



Title: A Randomized, Open-Label and Double-Blind, Placebo-Controlled, Single- and Multiple-Dose, Phase 1 Study of the Pharmacokinetics of TAK-491 40 mg and 80 mg in Healthy Chinese Subjects

NCT Number: NCT02541669

Protocol Approve Date: 05 August 2016

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PHASE 1 PROTOCOL

A Randomized, Open-Label and Double-Blind, Placebo-Controlled, Single- and Multiple-Dose, Phase 1 Study of the Pharmacokinetics of TAK-491 40 mg and 80 mg in Healthy Chinese Subjects

Pharmacokinetic Study of TAK-491 in Healthy Chinese Subjects

Sponsor: Takeda Development Center Asia, Pte. Ltd.
21 Biopolis Road, Nucleos North Tower,
Level 4, Singapore 138567

Study Number: TAK-491_112

IND Number: Not Applicable **EudraCT Number:** Not Applicable

Compound: TAK-491

Date: 05 August 2016 **Amendment Number: 04**

Amendment History:

Date	Amendment Number	Amendment Type	Region
27 April 2011	Initial Protocol	Not applicable	China
25 March 2013	1	Substantial	China
30 January 2014	2	Substantial	China
18 March 2016	3	Substantial	China
05 August 2016	4	Substantial	China

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1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

A separate contact information list will be provided.

Takeda sponsored European and Asian Pacific investigators will be provided with emergency medical contact information cards to be carried by each subject.

General advice on protocol procedures should be obtained through the monitor assigned to the study site. Information on service providers is given in Section 3.1 and relevant guidelines provided to the site.

Issue	Contact
Serious adverse event and pregnancy reporting	PPD
Medical Monitor (medical advice on protocol, compound, and medical management of subjects)	
Responsible Medical Officer (carries overall responsibility for the conduct of the study)	

1.2 Approval

REPRESENTATIVES OF TAKEDA

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonization E6 Good Clinical Practice Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical trial disclosure laws, and regulations.

SIGNATURES

Electronic signatures of the Responsible Medical Officer and other signatories are located on the last page of this document.

PPD



INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, 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 protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonization E6 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 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.
- [Appendix B](#) - Responsibilities of the Investigator.

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

Signature of Investigator

Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State)

Location of Facility (Country)

1.3 Protocol Amendment No. 4 Summary of Changes

This document describes the changes in reference to Protocol Amendment No. 3 dated 18 March 2016.

The primary purpose of this amendment was to add collection of plasma for pharmacokinetic sampling after Day 10, diet requirement update, and independent liver safety review. Full details on changes of text are given in [Appendix F](#) including detailed rationale. The following is a summary of the changes made in the amendment:

- The fat content of the diet of healthy volunteers during their hospital confinement was modified in Section [7.4](#).

Justification: To align the protocol with Ethic Committee's comments.

- The collection of plasma for pharmacokinetic sampling was added in Section [9.1.12.1](#).

Justification: To align the protocol with Ethic Committee's comments.

- The independent liver safety monitoring process was added in Section [11.0](#).

Justification: To align the protocol with Ethic Committee's comments.

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2.0 STUDY SUMMARY

Name of Sponsor: Takeda Development Center Asia, Pte. Ltd.	Compound: TAK-491			
Title of Protocol: A Randomized, Open-Label and Double-Blind, Placebo-Controlled, Single- and Multiple-Dose, Phase 1 Study of the Pharmacokinetics of TAK-491 40 mg and 80 mg in Healthy Chinese Subjects	IND No.: Not Applicable	EudraCT No.: Not Applicable		
Study Number: TAK-491_112	Phase: 1			
Study Design: This is a randomized, open-label and double-blind, placebo-controlled, single- and multiple-dose, phase 1 study involving 64 healthy Chinese subjects aged 18 to 45 years, inclusive, and considered eligible based on the inclusion and exclusion entry criteria. Subjects will receive TAK-491 or placebo as a single dose followed by multiple doses. Sixteen subjects were allocated equally and randomly to 1 of 2 TAK-491 regimens (40 mg or 80 mg) in the open-label part of the study; the 2 regimens were conducted in parallel.				
Open-Label Part				
Regimen	Regimen Description	Number of Subjects		
A	TAK-491 40 mg as a single dose followed by multiple doses	8		
B	TAK-491 80 mg as a single dose followed by multiple doses	8		
Forty-eight subjects will be allocated equally and randomly to 1 of 3 regimens (TAK-491 40 mg or 80 mg, or placebo) in the double-blind part of the study; the 3 regimens will be conducted in parallel. To maintain the study blind, subjects in this part of the study will receive a least 1 placebo tablet; the TAK-491 40 mg and TAK-491 80 mg tablets differ in size; therefore, placebo tablets for each size will be utilized as appropriate.				
Double-Blind Part				
Regime n	Regimen Description	Number of Subjects		
A	TAK-491 40 mg + placebo as a single dose followed by multiple doses	16		
B	TAK-491 80 mg + placebo as a single dose followed by multiple doses	16		
C	Placebo (2 tablets) as a single dose followed by multiple doses	16		
The pre-dosing study period will consist of a Screening Period (Study Days -28 to -2) and Check-in (Study Day -1). On Day 1, a single dose of TAK-491 or placebo will be administered followed by a pharmacokinetic sampling period through 72 hours postdose (Days 1-4). On Days 4 through 10, subjects will be on a daily dosing regimen, followed by a 24-hour pharmacokinetic assessment period prior to Study Exit (Day 11). The total confinement period for a subject who completes the study will be 12 days. All subjects will return to the site for PK sampling on Days 12-13 and follow-up serum chemistry tests on Day 14. A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 [± 2 days]) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since the final dose of study medication or Early Termination Visit.				

Screening	Check-in	Dosing and PK	72-hr PK	Dosing (a)	Dosing and AM Trough Level (b)	Dosing and 24-hr PK	Study Exit	PK collection	F/U Visit (c)	F/U Call
Days -28 to -2	Day -1	Day 1	Days 2-4	Day 4	Days 5-9	Day 10	Day 11	Days 12-13	Day 14	Day 24 (± 2)

PK=pharmacokinetics.

(a) Dosing will occur after the collection of the 72-hr pharmacokinetic sample.

(b) PK trough samples will be collected on Days 7 to 9.

(c) Serum chemistry testing.

Primary Objective:

To assess the pharmacokinetics of TAK-491 40 mg and 80 mg in healthy Chinese subjects after both single and multiple dose administration.

Secondary Objectives:

To evaluate the safety and tolerability of TAK-491 40 mg and 80 mg in healthy Chinese subjects after both single and multiple dose administration.

Subject Population: Healthy male and female subjects aged 18-45 years, inclusive.

Number of Subjects: Estimated total: 64	Number of Sites: 1 site in China
Dose Levels: TAK-491 40 mg TAK-491 80 mg Matched placebos	Route of Administration: oral
Duration of Treatment: Single dose with sample collection followed with 7 days of daily dosing and sample collection.	Period of Evaluation: Approximately 52 days (including Screening, Day -1 through Study Exit and Follow-up telephone call).
Main Criteria for Inclusion: Healthy male or non-pregnant, non-lactating female subjects of Chinese descent who are 18 to 45 years of age, inclusive, at signing of the informed consent form and first study medication dose. In addition, the subjects must be willing and able to comply with the protocol requirements, must be able to understand and sign a written informed consent form prior to the initiation of any study procedures, have a body mass index between $\geq 19.0 \text{ kg/m}^2$ and $< 24.0 \text{ kg/m}^2$; and must have clinical laboratory results within reference range unless the investigator deems the results not to be clinically significant.	
Main Criteria for Exclusion: The subject has a known hypersensitivity or allergy to any angiotensin type II receptor blocker or to any of the excipients in the TAK-491 formulation to be taken. Subjects will also be excluded if they are unwilling or unable to comply with the protocol or if they use any excluded medications, supplements, or food products outlined in the protocol. The subject has a systolic blood pressure < 110 and $\geq 160 \text{ mm Hg}$ or a diastolic blood pressure of < 60 and $\geq 100 \text{ mm Hg}$. Additionally, subjects will be excluded if they have abnormal Screening or Day -1 laboratory values that suggest a clinically significant underlying disease or have the following laboratory abnormalities: alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) that exceeds $> 1.5 \times$ the upper limit of normal, hemoglobin value $< 12 \text{ g/dL}$, or who has positive anti-HIV, anti-HCV, or positive HBsAg test results at Screening.	

Main Criteria for Evaluation and Analyses:

Pharmacokinetics:

Plasma pharmacokinetic parameters to be calculated for TAK-536 (the active moiety): area under the concentration-time curve from time 0 to 24 hours (AUC_{24}), area under the concentration-time curve from time 0 to the last quantifiable concentration (AUC_{last}) (after single dose only), area under the concentration-time curve from time 0 to infinity (AUC_{∞}) (after single dose only), average concentration during a dosing interval, at steady state ($C_{av,ss}$) (after multiple dose only), maximum observed concentration (C_{max}), minimum observed concentration during a dosing interval (C_{min}) (after multiple dose only), observed concentration at the end of a dosing interval (C_{trough}) (after multiple dose only), apparent clearance after extravascular administration (CL/F) (after single dose only), terminal disposition phase rate constant (λ_z) (after single dose only), terminal disposition phase half-life ($t_{1/2z}$) (after single dose only), time of first occurrence of C_{max} (t_{max}), and apparent volume of distribution during the terminal disposition phase after extravascular administration (V_z/F) (after single dose only).

Urine pharmacokinetic parameters to be calculated for TAK-536: amount of drug excreted in urine (Ae_{24} , Ae_{72} [after single dose only], Ae_{0-2} , Ae_{2-4} , Ae_{4-8} , Ae_{8-12} , Ae_{12-24} , Ae_{24-48} [after single dose only], Ae_{48-72} [after single dose only]), renal clearance (CL_R), and fraction of administered dose of drug excreted in urine during a dosing interval (f_{e24} and f_{e72}) (f_{e72} after single dose only).

Safety and Tolerability:

Safety and tolerability assessment will include physical examination findings, vital signs, 12-lead electrocardiogram (ECG) results, laboratory evaluations (hematology, serum chemistry, and urinalysis), and adverse events (AEs).

Statistical Considerations:

Pharmacokinetic Measures:

Concentrations of TAK-536 in plasma will be summarized by regimen over each scheduled sampling time using descriptive statistics for the open-label and double-blind parts separately and overall.

The amount of TAK-536 excreted in urine will be summarized by regimen over each scheduled sampling interval using descriptive statistics for each part separately and overall.

Plasma and urine PK parameters of TAK-536 will be summarized by regimen using descriptive statistics for each part separately and overall.

Safety:

AEs will be presented in the listings, and TEAEs will be summarized. Clinical laboratory variables, vital sign, and ECG parameters will be summarized with descriptive statistics for Baseline, postdose, and change from Baseline to postdose values. The percentage of subjects with at least 1 postdose laboratory variable, vital sign, and ECG parameter that meet Takeda's predefined criteria will be summarized. Physical examination findings will be presented in the data listings. All summaries will be performed by regimen for each part separately and overall.

Sample Size Justification:

A total sample size of 64 subjects will be enrolled in this study. Sixteen subjects, 8 per regimen (TAK-491 40 mg and 80 mg) were randomized to the open-label part of the study. Forty-eight subjects, 16 per regimen (TAK-491 40 mg, 80 mg, and placebo) will be randomized to the double-blind part of the study. The sample size is considered sufficient for evaluation of safety, tolerability, and PK of each part of the study, and was not based on statistical power considerations.

3.0 STUDY REFERENCE INFORMATION

3.1 Study-Related Responsibilities

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

3.2 Principal Investigator

PPD



3.3 List of Abbreviations

λ_z	terminal disposition phase rate constant
%CV	percent coefficient of variation
ACE	angiotensin-converting enzyme
AE	adverse event
Ae_t	amount of drug excreted in urine from time 0 to time t
Ae_{t1-t2}	amount of drug excreted in urine from time 1 to time 2
AII	angiotensin II
ALT	alanine aminotransferase
ARB	AT1 receptor blockers
AT1	angiotensin II type 1
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{24}	area under the concentration-time curve from time 0 to 24 hours
AUC_{∞}	area under the concentration-time curve from time 0 to infinity, calculated using the observed value of the last quantifiable concentration
AUC_{last}	area under the concentration-time curve from 0 to time of the last quantifiable concentration
BMI	body mass index
bpm	beats per minute
$C_{\text{av,ss}}$	average concentration during a dosing interval, at steady state
CFR	Code of Federal Regulations
CI	confidence interval
CL/F	apparent clearance after extravascular administration, calculated using the observed value of the last quantifiable concentration
CL _R	renal clearance
C _{max}	maximum observed concentration
C _{min}	minimum observed concentration during a dosing interval
CMV	cytomegalovirus
CRF	case report form
CRO	Contract Research Organization
CS	clinically significant
C_{trough}	observed concentration at the end of a dosing interval
CYP	cytochrome P-450
EBV VCA	Epstein-Barr virus viral capsid antigen
ECG	electrocardiogram
(e)CRF	electronic case report form
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
f_{et}	fraction of administered dose of drug excreted in urine from time 0 to time t

FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	γ -glutamyl transferase
HBcAb	hepatitis B core antibody
HBeAb	hepatitis B envelope antibody
HBeAg	hepatitis B envelope antigen
HBsAg	hepatitis B surface antigen
hCG	human chorionic gonadotropin
HCV	hepatitis C virus
HDL-C	high-density lipoprotein cholesterol
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IUD	intrauterine device
K ₂ EDTA	potassium ethylenediamine tetraacetic acid
LDL-C	low-density lipoprotein cholesterol
MedDRA	Medical Dictionary for Regulatory Activities
NCS	not clinically significant
PK	pharmacokinetic(s)
PTE	pretreatment event
RAAS	renin-angiotensin-aldosterone system
RCF	relative centrifugal force
SAE	serious adverse event
SAP	statistical analysis plan
SmPC	Summary of Product Characteristics
SUSAR	suspected unexpected serious adverse reaction
t _{1/2z}	terminal disposition phase half-life
TAK-491	azilsartan medoxomil
TAK-491F	salt free form of TAK-491
TAK-536	azilsartan
TAK-536 M-I	metabolite of TAK-536
TAK-536 M-II	metabolite of TAK-536
TEAE	treatment emergent adverse event
t _{max}	time of first occurrence of C _{max}
ULN	upper limit of normal
US	United States
V _{z/F}	apparent volume of distribution during the terminal disposition phase after extravascular administration, calculated using the observed value of the last quantifiable concentration
WHO	World Health Organization

3.4 Corporate Identification

TDC (Asia)	Takeda Development Center Asia Pte. Ltd.
TDC (EU)	Takeda Development Centre Europe Ltd.
TDC (US)	Takeda Development Center Americas, Inc.
TDC	TDC Asia, TDC Europe, and/or TDC Americas, as applicable
TPC	Takeda Pharmaceutical Company Limited
Takeda	TDC Asia, TDC Americas, TDC Europe, and/or TPC, as applicable

4.0 INTRODUCTION

4.1 Background

A major component of blood pressure regulation is the renin-angiotensin-aldosterone system (RAAS), a system of hormone-mediated feedback interactions that results in the relaxation or constriction of blood vessels in response to various stimuli. Angiotensin II (AII), a polypeptide hormone, is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE) as part of the RAAS. AII is the principal pressor agent of the RAAS with a myriad of effects on the cardiovascular system and on electrolyte homeostasis [1]. Two receptors for AII have been identified. Angiotensin II type 1 (AT1) receptors are located predominantly in vascular smooth muscle, where activation by AII results in vasoconstriction, hypertrophic proliferation, and inflammation. In contrast, stimulation of angiotensin II type 2 receptors by AII results in vasodilation, antiproliferative effects, and other effects that are opposite from those of AT1 receptor stimulation [2].

