Title: A Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of Escalating Multiple Doses of TAK-831 in Healthy Subjects

NCT Number: NCT03224325

SAP Approve Date: 27 September 2018

Certain information within this Statistical Analysis Plan has been redacted (ie, specific content is masked irreversibly from view with a black/blue bar) to protect either personally identifiable (PPD) information or company confidential information (CCI).

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

STATISTICAL ANALYSIS PLAN

STUDY NUMBER: TAK-831-1005

A Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of Escalating Multiple Doses of TAK-831 in Healthy Subjects

Phase 1, TAK-831-1005, Multiple Rising Dose

PHASE 1

Version**:** Amendment 1

Date**:** 27 September 2018

Prepared by:

PPD

Based on:

Protocol Version: Amendment 2[1]

Protocol Date: 19 March 2018

1.1 Approval Signatures

Study Title: A Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of Escalating Multiple Doses of TAK-831 in Healthy Subjects

Approvals:

2.0 TABLE OF CONTENTS

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

3.0 LIST OF ABBREVIATIONS

%CV	coefficient of variation
λ_z	terminal disposition phase rate constant
AE	adverse event
ANCOVA	analysis of covariance
AUC	area under the concentration-time curve
AUC_∞	Area under the concentration-time curve from time 0 to infinity
AUC_{24}	area under the plasma drug concentration-time curve from time 0 to 24hours postdose
$AUC_{24,CSF}$	area under the CSF drug concentration-time curve from time 0 to 24hours postdose
AUC_τ	area under the plasma drug concentration-time curve during a dosing interval
$AUC_{\tau,CSF}$	area under the CSF drug concentration-time curve during a dosing interval
AUC_t	area under the plasma drug concentration-time curve from time 0 to time t on Day 1
$AUC_{t,CSF}$	area under the CSF drug concentration-time curve from time 0 to time t on Day 1
$AUEC_{24}$	area under the plasma effect-time curve from time 0 to 24 hours postdose
$AUEC_{24,\ CSF}$	area under the CSF effect-time curve from time 0 to 24 hours postdose
BLQ	below the limit of quantification
BMI	body mass index
$C_{av,ss}$	average plasma drug concentration during a dosing interval, at steady state
$C_{av,ss,CSF}$	average CSF drug concentration during a dosing interval, at steady state
C-SSRS	Columbia-Suicide Severity Rating Scale
CI	confidence interval
CL/F	apparent clearance after extravascular administration
C_{max}	maximum observed plasma concentration
$C_{max,CSF}$	maximum observed CSF concentration
$C_{max,ss}$	maximum observed plasma concentration during a dosing interval, at steady state
$C_{max,ss,CSF}$	maximum observed CSF concentration during a dosing interval, at steady state
CNS	central nervous system
CSF	cerebrospinal fluid
CV	conventional units
ECG	Electrocardiogram
eCRF	electronic case report form
E_{max}	maximum observed effect
MAV	markedly abnormal value
MedDRA	Medical Dictionary for Regulatory Activities
MRD	multiple-rising dose
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	preferred term
PTE	pretreatment event
QD	once daily

$R_{ac(AUC)}$	accumulation ratio based on AUC
$R_{ac(Cmax)}$	accumulation ratio based on C_{max}
$R_{ac(AUC, CSF)}$	accumulation ratio based on CSF AUC
$R_{ac(Cmax, CSF)}$	accumulation ratio based on CSF C_{max}
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SI	International System of Units
SOC	system organ class
SRD	single-rising dose
TEAE	treatment emergent adverse event
$t_{1/2z}$	terminal disposition phase half-life
t_{max}	Time of first occurrence of C_{max}
$t_{max,CSF}$	time of first occurrence of C_{max} in CSF
V_z/F	apparent volume of distribution during the terminal disposition phase after extravascular administration
WHODrug	World Health Organization Drug Dictionary

4.0 SUMMARY OF CHANGES

This section describes the changes made about the SAP incorporating Amendment No. 1. Detailed description of amendments to the text is presented in Appendix E.

