Clinical Study Protocol

NCT Number: NCT05818956

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

Study Number: TAK-227-1001

Document Version and Date: Amendment 1.0, 15 February 2023

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TAKEDA PHARMACEUTICALS

PROTOCOL

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

Study Identifier: TAK-227-1001

Compound: TAK-227

Date: 15 February 2023

Version/Amendment Number: Amendment 1 Protocol

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Amendment History

Date	Amendment Number	Amendment Type	Region
14 November 2022	Initial Version		United States
15 February 2023	01	Not Applicable	United States

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1.0 STUDY SUMMARY

Name of Sponsor: Takeda Development Center Americas, Inc. (TDCA) 95 Hayden Avenue Lexington, Massachusetts 02421 Telephone: +1 (617) 679-7000	**Compound:** TAK-227
Study Identifier: TAK-227-1001	**Phase:** 1
Protocol 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	
Study Design: This is a single-center, open-label, single-dose, randomized, 3-period, 6-sequence, crossover study in healthy adults. Study schematic and dose regimens are shown in Table 2.a and Table 2.b. The schedule of assessments is shown in Schedule of Study Procedures (Section 3.0). On Day 1 of Treatment Period 1 participants will be randomly assigned to one of 6 possible treatment sequences (Table 8.a). On Day 1 of each treatment period, a single dose of TAK-227 will be administered orally under one of 3 different feeding conditions as per the randomization schedule: • Fasting (Treatment A), • Fed pre-dose, high-fat/high-calorie meal administered 30 minutes prior to dosing (Treatment B), and • Fed post-dose, high-fat/high-calorie meal administered ~30 minutes after dosing (Treatment C). For details on food and water consumption in the different treatments refer to Section 7.4.1 and Section 9.2.6. There will be a washout period of not less than 4 days between TAK-227 dosing in each period. Pharmacokinetic (PK) sample collections will be conducted pre-dose and up to 36 hours post-dose in each treatment period. Safety and tolerability will be assessed throughout the study by treatment-emergent adverse events (TEAEs), vital signs, electrocardiograms (ECGs), clinical laboratory evaluations, and physical examinations. 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.	
Study Primary Objective: • To assess the 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. **Study Secondary Objectives:** • 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. **Study Exploratory Objective:** • To evaluate other TAK-227 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.	

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Study Participant Population: Healthy male and female participants aged 18 to 55 years inclusive, at screening. Body Mass Index (BMI) 18.0-32.0 kg/m^2, inclusive, at screening.	
Planned Number of Participants: Twenty-four (24) participants may be enrolled	**Planned Number of Sites:** 1
Dose Levels: 50 mg TAK-227 capsule	**Route of Administration:** Oral
Duration of Treatment: Single-dose	**Planned Study Duration:** Approximately 40 days including screening period of up to 28 days and follow-up.

Criteria for Inclusion:

Participants must fulfill the following inclusion criteria to be eligible for participation in the study:

1. Healthy, adult, male or female, 18-55 years of age, inclusive, at screening.

   * Females of childbearing potential are defined as all females physiologically capable of becoming pregnant.

   Females of non-childbearing potential are defined as follows:

   - Females who have undergone one of the following sterilization procedures at least 6 months prior to the first dosing:
     - Hysteroscopic sterilization.
     - Bilateral tubal ligation or bilateral salpingectomy.
     - Hysterectomy.
     - Bilateral oophorectomy

   or

   - Females who are postmenopausal with amenorrhea for at least 12 month prior to the first dosing and follicle-stimulating hormone (FSH) serum levels consistent with postmenopausal status at screening.
2. BMI ≥ 18 and ≤ 32.0 kg/m^2 at screening visit.
3. Medically healthy with no clinically significant medical history, physical examination, laboratory profiles, vital signs or ECGs, as deemed by the Investigator or designee, including the following:
   - Seated blood pressure ≥ 90/40 mmHg and ≤ 140/90 mmHg at screening visit.
   - Seated heart rate ≥ 40 bpm and ≤ 99 bpm at screening visit.
   - QTcF interval ≤ 460 msec (males) or ≤ 470 msec (females) and has ECG findings considered normal or not clinical significance by the Investigator or designee at screening visit.
   - Estimated creatinine clearance ≥ 90 mL/min at screening visit.
   - Total bilirubin <1.25 × ULN at screening visit unless the participant has known Gilbert's syndrome that can explain the elevation of bilirubin.
   - Serum alanine aminotransferase (ALT) <1.25 × ULN at screening visit.
   - Creatinine ≤1.1 × ULN at screening visit.
4. A female of childbearing potential who is sexually active with a nonsterilized male partner agrees to use a highly effective/effective method of contraception from signing of the informed consent form (ICF) throughout the duration of the study and for 30 days after the last dose of study drug as described in Appendix D.
5. A male participant who is nonsterilized and sexually active with a female partner of childbearing potential agrees to use highly effective method of contraception from signing of the ICF throughout the duration of the study and for 100 days after last dose of study drug as described in Appendix D.
6. If male, must agree not to donate sperm from the first dosing until 100 days after the last dosing.
7. Continuous non-smoker who has not used nicotine and tobacco containing products for at least 3 months prior to the first dosing based on participant self-reporting.

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8. Understands the study procedures in the ICF, and be willing and able to comply with the protocol.

Criteria for Exclusion:

Participants must not be enrolled in the study if they meet any of the following criteria:

1. Is mentally or legally incapacitated or has significant emotional problems at the time of the screening visit or expected during the conduct of the study.
2. History or presence of clinically significant medical or psychiatric condition or disease in the opinion of the Investigator or designee.
3. History of any illness that, in the opinion of the Investigator or designee, might confound the results of the study or poses an additional risk to the participant by their participation in the study.
4. Drink alcohol in excess of 21 units per week for males or 14 units per week for females, with one unit = 150 mL of wine or 360 mL of beer or 45 mL of 45% alcohol.
5. History of alcoholism or drug abuse within the past 2 years prior to the first dosing.
6. Positive urine drug or alcohol results at screening or first check-in.
7. History or presence of hypersensitivity or idiosyncratic reaction to the study drug(s) or related compounds.
8. History or presence of Celiac disease (CeD) or other gastrointestinal inflammatory disorder such as inflammatory bowel disease or eosinophilic disorder.
9. History of lactose intolerance.
10. Female participants with a positive pregnancy test at screening visit or first check-in or who are lactating.
11. Positive results at screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV).
12. Unable to refrain from or anticipates the use of:
    - Any drug, including prescription and non-prescription medications, herbal remedies, or vitamin supplements within 14 days prior to the first dosing and throughout the study. Medication listed as part of acceptable birth control methods will be allowed (refer to Appendix D). Thyroid hormone replacement medication may be permitted if the participant has been on the same stable dose for the immediate 3 months prior to first study drug administration. Hormone replacement therapy will also be allowed.
    - Any drugs known to be significant inducers or inhibitors of Cytochrome P450 (CYP)3A4 enzymes and/or P-gp, including St. John's Wort, within 28 days prior to the first dosing and throughout the study. Appropriate sources (eg, Flockhart Table™) will be consulted to confirm lack of PK/pharmacodynamics interaction with study drug.
    - Chronic use of non-steroidal anti-inflammatory (define as more that 7 days of use) within 2 weeks prior to screening and throughout the study.
13. Has been on a diet incompatible with the on-study diet, in the opinion of the Investigator or designee, within the 30 days prior to the first dosing and throughout the study.
14. Donation of blood or significant blood loss within 56 days prior to the first dosing.
15. Plasma donation within 7 days prior to the first dosing.
16. Participation in another clinical study within 30 days prior to the first dosing. The 30-day window will be derived from the date of the last blood collection or dosing, whichever is later, in the previous study to Day 1 of Period 1 of the current study.
17. The participant is an immediate family member, study site employee, or is in a dependent relationship with a study site employee who is involved in conduct of this study (eg, spouse, parent, child, sibling) or may consent under duress.

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Main Criteria for Evaluation and Analyses:

Primary Endpoint:

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

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, ECGs, and clinical laboratory results (hematology, chemistry, and urinalysis) from baseline to post-baseline time points, and findings from physical examinations.

Exploratory Endpoints:

The exploratory endpoint 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)
- Area under the concentration-time curve (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:

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

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Statistical Considerations:

Pharmacokinetic analysis:

Individual TAK-227 and its metabolites plasma concentrations and PK parameters will be listed by participant and treatment, and summarized by treatment (TAK-227 plasma concentrations and PK parameters) and nominal time (TAK-227 plasma concentrations).

PK parameters (C_{max}, AUC_{last}, AUC_{∞}, $AUC_{extrap\%}$, t_{max}, λ_z, $t_{½z}$, CL/F, V_z/F, and t_{lag}) will be computed using standard non-compartmental analysis (NCA). Actual time of sampling will be used to calculate the PK parameters.

All individual concentration data and PK parameters will be listed with summary statistics such as geometric mean, median, arithmetic mean, standard deviation (SD), coefficient of variation (CV), minimum and maximum. Individual plasma concentration/time curves will be presented in linear/linear and log/linear scale. At least 3 PK time points within the terminal log-linear phase will be used to estimate the terminal rate constant for each participant in each treatment period. The appropriate PK analysis will be fully specified in the Clinical Pharmacology Analysis Plan (CPAP).

TAK-227 metabolites PK may be reported separately from the clinical study report as a standalone document.

A linear mixed-effect model will be applied to log-transformed C_{max}, AUC_{last}, and AUC_{∞} 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% confidence intervals (CIs) will be constructed for the differences between Treatment B (fed pre-dose) versus Treatment A (fasting), and Treatment C (fed post-dose) 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 pre-dose) versus Treatment A (fasting) and Treatment C (fed post-dose) versus Treatment A (fasting).

Analysis of t_{max} and t_{lag} will be performed by nonparametric Wilcoxon Signed Rank test.

Safety analysis:

The safety data will be summarized descriptively for TEAEs, and potentially clinically significant values for clinical laboratory tests, vital signs, 12-lead ECG, and use of concomitant medications by treatment.

Sample Size Justification:

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

Sample size calculations were 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
- 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 mean ratio (GMR) is at 0.90 or 1.10, the estimated 90% 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.

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2.0 STUDY SCHEMATIC

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 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 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.

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Table 2.b 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 pre-dose: after a high-fat/high-calorie meal [b], single dose, oral	Day 1
Treatment C	TAK-227 capsule	50 mg	Fed post-dose: 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.