Drugs that modulate the RAAS are used commonly worldwide for the treatment of hypertension. Of these, some block the synthesis of AII by inhibiting ACE (ACE inhibitors), while others (AT1 receptor blockers [ARBs]) inhibit the action of AII by binding directly to the AT1 receptor, thereby allowing blood vessels to dilate, resulting in a reduction in blood pressure [3]. The effects of ARBs on other conditions in which the RAAS plays an important role, such as congestive heart failure, postmyocardial infarction management, and diabetic nephropathy, have also been investigated. ARBs are generally considered to be more tolerable than other classes of antihypertensive agents and are often used to treat hypertension in patients who are intolerant to ACE inhibitors [4]. Cough and angioedema occur less frequently with ARBs than with ACE inhibitors, and common side effects such as symptomatic hypotension, flushing, tachycardia, palpitations, and dizziness, along with headache and lightheadedness, are generally mild and well tolerated [5].

There is still a need for compounds that improve tolerability and efficacy for the treatment of hypertension; therefore, azilsartan medoxomil, or TAK-491, a prodrug that is rapidly hydrolyzed to the active moiety, azilsartan, or TAK-536, which is a highly potent, long-acting ARB is being evaluated by Takeda to treat subjects with essential hypertension.

4.2 Summary of Nonclinical Data

Nonclinical studies have indicated that TAK-491 reduces blood pressure after single or multiple daily dosing without tachycardia and without rebound hypertension after the withdrawal of treatment. TAK-491 also demonstrated antiproteinuric effects in Wistar fatty rats with overt nephropathy and increased insulin sensitivity in spontaneously hypertensive rats. TAK-491 is not expected to have any untoward effects on the central nervous system or respiratory system. Its effect on the cardiovascular system in conscious dogs was limited to a reduction in systolic blood pressure, an observation consistent with the pharmacodynamic profile of the compound.

Please refer to the TAK-491 Global Investigator's Brochure for additional information [6].

4.3 Summary of Relevant Clinical Studies

TAK-491 is rapidly hydrolyzed to TAK-536, with no detectable TAK-491F (salt free form of TAK-491) plasma concentrations following doses of up to 320 mg for 7 days. TAK-536 undergoes extensive metabolism to TAK-536 M-I and M-II, the latter mainly by CYP2C9. Following both single and multiple dosing, dose proportionality was established for TAK-536 and M-II area under the plasma concentration-time curve (AUC) and maximum observed plasma concentration (C_{max}) values over the TAK-491 dose range of 20 mg to 320 mg. Approximately 15% of the TAK-491 dose is excreted as TAK-536 in urine.

Plasma exposure to TAK-536 following single doses of TAK-536 tablet and TAK-491 tablet under fasting conditions suggest that an equivalent dose of TAK-491 is approximately twice that of TAK-536 on a mg:mg basis. Administration of TAK-491 tablets with a high fat meal has no clinically significant effect on plasma exposure.

Several drug-drug interaction studies have been conducted with TAK-536, including warfarin, glyburide, pioglitazone, metformin, fluconazole, and ketoconazole; no clinically meaningful interactions were observed. Since TAK-491 is rapidly converted to TAK-536, it is not expected that coadministration of TAK-491 and these agents will result in any drug-drug interaction. An additional TAK-491 study with a cocktail of cytochrome P-450 (CYP) probes did not indicate the potential for drug-drug interactions with drugs metabolized by CYP3A4, CYP2C9, CYP2D6, or CYP1A2.

Administration of single doses of TAK-491 from 5 mg to 320 mg and of multiple doses from 20 mg to 320 mg was well tolerated in phase 1 studies. Adverse events (AEs) tended to be mild in intensity in these studies and no dose-related increase in the overall incidence of AEs was observed. In a study of age, gender, and race, no interactions based on these subject characteristics were observed. AEs with the greatest incidence that were considered to be related to TAK-491 in the phase 1 studies were dizziness and headache.

Steady-state total exposures to TAK-536 after multiple doses of TAK-491 in a phase 1 hepatic impairment study were approximately 28% and 64% greater in subjects with mild and moderate hepatic impairment, respectively, than in healthy subjects. These increases in exposure to TAK-536 are not considered to be clinically meaningful as TAK-491 was well-tolerated in other pharmacokinetic studies in which exposures to TAK-536 were greater than those observed in subjects with hepatic impairment. All AEs in the hepatic impairment study were mild, no subjects discontinued due to an AE, and neither mild nor moderate hepatic impairment had an impact on the relationship of AEs to study drug. Severe hepatic impairment was not studied.

Total exposure (AUC) to TAK-536, after a single dose of TAK-491 in a phase 1 renal impairment study, tended to be higher in subjects with renal impairment than in healthy subjects, with increases of 30%, 25%, 95%, and 4% in subjects with mild, moderate, and severe renal impairment, and ESRD, respectively. These increases in exposure to TAK-536 are not considered to be clinically meaningful, as TAK-491 was well-tolerated in a high-dose (TAK-491 160 to 320 mg) pharmacokinetic study in healthy subjects in which exposures 2- to 5-fold greater than those observed in subjects with renal impairment were observed. The mean $t_{1/2z}$ of TAK-536 was

not substantially different in subjects with renal impairment than in healthy subjects; therefore, daily dosing of TAK-491 is not expected to result in clinically significant accumulation of TAK-536 in patients with renal impairment. All AEs in the renal impairment study were mild, no subjects discontinued due to an AE, and no degree of renal impairment had an impact on the relationship of AEs to study drug.

In humans, the effects of TAK-491 on the RAAS are consistent with its mechanism of action and include increases in plasma renin activity and decreases in plasma aldosterone concentrations. In a phase 2 and multiple phase 3 studies in hypertensive subjects, TAK-491 administration resulted in significant reductions of blood pressure compared with placebo and active comparators. Further, incremental reductions in blood pressure were observed when TAK-491 was coadministered with other antihypertensive agents (eg, thiazide-like diuretics and calcium channel blockers). Additionally, TAK-491 was well tolerated, with an adverse event profile similar to that of placebo and active comparators.

TAK-491 (Edarbi) is approved in many countries and regions, including the United States (US) and the European Union, for the treatment of hypertension, either alone or in combination with other antihypertensive agents.

Please refer to the TAK-491 Global Investigator's Brochure and the US and EU Edarbi package inserts for additional information [\[7-9\]](#).

4.4 Rationale for the Study

The clinical pharmacology program of TAK-491 was extensive and designed to evaluate pharmacokinetics, pharmacodynamics, extrinsic factors (ie, drug-drug interactions and food effect), and intrinsic factors (ie, age, gender, race, renal impairment, and hepatic impairment). Studies were conducted primarily in the United States with some studies conducted in Europe; additional studies are needed to bridge between the data previously acquired and the populations of other countries. Though Asian populations are known to have differences in drug metabolizing enzymes compared to other populations, these differences are not expected to affect the pharmacokinetics of TAK-491 as TAK-536, the active moiety, is mainly metabolized by CYP2C9, a CYP enzyme not known to be significantly different between populations. In addition, an analysis of TAK-536 pharmacokinetic parameters between Japanese and Caucasian subjects did not detect a difference. The main objectives of this open-label and double-blind, randomized, placebo-controlled, single- and multiple-dose, phase 1 study are to assess the pharmacokinetics, and safety of TAK-491 in healthy Chinese subjects.

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

To assess the pharmacokinetics of TAK-491 40 mg and 80 mg in healthy Chinese subjects after both single and multiple dose administration.

5.1.2 Secondary Objectives

To evaluate the safety and tolerability of TAK-491 40 mg and 80 mg in healthy Chinese subjects after both single and multiple dose administration.

5.2 Endpoints

5.2.1 Primary Endpoints

Primary endpoints for TAK-536 (the active moiety derived from TAK-491) are as follows:

- Maximum observed concentration (C_{\max}).
- Time of first occurrence of C_{\max} (t_{\max}).
- Area under the concentration-time curve from time 0 to infinity (AUC_{∞}).
- Area under the concentration-time curve from time 0 to 24 hours (AUC_{24})
- Terminal disposition phase half-life ($t_{1/2z}$).

5.2.2 Additional Endpoints

Additional endpoints for TAK-536 are as follows:

- Area under the concentration-time curve from time 0 to time of last quantifiable concentration (AUC_{last}).
- Terminal disposition phase rate constant (λ_z).
- Observed concentration at the end of a dosing interval (C_{trough}).
- Minimum observed concentration during a dosing interval (C_{\min}).
- Average concentration during a dosing interval, at steady state ($C_{\text{av,ss}}$).
- Amount of drug excreted in urine from time 1 to time 2 (Ae_{t1-t2}).
- Renal clearance, (CL_R).
- Fraction of administered dose of drug excreted in urine from time 0 to 24 hours (f_{e0-24}) and time 0 to 72 hr (f_{e0-72}). Molecular weight adjustment needed from TAK-491 to TAK-536.

5.2.3 Safety Endpoints

The safety and tolerability endpoints of TAK-491 40 mg and 80 mg for the study will be determined from the primary safety variables that will include: physical examination findings, vital signs, 12-lead electrocardiogram (ECG) results, laboratory evaluations (hematology, serum chemistry, and urinalysis), and adverse events (AEs).

6.0 STUDY DESIGN AND DESCRIPTION

6.1 Study Design

This is a randomized, open-label and double-blind, placebo-controlled, single- and multiple-dose, phase 1 study involving 64 healthy Chinese subjects aged 18 to 45 years, inclusive, and considered eligible based on the inclusion and exclusion entry criteria. Subjects will receive TAK-491 or placebo as a single dose followed by multiple doses.

Sixteen subjects were allocated equally and randomly to 1 of 2 TAK-491 regimens (40 mg or 80 mg) in the open-label part of the study; the 2 regimens were conducted in parallel as shown in [Table 6.a](#).

Table 6.a Open-Label Study Regimen and Subject Allocation

Regimen	Regimen Description	Number of Subjects
A	TAK-491 40 mg as a single dose followed by multiple doses	8
B	TAK-491 80 mg as a single dose followed by multiple doses	8

Forty-eight subjects will be allocated equally and randomly to 1 of 3 regimens (TAK-491 40 mg or 80 mg, or placebo) in the double-blind part of the study; the 3 regimens will be conducted in parallel as shown in [Table 6.b](#). To maintain the study blind, subjects in this part of the study will receive a least 1 placebo tablet; the TAK-491 40 mg and TAK-491 80 mg tablets differ in size; therefore, placebo tablets for each size will be utilized as appropriate.

Table 6.b Double-Blind Study Regimen and Subject Allocation

Regimen	Regimen Description	Number of Subjects
A	TAK-491 40 mg + placebo as a single dose followed by multiple doses	16
B	TAK-491 80 mg + placebo as a single dose followed by multiple doses	16
C	Placebo (2 tablets) as a single dose followed by multiple doses	16

The pre-dosing study period will consist of a Screening Period (Study Days -28 to -2) and Check-in (Study Day -1). On Day 1, a single dose of TAK-491 or placebo will be administered followed by a pharmacokinetic sampling period through 72 hours postdose (Days 1-4). On Days 4 through 10, subjects will be on a daily dosing regimen, followed by a 24-hour pharmacokinetic assessment period prior to Study Exit (Day 11). The total confinement period for a subject who completes the study will be 12 days. All subjects will return to the site for PK sampling on Days 12 to 13 and follow-up serum chemistry tests on Day 14. A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 ± 2 days) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since the final dose of study medication or Early Termination Visit.

A schematic of the study design is included as [Figure 6.a](#). A schedule of assessments is listed in [Appendix A](#).

Figure 6.a Schematic of Study Design

Screening	Check-in	Dosing and PK	72-hr PK	Dosing (a)	Dosing and AM Trough Level (b)	Dosing and 24-hr PK	Study Exit	PK Collection	F/U Visit (c)	F/U Call
Days -28 to -2	Day -1	Day 1	Days 2-4	Day 4	Days 5-9	Day 10	Day 11	Days 12-13	Day 14	Day 24 (± 2)

PK=pharmacokinetic.

(a) Dosing will occur after the collection of the 72-hr pharmacokinetic sample.

(b) PK Trough samples will be collected on Days 7 to 9.

(c) Serum chemistry testing.

6.2 Justification for Study Design, Dose, and Endpoints

This randomized, open-label and double-blind, placebo-controlled, single- and multiple-dose, phase 1 study was designed with the following justification. The parameters listed as primary endpoints are considered appropriate for evaluation of pharmacokinetic profiles associated after single- and multiple-doses, and the additional safety endpoints comprise established safety assessments recognized as reliable, accurate, and relevant.