1. CCI [redacted]
2. CCI [redacted]

   CCI [redacted]
3. Approver changes

   Justification: SAP signatories changed in accordance with personal changes.

5.0 OBJECTIVES

5.1 Primary Objectives

To evaluate the safety and tolerability of TAK-831 when administered as multiple oral doses at escalating dose levels in healthy subjects.

5.2 Secondary Objectives

To evaluate the PK of TAK-831 when administered as multiple oral doses at escalating dose levels in healthy subjects.

5.3 Additional Objectives

CCI [redacted]

5.4 Study Design

The study is a investigator and subject blinded, sponsor-unblinded, placebo-controlled clinical study progressively assessing independent cohorts of healthy subjects to continue evaluation of the safety, tolerability, PK of escalating single doses (SD) and multiple doses (MD) of TAK-831, as either a suspension or T2 tablet formulation, at levels higher than those achieved in the initial single and multiple rising dose (SRD/MRD) Study TAK-831-1001.

Up to 6 cohorts will be run either singly or in parallel as non- cerebrospinal fluid (CSF) cohorts with plasma analysis or CSF cohorts with CSF analysis in addition to plasma analysis to assess TAK-831 exposure and CCI [redacted] levels in the central nervous system (CNS). After meeting all the selection criteria, there will be 8 subjects enrolled in each cohort. They will be randomized on Day 1 in a ratio of 1:3 to receive placebo or TAK-831.

All cohorts will start with an initial, in-house SD period to include predose PD assessments on Day -1, Day 1 TAK-831 dosing, and a 48-hour in-house observation period with full PK/PD collections. The SD PK/PD collection and observation phase will be followed by 14 days of in-house MD.

In CSF cohorts, lumbar CSF samples will be collected through either a single lumbar puncture or an indwelling temporary catheter on 2 separate occasions. In CSF cohorts that use an indwelling temporary catheter on both occasions, the first collection will occur on Day 1, and the second on Day 16. In CSF cohorts where the predose CSF sample is collected through a single lumbar puncture, the first CSF sample will be collected on Day -1 prior to the start of CCI [redacted]

monitoring and the second collection will be obtained through use of an indwelling temporary catheter on Day 16. If an indwelling temporary catheter is being used, it will collect serial CSF samples for 24 hours.

CCI [redacted]

Subjects in each cohort will be confined during dosing and be kept in the study unit for 24 hours after the last dose for non-CSF cohorts and 48 hours after catheter removal in CSF cohorts. The total confinement period in non-CSF cohorts will be 19 days (Day -2 to Day 17), 20 days in CSF cohorts where an indwelling temporary catheter is being used on Day 1 and Day 16 (confinement from Day -1 to Day 19); and 21 days in CSF cohorts where CSF is collected through a single predose lumbar puncture on Day -1 prior to the start of CCI [redacted] measurements and through an indwelling catheter on Day 16 (confinement from Day -2 to Day 19). Follow-up assessments will occur on Day 30 (±2) for all cohorts. An exception to these minimum confinement periods will be made if a determination is made to evaluate QT/QTc intervals for additional single doses of TAK-831 in non-CSF cohorts based on the established tolerated dose range.

A schematic of the study design is presented in Figure 5.a.

Figure 5.a Schematics of Study Design

Cohorts (up to 6 as needed)	Pretreatment Period		Treatment and Assessments (a)				Follow-up (b)
			SD Part				
	Screening	Check-in/ Baseline	Treatment	Evaluation	MD Part	Study Exit	
Non-CSF (or CSF cohorts that collect Day -1 CSF samples via a single predose lumbar puncture)	Days -28 to -3	Day -2	Day 1	Days 1 to 2	Days 3 to 16	Day 17 (non-CSF cohort) / Day 19 (CSF cohort where Day -1 CSF is collected via a single predose lumbar puncture)	Day 30 (±2)
CSF	Days -28 to -2	Day -1	Day 1	Days 1 to 2	Days 3 to 16	Day 19	Day 30 (±2)
		←		Confinement		→	

CSF=cerebrospinal fluid, MD=multiple dose, PD=pharmacodynamic, PK=pharmacodynamic, SD=single dose.
(a) TAK-831 dosing will be on Day 1 for SD and Days 3 through 16 for MD.
(b) The Follow-up Visit will occur by telephone unless abnormal, clinically significant findings are observed upon discharge. In these cases, subjects may then be brought back to the clinic for re-evaluation per investigator's discretion.