3.0 SCHEDULE OF STUDY PROCEDURES

Study Procedures [a]	S [b]	Study Days in Each of the 3 Treatment Periods																			ET [c]	FU [d]
Days →		-1	1															2		3-4 e		
Hours →		C-I [f]	0	0.25	0.5	0.75	1	1.25	1.5	2	3	4	5	6	8	10	12	24	36	Washout		
Administrative Procedures																						
Informed Consent	X																					
Inclusion/Exclusion Criteria	X	X g																				
Medical History	X																					
Demographics	X																					
Safety Evaluations																						
Physical Examination	X		X h															X i			X	
Height and Weight	X	X g,j																				
Vital Signs (PR, BP, T, and RR)	X		X h							X								X			X	
12-Lead Safety ECG	X		X h							X								X			X	
Hem, Serum Chem [k], and UA	X	X																X			X	
Serum Pregnancy Test (♀only)	X	X																				
Serum FSH (PMP ♀ only)	X																					
Urine Drug and Alcohol Screen	X	X g																				
HIV/Hepatitis Screen	X																					
AE Monitoring	X	<---------- X ---------->																				X
ConMeds Monitoring	X	<---------- X ---------->																				X
Study Drug Administration / PK																						
TAK-227 Administration			X																			
Blood for TAK-227 and metabolites PK			X l	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			

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Study Procedures [a]		**Study Days in Each of the 3 Treatment Periods**																			**ET** [c]	**FU** [d]
Days →	**S** [b]	**-1**	**1**															**2**		**3-4** [e]		
Hours →		**C-I** [f]	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**	**24**	**36**	**Washout**		
Other Procedures																						
Confinement in the CRU		<---------- X ---------->																				
Visit	X																					

a. The study procedures will be detailed in Section 9.0.
b. Screening will start within 28 days prior to the first TAK-227 administration on Day 1 of Treatment Period 1.
c. To be performed prior to early termination from the study.
d. 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 ConMeds 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.
e. There will be a washout of not less than 4 days between TAK-227 dosing in each treatment period. Day 4 of Treatment Periods 1 and 2 may be the same as Day -1 of Treatment Periods 2 and 3, respectively.
f. Participants will be admitted to the CRU on Day -1 of Treatment Period 1, at the time indicated by the CRU, until completion of study procedures on Day 2 of Treatment Period 3.
g. To be performed in Treatment Period 1 only.
h. To be performed within 24 hours prior to TAK-227 dosing.
i. To be performed in Treatment Period 3 only.
j. Weight only.
k. Samples for serum clinical chemistry will be obtained following a fast of at least 8 hours, however, in case of dropouts or rechecks, participants may not have fasted for 8 hours prior to the serum chemistry sample is taken.
l. To be performed prior to the dosing.

Abbreviations: ♀ = Females, AE = Adverse events, BP = Blood pressure, C-I = Check-in, Chem = Chemistry, ConMeds = Concomitant medication, CRU = Clinical research unit, ECG = Electrocardiogram, ET = Early termination, FSH = Follicle-stimulating hormone, FU = Follow-up, Hem = Hematology, HIV = Human immunodeficiency virus, PK = Pharmacokinetics, PMP = Premenopausal, PR = Pulse rate, RR = Respiration rate, S = Screening, T = Temperature, UA = Urinalysis.

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4.0 INTRODUCTION

4.1 Background

Celiac disease (CeD) is a common intestinal inflammatory disease characterized by an altered immune response to dietary wheat gluten and related proteins from rye and barley. CeD is triggered and maintained in genetically predisposed patients carrying human lymphocyte antigen (HLA) type DQ2 or DQ8, and it is dominated by an acquired T cell mediated immune response to gluten. Currently the only therapeutic option is a strictly followed gluten-free diet (GFD), and many patients do not achieve symptomatic and histologic remission with dietary modification alone. Thus, there is a great unmet medical need for a pharmacological treatment of CeD.

Extracellular transglutaminase 2 (TG2) in the small intestinal mucosa plays a key role in the pathogenesis of CeD. TG2 can either cross-link or deamidate glutamine-residues in gliadin peptides derived from gluten. The deamidated or cross-linked peptides bind strongly to the HLA types DQ2 or DQ8 which results in T helper 1 T-cell activation, inflammation and mucosal destruction. Given its pivotal role in the pathophysiology of this disease, TG2 is a rational target for therapeutic approaches in CeD. Inhibition of the enzymatic activity of TG2 would prevent deamidation of gliadin and could therefore block an initial and crucial step in CeD pathogenesis.

The compound TAK-227 (also known as ZED1227) is a newly developed peptidomimetic showing an efficient and highly selective inhibition of TG2. In preclinical and early clinical studies, TAK-227 demonstrated a favorable safety profile, justifying its further clinical development.

Through to 8-Sep-2022, a total of 270 healthy volunteers and participants with CeD were exposed to TAK-227 in 5 completed clinical studies: Phase 1 single ascending dose study in healthy participants (CEC-1), Phase 1 multiple ascending dose study in healthy participants (CEC-2), Phase 2a efficacy and tolerability, proof-of-concept, study in participants with well-controlled CeD undergoing gluten challenge (CEC-3), Phase 1 PK study in healthy participants (CEC-5), and Phase 1 mass balance study in healthy participants (CEC-6). A total of 26 additional participants have received either TAK-227 or placebo in the double blinded ongoing Phase 2b dose-finding study of efficacy and tolerability in adults with CeD experiencing symptoms and with intestinal injury despite gluten-free diet (CEC-4).

Following single administration of 5 to 500 mg doses and multiple dose administration of 10 to 100 mg doses, TAK-227 was rapidly absorbed, reaching peak plasma concentrations in approximately 1 to 2 hours. Multiple dose administration had no relevant effect on exposure at the dose levels 10, 20, and 100 mg, and elimination half-life ranged between 4 to 9 hours post-dose.

Following single dose administration in rats and minipigs, TAK-227 displayed low absolute bioavailability (1.5% to 4% in rat and 3.2% to 6.0% in minipigs). Due to the large differences in abundance of the biotransformation products detected in vivo to the in vitro profile of TAK-227 in hepatocytes, it is currently assumed that the biotransformation of TAK-227 occurs mainly extra-hepatically. The metabolism of TAK-227 in human liver microsomes was significantly

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reduced in the presence of the CYP3A4 inhibitor ketoconazole, with the conclusion of a possible drug-drug interaction risk due to inhibition of CYP3A4 in the liver and the intestines.

In the course of the two Phase 1 studies (CEC-1; CEC-2) no serious adverse events (SAEs) have been documented. In the recently completed phase 2a study CEC-3, a total of 4 SAEs were reported. Two (2) out of 4 were reported as suspected unexpected serious adverse reactions (SUSARs). The remaining 2 were reported after completion of study and they were considered unrelated to TAK-227.

The majority of adverse drug reactions were observed in the system organ classes Gastrointestinal Disorders and Nervous System Disorders. They were mostly mild in nature, with the most frequent events being diarrhea and headaches. Overall, the Phase 1 studies in healthy volunteers and the Phase 2 study in CeD patients demonstrated a favorable safety profile, which was comparable to placebo.

Refer to the Investigator's Brochure (IB) for detailed background information on TAK-227.

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4.2 Rationale for the Proposed Study

The purpose of this single-center, open-label, single-dose, randomized, 3-way crossover study in healthy adult participants is to determine if the systemic exposure to TAK-227 will be altered when dosed with a high-fat/high-calorie meal or under fasting conditions. TAK-227 is currently administered 30 minutes prior to a meal to allow for local drug exposure prior to any dietary gluten intake. This study will evaluate a potential food effect scenario if the dosing is conducted prior to a meal (current dosing conditions are followed; Treatment C) and following a meal (current dosing conditions are not followed; Treatment B). Enrolled participants will each receive a single oral dose of TAK-227 in each of the 3 treatment periods. The dosing in each treatment period will be separated by a washout period of not less than 4 days (single-dose plasma TAK-227 half-life of 5 to 8 hours). Participants will be dosed at approximately the same time each morning on Day 1 of each treatment period, with the dosing of each participant timed for sequential PK sampling. Thus, TAK-227 PK will be evaluated under 3 different feeding conditions:

- Fasting (Treatment A)
- Fed pre-dose, high-fat/high-calorie meal administered 30 minutes prior to dosing (Treatment B)
- Fed post-dose, high-fat/high-calorie meal administered ~30 minutes after dosing (Treatment C)

The high-fat/high-calorie meal should contain in total 800-1000 kCal with 500-600 kCal from fat, 250 kCal from carbohydrates, and 150 kCal from proteins [FDA, 2022].

4.3 Benefit/Risk Profile

The 50 mg dose of TAK-227 will be administered according to the adult dosing regimen used in the previous studies, as detailed in the IB.

There will be no direct health benefit for study participants from receipt of TAK-227. An indirect health benefit to the healthy participants enrolled in this study is the free medical tests received at screening and during the study.

The inclusion and exclusion criteria, screening, and safety monitoring practices employed in this protocol (ie, 12-lead ECG, vital signs, clinical laboratory tests, AE monitoring, and physical examination) are considered adequate to protect the participant's safety and should detect all TEAEs.

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 Hypothesis

Not applicable

5.2 Study Objectives

5.2.1 Study Primary Objective

- To assess the 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.

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5.2.2 Study 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.

5.2.3 Study Exploratory Objectives

- To evaluate other pharmacokinetic 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.

5.3 Endpoints

5.3.1 Primary Endpoint

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

5.3.2 Secondary Endpoints

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

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

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5.3.3 Exploratory Endpoint

The exploratory endpoint will be assessed through evaluation of the following PK parameters for TAK-227 and its metabolites in plasma (as 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)
- Apparent clearance after oral administration, calculated using the observed value of the last quantifiable concentration (CL/F)
- Area under the concentration-time curve (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:

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

6.0 STUDY DESIGN AND DESCRIPTION

6.1 Study Design

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

Study schematic and dose regimens are shown in Table 2.a and Table 2.b. The schedule of assessments is shown in Schedule of Study Procedures (Section 3.0).

On Day 1 of Treatment Period 1 participants will be randomly assigned to one of 6 possible treatment sequences (Table 8.a). On Day 1 of each treatment period, a single dose of TAK-227 will be administered orally under one of 3 different feeding conditions as per the randomization schedule:

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

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For details on food and water consumption in the different treatments refer to Section 7.4.1 and Section 9.2.6.

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 pre-dose and up to 36 hours post-dose 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 Investigator's discretion.

6.2 Dose Escalation

Not applicable.

6.3 Stopping Rules

Not applicable.

6.4 Rationale for Study Design, Dose, and Endpoints

6.4.1 Rationale of Study Design

TAK-227 is currently administered 30 minutes prior to a meal to allow for local drug exposure prior to any dietary gluten intake. This study is being conducted to assess the effect of food on the PK profile of TAK-227 in 2 clinical scenarios: either dosing instructions are followed and TAK-227 is administered prior to food (fed post-dose; Treatment C) or dosing instructions are not followed and TAK-227 is administered after the meal (fed pre-dose; Treatment B).

Participants will be randomized to treatment sequences to minimize assignment bias. A crossover design is being used to reduce the residual variability as every participant acts as their own control.

The washout period of not less than 4 days between doses is considered sufficient to prevent carryover effects of the preceding treatment.

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6.4.2 Rationale for Dose

The 50 mg TAK-227 dose was selected because it is the highest single dose currently being evaluated in clinical studies. Single doses of up to 500 mg were evaluated in healthy participants and were found well tolerated. As such, this dose is expected to provide a robust PK profile under the different food condition in this study while providing sufficient safety margin in case of increased exposure either one or more of the treatment conditions.

6.4.3 Rationale for Endpoints

6.4.3.1 Pharmacokinetic Endpoints

The PK endpoints are standard for this type of study.

6.4.3.2 Safety Endpoints

The key safety endpoints are typical for Phase 1 studies and will be assessed through monitoring of TEAEs, vital signs, ECGs, laboratory assessments, and physical examinations.

6.4.4 Future Biomedical Research

No additional analysis is planned to be performed on the PK plasma samples for possible future research. Any additional research on these samples unspecified by this protocol will require approval from the participants.