The pharmacokinetics of TAK-536 after administration of TAK-491 has been thoroughly characterized from data collected during the phase 1 clinical pharmacology program conducted in the United States and Europe. The $t_{1/2z}$ for TAK-536 following TAK-491 administration is approximately 11 hours; the planned sampling scheme after the single dose is approximately 6 half-lives and appropriate. Steady state of TAK-536 is achieved within 5 days of dosing of TAK-491; the multiple-dose duration is appropriate. A total of 64 healthy subjects will be enrolled into the study. An open-label design was used during the dosing of the first 16 subjects (in 2 consecutive cohorts) because the primary outcomes are based on objective measurements, that is, plasma concentrations of TAK-536. Fifteen of these first 16 subjects had a higher ALT and/or AST level on Day 11 than on Day -1. While none of these subjects had Day 11 ALT and/or AST values that exceeded 3×ULN of protocol mandated monitoring criteria (see [Section 7.5](#) [No.1] and [Section 10.2.3](#)), 7 of these exceeded the ULN. None of these ALT/AST elevations were accompanied by symptoms or signs of liver injury or by bilirubin elevation, and resolved without intervention. High frequency of subjects with elevations in ALT and/or AST levels were unexpected and unprecedented, as no similar findings were observed in previous TAK-491 phase 1 through 3 studies with the enrollment of subjects from different races and ethnicities (ie, Caucasians, African Americans, Mexican Americans, Native Americans, Asians, and other ethnicities). While other procedure-related factors, such as high-carbohydrate diet and minimal physical activity during the confinement period in the phase 1 unit, as described in the medical literature [\[10-12\]](#), might have been accountable for these asymptomatic ALT/AST elevations in the present study, a potential drug-related phenomenon has to be definitively ruled out. Hence, a

double-blind, placebo-controlled study design was adopted to complete the study. A randomization schedule for dose assignment will be used to reduce the potential for selection bias.

6.3 Premature Termination or Suspension of Study or Investigational Site

6.3.1 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 medication that indicates a change in the known risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.

6.3.2 Criteria for Premature Termination or Suspension of Investigational Site

A study site may be terminated prematurely or suspended if the site (including the investigator) is found in significant violation of GCP, protocol, or contractual agreement, is unable to ensure adequate performance of the study, or as otherwise permitted by the contractual agreement.

6.3.3 Procedures for Premature Termination or Suspension of the Study or the Participation of Investigational Site

In the event that the sponsor, an institutional review board (IRB)/independent ethics committee (IEC), or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

All entry criteria, including test results, need to be confirmed prior to the first dose of study drug on Day 1.

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. In the opinion of the investigator, the subject is capable of understanding and complying with protocol requirements.
2. The subject or, when applicable, the subject's legally acceptable representative signs and dates a written, informed consent form and any required privacy authorization prior to the initiation of any study procedures.
3. The subject is a healthy adult male or female subject of Chinese descent.
4. The subject is aged 18 to 45 years, inclusive, at the time of informed consent and first study medication dose.
5. The subject has a body mass index (BMI) $\geq 19.0 \text{ kg/m}^2$ and $< 24.0 \text{ kg/m}^2$, at Screening.
6. The subject has clinical laboratory evaluations (including clinical chemistry [fasted for at least 8 hours for the screening assessment], hematology, and complete urinalysis) within the reference range for the testing laboratory, unless the results were deemed by the investigator to be not clinically significant at Screening and Check-in (Day -1).
7. The subject is willing to refrain from strenuous exercise, from 72 hours before Check-in (Day-1) until after Final Visit.
8. A female subject of childbearing potential* who is sexually active with a nonsterilized* male partner agrees to use routinely adequate contraception* from signing of informed consent throughout the duration of the study and for 30 days after the last dose of study drug.

*Definitions and acceptable methods of contraception are defined in Section 9.1.9
Contraception and Pregnancy Avoidance Procedure and reporting responsibilities are defined in Section 9.1.10 Pregnancy.

7.2 Exclusion Criteria

Any subject who meets any of the following criteria will not qualify for entry into the study:

1. The subject has received any investigational compound within 30 days prior to Screening.
2. The subject has received TAK-491 in a previous clinical study or as a therapeutic agent.
3. The subject is an immediate family member, study site employee, or in a dependant relationship with a study site employee who is involved in the conduct of this study (eg, spouse, parent, child, sibling) or may consent under duress.

4. The subject has history of uncontrolled, clinically significant manifestations of metabolic (including diabetes mellitus, hypercholesterolemia, or dyslipidemia), endocrine, hematologic, pulmonary, cardiovascular, gastrointestinal, neurological, rheumatologic, skin and subcutaneous tissue disorders, infectious, hepatic, renal, urologic, immunologic, psychiatric or mood disorders (including any past history of suicide attempt), or a history of lactose intolerance, which may impact the ability of the subject to participate or potentially confound the study results.
5. The subject has a known hypersensitivity to any component of the formulation of TAK-491 or other AII inhibitors or related compounds.
6. The subject has a positive urine drug result for drugs of abuse or breath alcohol test at Screening or Check-in (Day -1).
7. The subject has a history of drug abuse (defined as any illicit drug use) or a history of alcohol abuse within 1 year prior to the Screening visit or is unwilling to agree to abstain from alcohol and drugs throughout the study.
8. Subject has taken any excluded medication, supplements, or food products listed in the Excluded Medications and Dietary Products table.
9. If female, the subject is pregnant or lactating or intending to become pregnant before, during, or within 30 days after participating in this study; or intending to donate ova during such time period.
10. Subject has current or recent (within 6 months) gastrointestinal disease that would be expected to influence the absorption of drugs (ie, a history of malabsorption, esophageal reflux, peptic ulcer disease, erosive esophagitis frequent [more than once per week] occurrence of heartburn, or any surgical intervention [eg, cholecystectomy]).
11. Subject has a history of cancer, except basal cell carcinoma that has been in remission for at least 5 years prior to Day 1.
12. Subject has positive test result for anti-HIV, anti-HCV antibodies, or for HBsAg at Screening.
13. Subject has used nicotine-containing products (including but not limited to cigarettes, pipes, cigars, chewing tobacco, nicotine patch or nicotine gum) within 28 days prior to Check-in (Day -1). Cotinine test is positive at Screening or Check-in (Day -1).
14. The subject has poor peripheral venous access.
15. Subject has donated or lost 450 mL or more of his or her blood volume (including plasmapheresis), or had a transfusion of any blood product within 30 days prior to Day 1.
16. Subject has a Screening or Check-in (Day -1) abnormal (clinically significant) ECG. Entry of any subject with an abnormal (not clinically significant) ECG must be approved, and documented by signature by the principal investigator.
17. Subject has abnormal Screening or Check-in (Day -1) laboratory values that suggest a clinically significant underlying disease or subject with the following laboratory

abnormalities: alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) $>1.5\times$ the upper limit of normal.

18. Subject has a hemoglobin value <12 g/dL at Screening.

19. The subject has a systolic blood pressure <110 and ≥160 mm Hg or a diastolic blood pressure <60 and ≥100 mm Hg at Screening or Check-in (Day -1).

7.3 Excluded Medications and Dietary Products

Use of the agents (prescription or nonprescription), foods, and supplements listed in [Table 7.a](#) is prohibited from the time points specified until completion of all study activities.

Table 7.a Prohibited Medications, Foods, and Supplements

28 days prior to Check-in (Day -1)	7 days prior to Check-in (Day -1)	72 hours prior to Check-in (Day -1)
Prescription medications	OTC medications (a)	Products containing caffeine or xanthine
Nicotine-containing products	Vitamin supplements	
Nutraceuticals (eg, St. John's wort, ginseng, kava kava, ginkgo biloba, Chinese herbs, and melatonin)	Foods or beverages containing grapefruit or grapefruit juice, star fruit or star fruit juice, Seville-type (sour) oranges and marmalade, apple, orange, or pineapple juice, vegetables from the mustard green family (eg, kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard), and charbroiled meats	Poppy seeds
Immunization/Vaccines (b)		
Known OTC inhibitors/inducers of CYPs 3A4/5, 2C9, 2C19, 2D6, 1A2, 2B6, 2E1, and 2A6 (c)	Alcohol-containing products	

OTC=over-the-counter.

(a) Occasional use of acetaminophen (≤1 g/day) or other medication as approved by Takeda on a case-by-case basis is allowed except on Day 1.

(b) Inclusive of but not limited to flu vaccinations.

(c) Omeprazole, lansoprazole, cimetidine, ranitidine, and chlorpheniramine.

Subjects must be instructed not to take any medications, including over-the-counter medications, without first consulting with the investigator.

7.4 Diet, Fluid, and Activity Control, and Subject Housing

Subjects will be confined to the clinic from Day -1 through Day 11, and will return to the clinic for PK sampling on Days 12 to 13 and follow-up serum chemistry testing on Day 14.

During the confinement period, subjects will be given a menu consistent with local guideline and local dietary practice. Subjects will be given 3 meals and an evening snack every day, each containing approximately **20%** fat (relative to the total calories). The meals served on Day 1 and Day 10 should be identical between study days and for each subject in the study. The study menu

should be recorded and submitted to the study file with a copy provided to the sponsor prior to the start of the study.

If a blood draw or any study procedure coincides with a meal, the blood draw will take precedence followed by the study procedure and then the meal.

TAK-491 will be administered on Day 1 and Day 10 with 240 mL of water after a fast of at least 8 hours. On Day 1 and Day 10, subjects will also continue to fast for an additional 4 hours after dosing and subjects may consume water ad libitum with the exception of 1 hour before and 1 hour after drug administration. For Days 4 to 9, fasting is not required for dosing.

Subjects will remain upright (seated, standing, or ambulatory) for 1 hour following the dose administration, except as necessitated by the occurrence of an adverse event /or study procedures (eg, obtaining 12-lead ECG). Subjects will refrain from strenuous exercise throughout the entire course of the study.

7.5 Criteria for Discontinuation or Withdrawal of a Subject

The primary reason for discontinuation or withdrawal of the subject from the study should be recorded in the case report form (CRF) using the following categories. For screen failure subjects, refer to Section 9.1.14.

1. Pretreatment event (PTE) or adverse event (AE). The subject has experienced a PTE or AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health or the subject is unwilling to continue because of the PTE or AE.
 - Liver Function Test Abnormalities
 - ALT or AST $>8 \times$ ULN, or
 - ALT or AST $>5 \times$ ULN and persists for more than 2 weeks, or
 - ALT or AST $>3 \times$ ULN in conjunction with elevated total bilirubin $>2 \times$ ULN or international normalized ratio (INR) >1.5 , or ALT or AST $>3 \times$ ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia ($>5\%$).
2. Significant protocol deviation. The discovery after administration of the first dose of study medication that the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the subject's health.
3. Lost to follow-up. The subject did not return to the clinic and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.

4. Voluntary withdrawal. The subject (or subject'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).

5. Study termination. The sponsor, IRB, IEC, or regulatory agency terminates the study.

6. Pregnancy. The subject is found to be pregnant.

Note: If the subject is found to be pregnant, the subject must be withdrawn immediately. The procedure is described in Section [9.1.10](#).

7. Other.

Note: The specific reasons should be recorded in the "specify" field of the CRF.

7.6 Procedures for Discontinuation or Withdrawal of a Subject

The investigator may terminate a subject's study participation at any time during the study when the subject meets the study termination criteria described in Section [7.5](#). In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Should a subject's participation be discontinued, the primary criterion for termination must be recorded. In addition, efforts should be made to perform all procedures scheduled for the Study Exit Visit.

Discontinued or withdrawn subjects will not be replaced after the first dose of study drug.

8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

This section contains information regarding all medication and materials provided directly by the sponsor, and/or sourced by other means, that are required by the study protocol, including important sections describing the management of clinical trial material.

8.1 Study Medication and Materials

A sufficient quantity of the following study drug for study conduct will be provided to the investigator.

- TAK-491 40 mg and matching placebo tablets.
- TAK-491 80 mg and matching placebo tablets.

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

Dosage Form	TAK-491 40 mg and 80 mg white, round biconvex tablets and matching placebo. Tablets of the TAK-491 investigational drug for oral administration contain 40 mg or 80 mg of TAK-491F (the salt-free form of TAK-491) and will be provided in blister cards, matching placebo will also be provided in blister cards. The active formulation contains drug substance and excipients; placebo contains excipients only.
Manufacturing	Takeda Pharmaceutical Company Limited, Osaka, Japan.
Packaging	TAK-491 investigational medicinal product and matching placebo will be foil/foil blistered and packaged in 10-day (8 daily doses+2 extra doses) child-resistant blister cards. Each tablet is connected to a desiccant through a linked channel. Each blister card contains 10 TAK-491 tablets and 10 desiccants for the open-label part, and 20 TAK-491 or matching placebo tablets and 20 desiccants for the double-blind part. The tablets should not be removed from the blister card until immediately prior to dosing.
Labeling	Each blister card provided to the clinical site will be labeled with a single panel label with pertinent study information and appropriate country specific regulatory caution statement. Each label will include a space for the pharmacist to include a subject specific reference number. The blister cards will be labeled in an open labeled fashion for the open-label part and in a double-blind fashion for the double-blind part of the study.

8.1.2 Storage

TAK-491 investigational medicinal product and matching placebo should be stored at 25°C (77°F); with excursions permitted to 15°C to 30°C (59°F to 86°F). The blister cards in which the tablets are contained should not be opened until immediately prior to dosing in order to protect from moisture and humidity.

All investigational medicinal product must be kept in an appropriate, limited-access, secure place until it is used or returned to the sponsor or designee for destruction. All study medication must be stored under the conditions specified on the label, and remain in the original container until dispensed. A daily temperature log of the drug storage area must be maintained every working day.

8.1.3 Dose and Regimen

Table 8.a describes the dose and tablet count that will be provided to each group.

Table 8.a Study Medication Supplies

Treatment Description			
Regimen	Dose	Active	Placebo (a)
A	TAK-491 40 mg	One TAK-491 40 mg tablet	One placebo for TAK-491 80 mg tablet
B	TAK-491 80 mg	One TAK-491 80 mg tablet	One placebo for TAK-491 40 mg tablet
C (a)	TAK-491 placebo	Not applicable	One placebo for TAK-491 40 mg tablet One placebo for TAK-491 80 mg tablet

(a) Double-blind part of study only.

On Days 1 and 4 through 10, study drug will be administered. The investigator or investigator's designee will instruct the subject on dosing procedures.

Following administration of study drug, hand-and-mouth checks will be performed to ensure that the dose was swallowed and will be recorded on the source document. Although timing of events requires that each subject receive the appropriate dose at specific times, the exact dose time of consecutive subjects may be staggered to obviate the need to have all subjects on precisely the same study schedule. The actual date and time of administration of the dose of study drug will be recorded on the source documents and the appropriate CRF.