6.0 ANALYSIS ENDPOINTS

6.1 Primary Endpoints

Primary endpoints for the study include:

- Percentage of subjects who experience at least 1 treatment emergent adverse event (TEAE).
- Percentage of subjects who meet the markedly abnormal criteria for clinical safety laboratory tests at least once postdose.
- Percentage of subjects who meet the markedly abnormal criteria for vital sign measurements at least once post dose.
- Percentage of subjects who meet the markedly abnormal criteria for 12-lead safety electrocardiogram (ECG) parameters at least once post dose.

6.2 Secondary Endpoints

Plasma PK Parameters of TAK-831:

1. Maximum observed plasma concentration (C_{max}) (Day 1).
2. Maximum observed steady-state plasma concentration during a dosing interval (Day 16).
3. Time of first occurrence of C_{max} (Days 1 and 16).
4. Area under the plasma concentration-time curve during a dosing interval (Days 1 and 16).

6.3 Additional Endpoints

CCI

[redacted]

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7.0 DETERMINATION OF SAMPLE SIZE

The sample size chosen of 8 subjects for all Cohorts (6 active : 2 placebo) is considered to be sufficient for evaluation of safety, tolerability, and PK of each cohort to determine the dose for the next cohort. The sample size was not based on statistical power considerations.

8.0 METHODS OF ANALYSIS AND PRESENTATION

8.1 General Principles

All study-related raw and derived data for randomized subjects will be presented in listings by cohort. Randomized subjects are the subjects who are enrolled and received a randomization number.

Continuous data will be summarized using the following descriptive statistics: number of subjects (N), mean, standard deviation (SD), median, minimum, and maximum, where appropriate. Where indicated, the coefficient of variation (%CV) and geometric mean will also be included in the summary of continuous data. Categorical data will be summarized using the number and percent of subjects for each category, where appropriate.

Unless otherwise stated, baseline is defined as the last observed value before the first dose of study medication.

As applicable, summaries will be presented by pooled placebo, each combination of TAK-831 dose level /formulation, TAK-831 overall and overall.

All data analyses and figures will be generated using SAS System® Version 9.2 or higher.

8.1.1 Study Definitions

There are no study-specific definitions.

8.1.2 Definition of Study Days

Study day will be calculated relative to the date of the first dose of the study drug in each study part. Study days prior to the first dose of study drug in each study part will be calculated as: {date of assessment/event – date of first dose of study drug of the subject}. Study days on or after the first dose of study drug will be calculated as: {date of assessment/event – date of first dose of study drug of the subject + 1}.

8.1.3 Definition of Study Visit Windows

There will be no visit windows.

8.1.4 Conventions for Missing Adverse Event Dates

There will be no imputation of incomplete or missing adverse event dates.

8.1.5 Conventions for Missing Concomitant Medication Dates

There will be no imputation of incomplete or missing concomitant medication dates.

8.1.6 Conventions for Missing Data

There will be no imputation of incomplete or missing data.

Plasma or CSF concentrations that are below the limit of quantification (BLQ) will be treated as zero in the summarization of concentration values and derivation of PK parameters. These values will be flagged in the data listings and deviations from this convention may be considered on a case-by-case basis as deemed appropriate.

8.2 Analysis Sets

Safety Set

The Safety Set will include all randomized subjects who receive at least 1 dose of study drug in a given part of the study. Subjects in this set will be used for demographic and safety summaries.