6.4.5 Critical Procedures Based on Study Objectives: Timing of Procedures

For this study, the critical component is the blood collection for plasma concentrations of TAK-227, and is to be collected as close to the scheduled times defined in this protocol as possible.

6.5 Study Design/Dosing/Procedures Modifications Permitted Within Protocol Parameters

The dose and administration of TAK-227 to any participant may not be modified. If necessary, a participant may be discontinued for the reasons described in Section 7.5 and Section 7.6

6.6 Study Beginning and End/Completion

6.6.1 Definition of Beginning of the Study

The beginning of the study will be defined as the beginning of the screening (ie, signing of the ICF) of the first participant.

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6.6.2 Definition of End of the Study

The end of study is defined as the date of the last scheduled procedure ie, the follow-up contact, as outlined in the Schedule of Study Procedures (Section 3.0).

A participant is considered to have completed the study if the participant completed the last scheduled procedure ie, the follow-up contact shown in the Schedule of Study Procedures (Section 3.0).

6.6.3 Definition of Study Discontinuation

In consultation with the Sponsor, Celerion reserves the right to terminate the study in the interests of participants' welfare.

The Sponsor reserves the right to suspend or terminate the study at any time and not allow additional enrollment, but to continue the study safety.

6.6.4 Criteria for Premature Termination or Suspension of the Study

The study will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the study:

- New information or other evaluation regarding the safety or efficacy of the study drug that indicates a change in the known risk/benefit profile for the product, such that the risk is no longer acceptable for participants participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises participants' safety.

6.6.5 Criteria for Premature Termination or Suspension of a Site

Not applicable.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS

7.1 Inclusion Criteria

Participants must fulfill the following inclusion criteria to be eligible for participation in the study:

1. Healthy, adult, male or female, 18-55 years of age, inclusive, at screening.

   * Females of childbearing potential are defined as all females physiologically capable of becoming pregnant.

   Females of non-childbearing potential are defined as follows:

   - Females who have undergone one of the following sterilization procedures at least 6 months prior to the first dosing:
     - Hysteroscopic sterilization.
     - Bilateral tubal ligation or bilateral salpingectomy.
     - Hysterectomy.
     - Bilateral oophorectomy

     or
   - Females who are postmenopausal with amenorrhea for at least 12 month prior to the first dosing and follicle-stimulating hormone (FSH) serum levels consistent with postmenopausal status at screening.

2. BMI ≥ 18 and ≤ 32.0 kg/m^2 at screening visit.
3. Medically healthy with no clinically significant medical history, physical examination, laboratory profiles, vital signs or ECGs, as deemed by the Investigator or designee, including the following:
   - Seated blood pressure ≥ 90/40 mmHg and ≤ 140/90 mmHg at screening visit.
   - Seated heart rate ≥ 40 bpm and ≤ 99 bpm at screening visit.
   - QTcF interval ≤ 460 msec (males) or ≤ 470 msec (females) and has ECG findings considered normal or not clinical significance by the Investigator or designee at screening visit.
   - Estimated creatinine clearance ≥ 90 mL/min at screening visit.
   - Total bilirubin <1.25 × ULN at screening visit unless the participant has known Gilbert's syndrome that can explain the elevation of bilirubin

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- Serum ALT <1.25 × ULN at screening visit.
- Creatinine ≤1.1 × ULN at screening visit.

4. A female of childbearing potential who is sexually active with a nonsterilized male partner agrees to use a highly effective/effective method of contraception from signing of the ICF throughout the duration of the study and for 30 days after the last dose of study drug as described in Appendix D.
5. A male participant who is nonsterilized and sexually active with a female partner of childbearing potential agrees to use highly effective method of contraception from signing of the ICF throughout the duration of the study and for 100 days after last dose of study drug as described in Appendix D.
6. If male, must agree not to donate sperm from the first dosing until 100 days after the last dosing.
7. Continuous non-smoker who has not used nicotine and tobacco containing products for at least 3 months prior to the first dosing based on participant self-reporting.
8. Understands the study procedures in the ICF and be willing and able to comply with the protocol.

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7.2 Exclusion Criteria

Participants must not be enrolled in the study if they meet any of the following criteria:

1. Is mentally or legally incapacitated or has significant emotional problems at the time of the screening visit or expected during the conduct of the study.
2. History or presence of clinically significant medical or psychiatric condition or disease in the opinion of the Investigator or designee.
3. History of any illness that, in the opinion of the Investigator or designee, might confound the results of the study or poses an additional risk to the participant by their participation in the study.
4. Drink alcohol in excess of 21 units per week for males or 14 units per week for females, with one unit = 150 mL of wine or 360 mL of beer or 45 mL of 45% alcohol.
5. History of alcoholism or drug abuse within the past 2 years prior to the first dosing.
6. Positive urine drug or alcohol results at screening or first check-in.
7. History or presence of hypersensitivity or idiosyncratic reaction to the study drug(s) or related compounds.
8. History or presence of CeD or other gastrointestinal inflammatory disorder such as inflammatory bowel disease or eosinophilic disorder.
9. History of lactose intolerance.

10. Female participants with a positive pregnancy test at screening visit or first check-in or who are lactating.
11. Positive results at screening for HIV, HBsAg, or HCV.
12. Unable to refrain from or anticipates the use of:
    - Any drug, including prescription and non-prescription medications, herbal remedies, or vitamin supplements within 14 days prior to the first dosing and throughout the study. Medication listed as part of acceptable birth control methods will be allowed (refer to Appendix D). Thyroid hormone replacement medication may be permitted if the participant has been on the same stable dose for the immediate 3 months prior to first study drug administration. Hormone replacement therapy will also be allowed.
    - Any drugs known to be significant inducers or inhibitors of CYP3A4 enzymes and/or P-gp, including St. John's Wort, within 28 days prior to the first dosing and throughout the study. Appropriate sources (eg, Flockhart Table™) will be consulted to confirm lack of PK/pharmacodynamics interaction with study drug.
    - Chronic use of non-steroidal anti-inflammatory (define as more that 7 days of use) within 2 weeks prior to screening and throughout the study.
13. Has been on a diet incompatible with the on-study diet, in the opinion of the Investigator or designee, within the 30 days prior to the first dosing and throughout the study.
14. Donation of blood or significant blood loss within 56 days prior to the first dosing.
15. Plasma donation within 7 days prior to the first dosing.
16. Participation in another clinical study within 30 days prior to the first dosing. The 30-day window will be derived from the date of the last blood collection or dosing, whichever is later, in the previous study to Day 1 of Treatment Period 1 of the current study.
17. The participant is an immediate family member, study site employee, or is in a dependent relationship with a study site employee who is involved in conduct of this study (eg, spouse, parent, child, sibling) or may consent under duress.

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7.3 Excluded Medications, Supplements, Dietary Products

Concomitant medications will be prohibited as listed in the exclusion criteria in Section 7.2. After the first dose, acetaminophen (up to 2 g per 24-hour period) may be administered at the discretion of the Investigator or designee. Thyroid hormone replacement medication may be permitted if the participant has been on the same stable dose for the immediate 3 months prior to the first dosing. Hormonal contraceptives (refer to Appendix D) and hormonal replacement medication may be permitted if the female participant has been on the same stable dose for at least 3 months prior to the first dose of the study drug.

If deviations occur, the Investigator or designee in consultation with the Sponsor if needed will decide on a case by case basis whether the participant may continue participation in the study.

All medications taken by participants during the course of the study will be recorded.

Use of excluded agents (prescription or non-prescription) or dietary products is outlined in Table 7.a.

Table 7.a Excluded Medications, Supplements, and Dietary Products

Category	Between Screening and First Dosing (Days -28 to pre-dose [Day 1])	After First Dosing (Day 1) to Follow-Up
Alcohol	Prohibited from 48 hours prior to first dosing	Prohibited from first dosing until the last PK sample on Day 2 of Treatment Period 3.
Xanthine and/or caffeine	Prohibited from 24 hours prior to first dosing [a]	Prohibited from first dosing until the last PK sample on Day 2 of Treatment Period 3.[a]
Medications	See Sections 7.1 and 7.2	See Sections 7.1 and 7.2
Nicotine- and tobacco-containing and/or cannabis products	Prohibited from 3 month prior to first dosing	Prohibited from first dosing until the follow-up visit.
Food substance		
Grapefruit/Seville orange	Prohibited from 14 days prior to first dosing	Prohibited from first dosing until the follow-up visit.

(a) small amounts of caffeine derived from normal foodstuffs eg, 250 mL/8 oz./1 cup decaffeinated coffee or other decaffeinated beverage, per day, with the exception of espresso; 45 g/1.5 oz. chocolate bar, per day, would not be considered a deviation to this restriction.

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7.4 Diet, Fluid, Activity

7.4.1 Diet and Fluid

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.

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 post-dose.

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 post-dose.

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 post-dose, 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.

* 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].

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.

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7.4.2 Activity

Participants will remain ambulatory or seated upright for the first 4 hours post-dose, except when they are supine or semi reclined for study procedures.

Should TEAEs occur at any time, participants may be placed in an appropriate position or will be permitted to lie down on their right side.

Participants will be instructed to refrain from strenuous physical activity which could cause muscle aches or injury, including contact sports at any time from screening until completion of the study.

7.5 Criteria for Discontinuation or Withdrawal of a Participant

The primary reason for discontinuation or withdrawal of the participant from the study or TAK-227 administration should be recorded in the case report form (CRF) using the following categories:

1. PTE and TEAE: The participant has experienced a PTE or a TEAE that requires early termination because continued participation imposes an unacceptable risk to the participant's health or the participant is unwilling to continue because of the PTE or TEAE.

   Liver Function Test (LFT) Abnormalities:

   Study drug should be discontinued immediately with appropriate clinical follow-up (including repeat laboratory tests, until a participant's laboratory profile has returned to normal/baseline status, see Section 9.2.7), if the following circumstances occur at any time during study drug treatment:

- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >8 × upper limit of normal (ULN), or
- ALT or AST >5 × ULN and persists for more than 2 weeks, or
- ALT or AST >3 × ULN in conjunction with elevated total bilirubin >2 × ULN, or
- ALT or AST >3 × ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (>5%).
- QTcF interval:

  TAK-227 should be discontinued immediately with appropriate clinical follow-up if a QTcF interval >500 msec or if there is an increase of QTcF >60 msec above baseline detected by ECG and confirmed with a repeat ECG. Appropriate clinical follow-up includes a repeat ECG.

2. Significant protocol deviation: The discovery post-enrollment that the participant failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the participant's health.
3. Lost to follow-up: Attempts to contact the participants were unsuccessful. Attempts to contact the participant must be documented in the participant's source documents.
4. Voluntary withdrawal: The participant (or participant's legally acceptable representative) wishes to withdraw from the study. The reason for withdrawal, if provided, should be recorded in the CRF.

   Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (ie, withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category). If a participant chooses to withdraw from study participation due to personal concerns related to the coronavirus disease 2019 (COVID-19) pandemic (other than a COVID-19-related AE), this should be specified as the reason for participant withdrawal in the CRF.
5. Study termination: The Sponsor, Institutional Review Board (IRB)/ Independent Ethics Committee (IEC), or regulatory agency terminates the study.
6. Pregnancy: as described in Appendix D.
7. Participants may be withdrawn from the study by the Investigator or designee for the following reasons:
   - Difficulties in blood collection.
   - Positive urine drug or alcohol test.
8. Other. The specific reasons for discontinuation should be entered into the CRF including unavoidable circumstances such as the COVID-19 pandemic. Participants may be withdrawn from the study at any time at the discretion of the Investigator or Sponsor for safety reasons which should be entered into the CRFs.