8.1.4 Overdose

An overdose is defined as a **known** deliberate or accidental administration of investigational drug, to or by a study subject, at a dose above that which is assigned to that individual subject according to the study protocol.

All cases of overdose (with or without associated adverse events) will be documented on an Overdose page of the CRF, in order to capture this important safety information consistently in the database. Adverse events associated with an overdose will be documented on AE CRF(s) according to Section 10.0, Pretreatment Events and Adverse Events.

Serious adverse events (SAEs) of overdose should be reported according to the procedure outlined in Section 10.2.2, Collection and Reporting of SAEs.

In the event of drug overdose, the subject should be treated symptomatically.

8.2 Study Medication Assignment and Dispensing Procedures

Subjects will be assigned, in the order in which they are randomized into the study to receive their treatment according to the randomization schedule allocated to the site. The Randomization Sequence Number will be entered onto the CRF.

Subjects will be assigned to receive a 4-digit randomization sequence number. The number will be assigned by the clinic site personnel in sequential order beginning with 1001 and ending with 1016 for the open-label part, and beginning with 2001 and ending with 2048 for the double-blind part.

This 4-digit number will be used by the clinical site to facilitate the prelabeling of pharmacokinetic samples, and will be the only subject identifier used on all sample collections. It should also be contained on the pharmacokinetic transport vials shipped to the bioanalytical laboratory, and will be used by the laboratory to report the subject data results. This 4-digit number should only be used for the purposes described in this section. It does not replace the 3-digit subject number which is assigned at the time the informed consent is obtained and which is used for all other procedures to identify the subjects throughout the study.

8.3 Randomization Code Creation and Storage

Randomization personnel of the sponsor or designee will generate the randomization schedule, which will be provided to the site pharmacist prior to the start of this study. All randomization information will be stored in a secured area, accessible only by authorized personnel.

8.4 Investigational Drug Blind Maintenance

For the double-blind part of the study, the investigational drug blind information is maintained through a randomization schedule held by the dispensing pharmacist.

8.5 Unblinding Procedure

For the double-blind part of the study, the investigational drug blind shall not be broken by the investigator unless information concerning the investigational drug is necessary for the medical treatment of the subject. All study assessments and causality should be performed, if possible, prior to unblinding. In the event of a medical emergency, if possible, the medical monitor should be contacted before the investigational drug blind is broken to discuss the need for unblinding.

For unblinding a subject, the investigational drug blind can be obtained by opening sealed envelope, or contacting the dispensing pharmacist.

The sponsor must be notified as soon as possible if the investigational drug blind is broken. The date, time, and reason the blind is broken must be recorded in the source documents and the same information (except the time) must be recorded on the (e)CRF.

If any site personnel are unblinded, investigational drug must be stopped immediately and the subject must be withdrawn from the study.

No change should be made to any assessment of the subject after unblinding.

8.6 Accountability and Destruction of All Study Medication

Drug supplies will be counted and reconciled at the site before being returned to Takeda or designee or being destroyed.

The investigator or designee must ensure that the study medication is used in accordance with the approved protocol and is dispensed only to subjects enrolled in the study. To document appropriate use of study medication (TAK-491 or placebo) the investigator must maintain records of all study medication delivery to the site, site inventory, use by each subject, and return to the sponsor or designee.

Upon receipt of study medication, the investigator or designee must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, the medication is received within the labeled storage conditions, and is in good condition. If quantity and conditions are acceptable, investigator or designee should acknowledge the receipt of the shipment. If there are any discrepancies between the packing list versus the actual product received, Takeda must be contacted to resolve the issue. The packing list should be filed in the investigator's essential document file.

The investigator must maintain 100% accountability for all study medication received and dispensed during his or her entire participation in the study. Proper drug accountability includes, but is not limited to:

- Continuously monitoring expiration dates if expiration date is provided to the investigator.
- Frequently verifying that actual inventory matches documented inventory.
- Verifying that the log is completed for the drug lot used to prepare each dose.
- Verifying that all containers used are documented accurately on the log.
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, the sponsor must be notified immediately.

The investigator must record the current inventory of all study medication (TAK-491 or placebo) on a sponsor-approved drug accountability log. The following information will be recorded at a minimum: protocol number and title, name of investigator, site identifier and number, description of study medication, expiry and/or retest date, date and amount dispensed, including the initials of the person dispensing and receiving the study medication. The log should include all required information as a separate entry for each subject to whom study medication is dispensed.

Prior to site closure or at appropriate intervals, a representative from the sponsor or its designee will perform clinical study material accountability and reconciliation before clinical study materials are returned to the sponsor or its designee for destruction or destroyed at the site, as applicable. The investigator will retain the original documentation regarding clinical study material accountability, return, and/or destruction, and copies will be sent to the sponsor or designee.

The investigator will be notified of any expiry date or retest date extension of clinical study material during the study conduct. On expiry date notification from the sponsor or designee, the site must complete all instructions outlined in the notification, including segregation of expired clinical study material for return to the sponsor or its designee for destruction.

In the event of expiry date extension of supplies already at the study site, supplies may be relabeled with the new expiry date at that site. In such cases, Takeda or its designee will prepare additional labels and all necessary documentation for completion of the procedure at the sites.

9.0 STUDY PLAN

9.1 Study Procedures

The following sections describe the study procedures and data to be collected. For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. The Schedule of Study Procedures is located in [Appendix A](#).

9.1.1 Informed Consent Procedure

The requirements of the informed consent are described in Section [15.2](#).

Informed consent must be obtained prior to the subject entering into the study, and before any protocol-directed procedures are performed.

A unique subject identification number (subject number) will be assigned to each subject at the time that informed consent is obtained; this subject number will be used throughout the study.

9.1.2 Demographics, Medical History, and Medication History Procedure

Demographic information to be obtained will include date of birth, sex, race as described by the subject, and smoking status of the subject at Screening.

Medical history to be obtained will include determining whether the subject has any significant conditions or diseases relevant to the condition/disease under study that stopped at or prior to informed consent. Ongoing conditions are considered concurrent medical conditions (see Section [9.1.7](#)).

Medication history information to be obtained includes any medication relevant to eligibility criteria stopped at or within 28 days prior to signing of informed consent.

9.1.3 Physical Examination Procedure

A physical examination will consist of the following body systems: (1) eyes; (2) ears, nose, throat; (3) cardiovascular system; (4) respiratory system; (5) gastrointestinal system; (6) dermatologic system; (7) extremities; (8) musculoskeletal system; (9) nervous system; (10) lymph nodes; and (11) other.

Any abnormal finding on a pretreatment physical examination assessment must be assessed as Not Clinically Significant (NCS) or Clinically Significant (CS) by the investigator and recorded in the source document and CRF. All CS findings/changes will be recorded as a PTE or concurrent medical condition in the source document and on the appropriate CRF described in Section [9.1.7](#) or Section [10.0](#).

On subsequent examinations, any abnormal change from the pretreatment physical examination assessment occurring immediately prior to the start of the investigational drug (Day 1) must be assessed as NCS or CS by the investigator and recorded in the source document and CRF. Any CS change, as determined by the investigator, will be recorded as an AE in source documentation and on the PTE/AE CRF described in Section [10.0](#).

9.1.4 Weight, Height, and BMI

A subject should have weight and height measured while wearing indoor clothing and with shoes off. The BMI is calculated using metric units with the formula provided below:

$$\text{Metric: } \text{BMI} = \text{weight (kg)}/\text{height (m)}^2$$

Height will be collected in centimeters without decimal places and weight will be collected in kilograms to 1 decimal place. Results for BMI will be expressed with 1 decimal place.

Example:

Height=176 cm (or 1.76 m), weight=79.2 kg; $\text{BMI}=79.2/1.76^2=25.6 \text{ kg/m}^2$.

9.1.5 Vital Sign Procedure

Vital signs will include body temperature (oral) measurement, sitting blood pressure (after 5 minutes resting), respiration rate and pulse (bpm).

When vital signs are scheduled at the same time as blood draws, the blood draw will take priority and vital signs will be obtained within 0.5 hour before or after the scheduled blood draw.

9.1.6 Documentation of Concomitant Medications

Concomitant medication is any drug given in addition to the study medication. These may be prescribed by a physician or obtained by the subject over the counter. Concomitant medication is not provided by Takeda. At each study visit, subjects will be asked whether they have taken any medication other than the study medication (used from signing of informed consent through the end of the study), and all medication, including vitamin supplements, over-the-counter medications, and oral herbal preparations, must be recorded in the CRF. Documentation will include generic medication name, dose, unit, frequency, route of administration, start and end dates, and reason for use.

9.1.7 Documentation of Concurrent Medical Conditions

Concurrent medical conditions are those significant ongoing conditions or diseases that are present at signing of informed consent. This includes clinically significant laboratory, electrocardiogram (ECG), or physical examination abnormalities noted at the Screening examination. The condition (ie, diagnosis) should be described.

9.1.8 Procedures for Clinical Laboratory Samples

All samples will be collected in accordance with acceptable laboratory procedures. The approximate total volume of blood for the study is 243 mL. Laboratory samples will be taken following a minimum 8-hour overnight fast on the days stipulated in the Schedule of Study Procedures ([Appendix A](#)).

[Table 9.a](#) lists the tests that will be obtained for each laboratory specimen.

Table 9.a Clinical Laboratory Tests

Hematology	Serum Chemistry	Urinalysis
Red blood cells	Alanine aminotransferase	pH
White blood cells with differential (neutrophils, eosinophils, basophils, lymphocytes, monocytes)	Albumin	Specific gravity
Hemoglobin	Alkaline phosphatase	Protein
Hematocrit	Aspartate aminotransferase	Glucose
Platelets	Total bilirubin	Blood
	Total protein	Ketones
	Creatinine	Microscopic Analysis (a):
	Blood urea nitrogen	RBC/high power field WBC/high
	Creatine kinase	power field
	γ -Glutamyl transferase	Epithelial cells, casts etc.
	Potassium	
	Sodium	
	Glucose	
	Chloride	
	Triglycerides	
	HDL-C	
	LDL-C	
	Total cholesterol	

Diagnostic Screening:

Serum	Urine	Breath
HBsAg, anti-HCV, anti-HIV (b)	Drug screen, including amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, and cotinine	Breath alcohol test
<u>Additional Virology Tests:</u>		
Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM (c)		

Female Subjects only:

Serum hCG at Screening, Check-in, and Study Exit or Early Termination

EBV= Epstein-Barr virus viral capsid antigen, hCG=human chorionic gonadotropin, RBC=red blood cell, WBC=white blood cell.

(a) Microscopic analysis should be performed only if urine evaluations are abnormal.

(b) HBsAg, anti-HCV, and anti-HIV test results will be collected for all subjects.

(c) The results for the additional virology tests performed at Screening will be recorded in the (e)CRF for randomized subjects only.

The local laboratory will perform laboratory tests for hematology, serum chemistries, and urinalysis. The results of laboratory tests will be returned to the investigator, who is responsible for reviewing and filing these results.

If subjects experience ALT or AST $>3 \times$ ULN, follow-up laboratory tests (at a minimum, serum alkaline phosphatase, ALT, AST, total bilirubin, GGT, and INR) should be performed within a maximum of 7 days and preferably within 48 to 72 hours after the abnormality was found. (Please refer to Section 7.5 for discontinuation criteria, and Section 10.2.3 for the appropriate guidance on Reporting of Abnormal Liver Function Tests in relation to ALT or AST $>3 \times$ ULN in conjunction with total bilirubin $>2 \times$ ULN.)

If the ALT or AST remains elevated $>3 \times \text{ULN}$ on these 2 consecutive occasions, the investigator must contact the Medical Monitor for consideration of additional testing, close monitoring, possible discontinuation of study medication, discussion of the relevant subject details and possible alternative etiologies.

Subjects with ALT/AST elevations $>1 \times \text{ULN}$ on Day 11 and/or 14 will be followed-up every 5 to 7 days until resolution.

All laboratory safety data will be transferred electronically to Takeda or designee in the format requested by Takeda. If the laboratory is unable to electronically transfer data, the study site coordinator or designee is responsible for transcribing laboratory results to the CRF. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

Laboratory reports must be signed and dated by the principal investigator or subinvestigator indicating that the report has been reviewed and any abnormalities have been assessed for clinical significance.

All clinically significant laboratory abnormalities must be recorded as a PTE/AE in the subject's source documents and on the appropriate CRF. A clinically significant laboratory abnormality that has been verified by retesting will be followed until the abnormality returns to an acceptable level or a satisfactory explanation has been obtained. Please refer to Section 10.2.3 Reporting of Abnormal Liver Function Tests for reporting requirements.

9.1.9 Contraception and Pregnancy Avoidance Procedure

From signing of informed consent, throughout the duration of the study, and for 30 days after last dose of study medication, female subjects of childbearing potential* who are sexually active with a nonsterilized male partner** must use adequate contraception. In addition they must be advised not to donate ova during this period.

*Females NOT of childbearing potential are defined as those who have been surgically sterilized (hysterectomy, bilateral oophorectomy or tubal ligation) or who are postmenopausal (eg, defined as at least 1 year since last regular menses with an FSH $>40 \text{ IU/L}$ or at least 5 years since last regular menses, confirmed before any study medication is implemented).

**Sterilized males should be at least 1 year postvasectomy and have confirmed that they have obtained documentation of the absence of sperm in the ejaculate.

An acceptable method of contraception is defined as one that has no higher than a 1% failure rate. In this study, where medications and devices containing hormones are included, the only acceptable methods of contraception are:

Barrier methods (each time the Intrauterine devices (IUDs): Hormonal contraceptives: subject has intercourse):

- Male condom PLUS spermicide
- Cap (plus spermicidal cream or jelly) PLUS male condom and spermicide
- Diaphragm (plus spermicidal cream or jelly) PLUS male condom and spermicide
- Copper T PLUS condom or spermicide
- Progesterone T PLUS condom or spermicide
- Implants
- Hormone shot/injection
- Combined pill
- Mini pill
- Patch
- Vaginal ring PLUS male condom and spermicide

Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova during the course of the study.