Pharmacokinetic Set

The PK set will include all randomized subjects with at least 1 measurable concentration for TAK-831. All subjects with valid PK parameter estimates will be included in the summaries and analyses for that parameter.

Pharmacodynamic Set

CCI

If any subject is found to be noncompliant with the dosing schedule or has incomplete data, a decision will be made on a case-by-case basis as to whether that subject should be included in the PK and PD analyses; however, data for all subjects will be presented in the data listings.

8.3 Disposition of Subjects

Disposition of all screened subjects (denominator) will be tabulated (count and percent); there will be no inferential analysis of subject disposition data.

Summaries will be presented by pooled placebo, each combination of TAK-831 dose level/formulation, TAK-831 overall and overall.

Disposition of all randomized subjects will be tabulated for each part of the study:

- All subjects received at least one dose of study drug (denominator).
- Subjects who completed the study.
- Subjects who prematurely discontinued study.

Primary reasons for discontinuation of study, as entered on the electronic case report form (eCRF), will be tabulated. Reasons for discontinuation include adverse event, significant protocol deviation, lost to follow-up, withdrawal by subject, study termination, and other. The date of first dose, date of last dose, duration of treatment and the reason for premature discontinuation of study drug/study visit will be presented for each subject in listings.

Disposition of screen failure subjects will be summarized descriptively. Primary reasons for failure will be summarized and will be presented in a data listing.

8.4 Demographic and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized using the Safety Set. Summaries will be presented by pooled placebo, each combination of TAK-831 dose level and formulation, TAK-831 overall and overall.

Summary statistics will be presented for continuous variables (age, height, weight, and body mass index [BMI]). The number and percentage subjects within each category will be presented for categorical variables (for example, gender, race, etc.). Individual subject demographic and baseline characteristic data will be listed.

Demographic variables of screen failure subjects and reasons for screen failures will be summarized overall for subjects who are screened but not enrolled in the study.

8.5 Medical History and Concurrent Medical Conditions

Medical history and concurrent medical conditions will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA, version 18 or higher) coding system.

Medical history includes any significant conditions or diseases relevant to the disease under study that stopped at or prior to signing of informed consent. Ongoing conditions are considered concurrent medical conditions.

All medical history and concurrent medical condition data will be listed by site (study center) and subject number. The listing will contain subject identifier, treatment, system organ class (SOC), preferred term (PT), whether there was any medical history or concurrent condition, and, if yes, a detail of the medical history or concurrent condition. No inferential statistics will be presented.

8.6 Medication History and Concomitant Medications

Medication history information includes any medication relevant to eligibility criteria stopped at or within 28 days prior to signing of informed consent. Concomitant medications are recorded on the eCRF and include any medications, other than study drug, taken at any time between informed consent and the end of the study.

All medication history and concomitant medications will be listed by site (study center) and subject number. The listings will contain subject identifier, treatment, World Health Organization Drug Dictionary (WHODrug) preferred medication name, dose, unit, frequency, route, start date, stop date, whether the medication was ongoing, and reason for use. No inferential statistics will be presented.

Medication history and concomitant medications will be coded using the WHODrug Version 01March 2017 or higher.

8.7 Study Drug Exposure and Compliance

The date and time of each dose for each subject will be reported in the data listing for all subjects. Meal consumptions and timing will be reported in the data listing as well. Summaries

of PK data will be provided by dose and formulation group. No other summary statistics for the extent of exposure to study drug or compliance calculations will be performed for this study.

8.8 Efficacy Analysis

Not applicable.

8.9 Pharmacokinetic/Pharmacodynamic Analysis

8.9.1 Pharmacokinetic Analysis

All PK summaries and analyses will be based on the PK set. PK parameters of TAK-831 will be derived using non-compartmental analysis methods. The PK parameters of TAK-831 will be determined from the concentration-time data for all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times.

Serial blood samples for determination of TAK-831 concentrations will be collected on Day 1 (within 30 minutes before dosing) and at 0.5, 1, 1.5, 2, 4, 8, 12, 24, hours after the morning dose; on Day 10 (predose), Day 13 (predose), and on Day 16 at predose (within 30 minutes before dosing), and at 0.5, 1, 2, 4, 8, 12, 16, and 24 hours after the morning dose and at Early Termination.