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7.6 Procedures for Discontinuation or Withdrawal of a Participant

The Investigator may discontinue a participant's study participation at any time during the study when the participant meets the study termination criteria described in Section 7.5. In addition, a participant may discontinue his or her participation without giving a reason at any time during the study. Should a participant's participation be discontinued, the primary criterion for termination must be recorded by the Investigator. In addition, efforts should be made to perform all procedures scheduled for the end-of-study or early termination as described in Section 3.0.

7.7 Participant Replacement

Replacement of discontinued or withdrawn participants due to any reason will be assessed on a case by case basis by the Sponsor and Investigator to ensure a minimum of 18 PK-evaluable participants complete the study.

8.0 CLINICAL STUDY MATERIAL MANAGEMENT

8.1 Clinical Study Drug

Drug Name (add this bold when multiple drugs):

Product Name:	TAK-227
Strength:	50 mg
Dose:	50 mg
Dosage Form/Formulation:	Capsule
Dosing regimen:	Single dose
Route of Administration:	Oral

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8.1.1 Clinical Study Drug Labeling

TAK-227 blister strips will be labeled for the study and placed in cartons. Each carton will be labeled with a study label in accordance with local regulatory requirements and tamper sealed.

8.1.2 Clinical Study Drug Inventory and Storage

The Sponsor will supply sufficient quantities of TAK-227 to allow completion of this study.

The same lot number will be used throughout the study. The lot numbers and expiration dates (where available) of the study drugs supplied will be recorded in the final report. Study drugs will be stored according to the product labels provided with the product.

Records will be made of the receipt, preparation, dispensing, and final disposition of the study drugs supplied.

8.1.3 Clinical Study Drug Blinding

This is an open-label study.

8.1.4 Randomization Code Creation and Storage

A computerized randomization scheme will be created by a Celerion statistician.

Participants will be randomized to 1 of 6 sequences as indicated in Table 8.a.

Table 8.a 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 pre-dose, high-fat/high-calorie meal administered 30 minutes prior to dosing.
Treatment C: Fed post-dose, high-fat/ high-calorie meal administered ~30 minutes after dosing.

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8.1.5 Clinical Study Blind Maintenance/Unblinding Procedure

Not applicable

8.1.6 Accountability and Destruction of Sponsor-Supplied Drugs

Records will be made of the receipt and dispensing of the study drugs supplied. At the conclusion of the study, any unused TAK-227 will be retained by Celerion, returned to the Sponsor or designee, or destroyed, as per Sponsor instructions. Any remaining supplies that were purchased by Celerion will be destroyed, if appropriate. If no supplies remain, this fact will be documented in the pharmacy product accountability records.

9.0 STUDY PROCEDURES

9.1 Administrative Procedures

9.1.1 Informed Consent Procedure

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the participants in non-technical terms. Participants will be required to read, sign,

and date an ICF summarizing the discussion prior to screening, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

Participants will be given a copy of their signed ICF.

9.1.1.1 Assignment of Screening and Randomization Numbers

Each participant will be assigned a unique identification number upon screening. Participants who complete the study screening assessments and meet all the eligibility criteria will be assigned a unique randomization number at the time of first dosing, different from the screening number.

If replacement participants are used, the replacement participant number will be 100 more than the original (eg, Participant No. 101 will replace Participant No. 1).

9.1.1.2 Study Drug Assignment

All participants will receive the treatments as detailed in Section 9.2.6.

9.1.2 Inclusion and Exclusion

Please refer to Section 7.1 and Section 7.2.

9.1.3 Medical History/Demography

Medical history and demographic data, including name, sex, age, race, ethnicity, and history of tobacco use will be recorded.

All medication taken by the participants within 6 month prior to first dosing will be recorded.

9.1.4 Concomitant Medications

Concomitant medications will be prohibited as listed in Section 7.3. All medications taken by participants during the course of the study will be recorded.

9.2 Clinical Procedures and Assessments

The Schedule of Study Procedures (Section 3.0) summarizes the clinical procedures to be performed at each visit. Individual clinical procedures are described in detail below. Additional evaluations/testing may be deemed necessary by the Investigator or designee and/or the Sponsor for reasons related to participant's safety.

For this study, collection of blood for TAK-227 PK is the critical parameter and needs to be collected as close to the exact time point as possible. All other procedures should be completed as close to the prescribed/scheduled time as possible but can be performed prior to or after the prescribed/scheduled time.

Any nonscheduled procedures required for urgent evaluation of safety concerns will take precedence over all routine scheduled procedures.

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9.2.1 Full Physical Exam

A full physical examination will be performed as outlined in the Schedule of Study Procedures (Section 3.0). Additional physical examinations may be performed at other times, if deemed necessary by the Investigator or designee.

9.2.2 Height and Weight

Body height (cm) and weight (kg) will be reported as outlined in the Schedule of Study Procedures (Section 3.0).

9.2.3 BMI

BMI will be calculated based on the height and weight measured at screening.

9.2.4 Vital Signs

Single measurements of temperature, respiratory rate, BP, and pulse rate, will be measured as outlined in the Schedule of Study Procedures (Section 3.0). Additional vital signs may be taken at any other times, if deemed necessary by the Investigator or designee.

BP and pulse rate measurements will be performed with participants in a seated position, except when they are supine or semi-reclined because of study procedures and/or AEs (e.g. nausea, dizziness) or if deemed necessary by the Investigator or designee.

Vital signs will be measured within 24 hours prior to Day 1 dosing of each treatment period for the pre-dose time point. When scheduled post-dose, vital signs will be performed within approximately 15 minutes of the scheduled time point.

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9.2.5 12-Lead ECG

Single 12-lead ECGs will be performed as outlined in the Schedule of Study Procedures (Section 3.0). Additional ECGs may be taken at any other times, if deemed necessary by the Investigator or designee.

ECGs will be performed with participants in a supine position. All ECG tracings will be reviewed by the Investigator or designee.

ECGs will be measured within 24 hours prior to Day 1 dosing of each treatment period for the pre-dose time point. When scheduled post-dose, ECGs will be performed within approximately 20 minutes of the scheduled time point.

9.2.6 Study Drug Administration

TAK-227 will be provided as described in Section 8.1.

Treatments are described in Table 2.b.

The pharmacy at the CRU will provide each dose in individual unit dose containers for each participant, as appropriate.

TAK-227 will be administered under fasting or fed conditions as detailed in Section 7.4.1. TAK-227 will be administered with approximately 240 mL of water. Water consumption will be restricted as detailed in Section 7.4.1.

Participants will be instructed not to crush, split, or chew the capsules.

The exact clock time of dosing will be recorded.

A qualified designee will be responsible for monitoring the administration of the timed oral doses. A mouth check will be performed by the qualified designee to ensure that the participants have swallowed the study drug. Once a participant has finished the dosing water, the qualified designee will use a flashlight and a tongue depressor to check the participant's mouth. Participants' hands will also be verified to ensure that the study drug was ingested.

9.2.7 AE Monitoring

Participants will be monitored throughout the study for adverse reactions to the study drug and/or procedures as described in Section 10.0.

9.2.8 Laboratory Procedures and Assessments

All tests listed below will be performed as outlined in the Schedule of Study Procedures (Section 3.0). In addition, laboratory safety tests may be performed at various unscheduled time points, if deemed necessary by the Investigator or designee.

9.2.8.1 Clinical Laboratory Tests

Hematology

Hematology will consist of the following tests:

Hemoglobin	Red blood cell count
Hematocrit	Platelet count
Total white blood cell count and differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and leukocytes)	

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Chemistry

Serum chemistry tests will be performed after at least an 8-hour fast; however, in case of dropouts or rechecks, participants may not have fasted for 8 hours prior to when the serum chemistry sample being taken.

Chemistry evaluations will consist of the following standard chemistry panel:

Blood Urea Nitrogen	Sodium
Bilirubin (total and direct)	Potassium
Alkaline phosphatase (ALP)	Chloride
Aspartate aminotransferase (AST)	Glucose
Alanine aminotransferase (ALT)	Creatinine *
Albumin	

* At screening, creatinine clearance will be calculated using the Cockcroft-Gault formula.

Urinalysis

Urinalysis will consist of the following tests:

pH	Bilirubin
Specific gravity	Blood *
Protein *	Nitrite *
Glucose	Urobilinogen
Ketones	Leukocyte esterase *

* If urinalysis is positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination (for red blood cells, white blood cells, bacteria, casts, and epithelial cells) will be performed.

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9.2.8.1.1 Other

HIV test	Urine drug screen
HBsAg	Opiates (includes morphine, heroin [diacetylmorphine], codeine, 6-acetylmorphine, dihydrocodeine, hydrocodone, thebaine, and, hydromorphone)
HCV	Opioids
Urine alcohol screen	Amphetamines
Serum pregnancy test (for females only)	Barbiturates
FSH (for postmenopausal females only)	Benzodiazepines
COVID-19 (Severe acute respiratory syndrome-Coronavirus-1 [SARS-CoV-2] polymerase chain reaction test or equivalent)	Cocaine
	Cannabinoids
	Methadone
	Phencyclidine

9.3 PK Samples

Samples for TAK-227 and its metabolites PK assessment will be collected as outlined in the Schedule of Study Procedures (Section 3.0).

Instructions for sample collection, processing, and shipping will be provided in separate documents.

Primary specimen collection parameters are provided in Table 9.a.

Table 9.a Primary Specimen Collections

Specimen Name	Primary Specimen	Primary Specimen Derivative	Description of Intended Use	Sample Collection
Plasma sample for PK	Blood	Plasma	Plasma sample for PK analysis	Mandatory

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9.3.1 PK Measurements

Samples from all participants will be assayed even if the participants do not complete the study. Samples for determination of plasma TAK-227 and its metabolites, will be analyzed using validated bioanalytical methods.

PK parameters of TAK-227 and its metabolites will be calculated from the individual concentration-time profiles from all evaluable participants using NCA methods. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times.

9.3.1.1 Plasma PK Measurements

The following PK parameters will be calculated from plasma concentrations of TAK-227 and its metabolites, unless otherwise specified:

AUC_{last}: Area under the concentration-time curve, from time 0 to the last quantifiable concentration.

AUC_{∞}: Area under the concentration-time curve, from time 0 extrapolated to infinity, calculated using the observed 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_{∞}.

CL/F: Apparent clearance after extravascular administration (parent only).

C_{max}: Maximum observed concentration.

t_{max}: Time of first occurrence of C_{max}

t_{lag}: Lag time to first quantifiable concentration

$t_{½z}$:	Terminal disposition phase half-life
λ_z	Terminal disposition phase rate constant
V_z/F:	Apparent volume of distribution during the terminal disposition phase after extravascular administration (parent only).

No value for λ_z, AUC_∞, $AUC_{extrap}\%$, CL/F, V_z/F, or $t_{½z}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

No PK parameters will be calculated for participants with detectable concentrations at 2 or fewer consecutive time points.

Individual and mean plasma concentration-curves (both linear and log-linear) will be included in the final report.

Additional PK parameters may be estimated as appropriate.