During the course of the study, regular serum human chorionic gonadotropin (hCG) pregnancy tests will be performed only for women of childbearing potential and subjects will receive continued guidance with respect to the avoidance of pregnancy as part of the study procedures. In addition to a negative serum hCG pregnancy test at Screening, subjects also must have a negative serum hCG pregnancy test at Check-in (Day -1) prior to receiving any dose of study medication on Day 1.

9.1.10 Pregnancy

If any subject is found to be pregnant during the study she should be withdrawn and any sponsor-supplied drug (TAK-491 or placebo) should be immediately discontinued.

If the pregnancy occurs during administration of active study medication, eg, after Day 1 or within 30 days of the last dose of study medication, the pregnancy should be reported immediately, using a pregnancy notification form, to the contact listed in Section 1.0.

If the female subject agrees to the primary care physician being informed, the investigator should notify the primary care physician that the subject was participating in a clinical study at the time she became pregnant and provide details of treatment the subject received.

All reported pregnancies will be followed up to final outcome, using the pregnancy form. The outcome, including any premature termination, must be reported to the sponsor.

9.1.11 ECG Procedure

A standard 12-lead ECG will be recorded. The investigator (or a qualified physician at the investigational site) will interpret the ECG using 1 of the following categories: within normal limits, abnormal but not clinically significant, or abnormal and clinically significant. The time that the ECG was performed will be recorded. The following parameters will be recorded on the CRF

from the subject's ECG trace: heart rate, QT interval, PR interval, QRS interval, and QT (Fridericia corrected).

9.1.12 Pharmacokinetic Sample Collection

9.1.12.1 Collection of Plasma for Pharmacokinetic Sampling

Blood samples (one 6-mL sample per scheduled time) for determination of TAK-536 plasma concentrations will be collected according to the schedule in [Table 9.b](#). Instructions for sample processing and shipment are provided in [Appendix E](#).

Table 9.b Collection of Blood Samples for TAK-536 Pharmacokinetic Analysis

Matrix	Dosing Day(s)	Scheduled Time
Plasma	1	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose
Plasma	7, 8, 9	Predose (up to 15 minutes prior to dose [0 hour])
Plasma	10	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24, 36, 48, and 72 hours postdose

The actual time of sample collection will be recorded on the source document and CRF. Instructions for sample processing and shipment are provided in [Appendix E](#).

Placebo samples will not be analyzed by the bioanalytical laboratory except 4 samples per subject receiving placebo, 2 on Day 1 and 2 on Day 10 at predose and the other around the expected time at which Cmax occurred (3 hours), to ensure from a safety perspective that no additional subjects could have been on active treatment.

9.1.12.2 Collection of Urine for Pharmacokinetic Sampling

Serial urine samples for determination of TAK-536 will be collected according to [Table 9.c](#).

Table 9.c Collection of Urine Samples for Pharmacokinetic Analysis

Matrix	Dosing Day	Scheduled Time
Urine	1	Predose (-12 to 0 hour) and at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose
Urine	10	0 to 2, 2 to 4, 4 to 8, 8 to 12, and 12 to 24 hours postdose

Instructions for sample processing and shipment are provided in [Appendix E](#).

Urine samples for subjects randomized to placebo will not be analyzed.

9.1.12.3 Bioanalytical Methods

Plasma and urine concentrations of TAK-536 will be measured by high-performance liquid chromatography with tandem mass spectrometry. The bioanalytical method is a validated LC-MS/MS method for TAK-536.

9.1.13 Pharmacokinetic Parameters

The pharmacokinetic parameters of TAK-536 will be determined from the concentration-time profiles for all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. The following pharmacokinetic parameters will be calculated from plasma concentration values of TAK-536:

Symbol/Term	Definition
Plasma	
AUC ₂₄ (ng·hr/mL)	Area under the concentration-time curve from time 0 to 24 hours (multiple dose only).
AUC _{last} (ng·hr/mL)	Area under the concentration-time curve from time 0 to time of the last quantifiable concentration (single dose only).
AUC _∞ (ng·hr/mL)	Area under the concentration-time curve from time 0 to infinity (single dose only), calculated using the observed value of the last quantifiable concentration.
C _{av,ss} (ng/mL)	Average concentration during a dosing interval at steady state.
C _{max} (ng/mL)	Maximum observed concentration.
C _{min} (ng/mL)	Minimum observed concentration during a dosing interval.
C _{trough} (ng/mL)	Observed concentration at the end of a dosing interval.
λ _z (1/hr)	Terminal disposition phase rate constant (single dose only).
t _{1/2z} (hr)	Terminal disposition phase half-life (single dose only).
t _{max} (hr)	Time of first occurrence of C _{max} .

The following pharmacokinetic parameters will be derived from urine concentrations of TAK-536:

Symbol/Term	Definition
Urine	
Ae _{t1-t2}	Amount of drug excreted in urine from time 1 to time 2.
Ae _t	Amount of drug excreted in urine from time 0 to time t.
f _{et}	Fraction of administered dose of drug excreted in urine from time 0 to time t. Molecular weight adjustment needed for metabolites
CL _R	Renal clearance.

9.1.14 Documentation of Screen Failure

Investigators must account for all subjects who sign informed consent. If the subject is found to be not eligible at this visit, the investigator should complete the CRF.

The primary reason for screen failure is recorded in the CRF using the following categories:

- PTE/AE.
- Did not meet inclusion criteria or did meet exclusion criteria.
- Major protocol deviation.
- Lost to follow-up.
- Voluntary withdrawal, specify reason.
- Study termination.
- Other, specify reason.

Subject numbers assigned to subjects who fail screening should not be reused.

9.1.15 Documentation of Study Entrance/Randomization

Only subjects who meet all of the inclusion criteria and none of the exclusion criteria are eligible for entrance into the treatment phase.

If the subject is found to be not eligible for entrance, the investigator should record the primary reason for failure on the applicable CRF.

9.2 Monitoring Subject Treatment Compliance

Study medication will be administered while subjects are under observation in the clinical research unit. Following administration of the study medication, appropriate mouth and/or hand checks will be performed to ensure that the dose is swallowed and noted in the source document. The date and time of each dose will be recorded in the source documents and on the (e)CRFs. An inventory of the study medication supplies dispensed will be performed by the site pharmacist or authorized study designee and recorded onto the Drug Accountability Log in the subject's source document records or equivalent. The exact dose time of consecutive subjects may be staggered to facilitate logistics at the site.

9.3 Schedule of Observations and Procedures

The schedule for all study-related procedures for all evaluations is shown in [Appendix A](#). Assessments should be completed at the designated visit/time point(s).

9.3.1 Screening

Subjects will be screened for the study within 28 days prior to the first dose of study medication. Subjects will be screened in accordance with predefined inclusion and exclusion criteria as described in Section [7.0](#). See Section [9.1.14](#) for procedures for documenting screening failures.

Procedures to be completed at Screening Visit include:

- Informed consent.
- Demographics, medical history, and medication history.
- Physical examination.
- Vital signs.
- Weight, height and BMI.
- Concomitant medications.
- Concurrent medical conditions.
- PTE assessment.
- HBsAg, anti-HCV, and anti-HIV tests (exclusion criteria)
- Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG and EBV VCA IgM, and Anti-CMV IgG and Anti-CMV IgM.
- Screening clinical laboratory tests (including pregnancy test for all female subjects).
- Urine drug and cotinine
- Breath alcohol screen.
- 12-lead ECG.

9.3.2 Day -1 (Check-in)

- Physical examination.
- Vital signs.
- Concomitant medications assessment.
- Concurrent medical conditions.
- PTE assessment.
- Clinical laboratory tests (including pregnancy test for all female subjects).
- Urine drug and cotinine
- Breath alcohol screen.
- 12-lead ECG.
- PK urine collection.

9.3.3 Day 1: Study Entrance

Study entrance will take place on Day 1. The following procedures will be performed and documented:

- Vital signs.
- PK urine collection.
- PK blood collection.
- Administration of study drug.
- AE assessment.
- Concomitant medications assessment.

Subjects will be administered study drug in the unit under the supervision of the investigator or designee, as described in Section 8.2. The procedure for documenting Screening failures is provided in Section 9.1.14.

9.3.4 Days 2 and 3

- Vital signs.
- PK urine collection.
- PK blood collection.
- AE assessment.
- Concomitant medications assessment.

9.3.5 Day 4

- Vital signs.
- Clinical laboratory tests.
- PK urine collection.
- PK blood collection.
- Administration of study drug.
- AE assessment.
- Concomitant medications assessment.

9.3.6 Days 5 and 6

- Vital signs.
- Administration of study drug.

- AE assessment.
- Concomitant medications assessment.

9.3.7 Days 7 through 10

- Vital signs.
- PK urine collection (Day 10 only).
- PK blood collection.
- Administration of study drug.
- AE assessment.
- Concomitant medications assessment.

9.3.8 Day 11

- Physical examination.
- Vital signs.
- Weight measurement.
- Clinical laboratory tests (including pregnancy test for all female subjects).
- 12-lead ECG.
- PK urine collection.
- PK blood collection.
- Concomitant medications assessment.
- AE assessment.

Subjects will be discharged from the clinic after the completion of all scheduled procedures and at the investigator's discretion.

For all subjects receiving study medication, the investigator must complete the End of Study CRF page.

9.3.9 Days 12 through 13

- Vital signs.
- PK blood collection.
- Concomitant medications assessment.
- AE assessment.

9.3.10 Day 14: F/U Visit

- Clinical laboratory tests.
- Concomitant medications assessment.
- AE assessment.

9.3.11 Early Termination

The reason for discontinuation must be documented in the source document and CRF. The following procedures will be performed and documented:

- Physical examination.
- Vital signs.
- Weight measurement.
- Clinical laboratory tests (including pregnancy test for all female subjects).
- 12-lead ECG.
- Concomitant medications assessment.
- AE assessment.

For all subjects receiving study medication, the investigator must complete the End of Study CRF page.

9.3.12 Follow-up Telephone Call

A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 ± 2 days) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since final dose of study medication or Early Termination Visit.

9.4 Blood Volume

Total blood sampling volume for an individual subject is shown in [Table 9.d](#).

Table 9.d Approximate Blood Volume

Sample Type	Sample Volume (mL)	Number of Samples			Total Volume (mL)
		Screening	Single Dose	Multiple Dose with Study Exit	
Safety Laboratory Samples	15	2	1	2	75
Pharmacokinetic Samples	6	N/A	14	17	186
Total Blood Sampling Volume					261

Direct venipuncture is the method of blood collection. Additional blood samples may be collected at the discretion of the investigator to assess safety.

10.0 PRETREATMENT EVENTS AND ADVERSE EVENTS

10.1 Definitions

10.1.1 PTEs

A PTE is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study but prior to administration of any study medication; it does not necessarily have to have a causal relationship with study participation.

10.1.2 AEs

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a drug; it does not necessarily have to have a causal relationship with this 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.

10.1.3 Additional Points to Consider for PTEs and AEs

An untoward finding generally may:

- Indicate a new diagnosis or unexpected worsening of a pre-existing condition. (Intermittent events for pre existing conditions underlying disease should not be considered PTEs or 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.
- PTEs/AEs caused by a study procedure (eg, a bruise after blood draw) should be recorded as a PTE/AE.

Diagnoses vs 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 a PTE(s) or as an AE(s).

Laboratory values and ECG findings:

- Changes in laboratory values or ECG parameters are only considered to be PTEs or 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 a PTE or as an AE.

Pre-existing conditions:

- Pre-existing conditions (present at the time of signing of informed consent) are considered concurrent medical conditions and should NOT be recorded as PTEs or AEs. Baseline evaluations (eg, laboratory tests, ECG, X-rays etc.) should NOT be recorded as PTEs unless related to study procedures. However, if the subject experiences a worsening or complication of such a concurrent condition, the worsening or complication should be recorded appropriately as a PTE (worsening or complication occurs before start of study medication) or an AE (worsening or complication occurs after start of study medication). Investigators should ensure that the event term recorded captures the change in the condition (eg, “worsening of...”).
- If a subject has a pre-existing episodic condition (eg, asthma, epilepsy) any occurrence of an episode should only be captured as a PTE/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 in the condition from Baseline (eg “worsening of...”).
- If a subject has a degenerative concurrent condition (eg, cataracts, rheumatoid arthritis), worsening of the condition should only be captured as a PTE/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 PTEs or AEs:

- If the subject experiences a worsening or complication of a PTE after starting administration of the study medication, the worsening or complication should be recorded appropriately as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, “worsening of...”).
- If the subject experiences a worsening or complication of an AE 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 /Serious PTEs:

- If the subject experiences changes in severity of an AE/serious PTE, 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 informed consent are not considered PTEs or 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 a PTE or an AE. Complications resulting from any planned surgery should be reported as adverse events.

Elective surgeries or procedures:

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

Overdose:

- Cases of overdose with any medication without manifested side effects are NOT considered PTEs or AEs, but instead will be documented on an Overdose page of the CRF. Any manifested side effects will be considered PTEs or AEs and will be recorded on the AE page of the CRF.

10.1.4 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 subject 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 subject 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](#)).

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
	Confirmed or suspected transmission of infectious agent by a medicinal product
	Neuroleptic malignant syndrome / malignant hyperthermia
	Spontaneous abortion / stillbirth and fetal death

PTEs that fulfill 1 or more of the serious criteria above are also to be considered SAEs and should be reported and followed up in the same manner (see Sections 10.2.2 and 10.3).

10.1.5 Severity of PTEs and AEs

The different categories of intensity (severity) are characterized as follows:

Mild: The event is transient and easily tolerated by the subject.
Moderate: The event causes the subject discomfort and interrupts the subject's usual activities.
Severe: The event causes considerable interference with the subject's usual activities.

10.1.6 Causality of AEs

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

Yes: An AE that follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug), or for which possible involvement of the drug can be argued, although factors other than the drug, such as underlying diseases, complications, concomitant drugs and concurrent treatments, may also be responsible.
No: 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 drugs and concurrent treatments.

10.1.7 Relationship to Study Procedures

Relationship (causality) to study procedures should be determined for all PTEs and AEs.

The relationship should be assessed as Yes if the investigator considers that there is reasonable possibility that an event is due to a study procedure. Otherwise, the relationship should be assessed as No.

10.1.8 Start Date

The start date of the AE/PTE is the date that the first signs/symptoms were noted by the subject and/or physician.

10.1.9 Stop Date

The stop date of the AE/PTE is the date at which the subject recovered, the event resolved but with sequelae or the subject died.