In CSF cohorts, serial CSF samples will be collected On Day 1 and/or Day 16 at predose (within 30 minutes before dosing) and at 1, 4, 8, 12, and 24 hours after the morning dose.

The following pharmacokinetic parameters will be calculated for plasma (Day -1, Day 1 and Day 16) and CSF (Days 1 and/or 16) concentrations of TAK-831 when appropriate:

Symbol/Term	Definition
AUC_{τ} /$AUC_{\tau, CSF}$	Area under the concentration-time curve during a dosing interval.
AUC_t /$AUC_{t, CSF}$	Area under the concentration-time curve from time 0 to time t. (Day 1 only)
AUC_{∞}	Area under the concentration-time curve from time 0 to infinity, calculated using the observed value of the last quantifiable concentration (Day 1 only)
$R_{ac(AUC)}$ / $R_{ac(AUC, CSF)}$	Accumulation ratio based on AUC_{τ}.
$R_{ac(Cmax)}$ / $R_{ac(Cmax, CSF)}$	Accumulation factor based on C_{max}.
$C_{av,ss}$ /$C_{av,ss, CSF}$	Average concentration during a dosing interval, at steady state.
C_{max} /$C_{max, CSF}$	Maximum observed concentration on Day 1 and Day 16.
CL/F	Apparent clearance after extravascular administration, calculated as =Dose/AUC_{∞} after a single dose. Plasma only. (Day 1 only)
λ_z	Terminal disposition phase rate constant. Plasma only.
$t_{1/2z}$	Terminal disposition phase half-life. Plasma only.
t_{max}/ $t_{max, CSF}$	Time of first occurrence of C_{max}.
V_z/F	Apparent volume of distribution during the terminal disposition phase after extravascular administration, calculated using the observed value of the last quantifiable concentration. Plasma only. (Day 1 only)

Descriptive statistics (N, arithmetic mean, SD, %CV, median, minimum, and maximum) will be used to summarize the plasma and CSF PK parameters for TAK-831, including dose-normalized C_{max} and AUCs, by dose and formulation. In addition, geometric mean will be computed for C_{max} and AUCs, including those dose-normalized C_{max} and AUCs. Individual plasma PK parameters will be presented in a data listing.

Dose proportionality in plasma and CSF PK parameters (C_{max} and AUCs as well as dose-normalized C_{max} and AUCs) will be assessed graphically using box-plots for Day 1 and Day 16 plasma and CSF PK parameters for each formulation. Other figures needed for PK data are described in Clinical pharmacology Analysis Plan for this study.

Additional analyses will be included if appropriate.

8.9.2 Pharmacodynamic Analysis

CCI

[redacted]

CCI

[redacted]

8.10 Other Outcomes

Not applicable.

8.11 Safety Analysis

Safety analyses include AEs, clinical laboratory parameters, vital sign parameters, 12-lead ECG results, and other safety parameters. The Safety Set will be used for all summaries of safety parameters. The safety endpoints will be presented by pooled placebo, each TAK-831 dose level, TAK-831 overall, and overall.

8.11.1 Adverse Events

A pretreatment event (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.

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. PTE and AE verbatim terms will be coded by SOC and PT using MedDRA (version 20.0 or later).

TEAEs will be defined as any sign, symptom, syndrome, or new illness, regardless of relationship to study drug, that occur after the first dose of study drug and up to 30 days (onset date – last date of dose + 1 $\leq$ 30) after the last dose of study drug or early termination.

TEAEs are recorded in the eCRF as being related or not related to study drug and study procedure. TEAEs that are recorded as related to study drug and/or study procedure will be summarized separately. TEAEs will also be presented by intensity/severity (mild, moderate, and severe). Serious TEAEs, TEAEs leading to study drug discontinuation, and TEAEs leading to death will also be summarized using SOC and PT.

