of precision will be presented as follows: minimum/maximum in the same precision as in the database, mean/median in one more precision level than minimum/maximum, SD in one more precision level than mean/median, and n will be presented as an integer. Counts and percentages will be presented as integers. Baseline is defined as the last observation prior to dosing in each period, unless specified otherwise.

6.1.1 Handling of Treatment Misallocations

Participants with any treatment misallocations will be analyzed based on the treatment the participants actually received.

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6.2 Study Information

A study information table will be generated including the following items: date of first participant's signed informed consent form (ICF), date of dosing, date of last participant's last visit/contact, date of last participant's last procedure for collection of data for primary endpoint, the version of Medical Dictionary for Regulatory Activities (MedDRA®), the version of World Health Organization (WHO) Drug Dictionary, and SAS version used for creating the datasets.

6.3 Disposition of Participants

Disposition of participants (number of participants dosed, completed the study, discontinued from the study and/or study drug, and reason(s) for discontinuation(s)) will be summarized by randomized treatment sequence and overall. Study completion status, including reason for discontinuation of study and/or study drug, will be listed by participant.

6.4 Demographic and Other Baseline Characteristics

6.4.1 Demographics

Demographic and baseline characteristics will be summarized by randomized treatment sequence and overall based on the safety set. Summary statistics (n, mean, SD, minimum, median, and maximum) will be generated for continuous variables (age, weight, height, and body mass index [BMI]) and the number and percentages of participants within each category will be presented for categorical variables (sex, race, and ethnicity). Height, weight, and BMI measured at screening will be used in the summaries. Other demographic data will also be listed as recorded on the CRF, including date of informed consent and protocol version.

6.4.2 Medical History and Concurrent Medical Conditions

Medical history will include determining whether the participant has any significant conditions or diseases that resolved at or before signing the ICF. All medical history reported by the participant will be recorded regardless of when it may have occurred. Concurrent medical conditions are those significant ongoing conditions or diseases that are present at signing the ICF. Each participant's medical history and concurrent medical conditions will be listed.

Any medical condition starting or worsening after taking the first dose of study drug will be classified as a TEAE. All medical history will be coded using MedDRA® version specified in the data management plan (DMP). If available, the medical history and concurrent medical condition listings will include the coded term (preferred term and system organ class [SOC]), start date (if known) and end date (if known) or whether the condition was ongoing, and a description of the condition or event. No summaries or statistical analysis will be performed for these data.

6.5 Medication History and Concomitant Medications

Medication history includes any relevant medication stopped at or within 28 days before signing the ICF. Concomitant medication includes any medication other than the study drug taken at any time between screening and the end of the study (including follow-up contact). All medication history and concomitant medications recorded during the study will be coded with the WHO Drug Dictionary version specified in the DMP and listed. If available, the listings will include the medication name, coded term, dosage, route of administration, start date and time (if known), end date and time (if known), or whether it continued after study completion, and indication for use. No summaries or statistical analysis will be performed for these data.

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6.6 Efficacy Analysis

Not applicable.

6.7 Safety Analysis

Safety will be evaluated by the incidence of TEAEs, severity and relationship(s) of TEAEs, and changes from baseline in the participants' clinical laboratory results, vital signs, and 12-lead ECGs using the safety set. Clinically significant laboratory values and vital signs will be reported as AEs, as applicable. All safety data will be listed by participant, treatment, and assessment time points, including rechecks, unscheduled assessments, and early termination (ET), chronologically.

Continuous variables will be summarized using n, mean, SD, minimum, median, and maximum. Frequency counts and percentages will be reported for categorical data when appropriate. Where individual data points are missing because of dropouts or other reasons, the data will be summarized based on reduced denominators. Postdose recheck, unscheduled, or ET results will not be used in summaries.

Tables summarizing safety data by assessment time point will only include summaries for baseline and post-baseline time points.

6.7.1 Adverse Events

All AEs captured in the database will be listed in by-participant data listings including verbatim term, coded term, severity (mild, moderate, severe), relationship to study drug (related or not related), relationship to COVID-19 and COVID-19 vaccine, frequency, and action relative to the study drug as recorded in the CRF. Study procedure taken due to AE will also be listed. All AEs occurring during this study will be coded using the MedDRA® version specified in the DMP. Only TEAEs will be summarized.

A TEAE is defined as an AE that is starting or worsening at the time of or after the first dose of study drug. Each TEAE will be attributed to the treatment prior to and the closest to the AE based on the AE onset date and time.

If the onset time of an AE is missing and the onset date is the same as a treatment dosing date, then the AE will be counted under the treatment given on the same day. If onset time of an AE is missing and the onset date does not fall on a dosing date, then the AE will be considered treatment emergent for the most recent treatment administered. If the onset date of an AE is missing, then the AE will be considered treatment emergent and attributed to the first treatment received. If severity is missing, the AE will be counted as severe, and if relationship is missing, the AE will be counted as related.