10.1.10 Frequency

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

10.1.11 Action Concerning Study Medication

- Drug withdrawn – a study medication is stopped due to the particular AE.
- Dose not changed – the particular AE did not require 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 subject died, dosing with study medication was already stopped before the onset of the AE.

10.1.12 Outcome

- Recovered/Resolved – Subject returned to first assessment status with respect to the AE/PTE.
- Recovering/Resolving – the intensity is lowered by one or more stages: the diagnosis or signs/symptoms has almost disappeared; the abnormal laboratory value improved, but has not returned to the normal range or to baseline; the subject died from a cause other than the particular AE/PTE 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 got worse than when it started; is an irreversible congenital anomaly; the subject died from another cause with the particular AE/PTE state remaining “Not recovered/not resolved”.
- Resolved with sequelae – the subject recovered from an acute AE/PTE but was left with permanent/significant impairment (eg, recovered from a cardiovascular accident but with some persisting paresis).
- Fatal – the AEs/PTEs which are considered as the cause of death.
- Unknown – the course of the AE/PTE cannot be followed up due to hospital change or residence change at the end of the subject’s participation in the study.

10.2 Procedures

10.2.1 Collection and Reporting of AEs

10.2.1.1 PTE and AE Collection Period

Collection of PTEs will commence from the time the subject signs the informed consent to participate in the study and continue until the subject is first administered study medication (Day 1) or until screen failure. For subjects who discontinue prior to study medication administration, PTEs are collected until the subject discontinues study participation.

Collection of AEs will commence from the time that the subject is first administered study medication (Day 1). Routine collection of AEs will continue until 30 days after the last dose of study drug.

10.2.1.2 PTE and AE Reporting

At each study visit, the investigator 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. Subjects may report AEs occurring at any other time during the study. Subjects experiencing a serious PTE 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. Non-serious PTEs, related or unrelated to the study procedure, need not to be followed-up for the purposes of the protocol.

All subjects 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 PTEs and AEs will be documented in the PTE/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 stop date and time.
- Severity.
- Investigator's opinion of the causal relationship between the event and administration of study medication(s) (yes or no) (not completed for PTEs).
- Investigator's opinion of the causal relationship to study procedure(s), including the details of the suspected procedure.
- Action concerning study medication (not applicable for PTEs).
- Outcome of event.
- Seriousness.

10.2.2 Collection and Reporting of SAEs

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

A Takeda SAE form must be completed 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.
- Subject 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 Section 1.0.

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 Serious PTEs will follow the procedure described for SAEs.

10.2.3 Reporting of Abnormal Liver Function Tests

If a subject is noted to have ALT or AST elevated $>3 \times \text{ULN}$ on 2 consecutive occasions, the abnormality should be recorded as an AE if assessed as clinically significant. 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 subject is noted to have ALT or AST $>3 \times \text{ULN}$ and total bilirubin $>2 \times \text{ULN}$ for which an alternative etiology has not been identified, the event should be recorded as an SAE and reported as per Section 10.2.2. The investigator must contact the Medical Monitor for discussion of the relevant subject 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 9.1.8 must also be performed. In addition, an LFT Increases CRF must be completed and transmitted with the Takeda SAE form (as per Section 10.2.2).

10.3 Follow-up of SAEs

10.3.1 Safety Reporting of SAEs

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.

10.3.2 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, including the EMA, 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 trial. 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 STUDY-SPECIFIC COMMITTEES

No steering committee or clinical endpoint committee will be used in this study.

Takeda will designate an external hepatologist as an independent assessor of any liver enzyme elevation in the study. Takeda will also designate a Pharmacovigilance physician who is not involved in the study conduct as a sponsor liaison to participate and facilitate the safety communication between the external expert, the study site and Takeda.

All liver enzyme elevations occurring in the study subjects will be initially submitted to blinded review by the external hepatologist. The unblinded aggregate data from a series of patients will also be reviewed by the external hepatologist, a designated independent physician from the site, and a sponsor liaison before the next cohort is dosed with the support of an independent statistician, and a written assessment will be provided to Takeda liaison and the study PI in a real time.

12.0 DATA HANDLING AND RECORDKEEPING

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

12.1 (e)CRFs

Completed (e)CRFs are required for each subject who signs an informed consent.

The sponsor or its designee will supply investigative sites with access to (e)CRFs. These forms are used to transmit the information collected in the performance of this study to the sponsor and regulatory authorities. (e)CRFs must be completed in English.

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 to (e)CRFs 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 the change. Reasons for significant corrections should additionally be included. All new additions are to be made with the date and signature.

The principal investigator must review the (e)CRFs for completeness and accuracy and must sign and date the appropriate (e)CRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the (e)CRFs.

After submission of the (e)CRFs to the sponsor, any change of, modification of, or addition to the data on the (e)CRFs should be made by the investigator with use of change and modification records of (e)CRFs (Data Clarification Form) provided by the sponsor. The principal investigator must review the Data Clarification Form for completeness and accuracy, and must sign, and date the form.

(e)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 subject's medical and hospital records pertinent to the study to ensure accuracy of the (e)CRFs. The completed (e)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.

12.2 Record Retention

The investigator agrees to keep the records stipulated in Section 12.1 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating subjects, medical records, temporary media such as thermal sensitive paper should be copied and certified, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the

informed consent forms), electronic copy of (e)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 degradable thermal sensitive paper should be photocopied by the site and filed with the original in the subject's chart to ensure long-term legibility. Furthermore, International Conference on Harmonization (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 Phase 1 Site Specifications document for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized prior to unblinding of subjects' treatment assignment. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

13.1.1 Analysis Sets

13.1.1.1 Safety Set

The safety set will include all randomized subjects who receive at least 1 dose of study drug, including subjects who do not complete the study. Subjects in this set will be used for PK parameter summaries.

13.1.1.2 Pharmacokinetic Set

The pharmacokinetic set will consist of all subjects who received study drug and have at least 1 measurable plasma concentration for TAK-536.

If any subjects are found to have incomplete data, a decision will be made on a case-by-case basis as to their inclusion in the analysis, but will be presented in the subject listings.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized by regimen for each part separately and overall. Summary statistics (N, mean, SD, median, minimum, and maximum) will be generated for continuous variables (eg, age, height, weight and BMI), and the number and percentage of subjects within each category will be presented for categorical variables (eg, gender, race). Individual subject demographic and baseline characteristics data will be listed.

13.1.3 Pharmacokinetic Analysis

Descriptive statistics (N, mean, SD, SE, median, minimum, and maximum) will be used to summarize the plasma concentration values of TAK-536 at each sampling time point for each profile day by regimen for each part separately and overall. Individual plasma concentrations of TAK-536 will be presented in listing.

The concentration of TAK-536 excreted in urine will be summarized at each scheduled sampling interval using descriptive statistics (N, mean, SD, SE, median, minimum, and maximum) by regimen for each part separately and overall. Individual concentration of TAK-536 in urine excretion data will be presented in a data listing.

Descriptive statistics (N, mean, SD, SE, %CV, median, minimum, and maximum) will be used to summarize all pharmacokinetic parameters of TAK-536 by regimen for each part separately and overall. Geometric means will also be computed for AUCs, C_{\max} , and C_{\min} .

All derived pharmacokinetic parameters will be presented in the data listings.

Additional statistical methods will be considered if deemed appropriate.

A more detailed analysis will be presented in the SAP.

13.1.4 Safety Analysis

Safety analysis will be performed using the safety set. All safety assessments, including AEs, clinical laboratory evaluations, 12-lead ECG results, physical examination, and vital signs will be summarized with descriptive statistics by regimen for each part separately and overall, where appropriate, and presented in the data listings.

All PTE/AEs, and treatment emergent adverse events (TEAE) will be coded using the MedDRA.

A TEAE is defined as an AE or SAE that occurs after the subject receives the first dose of study drug and until either within 30 days after the last dose of study medication is received. A TEAE may also be a continuing AE reported prior to the date of the first dose of study medication, which increases in severity after the start of dosing.

Summary of TEAEs will include numbers and percentages of subjects experiencing AE by system organ class and preferred term. TEAEs will also be summarized based on their intensity and relationship to study drug by system organ class and preferred term. If a subject has more than 1 AE that codes to the same preferred term, the subject will be counted only once for that preferred term within treatment. Similarly, if a subject has more than 1 AE within a system organ class category, the subject will be counted only once in the system organ class category within the treatment. In the intensity summary, a subject will be counted only in the highest intensity category for each preferred term and each organ class. In the relationship to study drug summary, a subject will be counted only in the highest relationship category for each preferred term and each system organ class. Each summary table will include incidences of adverse events for each regimen and overall.

All TEAE summary tables will be listed in SAP.

PTE will be summarized by system organ class and preferred term and by regimen.

All PTEs/TEAEs will be presented in the data listings.

Clinical laboratory variables will be summarized with descriptive statistics for Baseline, postdose and change from Baseline to postdose values. Individual results for clinical hematology, serum chemistry, and urinalysis laboratory tests that are outside of normal range will be identified in a summary table as well as those that meet Takeda predefined criteria for markedly abnormal values if applicable. All laboratory values will be provided in the listing.

Vital signs will be summarized with descriptive statistics for Baseline, postdose, and change from Baseline to postdose values. Individual vital sign values which meet Takeda predefined criteria for markedly abnormal values will be identified in a summary table. All vital signs data will be presented in the data listing.

Individual ECG measurements that meet Takeda predefined criteria for markedly abnormal values will be flagged and displayed in a table. All ECG data will be listed in the data listings.

13.2 Interim Analysis and Criteria for Early Termination

No interim analysis is planned.

13.3 Determination of Sample Size

Total sample size of 64 subjects will be enrolled in this study. Sixteen subjects, 8 per regimen (TAK-491 40 mg and 80 mg), were randomized to the open-label part of the study. Forty-eight subjects, 16 per regimen (TAK-491 40 mg, 80 mg and placebo), will be randomized to the double-blind part of the study. The sample size is considered sufficient for evaluation of safety, tolerability, and PK of each part of the study, and was not based on statistical power considerations.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 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. Source documents will be reviewed for verification of data recorded on the (e)CRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or its designee (CRO) and by the IRB or IEC.

All aspects of the study and its documentation will be subject to review by the sponsor or designee (as long as blinding is not jeopardized), including but not limited to the Investigator's Binder, study medication, subject medical records, informed consent documentation, documentation of subject authorization to use personal health information (if separate from the informed consent forms), and review of (e)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.

14.2 Protocol Deviations

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

Every attempt will be made to collect each pharmacokinetic blood sample at the designated time point, and the actual time of each blood sample will be recorded on the source document and CRF. However, blood samples not collected within the interval specified for the scheduled sample time should be reported to Takeda using the Protocol Deviation Form.

Protocol Deviation Forms are to be completed for pharmacokinetic samples collected outside of the following intervals:

Table 14.a Windows for Pharmacokinetic Sample Collection

Minutes	Nominal Sampling Time
no more than 30 minutes predose	0 hour
±5	immediately postdose to ≤6 hours
±10	>6 hours to ≤12 hours postdose
±15	>12 hours to ≤24 hours
±30	>24 hours

14.3 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 and institution guarantee access for quality assurance auditors to all study documents as described in Section 14.1.

15.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonized Tripartite Guideline for GCP. Each investigator will conduct the study according to applicable local or regional regulatory requirements. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 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.

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 informed consent form, and, if applicable, subject 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 subject 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, informed consent form) 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 written permission from competent authority to begin the trial. 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 informed consent form, recruitment materials intended for viewing by subjects, 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.

Subject incentives should not exert undue influence for participation. Payments to subjects must be approved by the IRB or IEC and sponsor.

15.2 Subject Information, Informed Consent, and Subject 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 informed consent form, subject authorization form (if

applicable), and subject information sheet (if applicable) describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for purposes of conducting the study. The informed consent form and the subject 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 informed consent form 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 informed consent form and if applicable, the subject authorization form. The informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be approved by both the IRB or IEC and the sponsor prior to use.

The informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be written in a language fully comprehensible to the prospective subject. It is the responsibility of the investigator to explain the detailed elements of the informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) to the subject. 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 subject is not capable of rendering adequate written informed consent, then the subject's legally acceptable representative may provide such consent for the subject in accordance with applicable laws and regulations.

The subject, or the subject'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 subject, or the subject's legally acceptable representative, determines he or she will participate in the study, then the informed consent form and subject authorization form (if applicable) must be signed and dated by the subject, or the subject's legally acceptable representative, at the time of consent and prior to the subject entering into the study. The subject or the subject'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 informed consent form and subject authorization (if applicable) at the time of consent and prior to subject entering into the study; however, the sponsor may allow a designee of the investigator to sign to the extent permitted by applicable law.

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

All revised informed consent forms must be reviewed and signed by relevant subjects or the relevant subject'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 subject's medical record, and the subject should receive a copy of the revised informed consent form.

15.3 Subject Confidentiality

The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject'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 subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject'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, the State Food and Drug Administration, the FDA, the Medicines and Healthcare products Regulatory Agency, the Pharmaceuticals and Medical Devices Agency), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject'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 subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process (see Section 15.2).

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

15.4 Publication, Disclosure, and Clinical Trial Registration Policy

15.4.1 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 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.

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 Master Services Agreement or equivalent agreement. In the event of any discrepancy between the protocol and the Master Services Agreement or equivalent agreement the Master Services Agreement or equivalent agreement will prevail.

15.4.2 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable law, regulation and guidance, Takeda will, at a minimum, register all

clinical trials conducted in patients that it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites before trial initiation.

15.4.3 Clinical Trial Results Disclosure

Takeda will minimally post the results of clinical trials conducted in patients, regardless of outcome, on ClinicalTrials.gov or other publicly accessible websites, as required by applicable laws and/or regulations.