When calculating the frequency and percentage of subjects who reported TEAEs, a subject will be counted only once for each SOC or PT when multiple TEAEs are coded to the same SOC or PT. For the intensity or relatedness summaries, if a subject reports multiple TEAEs coded to the same SOC or PT, the TEAE with maximum intensity or strongest relationship will be included in the summary.

AEs with missing intensity will be listed as such in the AE listings, however, will be summarized as severe in summary tables. If the relationship of an event is missing, the relationship for the event will be considered to have been related.

In general, AEs will be tabulated at the following levels: overall summary (subjects with at least 1 AE in any dose or regimen), the MedDRA SOC, and the MedDRA PT. The tables will include the number and percentage (N[%]) of subjects. The following summary tables will be generated for each study part:

- Overview of Treatment-Emergent Adverse Events.
- Treatment-Emergent Adverse Events by System Organ Class and Preferred Term.
- Treatment-Emergent Adverse Events by Preferred Term.

- Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term.
- Relationship of Treatment-Emergent Adverse Events to Study Drug by System Organ Class and Preferred Term.
- Drug-Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term.
- Drug-Related Treatment-Emergent Adverse Events by Preferred Term.
- Intensity of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term.
- Pretreatment Adverse Events by System Organ Class and Preferred Term.

In addition, subject mappings for the TEAEs by SOC and PT will be generated.

Data listings will be provided for PTEs, TEAEs, TEAEs leading to study drug discontinuation, liver function abnormalities, SAEs, and AEs that resulted in death. AEs happened after 30 days post the last dose of the study drug will be listed as well.

8.11.2 Clinical Laboratory Evaluations

Clinical laboratory tests will be assessed using the Safety Set and will be evaluated and presented using International System of Units (SI) units unless otherwise stated. Refer to Protocol Section 9.2.9.1 and 9.2.9.2 as well as the schedule of the events for a list of all clinical laboratory tests.

All laboratory test parameters will be displayed in individual subject data listings in both SI units and conventional (CV) units. For test results not in SI units, the conversion to SI units will be done in the derived SDTM and ADaM datasets using the known conversion factors. If necessary, SI units from the central laboratory may be converted to Takeda's preferred SI units in the derived SDTM and ADaM datasets. All summaries and analyses will be based on the values using these preferred SI units.

Only observations within 7 days of the last dose of study drug will be included in the tables. No inferential statistics will be presented unless otherwise stated.

Descriptive statistics (N, mean, SD, median, minimum, and maximum) for the observed and change from baseline values will be presented. Study baseline will be used for change from baseline. Note that "character" urinalysis tests will only be listed.

Laboratory MAVs, identified by the criteria defined in Appendix A, will be tabulated. If a subject has a MAV for a particular laboratory test, all visits for that subject for that parameter will be listed. The number and percentage of subjects with at least 1 postdose markedly abnormal laboratory test result will be summarized. The mapping of the subjects who meet the MAV criteria will be listed as a table. All observations, including ones at unscheduled visits, will be included in the MAV evaluation and summaries.

Listings of all clinical safety laboratory data will be provided in Appendix 16.2 and will be presented in both SI and CV units. Laboratory data outside of the normal reference range will be

indicated in the listings. In addition, MAVs will be flagged. The listing will include site number, subject identifier, age, gender, treatment group, study visit, and sample collection date.

8.11.3 Vital Signs

Descriptive statistics (N, mean, SD, median, minimum, and maximum) will be used to summarize vital sign parameters at baseline, each post-baseline visit, and change from baseline to each post-baseline visit. Only observations within 7 days of the study drug will be included in the tables.

Vital sign MAVs, identified by the criteria defined in Appendix B, will be tabulated. If a subject has a MAV for a particular vital signs parameter, all visits for that subject for that parameter will be listed. The number and percentage of subjects with at least 1 postdose markedly abnormal vital signs measurement will be summarized. The mapping of the subjects who meet the MAV criteria will be listed as a table. All observations, including ones at unscheduled visits, will be included in the MAV evaluation and summaries.