9.3.2 Biomarker Measurements

Not applicable.

9.3.3 PGx Measurements

Not applicable.

9.3.4 Confinement

Participants will be housed on Day -1 of each treatment period, at the time indicated by the CRU, until after the 36-hour blood draw and/or study procedures of Treatment Period 3.

At all times, a participant may be required to remain at the CRU for longer at the discretion of the Investigator or designee.

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10.0 ADVERSE EVENTS

10.1 Definitions and Elements of AEs

An AE is defined as any untoward medical occurrence in a clinical investigation participant who has signed the ICF to participate in a study; it does not necessarily have to have a causal relationship with the treatment.

An AE can therefore be any unfavorable and unintended sign (eg, a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, whether or not it is considered related to the drug.

A pretreatment event (PTE) is defined as any untoward medical occurrence in a clinical investigation participant who has signed the ICF to participate in a study but before administration of any study drug; it does not necessarily have to have a causal relationship with study participation.

An untoward finding generally may:

- Indicate a new diagnosis or unexpected worsening of a preexisting condition. (Intermittent events for pre-existing conditions or underlying disease should not be considered AEs.)
- Necessitate therapeutic intervention.
- Require an invasive diagnostic procedure.
- Require discontinuation or a change in dose of study medication or a concomitant medication.
- Be considered unfavorable by the Investigator for any reason.

Diagnoses versus signs and symptoms:

- Each event should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory values or ECG findings) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, sign(s) or symptom(s) should be recorded appropriately as an AE(s).

Laboratory values and ECG findings:

- Changes in laboratory values or ECG parameters maybe considered AEs if they are judged to be clinically significant (ie, if some action or intervention is required or if the Investigator judges the change to be beyond the range of normal physiologic fluctuation). A laboratory re-test and/or continued monitoring of an abnormal value are not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.
- If abnormal laboratory values or ECG findings are the result of pathology for which there is an overall diagnosis (eg, increased creatinine in renal failure), the diagnosis only should be reported appropriately as an AE.

Pre-existing conditions:

- A pre-existing condition (present at the time of signing the ICF) is considered a concurrent medical history condition and should NOT be recorded as an AE. A baseline evaluation (eg, laboratory test, ECG, X-ray, etc) should NOT be recorded as an AE unless related to a study procedure. However, if the participant experiences a worsening or complication of such a concurrent medical history condition, the worsening or complication should be recorded appropriately as an AE (worsening or complication occurs after the ICF is signed). Investigators should ensure that the event term recorded captures the change in the condition (eg, “worsening of…”).

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- If a participant has a pre-existing episodic condition (eg, asthma, epilepsy), any occurrence of an episode should only be captured as an AE if the episodes become more frequent, serious, or severe in nature, that is, Investigators should ensure that the AE term recorded captures the change from Baseline in the condition (eg "worsening of…").
- If a participant has a degenerative concurrent condition (eg, cataracts, rheumatoid arthritis), worsening of the condition should only be captured as an AE if occurring to a greater extent to that which would be expected. Again, Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Worsening of AEs:

- If the participant experiences a worsening or complication of an AE after the first administration of study medication or after any change in study medication, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Changes in severity of AEs:

- If the participant experiences a change in the severity of an AE that is not associated with a change in study medication, the event should be captured once with the maximum severity recorded.

Preplanned surgeries or procedures:

- Preplanned procedures (surgeries or therapies) that were scheduled prior to signing of the ICF are not considered AEs. However, if a preplanned procedure is performed early (eg, as an emergency) due to a worsening of the pre-existing condition, the worsening of the condition should be captured appropriately as an AE. Complications resulting from any planned surgery should be reported as AEs.

Elective surgeries or procedures:

- Elective procedures performed where there is no change in the participant's medical condition should not be recorded as AEs but should be documented in the participant's source documents. Complications resulting from an elective surgery should be reported as AEs.

Overdose:

- An overdose is defined as a known deliberate or accidental administration of study drug, to or by a study participant, at a dose above the maximum scheduled dose being used in this study irrespective of the participant's assigned according to the study protocol. It is up to the Investigator or the reporting physician to decide whether a dose is to be considered an overdose or considered as a medication dosing error, in consultation with the Sponsor.
- All cases of overdose (with or without associated AEs) will be documented on an overdose page of the (e)CRF, in order to capture this important safety information consistently in the database. Cases of overdose in itself without manifested signs or symptoms are considered as

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AEs and should be captured with the appropriate Preferred Term of Overdose in the AE eCRF. AEs associated with an overdose will be documented on AE CRF(s) according to Section 10.2.8.

- Serious adverse events of overdose should be reported according to the procedure outlined in Section 10.2.8.
- In the event of drug overdose, the participant should be treated according to the standard practices of the Investigator and the site.

10.1.1 SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

1. Results in DEATH.
2. Is LIFE THREATENING.
   - The term "life threatening" 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.
3. Requires inpatient HOSPITALIZATION or prolongation of existing hospitalization.
4. Results in persistent or significant DISABILITY/INCAPACITY.
5. Is a CONGENITAL ANOMALY/BIRTH DEFECT.
6. Is an IMPORTANT MEDICAL EVENT that satisfies any of the following:
   - May require intervention to prevent items 1 through 5 above.
   - May expose the participant to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.
   - Includes any event or synonym described in the Takeda Medically Significant AE List (Table 10.a).

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Table 10.a Takeda Medically Significant AE List

Term	
Acute respiratory failure/acute respiratory distress syndrome	Hepatic necrosis
Torsade de pointes / ventricular fibrillation / ventricular tachycardia	Acute liver failure
Malignant hypertension	Anaphylactic shock
Convulsive seizures	Acute renal failure
Agranulocytosis	Pulmonary hypertension
Aplastic anemia	Pulmonary fibrosis
Toxic epidermal necrolysis/ Stevens-Johnson syndrome	Confirmed or suspected endotoxin shock
COVID-19-related disease	Confirmed or suspected transmission of infectious agent by a medicinal product
COVID-19 pneumonia	Neuroleptic malignant syndrome / malignant hyperthermia
	Spontaneous abortion / stillbirth and fetal death

Abbreviations: COVID-19 = Coronavirus disease-2019

AEs that fulfill 1 or more of the serious criteria above are to be considered SAEs and should be reported and followed up in the same manner (see Sections 10.1 and 10.1.1).

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10.2 AE Procedures

10.2.1 Assigning Severity/Intensity of AEs

The different categories of severity/intensity are:

Mild: An adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: An adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: An adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

10.2.2 Assigning Causality of AEs

The relationship of each AE to study medication(s) will be assessed using the following categories:

Related: An AE that follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug), or for which a causal relationship is at least a reasonable possibility, ie, the relationship cannot be

ruled out, although factors other than the drug, such as underlying diseases, complications, concomitant drugs and concurrent treatments, may also be responsible.

Not Related: An AE that does not follow a reasonable temporal sequence from administration of a drug and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant medications and concurrent treatments.

In addition, relationship (causality) to COVID-19 should be determined for all PTEs and TEAEs. The relationship should be assessed as related if the Investigator considers that there is reasonable possibility that an event is due to COVID-19. Otherwise, the relationship should be assessed as not related.

Similarly, relationship (causality) to COVID-19 vaccines should be determined for all PTEs and TEAEs. The relationship should be assessed as related if the Investigator considers that there is reasonable possibility that an event is due to COVID-19 vaccines. Otherwise, the relationship should be assessed as not related. If the AE has relationship to vaccination, specific verbatim term should be used, eg, post-vaccination fever, vaccination site burning.

In addition, if the causality assessment done by the Investigator determines that the event or events are related or possible related to COVID-19 or the COVID-19 vaccine, the events should be assessed as not related to the study drug. If the AE is related to COVID-19 vaccination, specific verbatim term(s) should be used, eg, post-vaccination fever, vaccination site burning.

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10.2.3 Start Date

The start date of the AE is the date that the first signs/symptoms were noted by the participant and/or Investigator.

10.2.4 End Date

The end date of the AE is the date at which the participant recovered, the event resolved but with sequelae or the participant died.

10.2.5 Pattern of Adverse Event (Frequency)

Episodic AEs (eg, headache) or those which occur repeatedly over a period of consecutive days are intermittent. All other events are continuous.

10.2.6 Action Taken With Study Treatment

- Drug withdrawn – a study medication is stopped due to the particular AE.
- Dose not changed – the particular AE did not require a dose change, including stopping a study medication.
- Unknown – only to be used if it has not been possible to determine what action has been taken.

- Not applicable – a study medication was stopped for a reason other than the particular AE eg, the study has been terminated, the participant died, dosing with study medication had not yet started or dosing with study medication was already stopped before the onset of the AE.

10.2.7 Outcome

- Recovered/resolved – participant returned to first assessment status with respect to the AE.
- Recovering/resolving – the intensity is lowered by one or more stages: the diagnosis has or signs/symptoms have almost disappeared; the abnormal laboratory value improved, but has not returned to the normal range or to the baseline value; the participant died from a cause other than the particular AE with the condition remaining "recovering/resolving."
- Not recovered/not resolved – there is no change in the diagnosis, signs or symptoms; the intensity of the diagnosis, signs/symptoms or laboratory value on the last day of the observed study period has become worse than when it started; is an irreversible congenital anomaly; the participant died from another cause with the particular AE state remaining "Not recovered/not resolved."
- Recovered/ Resolved with sequelae – the participant recovered from an acute AE but was left with permanent/significant impairment (eg, recovered from a cardiovascular accident but with some persisting paresis).
- Fatal – an AE that is considered as the cause of death.
- Unknown – the course of the AE cannot be followed up due to hospital change or residence change at the end of the participant's participation in the study.

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10.2.8 Collection and Reporting of AEs, SAEs, and Abnormal LFTs

10.2.8.1 Collection Period

Collection of AEs (ie, PTEs, AEs, SAEs, Overdose, and Abnormal LFTs) will commence at the time the participant signs the ICF. Routine collection of AEs will continue until the follow-up phone call on Day 7 (± 3 days), approximately 7 days after the last dose of study drug. For participants who discontinue prior to the administration of study medication, AEs will be followed until the participant discontinues study participation.

10.2.8.2 Reporting AEs

At each study visit, the Investigator or designee will assess whether any subjective AEs have occurred. A neutral question, such as "How have you been feeling since your last visit?" may be asked. Participants may report AEs occurring at any other time during the study. Participants experiencing an SAE prior to the first exposure to study drug must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to Baseline or there is a satisfactory explanation for the change.

All participants experiencing AEs, whether considered associated with the use of the study medication or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or until there is a satisfactory explanation for the changes observed. All AEs will be documented in the AE page of the CRF, whether or not the Investigator concludes that the event is related to the drug treatment. The following information will be documented for each event:

- Event term.
- Start and end date and time.
- Pattern of AE (frequency).
- Severity/Intensity.
- Causality (Investigator's opinion of the causal relationship between the event and administration of study drug[s]).
- Relationship to COVID-19.
- Relationship to COVID-19 vaccine.
- Action taken with study drug.
- Outcome of event.
- Seriousness.

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10.2.8.3 Reporting SAEs

When an SAE occurs through the AE collection period it should be reported according to the procedure outlined below:

A Takeda SAE form must be completed, in English and signed by the Investigator immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event and the reason why the event is categorized as serious.
- Participant identification number.
- Investigator's name.
- Name of the study medication(s).
- Causality assessment.