TEAEs will be tabulated by treatment (including overall), SOC, and preferred term. Summary tables will include number of participants reporting the TEAE as percent of safety set by treatment and overall. The most commonly reported non-serious TEAEs (i.e., those events reported by >5% of participants in any treatment, excluding serious adverse events (SAEs) will also be summarized. The denominators for percent calculations will be the number of participants dosed for each treatment. In addition, TEAEs will be summarized as number of TEAEs and percentage of TEAEs for each treatment and overall.

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Additional TEAE summary tables will be presented by severity and relationship to study drug. If a participant has multiple TEAEs with different severity levels within the same preferred term, the participant will be counted in the most severe category only. For relationship to study drug, if a participant has both related and unrelated TEAEs with the same preferred term, the participant will be counted as having related TEAEs.

An overview summary of TEAEs table, including number of participants with TEAEs, treatment-emergent SAEs, treatment-related TEAEs, treatment-related SAEs, TEAEs by severity, and AEs leading to discontinuation will be provided.

Should any SAEs (including all-cause mortalities) occur, they will be summarized the same way as TEAEs. All AEs will be displayed in the data listings and TEAEs will be discussed in the text of the clinical study report (CSR).

6.7.2 Adverse Events of Special Interest

Not applicable.

6.7.3 Clinical Laboratory Evaluation

Clinical laboratory tests will be measured as described in Table 6.a:

Table 6.a Collection of Laboratory Samples

Clinical Laboratory Panels	Time Point		
	Period	CRF/Listing Day and Hour	Table
Serum Chemistry, Hematology, Urinalysis	Screening		NA
	1	Day -1 PREDOSE Day 2 Hour 24	Baseline Period 1 Day 2
	2	Day 2 Hour 24	Period 2 Day 2
	3	Day 2 Hour 24	Period 3 Day 2

Time points in the CRF/Listing column are based on the protocol, and it should be noted that the data listings will reflect the data found in the final participant CRFs.
If applicable, an early termination assessment will be performed.
NA = Not Applicable

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For all numeric values of laboratory test results, summary statistics (n, mean, SD, minimum, median, and maximum) will be presented by randomized sequence at each scheduled visit using the units found in the source data. Change from baseline will be summarized in a similar manner. Baseline is defined as the last assessment including rechecks taken prior to the first dose in Period 1. The mean value calculated for each assessment time point will be compared to the reference range and flagged if outside of the reference range (* if above the reference range and ^ if below the reference range). In the event there is more than one reference range for a laboratory test due to sex or age, the comparison will be made against the lowest of the lower ranges and the highest of the higher ranges. Postdose unscheduled, recheck, or ET assessments will not be used in summaries. Only baseline and post-baseline time points will be summarized. All clinical laboratory data will be listed by participant. Urine drug screen will be performed at screening and check-in, and results will be listed by participant.

Out-of-normal range flags will be recorded as high (H) and low (L) for numerical results and did-not-match (*) for categorical results. For each laboratory test, a shift table will be developed comparing the frequency and percentage of the results at baseline (above reference range (H), within reference range (N), or below reference range (L)) with the postdose time points. For urinalysis tests, the categories are normal (N) and abnormal (A). Out-of-range values and corresponding recheck results will be listed.

Urine drug screen will be performed at screening and check-in. Results will be listed by participant.

6.7.4 Vital Signs

Vital signs will be measured as described in Table 6.b:

Table 6.b Collection of Vital Signs

Parameter	Time Point		
	Period	CRF/Listing Day and Hour	Table
Blood Pressure, Heart Rate, Respiration, Temperature	Screening		NA
	1, 2, 3	Day 1 PREDOSE	Baseline
		Day 1 Hour 2	Hour 2
		Day 2 Hour 24	Hour 24

Time points in the CRF/Listing column are based on the protocol, and it should be noted that the data listing will reflect the data found in the final participant CRFs.
If applicable, an early termination assessment will be performed.
NA = Not applicable

Summary statistics (n, mean, SD, minimum, median, and maximum) will be presented for vital sign results by treatment and time point. Change from baseline will be summarized in a similar manner. Baseline is defined as the last assessment including rechecks taken prior to dosing in each treatment. Postdose unscheduled or recheck assessments, or ET results, will not be used in analysis. Only baseline and postdose results will be summarized. Vital sign data will be listed by participant.

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6.7.5 12-Lead ECG

Single 12-lead ECGs will be measured as described in Table 6.c:

Table 6.c Collection of ECGs

Parameter	Time Point		
	Period	CRF/Listing Day and Hour	Table
HR, RR, PR, QRS, QT, QTcF	Screening		NA
	1, 2, 3	Day 1 PREDOSE	Baseline
		Day 1 Hour 2	Hour 2
		Day 2 Hour 24	Hour 24

Time points in the CRF/Listing column are based on the protocol, and it should be noted that the data listing will reflect the data found in the final participant CRFs.
If applicable, an early termination assessment will be performed.
NA = Not applicable

Summary statistics (n, mean, SD, minimum, median, and maximum) will be presented for ECG results by treatment and time point. Change from baseline will be summarized in a similar manner. Baseline is defined as the last assessment including rechecks taken prior to dosing in each treatment. Postdose unscheduled or recheck assessments, or ET results, will not be used in analysis. Only baseline and postdose results will be summarized. ECG data will be listed by participant.