Orthostatic hypotension, identified by the criteria defined in Appendix C, will be calculated at every time point where standing and supine measurements are available using the formula: standing vital measurement – supine vital measurement. The mapping of the subjects who meet the criteria for orthostatic hypotension will be listed by study visit as a table. All orthostatic hypotension observations, including ones at unscheduled visits, will be included in the subject mappings.

Listings of all vital signs data will be provided in Appendix 16.2. Vital sign MAVs will be flagged in the listings. The listing will include site number, subject identifier, age, gender, treatment group, study visit, and sample collection date.

8.11.4 12-Lead ECGs

Descriptive statistics (N, mean, SD, median, minimum, and maximum) of ECG parameters, including heart rate, PR interval, RR interval, QRS interval, QT interval, and QTc interval (Fredericia's and Bazett's corrections), will be presented for baseline, each post-baseline visit, and change from baseline to each post-baseline visit. Only the scheduled measurements will be included in the summary. Only observations within 7 days of the study drug will be included in the tables. No inferential statistics will be presented.

ECG MAVs, identified by the criteria defined in Appendix D, will be tabulated. If a subject has a MAV for a particular 12-lead ECG parameter, all visits for that subject for that parameter will be listed. The number and percentage of subjects with at least 1 postdose markedly abnormal 12-lead ECG measurement will be summarized. The mapping of the subjects who meet the MAV criteria will be listed as a table. All observations, including ones at unscheduled visits, will be included in the MAV evaluation and summaries.

Overall ECG interpretation category (normal, abnormal not clinically significant, abnormal clinically significant) is collected by eCRF at baseline and at each scheduled post-baseline visit. Shifts in ECG interpretation will be presented as cross-tabulations (baseline versus each post-

baseline visit) of numbers of subjects with normal, abnormal not clinically significant, and abnormal clinically significant interpretations with missing, if applicable, and total categories by regimen.

Listings of all 12-lead ECG data will be provided in Appendix 16.2. MAVs will be flagged in the listings. The listing will include site number, subject identifier, age, gender, treatment group, study visit, and sample collection date.

8.11.5 CCI

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[redacted]

8.11.6 Other Observations Related to Safety

Follow-up phone calls, DNA sample collections, physical and neurological examination and suicidality assessments (C-SSRS) will be presented in data listings.

8.12 Interim Analysis

This is an investigator-subject blinded, sponsor unblinded study. No formal interim analysis is planned.

CCI

[redacted]

8.13 Changes in the Statistical Analysis Plan

Not Applicable.

9.0 REFERENCES

1. A Phase 1, Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Safety, Tolerability and Pharmacokinetic Study of Escalating Single and Multiple Doses of TAK-831 in Healthy Subjects, Takeda Development Center Europe, Ltd., Protocol No. TAK-831-1005, Amendment No.2, dated 19 March 2018.
2. Garnett C, Bonate PL, Dang Q, Ferber G, et.al. (2018) Scientific white paper on concentration-QTc modeling. *J. Pharmacokinet Pharmacodyn*. 45, 383-397.

Appendix A Criteria for Identification of Markedly Abnormal Laboratory Values

Hematology—Criteria for Markedly Abnormal Values

Parameter	Unit	Low Abnormal	High Abnormal
Hemoglobin	Both	< 0.8 × LLN	> 1.2 × ULN
Hematocrit	Both	< 0.8 × LLN	> 1.2 × ULN
RBC count	Both	< 0.8 × LLN	> 1.2 × ULN
WBC count	Both	<0.5 x LLN	>1.5 x ULN
Platelet count	Conventional	<75 x 10^3/μL	>600 x 10^3/μL
	SI	<75 x 10^9/L	>600 x 10^9/L

LLN=lower limit of normal, RBC=red blood cell, ULN=upper limit of normal, WBC=white blood cell.