The SAE form should be transmitted within 24 hours to the attention of the contact listed in Appendix 14.1.1.

Any SAE spontaneously reported to the Investigator following the AE collection period should be reported to the Sponsor if considered related to study participation.

Reporting of SAEs that begin before first administration of study drug will follow the same procedure for SAEs occurring on treatment.

10.2.8.3.1 SAE Follow-Up

If information is not available at the time of the first report becomes available at a later date, the Investigator should complete a follow-up SAE form or provide other written documentation and fax it immediately within 24 hours of receipt. Copies of any relevant data from the hospital notes (eg, ECGs, laboratory tests, discharge summary, postmortem results) should be sent to the addressee, if requested.

All SAEs should be followed up until resolution or permanent outcome of the event. The timelines and procedure for follow-up reports are the same as those for the initial report.

10.2.8.4 Reporting Special Interest AEs

Not Applicable. There are no AEs of Special Interest for TAK-227.

10.2.8.5 Reporting of Abnormal LFTs

If a participant is noted to have ALT or AST elevated >3 ×ULN on 2 consecutive occasions, the abnormality should be recorded as an AE. In addition, an LFT Increases CRF must be completed providing additional information on relevant recent history, risk factors, clinical signs and symptoms and results of any additional diagnostic tests performed.

If a participant is noted to have ALT or AST >3 ×ULN and total bilirubin >2 ×ULN for which an alternative etiology has not been identified, the event should be recorded as an SAE and reported as per Section 10.2.8.3. The Investigator must contact the Medical Monitor for discussion of the relevant participant details and possible alternative etiologies, such as acute viral hepatitis A or B or other acute liver disease. Follow-up laboratory tests as described in Section 9.2.8 must also be performed. In addition, an LFT Increases CRF must be completed and transmitted with the Takeda SAE form (as per Section 10.2.9).

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10.2.9 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The Sponsor will be responsible for reporting all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, Investigators and IRBs or IECs, as applicable, in accordance with national regulations in the countries where the study is conducted. Relative to the first awareness of the event by/or further provision to the Sponsor or Sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other serious events, unless otherwise required by national regulations. The Sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the study. The investigational site also will forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations.

11.0 STATISTICAL METHODS

11.1 Statistical and Analytical Plans

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a statistical analysis plan (SAP). The SAP will be prepared by Celerion and agreed upon with the Sponsor. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoints definition and/or its analysis will also be reflected in a protocol amendment. Additional statistical analyses other than those described in this section may be performed if deemed appropriate.

11.1.1 Analysis Sets

11.1.1.1 Safety Set

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

11.1.1.2 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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11.1.2 Analysis of Demography and Other Baseline Characteristics

Continuous demographic data (ie, age, weight, height, and BMI) will be listed and summarized using appropriate summary statistics. Categorical demographic data (ie, sex, race, and ethnicity) will also be listed and tabulated.

11.1.3 PK Analysis

Statistical analysis of PK data will be based on the PK set.

Individual TAK-227 and its metabolites plasma concentrations and PK parameters will be listed by participant and treatment, and summarized by treatment (TAK-227 and metabolites plasma concentrations and PK parameters) and nominal time (TAK-227 and metabolites plasma concentrations).

PK parameters (C_{max}, AUC_{last}, AUC_{∞}, $AUC_{extrap\%}$, t_{max}, λ_z, $t_{½z}$, CL/F, V_z/F, and t_{lag}) will be computed using standard NCA. Actual time of sampling will be used to calculate the PK parameters.

All individual concentration data and PK parameters will be listed with summary statistics such as geometric mean, median, arithmetic mean, SD, CV, minimum and maximum. Individual plasma concentration/time curves will be presented in linear/linear and log/linear scale. At least 3 PK time points within the terminal log-linear phase will be used to estimate the terminal rate constant for each participant in each treatment period.

The appropriate PK analysis will be fully specified in the CPAP.

TAK-227 metabolites PK may be reported separately from the clinical study report as a standalone document.

11.1.3.1 Food-effect estimation

A linear mixed-effect model will be applied to log-transformed C_{max}, AUC_{last}, and AUC_{∞} 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 pre-dose) versus Treatment A (fasting), and Treatment C (fed post-dose) 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 pre-dose) versus Treatment A (fasting) and Treatment C (fed post-dose) versus Treatment A (fasting).

11.1.3.2 Non-Parametric Analysis

Analysis of t_{max} and t_{lag} will be performed by nonparametric Wilcoxon Signed-Rank test.

11.1.4 PD Analysis

Not applicable.

11.1.5 Safety Analysis

Dosing dates and times will be listed by participant.

TEAEs will be tabulated. The remaining quantitative safety data as well as the difference to baseline, when appropriate, will be summarized using the appropriate descriptive statistics.

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11.1.5.1 AEs

AEs will be coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA®) available at Celerion and summarized by treatment for the number of participants reporting the TEAE and the number of TEAEs reported. A by-participant AE data listing including verbatim term, coded term, treatment, severity, and relationship to the study drug will be provided.

11.1.5.2 Clinical Laboratory Evaluation

Clinical laboratory results will be summarized by treatment and point of time of collection and a shift table describing out of normal range shifts will be provided.

11.1.5.3 Vital Signs

Vital signs assessments will be summarized by treatment and point of time of collection.

11.1.5.4 Other Safety Parameters

ECGs will be summarized by treatment and point of time of collection.

Medical history, and concurrent conditions will be coded using the MedDRA® and concomitant medications will be coded using the World Health Organization (WHO) drug dictionary and will be listed by participant.

11.2 Interim Analysis and Criteria for Early Termination

Not applicable.

11.3 Determination of Sample Size

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
- 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 GMR is at 0.90 or 1.10, the estimated 90% 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.

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12.0 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Study-Site Monitoring Visits

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol are followed. Due to COVID-19, monitoring visits may also be conducted remotely. Source documents will be reviewed for verification of data recorded on the CRFs. Source documents are defined as original documents, data, and records. The Investigator and study site guarantee access to source documents by the Sponsor or its designee (contract research organization) and by the IRB or IEC.

All aspects of the study and its documentation will be subject to review by the Sponsor or the Sponsor's designee, including but not limited to the Investigator's Binder, study drug, participant medical records, informed consent documentation, and review of CRFs and associated source documents. It is important that the Investigator and other study personnel are available during the monitoring visits and that sufficient time is devoted to the process.

12.2 Protocol Deviations

The Investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study participants. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the Investigator should consult with the sponsor or designee (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

For COVID-19-related protocol deviations, the specific protocol deviation, the reason for the deviation, and the relationship to COVID-19 should be documented using CRU standard processes.

Significant deviations include, but are not limited to, those that involve fraud or misconduct, increase the health risk to the participant, or confound interpretation of primary study assessment.

12.3 Quality Assurance Audits and Regulatory Agency Inspections

The study site also may be subject to quality assurance audits by the Sponsor or designees. In this circumstance, the Sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the medication is stored and prepared, and any other facility used during the study. In addition, there is the possibility that this study may be inspected by regulatory agencies, including those of foreign governments (eg, the FDA, the United Kingdom Medicines and Healthcare products Regulatory Agency, the Pharmaceuticals and Medical Devices Agency of Japan). If the study site is contacted for an inspection by a regulatory body, the Sponsor should be notified immediately. The Investigator guarantees access for quality assurance auditors to all study documents as described in Section 12.1.

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13.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, participants) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the International Conference on Harminsation (ICH) Harmonised Tripartite Guideline for GCP. Each Investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix A. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and Investigator responsibilities.

13.1 IRB and/or IEC Approval

IRBs and IECs must be constituted according to the applicable state and federal/local requirements of each participating region. The Sponsor or designee will require documentation noting all names and titles of members who make up the respective IRB or IEC. If any member of the IRB or IEC has direct participation in this study, written notification regarding his or her

abstinence from voting must also be obtained. Those Americas sites unwilling to provide names and titles of all members due to privacy and conflict of interest concerns should instead provide a Federal Wide Assurance Number or comparable number assigned by the Department of Health and Human Services.

The Sponsor or designee will supply relevant documents for submission to the respective IRB or IEC for the protocol's review and approval. This protocol, the Investigator's Brochure, a copy of the ICF, and, if applicable, participant recruitment materials and/or advertisements and other documents required by all applicable laws and regulations, must be submitted to a central or local IRB or IEC for approval. The IRB's or IEC's written approval of the protocol and participant informed consent must be obtained and submitted to the Sponsor or designee before commencement of the study (ie, before shipment of the Sponsor-supplied drug or study specific screening activity). The IRB or IEC approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (eg, ICF) reviewed; and state the approval date. The Sponsor will ship drug/notify site once the Sponsor has confirmed the adequacy of site regulatory documentation and, when applicable, the Sponsor has received permission from competent authority to begin the study. Until the site receives drug/notification no protocol activities, including screening, may occur.

Sites must adhere to all requirements stipulated by their respective IRB or IEC. This may include notification to the IRB or IEC regarding protocol amendments, updates to the ICF, recruitment materials intended for viewing by participants, local safety reporting requirements, reports and updates regarding the ongoing review of the study at intervals specified by the respective IRB or IEC, and submission of the Investigator's final status report to IRB or IEC. All IRB and IEC approvals and relevant documentation for these items must be provided to the Sponsor or its designee.

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Participant incentives should not exert undue influence for participation. Payments to participants must be approved by the IRB or IEC and Sponsor.

13.2 Participant Information, Informed Consent, and Participant Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The ICF, participant authorization form (if applicable), and participant information sheet (if applicable) describe the planned and permitted uses, transfers, and disclosures of the participant's personal and personal health information for purposes of conducting the study. The ICF and the participant information sheet (if applicable) further explain the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is given. The ICF will detail the requirements of the participant and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

The Investigator is responsible for the preparation, content, and IRB or IEC approval of the ICF and, if applicable, the participant authorization form. The ICF, participant authorization form (if

applicable), and participant information sheet (if applicable) must be approved by both the IRB or IEC and the Sponsor prior to use.

The ICF, participant authorization form (if applicable), and participant information sheet (if applicable) must be written in a language fully comprehensible to the prospective participant. It is the responsibility of the Investigator to explain the detailed elements of the ICF, participant authorization form (if applicable), and participant information sheet (if applicable) to the participant. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by the IRB or IEC. In the event the participant is not capable of rendering adequate written informed consent, then the participant's legally acceptable representative may provide such consent for the participant in accordance with applicable laws and regulations.

The participant, or the participant's legally acceptable representative, must be given ample opportunity to: (1) inquire about details of the study, and (2) decide whether or not to participate in the study. If the participant, or the participant's legally acceptable representative, determines he or she will participate in the study, then the ICF and participant authorization form (if applicable) must be signed and dated by the participant, or the participant's legally acceptable representative, at the time of consent and prior to the participant entering into the study. The participant or the participant's legally acceptable representative should be instructed to sign using their legal names, not nicknames, using blue or black ballpoint ink. The Investigator must also sign and date the ICF and participant authorization (if applicable) at the time of consent and prior to participant entering into the study; however, the Sponsor may allow a designee of the Investigator to sign to the extent permitted by applicable law.

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Once signed, the original ICF, participant authorization form (if applicable), and participant information sheet (if applicable) will be stored in the Investigator's site file. The Investigator must document the date the participant signs the ICF in the participant's medical record. Copies of the signed ICF, the signed participant authorization form (if applicable), and participant information sheet (if applicable) shall be given to the participant.