6.7.6 Physical Examinations

Full physical examinations will be performed at screening, prior to dosing in each period, and 24 hours postdose in Period 3, or early termination. Additional physical examinations may be performed at other times at the discretion of the Investigator. Physical examination findings will be presented in the data listings by participant.

6.7.7 Overdose

All cases of overdose will be presented in a data listing by participant. Any AEs associated with overdose will be documented on the AE page of the CRF.

6.7.8 Extent of Exposure and Compliance

The dates, times, and doses of TAK-227 will be listed by participant and study period. Dates and times of critical meals for the fed treatments will also be listed.

6.8 Pharmacokinetic Analysis

Blood samples for assessment of plasma concentrations of TAK-227 and its metabolites will be collected as outlined in Table 6.d below:

Table 6.d Collection of Blood Samples for Pharmacokinetic Analysis

Analytes	Matrix	Scheduled Time (Hours)*
TAK-227 and metabolites	Plasma	Predose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 36 hours postdose.

*The actual date and time of sample collection will be recorded on the source document in the case report form.

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Plasma concentrations of TAK-227 and its metabolites will be listed and summarized descriptively by PK sampling time and treatment using the following descriptive statistics: n, mean, SD, CV%, SEM, minimum, median, and maximum. Excluded concentrations will be presented and footnoted as such in the concentration table listings, and those values will be excluded from the descriptive statistics.

Individual participant concentration-time curves will be plotted by treatment on linear and semi-log scales. The arithmetic mean profiles of the concentration-time data will be plotted by treatment on linear (with and without SD) and semi-log scales. For summary statistics and arithmetic mean plots by sampling time, the nominal PK sampling time will be used. For individual participant plots by time, the actual PK sampling time will be used.

PK parameters will be calculated from plasma concentration-time profiles using non-compartmental analysis methods where all calculations will be based on actual sampling times after dosing. The PK parameters will be summarized by treatment using the following descriptive statistics: n, mean, SD, CV%, SEM, minimum, median, maximum, geom mean, and geom CV%. Excluded parameters will be presented and footnoted as such in the PK parameter table listings, and those values will be excluded from descriptive statistics.

Food-Effect Estimation

A linear mixed-effects model will be applied to natural log-transformed C_{max}, AUC_{last}, AUC_{∞}, and AUC_{∞_pred} for TAK-227 with treatment, period, and sequence as fixed effects, and participant within sequence as a random effect. Point estimates and their associated 90% CIs will be constructed for the differences between Treatment B (fed predose) versus Treatment A (fasting), and Treatment C (fed postdose) versus Treatment A (fasting). The point estimates and their associated 90% CIs will be then back transformed to provide point estimates and 90% CIs for the ratios of Treatment B (fed predose) versus Treatment A (fasting) and Treatment C (fed postdose) versus Treatment A (fasting).

The food-effect estimation will be performed using the following SAS code:

```
PROC MIXED;
CLASS TREAT PERIOD SEQUENCE PARTICIPANT;
MODEL LN(PARAM) = TREAT PERIOD SEQUENCE / DDFM = KR;
RANDOM PARTICIPANT(SEQUENCE);
ESTIMATE “Treatment B vs Treatment A” TREAT -1 1 0 / CL ALPHA=0.1 E;
ESTIMATE “Treatment C vs Treatment A” TREAT -1 0 1 / CL ALPHA=0.1 E;
LSMEANS TREAT;
RUN;
```

Non-Parametric Analysis

Analysis of t_{max} and t_{lag} will be performed by nonparametric Wilcoxon Signed-Rank test. The Hodges-Lehmann method and Walsh averages will be used to estimate the median difference between treatments. The t_{max} and t_{lag} parameters will not be ln-transformed. The comparisons of interest are the same as in the linear mixed-effects model.

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6.9 Patient Reported Outcomes and Health Care Utilization Endpoints Analysis

Not applicable.

6.10 Interim Analysis

Not applicable.

6.11 Preliminary Analysis

A preliminary PK analysis will be completed as described in the CPAP and Section 6.8 of the statistical analysis plan (SAP), with the following changes: 1) Quality-controlled data (not quality-assured data) will be used; 2) nominal times (not actual sampling times) will be used to calculate PK parameters; 3) tables and figures will be created using Phoenix® WinNonlin® Version 8.3.4 or higher.

6.12 Data Monitoring Committee/Internal Review Committee/ [Other Data Review Committees]

Not applicable.

7.0 REFERENCES

Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Guidance for Industry: Assessing the Effects of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations. June 2022. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/assessing-effects-food-drugs-inds-and-ndas-clinical-pharmacology-considerations.

8.0 CHANGES TO PROTOCOL PLANNED ANALYSES

Additional PK parameters will be calculated as listed in Section 1.2.4.

9.0 APPENDIX

9.1 Changes From the Previous Version of the SAP

Not applicable.

9.2 Data Handling Conventions

Not applicable.

9.3 Analysis Software

SAS® Version 9.4 or higher will be used for all statistical analysis provided in the CSR.

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