15.5 Insurance and Compensation for Injury

Each subject in the study must be insured in accordance with the regulations applicable to the site where the subject 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 clinical study subjects. Refer to the Master Services Agreement or equivalent agreement regarding the sponsor's policy on subject 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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Appendix A Schedule of Study Procedures

Study Day	Screening	Check-in	Single and Multiple Dose							Study Exit (o)	PK collection	F/U Visit	ET Visit (a)	F/U Phone Call (b)
	Days -28 to -2	Day -1	Day 1	Days 2-3	Day 4	Days 5-6	Days 7-9	Day 10	Day 11	Days 12-13	Day 14			
Informed consent (c)	X													
Inclusion/exclusion criteria	X	X												
Demographics and medical history		X												
Medication history	X													
Physical examination	X	X									X			X
Vital signs (d)	X	X	X	X	X	X	X	X	X	X	X			X
Weight, height and BMI (e)	X	X									X			X
Concomitant medications (f)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concurrent medical conditions (g)		X												
Clinical laboratory tests (h)	X	X			X						X		X	X
Virology testing (i)	X													
Pregnancy test (hCG) (j)	X	X									X			X
Urine drug and cotinine screens and breath alcohol	X	X												
12-lead ECG	X	X									X			X
Study drug dosing (k)			X		X	X	X	X						
PK blood collection (l)			X	X	X		X	X	X	X	X			
PK urine collection (m)		X	X	X	X				X	X				
PTE assessment (n)	X	X	X											
AE assessment			X	X	X	X	X	X	X	X	X	X	X	X

Footnotes for Appendix A are on the next page.

Footnotes for Appendix A

ET=early termination.

- (a) Conduct procedures for subjects discontinued early. The PK sample collection should not be collected at the Early Termination Visit if a PK sample is not scheduled. Additional procedures may be performed at the discretion of the investigator to assess safety.
- (b) A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 ± 2) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since final dose of study medication or Early Termination Visit. Any spontaneously reported AEs will continue to be collected for 30 days after the last dose of study medication or Early Termination.
- (c) ICF must be signed before any study specific procedures are performed.
- (d) Sitting blood pressure, pulse, respiration rate and oral temperature will be measured on Day -1, and on confinement days, vitals are to be measured pre-dose or upon morning rising. Vitals will be collected prior to the PK blood collection.
- (e) Height (for calculation of BMI) will only be measured at Screening. Weight will be measured at Screening, Check-in (Day -1), and Study Exit or Early Termination Visit.
- (f) Record all ongoing medications.
- (g) Concurrent conditions are medical conditions that are present at Screening (time of informed consent).
- (h) Hematology, serum chemistries, and urinalysis tests. Blood and urine samples to be obtained before study drug dosing on Day 4. On Day 14, serum chemistry only; subjects with ALT/AST elevations on Day 11 and/or 14 will be followed-up every 5-7 days until resolution.
- (i) HBsAg, anti-HCV, anti-HIV, Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM. HBsAg, anti-HCV, and anti-HIV test results will be collected for all subjects. The results for Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM tests performed at Screening will be recorded in the (e)CRF for randomized subjects only.
- (j) Women of childbearing potential.
- (k) Study drug will be administered on Day 1 and Day 10 with 240 mL of water after a fast of at least 8 hours. On Day 1 and Day 10, subjects will also continue to fast for an additional 4 hours after dosing and subjects may consume water ad libitum with the exception of 1 hour before and 1 hour after drug administration. For Days 4 to 9, fasting is not required for dosing.
- (l) TAK-536 plasma PK: 6 mL blood samples will be collected on Day 1 at Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose. On Days 7, 8, and 9 at predose (up to 15 minutes prior to dose [0 hour]) and on Day 10 at predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72 hours postdose.
- (m) TAK-536 urine PK: urine will be collected starting on Day 1 at Predose (-12 to 0 hour) and at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose and on Day 10 at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose.
- (n) PTEs will be collected from the time of the informed consent to immediately prior to dosing on Day 1 of Period 1.
- (o) Subjects will be discharged from the clinic after completion of all scheduled procedures and at the investigator's discretion.

Appendix B Responsibilities of the Investigator

Clinical research studies sponsored by the sponsor are subject to ICH GCP and all applicable local laws and regulations.

The investigator agrees to assume the following responsibilities:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the staff who will assist in the protocol.
3. Ensure that study related procedures, including study specific (non routine/non standard panel) screening assessments are NOT performed on potential subjects, prior to the receipt of written approval from relevant governing bodies/authorities.
4. Ensure that all colleagues and employees assisting in the conduct of the study are informed of these obligations.
5. Secure prior approval of the study and any changes by an appropriate IRB/IEC that conform to ICH, and local regulatory requirements.
6. 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 subjects. 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.
7. Ensure that requirements for informed consent, as outlined in ICH and local regulations, are met.
8. Obtain valid informed consent from each subject who participates in the study, and document the date of consent in the subject's medical chart. Valid informed consent is the most current version approved by the IRB/IEC. Each informed consent form should contain a subject authorization section that describes the uses and disclosures of a subject's personal information (including personal health information) that will take place in connection with the study. If an informed consent form does not include such a subject authorization, then the investigator must obtain a separate subject authorization form from each subject or the subject's legally acceptable representative.
9. Prepare and maintain adequate case histories of all persons entered into the study, including (e)CRFs, hospital records, laboratory results, etc, and maintain these data for a minimum of 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.
10. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
11. Maintain current records of the receipt, administration, and disposition of sponsor-supplied drugs, and return all unused sponsor-supplied drugs to the sponsor.

12. Report adverse reactions to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.

Appendix C Elements of the Subject Informed Consent

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

1. A statement that the study involves research.
2. An explanation of the purposes of the research.
3. The expected duration of the subject'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 subjects involved in the study.
7. A description of the subject'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 subject may receive.
11. A description of any reasonably foreseeable risks or discomforts to the subject and, when applicable, to an embryo, fetus, or nursing infant.
12. A description of any benefits to the subject or to others that reasonably may be expected from the research. When there is no intended clinical benefit to the subject, the subject should be made aware of this.
13. Disclosures of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject and their important potential risks and benefits.
14. A statement describing the extent to which confidentiality of records identifying the subject 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 informed consent form, the subject or the subject'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 subject for participating in the study.
17. The anticipated expenses, if any, to the subject for participating in the study.
18. An explanation of whom to contact for answers to pertinent questions about the research (investigator), subject's rights, and IRB/IEC and whom to contact in the event of a research-related injury to the subject.
19. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject otherwise is entitled, and that the subject may discontinue

participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

20. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

21. A statement that the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the study.

22. The foreseeable circumstances or reasons under which the subject's participation in the study may be terminated.

23. A written subject authorization (either contained within the informed consent form or provided as a separate document) describing to the subject the contemplated and permissible uses and disclosures of the subject's personal information (including personal health information) for purposes of conducting the study. The subject authorization must contain the following statements regarding the uses and disclosures of the subject'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 subjects 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 subjects 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 subject's identity will remain confidential in the event that study results are published.

24. Female subjects of childbearing potential (eg, nonsterilized, premenopausal female subjects) who are sexually active must use adequate contraception (as defined in the informed consent) from Screening and throughout the duration of the study and for 30 days following the last dose of study drug. Regular pregnancy tests will be performed throughout the study for all

female subjects of childbearing potential. If a subject is found to be pregnant during study, study medication will be discontinued.

Appendix D Investigator Consent to Use 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 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.

Appendix E Collection, Storage, and Shipment of Bioanalytical Samples

Instructions for Processing of Plasma Samples for Pharmacokinetic Analysis of TAK-536

1. Collect 6 mL of venous blood into a chilled **CCI** [REDACTED] containing K₂EDTA.
2. Gently invert the Vacutainer several times to mix the additive with the collected blood prior to centrifugation and place immediately on ice.
3. Centrifuge the Vacutainers for 10 minutes at approximately 1100 to 1300 (RCF) at approximately 4°C in a refrigerated centrifuge. Note: if using a collection device other than **CCI** [REDACTED] refer to manufacturer's instruction for proper centrifugation force and time.
4. Immediately following centrifugation, gently remove plasma from the packed cells. To ensure a more homogeneous sample, all plasma should first be transferred into 1 aliquot. From there, split the plasma evenly between the 2 aliquots. A minimum of 1.0 mL needs to be obtained for each sample. Labeling will include protocol number (TAK-491_112), matrix (ie, plasma), analyte (TAK-536), subject randomization number, nominal day and time, and either "SET 1" (for original sample) or "SET 2" (for duplicate sample).
5. Cap the labeled storage tubes and freeze the plasma samples immediately at approximately -20°C or lower. No more than 60 minutes will elapse between blood collection and freezing the plasma sample.
6. Keep samples frozen at approximately -20°C or lower until shipment to bioanalytical laboratory. SET 1 samples will be shipped first on dry ice, followed by shipment of duplicate SET 2 samples after SET 1 samples have been received by the bioanalytical laboratory.

Instructions for Processing of Urine Samples for Pharmacokinetic Analysis of TAK-536

1. Collect urine into polypropylene containers. During the collection interval the urine will be stored at approximately 4°C.
2. Stir the urine in the polypropylene container vigorously.
3. Measure the urine volume at the end of the collection period.
4. Place two 10 mL aliquots of urine in polypropylene containers. Labeling may include protocol number (TAK-491_112), matrix (ie, urine), analyte (TAK-536), enrollment number, nominal day and time, and either "SET 1" (for original sample) or "SET 2" (for duplicate sample).
5. Freeze the urine samples within 4 hours of the end of the collection period and store frozen at approximately -20°C or lower.
6. Keep samples frozen at approximately -20°C or lower until shipment to bioanalytical laboratory. SET 1 samples will be shipped first on dry ice, followed by shipment of duplicate SET 2 samples after SET 1 samples have been received by the bioanalytical laboratory.

Instructions for Shipping of Samples for Pharmacokinetic Analysis

Site may adhere to the site's Standard Operating Procedures on shipping samples if the Standard Operating Procedure differs from the sample instruction provided.

1. Biological samples (ie, plasma or urine) should be shipped on dry ice to prevent thawing during transit. Ship samples only on Monday, Tuesday or Wednesday, and at least 2 days prior to a national holiday, to minimize the possibility of samples in transit over a weekend or holiday. If duplicate samples are to be shipped, send SET 1 samples and await confirmation of arrival before shipping the duplicate SET 2 samples.
2. Before shipping, make sure the sample tubes are tightly sealed. Separate each subject's samples as follows:
3. Separate the duplicate SET 2 samples from the SET 1 samples.
4. Place SET 1 samples for each subject into a self-sealing zipper-lock (eg, Ziploc[®]) bag containing additional absorbent material.
5. Using a permanent marker, write the subject randomization number, sample matrix (ie, plasma), analyte (TAK-536), number of samples, and "SET 1" on each Ziploc bag.
6. Place the bags of individual subject's samples into a larger plastic bag so that samples are double bagged. Duplicate SET 2 samples should be returned to the freezer for storage. Repeat steps 3-6 above when preparing duplicate samples for shipment, except Ziploc bags should be marked "SET 2."
7. An inventory of individual samples should accompany each shipment and should include the Sponsor's name (Takeda), study drug (TAK-491), protocol number TAK-491_112, investigator's name, sample type (ie, plasma, urine), subject's randomization number, nominal collection day and time, and intended sample storage conditions. When duplicate SET 2 samples are being shipped, make a copy of the original SET 1 sample inventory and mark as "SET 2." Place the inventory paperwork into a large Ziploc bag. SET 1 samples will be shipped first on dry ice, followed by shipment of duplicate SET 2 samples after SET 1 samples have been received by the analytical laboratory.
8. For sample packing, utilize dry ice generously (eg, 20-25 pounds per day of transit) to safeguard against longer than expected shipping times and delays. Use newspaper or other material to insulate the double-bagged samples from direct contact with the dry ice. Place the sample bundles into a styrofoam container (or other suitable container) and fill the excess space with dry ice slabs or ice pellets (preferably the latter). Make a note of the estimated weight of the dry ice used per shipping container.
9. Place the inventory paperwork (in a large Ziploc bag) on top of the dry ice in the styrofoam container. Place the lid on the styrofoam container and seal completely with strapping tape. Place the styrofoam container in a cardboard shipping carton and seal securely with strapping tape.
10. Mark the outside of shipping carton(s) with a tally number (eg, 1 of 5, 2 of 5).

11. Affix an address label to each shipping carton. Complete the address label with the following information: Contact name, telephone and fax number. The bioanalytical laboratory contact detail will be provided in a separate document.
12. Affix a carbon dioxide label on each carton, specifically:

Carbon Dioxide Solid UN-1845

Class 9 PKG GR III

Quantity _____

(fill in weight to nearest lb/kg and specify unit of measure used)

13. Affix 2 dry ice symbol labels on opposite sides of the carton. Mark **KEEP FROZEN** on each carton. Specify a return address and contact person on the carton.
14. Obtain the airway bill number and a receipt of shipment from the carrier.

After shipping of the samples, please contact the analytical site and Takeda Study Manager to notify them of next day delivery. When calling, provide the following information:

Name of courier or transport company

Time and date the shipment left the clinical site

Airway bill number

Appendix F Detailed Description of Amendments to Text

Rationale for Amendment

Protocol Amendment 3, dated 18 March 2016 was submitted to the EC and reviewed by the EC. The EC requested that Takeda update the protocol. The EC letter stated, “Among the 16 subjects, 15 subjects in the open-label trial were known to have increased ALT and AST levels on Day 11 and the exposure was increased correspondingly in case of moderate hepatic impairment, indicating that repeated-dose drug administration had a certain impact on hepatic function, conversely, hepatic function also affected the in vivo behavior of drug. Therefore, it is unreasonable to collect samples for calculating terminal elimination constant (λz) and terminal half-life ($T_{1/2}$) only after single-dose administration; sample collection should also be carried out after multiple-dose administration.”

Takeda agrees to add PK sample collection at 36, 48, and 72 hours after the Day 10 dose to address this EC comment.

Pages 12 and 24, Study Summary: Study Design and Section 6.1

Existing Text

The pre-dosing study period will consist of a Screening Period (Study Days -28 to -2) and Check-in (Study Day -1). On Day 1, a single dose of TAK-491 or placebo will be administered followed by a pharmacokinetic sampling period through 72 hours postdose (Days 1-4). On Days 4 through 10, subjects will be on a daily dosing regimen, followed by a 24-hour pharmacokinetic assessment period prior to Study Exit (Day 11). The total confinement period for a subject who completes the study will be 12 days. All subjects will return to the site for follow-up serum chemistry tests on Day 14. A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 [± 2 days]) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since the final dose of study medication or Early Termination Visit.

A schematic of the study design is included as [Figure 6.a](#). A schedule of assessments is listed in [Appendix A](#).

Figure 6.a Schematic of Study Design

Screening	Check-in	Dosing and PK	72-hr PK	Dosing (a)	Dosing and AM Trough Level (b)	Dosing and 24-hr PK	Study Exit	F/U Visit (c)	F/U Call
Days -28 to -2	Day -1	Day 1	Days 2-4	Day 4	Days 5-9	Day 10	Day 11	Day 14	Day 24 (± 2)

PK=pharmacokinetics.