All revised ICFs must be reviewed and signed by relevant participants or the relevant participant's legally acceptable representative in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the participant's medical record, and the participant should receive a copy of the revised ICF.

13.3 Participant Confidentiality

The Sponsor and designees affirm and uphold the principle of the participant's right to protection against invasion of privacy. Throughout this study, a participant's source data will only be linked to the Sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited participant attributes, such as sex, age, or date of birth, and participant initials may be used to verify the participant and accuracy of the participant's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the Sponsor requires the Investigator to permit its monitor or designee's monitor, representatives

from any regulatory authority (eg, FDA, Medicines and Healthcare products Regulatory Agency, Pharmaceuticals and Medical Devices Agency), the Sponsor's designated auditors, and the appropriate IRBs and IECs to review the participant's original medical records (source data or documents), including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a participant's study participation, and autopsy reports. Access to a participant's original medical records requires the specific authorization of the participant as part of the informed consent process (see Section 13.2).

Copies of any participant source documents that are provided to the Sponsor must have certain personally identifiable information removed (ie, participant name, address, and other identifier fields not collected on the participant's CRF).

13.4 Publication, Disclosure, and Clinical Study Registration Policy

13.4.1 Publication and Disclosure

The Investigator is obliged to provide the Sponsor with complete test results and all data derived by the Investigator from the study. During and after the study, only the Sponsor may make study information available to other study Investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the Sponsor.

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The Sponsor may publish any data and information from the study (including data and information generated by the Investigator) without the consent of the Investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

13.4.2 Clinical Study Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, Takeda will, at a minimum register all interventional clinical trials it Sponsors anywhere in the world on ClinicalTrials.gov and/or other publicly accessible websites before start of study, as defined in Takeda Policy/Standard. Takeda contact information, along with Investigator's city, state (for Americas Investigators), country, and recruiting status will be registered and available for public viewing.

For some registries, Takeda will assist callers in locating study sites closest to their homes by providing the Investigator name, address, and phone number to the callers requesting trial information. Once participants receive Investigator contact information, they may call the site requesting enrollment into the trial. The investigative sites are encouraged to handle the trial

inquiries according to their established participant screening process. If the caller asks additional questions beyond the topic of trial enrollment, they should be referred to the Sponsor.

Any Investigator who objects to the Sponsor providing this information to callers must provide the Sponsor with a written notice requesting that their information not be listed on the registry site.

13.4.3 Clinical Study Results Disclosure

Takeda will post the results of clinical studies on ClinicalTrials.gov or other publicly accessible websites, as required by Takeda Policy/Standard, applicable laws and/or regulations.

13.5 Insurance and Compensation for Injury

Each participant in the study must be insured in accordance with the regulations applicable to the site where the participant is participating. If a local underwriter is required, then the Sponsor or Sponsor's designee will obtain clinical study insurance against the risk of injury to study participants. Refer to the study site agreement regarding the Sponsor's policy on participant compensation and treatment for injury. If the Investigator has questions regarding this policy, he or she should contact the Sponsor or Sponsor's designee.

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14.0 ADMINISTRATIVE AND REFERENCE INFORMATION

14.1 Administrative Information

14.1.1 Study Contact Information

Contact Type / Role	Contact
Serious adverse event and pregnancy reporting	Pharmacovigilance Takeda Development Center Americas, Inc. Fax: 224-554-1052

14.1.2 INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, package insert and any other product information provided by the Sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also to protect the rights, safety, privacy, and well-being of study participants in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonisation, E6[R2] Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in Section 10.2.9 of this protocol.
- Terms outlined in the study site agreement.
- Responsibilities of the Investigator (Appendix A).

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Appendix D of this protocol.

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_____

Signature of Investigator Date

_____

Investigator Name (print or type)

_____

Investigator's Title

_____

Location of Facility (City, State/Provence)

_____

Location of Facility (Country)

14.1.3 Study-Related Responsibilities

The Sponsor will perform all study-related activities with the exception of those identified in the Study-Related Responsibilities template. The vendors identified for specific study-related activities will perform these activities in full or in partnership with the Sponsor.

14.1.4 List of Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{∞}	The area under the concentration-time curve, from time 0 to infinity
$AUC_{\%extrap}$	Percent of AUC_{∞} extrapolated
AUC_{last}	The area under the concentration-time curve, from time 0 to the last quantifiable concentration, as calculated by the linear-log trapezoidal method.
BMI	Body mass index
bpm	Beats per minute
CeD	Celiac disease
CFR	Code of Federal Regulations
CI	Confidence interval
CL/F	Apparent clearance after extravascular administration
cm	Centimeter
C_{max}	Maximum observed concentration
COVID-19	Coronavirus disease 2019
CRF	Case report form
CRU	Clinical Research Unit
CV	Coefficient of variance
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GMR	Geometric mean ratio
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human lymphocyte antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee

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INR	International normalized ratio
IRB	Institutional Review Board
kg	Kilogram
LFT	Liver function test
m^2	Meters squared
MedDRA®	Medical Dictionary for Regulatory Activities®
mg	Milligram
mL	Milliliter
mmHg	Millimeter of mercury
msec	Millisecond
NCA	Non-compartmental analysis
PK	Pharmacokinetic(s)
QTcF	QT interval corrected for heart rate using Fridericia's formula
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SUSAR	Suspected unexpected serious adverse reaction
$t_{½z}$	Terminal disposition phase half-life
t_{lag}	Lag time to first quantifiable concentration in plasma
t_{max}	Time to first occurrence of maximum observed concentration
TDCA	Takeda Development Center Americas
TEAE	Treatment-emergent adverse event
TG2	Transglutaminase 2
ULN	Upper limit of normal
US	United States
USA	United States of America
V_z/F	Apparent volume of distribution during the terminal disposition phase after extravascular administration
λ_z	Terminal disposition phase rate constant

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15.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs, medical history, and concurrent conditions will be coded using the MedDRA®. Drugs will be coded using the World Health Organization Drug Dictionary.

15.1 CRFs (Electronic and Paper)

Celerion standard CRFs will be supplied. CRFs are produced, stored electronically, and are available to the designated study team members. Each CRF is reviewed and signed by the Investigator. The final signed CRFs are provided to the Sponsor in the format as decided upon between Celerion and the Sponsor (eg, compact disc, flashdrive, secure file transfer protocol). This will be documented in the Data Management Plan (if applicable).

After completion of the entry process, computer logic checks will be run to identify items, such as inconsistent dates, missing data, and questionable values. Queries may be issued by Takeda personnel (or designees) and will be answered by the site.

Corrections are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for change. Reasons for significant corrections should additionally be included.

The Principal Investigator must review the CRFs for completeness and accuracy and must sign and date the appropriate CRFs as indicated. Furthermore, the Investigator must retain full responsibility for the accuracy and authenticity of all data entered on the CRFs.

After the lock of the clinical study database, any change of, modification of, or addition to the data on the CRFs should be made by the Investigator with use of change and modification records of the CRFs. The Principal Investigator must review the data change for completeness and accuracy, and must sign and date.

CRFs will be reviewed for completeness and acceptability at the study site during periodic visits by study monitors. The Sponsor or its designee will be permitted to review the participant's medical and hospital records pertinent to the study to ensure accuracy of the CRFs. The completed CRFs are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the Sponsor.

15.2 Record Retention

The Investigator agrees to keep the records stipulated in Section 15.1 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating participants, medical records, temporary media such as thermal sensitive paper, source worksheets, all original signed and dated ICFs, participant authorization forms regarding the use of personal health information (if separate from the ICFs), electronic copy of CRFs, including the audit trail, and detailed records of drug disposition to enable evaluations or audits from regulatory authorities, the Sponsor or its designees. Any source documentation printed on

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degradable thermal sensitive paper should be photocopied by the site and filed with the original in the participant's chart to ensure long-term legibility. Furthermore, ICH E6 Section 4.9.5 requires the Investigator to retain essential documents specified in ICH E6 (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH E6 Section 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the Clinical Study Site Agreement between the Investigator and Sponsor.

Refer to the Clinical Study Site Agreement for the Sponsor's requirements on record retention. The Investigator and the head of the institution should contact and receive written approval from the Sponsor before disposing of any such documents.

16.0 REFERENCES

1. 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.

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17.0 APPENDICES

Appendix A Responsibilities of the Investigator

Clinical research studies sponsored by the Sponsor are subject to ICH GCP and all the applicable local laws and regulations. The responsibilities imposed on Investigators by the FDA are summarized in the "Statement of Investigator" (Form FDA 1572), which must be completed and signed before the Investigator may participate in this study.

The Investigator agrees to assume the following responsibilities by signing a Form FDA 1572:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the staff that will assist in the protocol.
3. If the Investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the Investigator/institution should ensure that this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.
4. Ensure that study related procedures, including study specific (nonroutine/nonstandard panel) screening assessments are NOT performed on potential participants, prior to the receipt of written approval from relevant governing bodies/authorities.
5. Ensure that all colleagues and employees assisting in the conduct of the study are informed of these obligations.
6. Secure prior approval of the study and any changes by an appropriate IRB/IEC that conform to 21 CFR Part 56, ICH, and local regulatory requirements.
7. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to participants. Make at least yearly reports on the progress of the study to the IRB/IEC, and issue a final report within 3 months of study completion.
8. Ensure that requirements for informed consent, as outlined in 21 CFR Part 50, ICH and local regulations, are met.
9. Obtain valid informed consent from each participant who participates in the study, and document the date of consent in the participant's medical chart. Valid informed consent is the most current version approved by the IRB/IEC. Each ICF should contain a participant authorization section that describes the uses and disclosures of a participant's personal information (including personal health information) that will take place in connection with the study. If an ICF does not include such a participant authorization, then the Investigator must obtain a separate participant authorization form from each participant or the participant's legally acceptable representative.
10. Prepare and maintain adequate case histories of all persons entered into the study, including CRFs, hospital records, laboratory results, etc, and maintain these data for a minimum of

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2 years following notification by the Sponsor that all investigations have been discontinued or that the regulatory authority has approved the marketing application. The Investigator should contact and receive written approval from the Sponsor before disposing of any such documents.

11. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
12. Maintain current records of the receipt, administration, and disposition of Sponsor-supplied drugs, and return all unused Sponsor-supplied drugs to the Sponsor.
13. Report adverse reactions to the Sponsor promptly. In the event of an SAE, notify the Sponsor within 24 hours.

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Appendix B Elements of the Participant Informed Consent

In seeking informed consent, the following information shall be provided to each participant:

1. A statement that the study involves research.
2. An explanation of the purposes of the research.
3. The expected duration of the participant's participation.
4. A description of the procedures to be followed, including invasive procedures.
5. The identification of any procedures that are experimental.
6. The estimated number of participants involved in the study.
7. A description of the participant's responsibilities.
8. A description of the conduct of the study.
9. A statement describing the treatment(s) and the probability for random assignment to each treatment.
10. A description of the possible side effects of the treatment that the participant may receive.
11. A description of any reasonably foreseeable risks or discomforts to the participant and, when applicable, to an embryo, fetus, or nursing infant.
12. A description of any benefits to the participant or to others that reasonably may be expected from the research. When there is no intended clinical benefit to the participant, the participant should be made aware of this.
13. Disclosures of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant and their important potential risks and benefits.
14. A statement describing the extent to which confidentiality of records identifying the participant will be maintained, and a note of the possibility that regulatory agencies, auditor(s), IRB/IEC, and the monitor may inspect the records. By signing a written ICF, the participant or the participant's legally acceptable representative is authorizing such access.
15. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of or where further information may be obtained.
16. The anticipated prorated payment(s), if any, to the participant for participating in the study.
17. The anticipated expenses, if any, to the participant for participating in the study.
18. An explanation of whom to contact for answers to pertinent questions about the research (Investigator), participant's rights, and IRB/IEC and whom to contact in the event of a research-related injury to the participant.
19. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the participant otherwise is entitled, and that the participant or the

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participant's legally acceptable representative may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled.

20. The consequences of a participant's decision to withdraw from the research and procedures for orderly termination of participation by the participant.
21. A statement that the participant or the participant's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the participant's willingness to continue participation in the study.
22. The foreseeable circumstances or reasons under which the participant's participation in the study may be terminated.
23. A written participant authorization (either contained within the ICF or provided as a separate document) describing to the participant the contemplated and permissible uses and disclosures of the participant's personal information (including personal health information) for purposes of conducting the study. The participant authorization must contain the following statements regarding the uses and disclosures of the participant's personal information:

    a) that personal information (including personal health information) may be processed by or transferred to other parties in other countries for clinical research and safety reporting purposes, including, without limitation, to the following: (1) Takeda, its affiliates, and licensing partners; (2) business partners assisting Takeda, its affiliates, and licensing partners; (3) regulatory agencies and other health authorities; and (4) IRBs/IECs;

    b) it is possible that personal information (including personal health information) may be processed and transferred to countries that do not have data protection laws that offer participants the same level of protection as the data protection laws within this country; however, Takeda will make every effort to keep your personal information confidential, and your name will not be disclosed outside the clinic unless required by law;

    c) that personal information (including personal health information) may be added to Takeda's research databases for purposes of developing a better understanding of the safety and effectiveness of the study medication(s), studying other therapies for patients, developing a better understanding of disease, and improving the efficiency of future clinical studies;

    d) that participants agree not to restrict the use and disclosure of their personal information (including personal health information) upon withdrawal from the study to the extent that the restricted use or disclosure of such information may impact the scientific integrity of the research; and

    e) that the participant's identity will remain confidential in the event that study results are published.

24. Female participants of childbearing potential (eg, nonsterilized, premenopausal female participants) who are sexually active must use highly effective contraception (as defined in the ICF) from signing the ICF and throughout the duration of the study, and for 30 days after

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the last dose of study drug. Regular pregnancy tests will be performed throughout the study for all female participants of childbearing potential. If a participant is found to be pregnant during study, study drug will be discontinued, and the Investigator will offer the participant the choice to receive treatment information.

25. Male participants must use highly effective contraception (as defined in the ICF) from signing the informed consent throughout the duration of the study and for 100 days after the last dose of study drug. If the partner of the participant is found to be pregnant during the study, the Investigator will offer the participant the choice to receive treatment information.
26. A statement that clinical trial information from this trial will be publicly disclosed in a publicly accessible website, such as ClinicalTrials.gov.

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Appendix C Investigator Consent to the Use of Personal Information

Takeda will collect and retain personal information of Investigator, including his or her name, address, and other personally identifiable information. In addition, Investigator's personal information may be transferred to other parties located in countries throughout the world (eg, the United Kingdom, United States, and Japan), including the following:

- Takeda, its affiliates, and licensing partners.
- Business partners assisting Takeda, its affiliates, and licensing partners.
- Regulatory agencies and other health authorities.
- IRBs and IECs.

Investigator's personal information may be retained, processed, and transferred by Takeda and these other parties for research purposes including the following:

- Assessment of the suitability of Investigator for the study and/or other clinical studies.
- Management, monitoring, inspection, and audit of the study.
- Analysis, review, and verification of the study results.
- Safety reporting and pharmacovigilance activities relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to other medications used in other clinical studies that may contain the same chemical compound present in the study medication.
- Inspections and investigations by regulatory authorities relating to the study.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of study records.
- Posting Investigator site contact information, study details and results on publicly accessible clinical trial registries, databases, and websites.

Investigator's personal information may be transferred to other countries that do not have data protection laws that offer the same level of protection as data protection laws in Investigator's own country.

Investigator acknowledges and consents to the use of his or her personal information by Takeda and other parties for the purposes described above.

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Appendix D Pregnancy and Contraception

Contraception and Pregnancy Avoidance Procedure

Male Participants

From signing of the ICF, throughout the duration of the study, and for 100 days after last dose of study drug, nonsterilized** male participants who are sexually active with a female partner of childbearing potential* must use barrier contraception (eg, condom with or without spermicidal cream or jelly). In combination with this measures, females of childbearing potential who are partners of male subjects are also advised to use additional contraception as shown in the list containing highly effective/effective contraception below. In addition, male subjects must be advised not to donate sperm during this period.

Female Participants and Their Male Partners

From signing of the ICF, throughout the duration of the study, and for 30 days after last dose of study drug, female participants of childbearing potential* who are sexually active with a nonsterilized male partner** must use a highly effective method (from the list below).

In addition they must be advised not to donate ova during this period.

Definitions and Procedures for Contraception and Pregnancy Avoidance

The following definitions apply for contraception and pregnancy avoidance procedures.

* A female is considered a female of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral tubal ligation, bilateral salpingectomy, hysteroscopic sterilization, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range (FSH >40 IU/L) may be used to confirm a post-menopausal state in younger females (eg, those <45 year old) or females who are not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

** Sterilized males should be at least 1 year post-bilateral vasectomy and have confirmed that they have obtained documentation of the absence of sperm in the ejaculate or have had bilateral orchidectomy.

The following procedures apply for contraception and pregnancy avoidance.

1. Highly effective methods of contraception are defined as "those, alone or in combination, that result in a low failure rate (ie, less than 1% failure rate per year when used consistently and correctly). In this study, where medications and devices containing hormones are included, the only acceptable methods of contraception are:
    - Non-Hormonal Methods:
        - Intrauterine device (IUD).

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  - Bilateral tubal occlusion.
  - Vasectomised partner (provided that partner is the sole sexual partner of the study participant and that the vasectomised partner has received medical assessment of the surgical success.

- Hormonal Methods: Hormonal contraception may be susceptible to interaction with the investigative compound, comparator, concomitant medications, which may reduce the efficacy of the contraception method.
  - Combined (estrogen and progestogen) hormonal contraception associated with inhibition of ovulation initiated at least 3 months prior to the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if for shorter duration until she has been on contraceptive for 3 months;
    - Oral.
    - Intravaginal (eg, ring).
    - transdermal.
  - Progestogen-only hormonal contraception associated with inhibition of ovulation initiated at least 3 months prior to the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if shorter until she has been on contraceptive for 3 months;
    - oral.
    - Injectable.
    - Implantable.

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2. In addition, effective methods of contraception (there may be a higher than 1% failure rate) are:
   - Double-barrier method (contraceptive sponge, diaphragm or cervical cap with spermicidal jellies or creams PLUS male condom).
3. Unacceptable methods of contraception are:
   - Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods).
   - Spermicides only.
   - Withdrawal.
   - No method at all.
   - Use of female and male condoms together.
   - Cap/diaphragm/sponge without spermicide and without condom.
4. Participants will be provided with information on highly effective/effective methods of contraception as part of the participant informed consent process and will be asked to sign a

consent form stating that they understand the requirements for avoidance of pregnancy, donation of ova, and sperm donation during the course of the study.

5. During the course of the study, regular serum human chorionic gonadotropin (hCG) pregnancy tests will be performed only for females of childbearing potential and all participants (male and female) will receive continued guidance with respect to the avoidance of pregnancy and sperm donation as part of the study procedures. Such guidance should include a reminder of the following:
   a) contraceptive requirements of the study
   b) reasons for use of barrier methods (ie, condom) in males with pregnant partners
   c) assessment of participant compliance through questions such as
      i. Have you used the contraception consistently and correctly since the last visit?
      ii. Have you forgotten to use contraception since the last visit?
      iii. Are your menses late (even in females with irregular or infrequent menstrual cycles a pregnancy test must be performed if the answer is "yes")
      iv. Is there a chance you could be pregnant?
6. In addition to a negative serum hCG pregnancy test at Screening, female participants of childbearing potential must also have confirmed menses in the month before first dosing (no delayed menses; with the exception of female participants using a protocol acceptable contraception method that has a known side effect of delayed or irregular menses). In addition, participants must also have a negative serum hCG pregnancy test at first check-in, within approximately 24 hours prior to receiving first dose of stud drug as close as possible and prior to first dose of study drug, preferably on the same day.

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General Guidance With Respect to the Avoidance of Pregnancy

Such guidance should include a reminder of the following:

7. contraceptive requirements of the study.
8. reasons for use of barrier methods (ie, condom) in males with pregnant partners.
9. assessment of participant compliance through questions such as:
   – Have you used the contraception consistently and correctly since the last visit?
   – Have you forgotten to use contraception since the last visit?
   – Are your menses late (even in females with irregular or infrequent menstrual cycles a pregnancy test must be performed if the answer is "yes")
   – Is there a chance you could be pregnant?

Pregnancy

If any participant is found to be pregnant during the study she should be withdrawn and any sponsor-supplied drug should be immediately discontinued. In addition, any pregnancies in the female partner of a male participant during the study or for 165 days after the last dose, should also be recorded following authorization from the participant's partner.

If a female subject's pregnancy occurs within 105 days of administration of TAK-227, the pregnancy should be reported immediately, using a pregnancy notification form, to the contact listed in the medical monitoring plan

If the female participant and/or female partner of a male participant agrees to the primary care physician being informed, the Investigator should notify the primary care physician of her or her male partner (ie, male participant) participation in a clinical study at the time she became pregnant and provide details of the study drug the female participant or her male partner (ie, male participant) received.

All pregnancies, including female participants and female partners of male participants on active study drug (including comparator, if applicable) will be followed up to final outcome, using the pregnancy form. Pregnancies will remain blinded to the study team. The outcome, including any premature termination, must be reported to the sponsor. An evaluation after the birth of the child will also be conducted.

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Appendix E Summary of Changes from Previous Version

A summary of changes incorporated into Amendment 1 is provided in the table below.

Summary of Changes(s) Since Last Version of Approved Protocol	
Description of Change	**Section(s) Affected by Change**
Change creatine clearance in inclusion criteria from 80 mL/min to 90 mL/min	Section 1.0 (Study Summary) Section 7.1 (Inclusion Criteria)
Change washout period in table from "Days 3-4" to "Not less than 4 days". Also, change "at least 96 hours" to "not less than 4 days".	Section 1.0 (Study Summary) Table 2.a (Study Schematic) Table 2.b (Study Treatments for Study Drug) Section 3.0 (Schedule of Study Procedures) Section 4.2 (Rationale for the Proposed Study) Section 6.1 (Study Design) Section 6.4.1 (Rationale of Study Design)

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ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
[redacted]	Clinical Science Approval	17-Feb-2023 16:52 UTC
[redacted]	Biostatistics Approval	17-Feb-2023 16:54 UTC
[redacted]	Clinical Pharmacology Approval	18-Feb-2023 02:50 UTC

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