(a) Dosing will occur after the collection of the 72-hr pharmacokinetic sample.

(b) PK trough samples will be collected on Days 7 to 9.

(c) Serum chemistry testing.

Revised Text

The pre-dosing study period will consist of a Screening Period (Study Days -28 to -2) and Check-in (Study Day -1). On Day 1, a single dose of TAK-491 or placebo will be administered followed by a pharmacokinetic sampling period through 72 hours postdose (Days 1-4). On Days 4 through 10, subjects will be on a daily dosing regimen, followed by a 24-hour pharmacokinetic assessment period prior to Study Exit (Day 11). The total confinement period for a subject who completes the study will be 12 days. **All subjects will return to the site for PK sampling on Days 12 to 13 and follow-up serum chemistry tests on Day 14.** A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study drug (Day 24 ± 2 days) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since the final dose of study drug or Early Termination Visit.

A schematic of the study design is included as [Figure 6.a](#). A schedule of assessments is listed in [Appendix A](#).

Screening	Check-in	Dosing and PK	72-hr PK	Dosing (a)	Dosing and AM Trough Level (b)	Dosing and 24-hr PK	Study Exit	PK collection	F/U Visit (c)	F/U Call
Days -28 to -2	Day -1	Day 1	Days 2-4	Day 4	Days 5-9	Day 10	Day 11	Days 12-13	Day 14	Day 24 (± 2)

PK=pharmacokinetic.

(a) Dosing will occur after the collection of the 72-hr pharmacokinetic sample.
(b) PK Trough samples will be collected on Days 7 to 9.
(c) Serum chemistry testing.

GLOBAL CHANGES TO ADD ASSOCIATED CHANGES TO BLOOD VOLUMES FOR ALL SUBJECTS

Rationale for Amendment

To add mandatory PK collection from Days 12 to 13 to address the EC's comments.

Page 41, Section 9.1.12 Pharmacokinetic Sample Collection

Existing Text

Table 9b Collection of Blood Samples for TAK-536 Pharmacokinetic Analysis

Matrix	Dosing Day(s)	Scheduled Time
Plasma	1	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose
Plasma	7, 8, 9	Predose (up to 15 minutes prior to dose [0 hour])
Plasma	10	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose

Revised Text

Table 9.b Collection of Blood Samples for TAK-536 Pharmacokinetic Analysis

Matrix	Dosing Day(s)	Scheduled Time
Plasma	1	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose
Plasma	7, 8, 9	Predose (up to 15 minutes prior to dose [0 hour])
Plasma	10	Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24, 36, 48, and 72 hours postdose

Page 46, Section 9.3.9: Days 12 -13

Existing Text

Not applicable.

Revised Text

- Vital signs.**
- PK blood collection.**
- Concomitant medications assessment.**
- AE assessment.**

Page 47, Section 9.4: Blood Volume (Table 9.d)

Existing Text

Sample Type	Sample Volume (mL)	Screening	Number of Samples		Total Volume (mL)
			Single Dose	Multiple Dose with Study Exit	
Safety Laboratory Samples	15	2	1	2	75
Pharmacokinetic Samples	6	N/A	14	14	168
Total Blood Sampling Volume					243

Revised Text

Sample Type	Sample Volume (mL)	Screening	Number of Samples		Total Volume (mL)
			Single Dose	Multiple Dose with Study Exit	
Safety Laboratory Samples	15	2	1	2	75
Pharmacokinetic Samples	6	N/A	14	17	186
Total Blood Sampling Volume					261

Page 70, Appendix A: Schedule of Study Procedures

Existing Text

Study Day	Screening	Check-in	Single and Multiple Dose							Study Exit	F/U Visit	ET Visit (a)	F/U Phone Call (b)
	Days -28 to -2	Day -1	Day 1	Days 2-3	Day 4	Days 5-6	Days 7-9	Day 10	Day 11	Day 14			
Informed consent (c)	X												
Inclusion/exclusion criteria	X	X											
Demographics and medical history	X												
Medication history	X												
Physical examination	X	X								X		X	
Vital signs (d)	X	X	X	X	X	X	X	X	X			X	
Weight, height and BMI (e)	X	X								X		X	
Concomitant medications (f)	X	X	X	X	X	X	X	X	X	X	X	X	X
Concurrent medical conditions (g)	X	X											
Clinical laboratory tests (h)	X	X			X					X	X	X	
Virology testing (i)	X												
Pregnancy test (hCG) (j)	X	X								X		X	
Urine drug and cotinine screens and breath alcohol	X	X											
12-lead ECG	X	X								X		X	
Study drug dosing (k)			X		X	X	X	X					
PK blood collection (l)			X	X	X		X	X	X				
PK urine collection (m)			X	X	X	X			X	X			
PTE assessment (n)	X	X	X										
AE assessment			X	X	X	X	X	X	X	X	X	X	X

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ET=early termination.

- (a) Conduct procedures for subjects discontinued early. The PK sample collection should not be collected at the Early Termination Visit if a PK sample is not scheduled. Additional procedures may be performed at the discretion of the investigator to assess safety.
- (b) A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 ± 2) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since final dose of study medication or Early Termination Visit. Any spontaneously reported AEs will continue to be collected for 30 days after the last dose of study medication or Early Termination.
- (c) ICF must be signed before any study specific procedures are performed.
- (d) Sitting blood pressure, pulse, respiration rate and oral temperature will be measured on Day -1, and on confinement days, vitals are to be measured pre-dose or upon morning rising. Vitals will be collected prior to the PK blood collection.
- (e) Height (for calculation of BMI) will only be measured at Screening. Weight will be measured at Screening, Check-in (Day -1), and Study Exit or Early Termination Visit.
- (f) Record all ongoing medications.
- (g) Concurrent conditions are medical conditions that are present at Screening (time of informed consent).
- (h) Hematology, serum chemistries, and urinalysis tests. Blood and urine samples to be obtained before study drug dosing on Day 4. On Day 14, serum chemistry only; subjects with ALT/AST elevations on Day 11 and/or 14 will be followed-up every 5-7 days until resolution.
- (i) HBsAg, anti-HCV, anti-HIV, Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM. HBsAg, anti-HCV, and anti-HIV test results will be collected for all subjects. The results for Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM tests performed at Screening will be recorded in the (e)CRF for randomized subjects only.
- (j) Women of childbearing potential.
- (k) Study drug will be administered on Day 1 and Day 10 with 240 mL of water after a fast of at least 8 hours. On Day 1 and Day 10, subjects will also continue to fast for an additional 4 hours after dosing and subjects may consume water ad libitum with the exception of 1 hour before and 1 hour after drug administration. For Days 4 to 9, fasting is not required for dosing.
- (l) TAK-536 plasma PK: 6 mL blood samples will be collected on Day 1 at Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose. On Days 7, 8, and 9 at predose (up to 15 minutes prior to dose [0 hour]) and on Day 10 at predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24 hours postdose.
- (m) TAK-536 urine PK: urine will be collected starting on Day 1 at Predose (-12 to 0 hour) and at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose and on Day 10 at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24 hours postdose.
- (n) PTEs will be collected from the time of the informed consent to immediately prior to dosing on Day 1 of Period 1.

Revised Text

Study Day	Screening	Check-in	Single and Multiple Dose								Study Exit (o)	PK collection	F/U Visit	ET Visit (a)	F/U Phone Call (b)
	Days -28 to -2	Day -1	Day 1	Days 2-3	Day 4	Days 5-6	Days 7-9	Day 10	Day 11	Days 12-13	Day 14				
Informed consent (c)	X														
Inclusion/exclusion criteria	X	X													
Demographics and medical history		X													
Medication history	X														
Physical examination	X	X									X			X	
Vital signs (d)	X	X	X	X	X	X	X	X	X	X	X			X	
Weight, height and BMI (e)	X	X									X			X	
Concomitant medications (f)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concurrent medical conditions (g)		X													
Clinical laboratory tests (h)	X	X			X						X		X	X	
Virology testing (i)	X														
Pregnancy test (hCG) (j)	X	X									X			X	
Urine drug and cotinine screens and breath alcohol	X	X													
12-lead ECG	X	X									X			X	
Study drug dosing (k)			X		X	X	X	X							
PK blood collection (l)			X	X	X		X	X	X	X	X				
PK urine collection (m)		X	X	X	X				X	X					
PTE assessment (n)	X	X	X												
AE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes for Appendix A are on the next page.

Footnotes for Appendix A

ET=early termination.

- (a) Conduct procedures for subjects discontinued early. The PK sample collection should not be collected at the Early Termination Visit if a PK sample is not scheduled. Additional procedures may be performed at the discretion of the investigator to assess safety.
- (b) A Follow-up telephone call will be made by the site at 14 (± 2) days following the last dose of study medication (Day 24 ± 2) to inquire for any ongoing adverse events or serious adverse events (SAEs), new adverse events or SAEs, and concomitant medications taken since final dose of study medication or Early Termination Visit. Any spontaneously reported AEs will continue to be collected for 30 days after the last dose of study medication or Early Termination.
- (c) ICF must be signed before any study specific procedures are performed.
- (d) Sitting blood pressure, pulse, respiration rate and oral temperature will be measured on Day -1, and on confinement days, vitals are to be measured pre-dose or upon morning rising. Vitals will be collected prior to the PK blood collection.
- (e) Height (for calculation of BMI) will only be measured at Screening. Weight will be measured at Screening, Check-in (Day -1), and Study Exit or Early Termination Visit.
- (f) Record all ongoing medications.
- (g) Concurrent conditions are medical conditions that are present at Screening (time of informed consent).
- (h) Hematology, serum chemistries, and urinalysis tests. Blood and urine samples to be obtained before study drug dosing on Day 4. On Day 14, serum chemistry only; subjects with ALT/AST elevations on Day 11 and/or 14 will be followed-up every 5-7 days until resolution.
- (i) HBsAg, anti-HCV, anti-HIV, Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM. HBsAg, anti-HCV, and anti-HIV test results will be collected for all subjects. The results for Anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcAb, EBV VCA IgG, EBV VCA IgM, Anti-CMV IgG, and Anti-CMV IgM tests performed at Screening will be recorded in the (e)CRF for randomized subjects only.
- (j) Women of childbearing potential.
- (k) Study drug will be administered on Day 1 and Day 10 with 240 mL of water after a fast of at least 8 hours. On Day 1 and Day 10, subjects will also continue to fast for an additional 4 hours after dosing and subjects may consume water ad libitum with the exception of 1 hour before and 1 hour after drug administration. For Days 4 to 9, fasting is not required for dosing.
- (l) TAK-536 plasma PK: 6 mL blood samples will be collected on Day 1 at Predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose. On Days 7, 8, and 9 at predose (up to 15 minutes prior to dose [0 hour]) and on Day 10 at predose (up to 15 minutes prior to dose [0 hour]) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, **36, 48, 72** hours postdose.
- (m) TAK-536 urine PK: urine will be collected starting on Day 1 at Predose (-12 to 0 hour) and at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose and on Day 10 at 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose.
- (n) PTEs will be collected from the time of the informed consent to immediately prior to dosing on Day 1 of Period 1.
- (o) **Subjects will be discharged from the clinic after completion of all scheduled procedures and at the investigator's discretion.**

GLOBAL CHANGE TO MODIFY DIET FOR ALL SUBJECTS

Page 28, Section 7.4 Diet, Fluid, and Activity Control, and Subject Housing: Second paragraph

Rationale for Amendment

The fat content of the diet of healthy volunteers during their hospital confinement was modified to align the protocol with Ethic Committee's comments.

Existing Text

During the confinement period, subjects will be given a menu *for the dosing period that includes 3 meals and an evening snack, each containing approximately 30% fat (relative to the total calories)*. The meals served on Day 1 and Day 10 should be identical between study days and for each subject in the study. The study menu should be recorded and submitted to the study file with a copy provided to the sponsor prior to the start of the study.

Revised Text

During the confinement period, subjects will be given a menu **consistent with local guideline and local dietary practice. Subjects will be given 3 meals and an evening snack every day, each containing approximately 20% fat (relative to the total calories)**. The meals served on Day 1 and Day 10 should be identical between study days and for each subject in the study. The study menu should be recorded and submitted to the study file with a copy provided to the sponsor prior to the start of the study.

GLOBAL CHANGES TO ADD LIVER SAFETY MONITORING PROCESS FOR ALL SUBJECTS

Rationale for Amendment

To add liver safety monitoring process to address the EC's comments.

Page 57, Section 11.0 STUDY-SPECIFIC COMMITTEES

Existing Text

No steering committee, *data safety monitoring committee*, or clinical endpoint committee will be used in this study.

Revised Text

No steering committee or clinical endpoint committee will be used in this study.

Takeda will designate an external hepatologist as an independent assessor of any liver enzyme elevation in the study. Takeda will also designate a Pharmacovigilance physician who is not involved in the study conduct as a sponsor liaison to participate and facilitate the safety communication between the external expert, the study site and Takeda.

All liver enzyme elevations occurring in the study subjects will be initially submitted to blinded review by the external hepatologist. The unblinded aggregate data from a series of patients will also be reviewed by the external hepatologist, a designated independent physician from the site, and a sponsor liaison before the next cohort is dosed with the support of an independent statistician, and a written assessment will be provided to Takeda liaison and the study PI in a real time.

OTHER TEXT CHANGES

Page 3, Section 1.2: Approval

Existing Text

SIGNATURES

Electronic signatures of the Responsible Medical Officer and other signatories are located on the last page of this document.

PPD



Revised Text

SIGNATURES

Electronic signatures of the Responsible Medical Officer and other signatories are located on the last page of this document.

PPD



Amendment 4-A Randomized, Open-Label and Double-Blind, Placebo-Controlled, Single- and Multiple-Dose, Phase 1 Study of the Pharmacokinetics of TAK-491 40 mg and 80 mg in Healthy Chinese Subjects

ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
PPD	Statistical Approval	08-Aug-2016 14:12 UTC
	Clinical VP Approval	08-Aug-2016 14:21 UTC
	Clinical Pharmacology Approval	08-Aug-2016 14:33 UTC
	Pharmacovigilance Approval	08-Aug-2016 14:37 UTC
	Clinical Operations Approval	10-Aug-2016 02:01 UTC