Serum Chemistry—Criteria for Markedly Abnormal Values

Parameter	Unit	Low Abnormal	High Abnormal
ALT	Both	--	>3x ULN
AST	Both	--	>3x ULN
GGT	Both	--	>3x ULN
Alkaline phosphatase	Both	--	>3x ULN
Total bilirubin	Conventional	--	>2.0 mg/dL
	SI	--	>34.2 μmol/L
Albumin	Conventional	<2.5 g/dL	--
	SI	<25 g/L	--
Total protein	Both	<0.8x LLN	>1.2x ULN
Creatinine	Conventional	--	>2.0 mg/dL
	SI		>177 μmol/L
Blood urea nitrogen	Conventional		>30 mg/dL
	SI		>10.7 mmol/L
Sodium	Conventional	<130 mEq/L	>150 mEq/L
	SI	<130 mmol/L	>150 mmol/L
Potassium	Conventional	<3.0 mEq/L	>6.0 mEq/L
	SI	<3.0 mmol/L	>6.0 mmol/L
CPK	Both	--	>5x ULN
Glucose	Conventional	< 50 mg/dL	>350 mg/dL
	SI	< 2.8 mmol/L	>19.4 mmol/L

ALT=alanine aminotransferase, AST=aspartate aminotransferase, CPK=creatine phosphokinase, GGT=γ-glutamyl transferase, LLN=lower limit of normal, ULN=upper limit of normal.

Appendix B Criteria for Abnormal Changes from Baseline of Vital Signs

Parameter	Unit	Lower Criteria	Upper Criteria
Pulse	bpm	<50	>120
Systolic blood pressure	mm Hg	<85	>180
Diastolic blood pressure	mm Hg	<50	>110
Body temperature	^{o}C	< 35.6	>37.7

Appendix C Criteria for Identification of Markedly Abnormal Orthostatic Changes

Parameter	Criteria
Orthostatic Hypotension	(Orthostatic Systolic Blood Pressure < -20 mm Hg OR Orthostatic Diastolic Blood Pressure < -10 mm Hg) AND Heart Rate Increase > 20 beats/min

Note: Orthostatic measurement = standing vital measurement – supine vital measurement.

Appendix D Criteria for Out-of-Range Values for the 12-Lead ECG Parameters

Parameter	Lower Criteria	Upper Criteria
Heart rate	<50 beats per minute	>120 beats per minute
PR	≤80 milliseconds	≥200 milliseconds
QT Interval	≤300 milliseconds	≥460 milliseconds
QTcB Interval	≤300 milliseconds	≥500 milliseconds OR ≥30 milliseconds change from baseline and ≥450 milliseconds
QTcF Interval	≤300 milliseconds	≥500 milliseconds OR ≥30 milliseconds change from baseline and ≥450 milliseconds
QRS	≤80 milliseconds	≥180 milliseconds

Appendix E Detailed Description of Amendment to Text

This section describes changes about SAP incorporating Amendment No. 1.

Page 2 Section 1.1 Approval Signatures

Existing Text

Approvals:

PPD

Revised Text

Approvals:

Rational for Amendment

SAP signatories updated in accordance with personnel changes.

Page 8, Section 5.3 Additional Objectives:

Existing Text:

…4. To explore the effect of a single oral dose of TAK-831 on the QT/QTc interval in non-CSF cohorts.

Revised Text:

… 4. To explore the effect of a single oral dose of TAK-831 on the QT/QTc interval in non-CSF cohorts **(or in CSF cohorts that collect baseline CSF samples via a single pre-dose lumbar puncture on Day -1 prior to** CCI [redacted] **measurements).**

Rational for Amendment

For the last 2 dosing Cohorts 5 and 6, The serial CSF samples are not to be collected on Day 1 to allow CCI [redacted] monitoring in the CSF cohorts where, and the first single baseline CSF sample is to be collected instead through a single lumbar puncture on Day -1 based on the protocol Amendment 2. The analysis of CSF samples is revised in the SAP to reflect this study change.

Page 8 Section 5.4 Study Design
Existing Text:

The study is an investigator and subject blinded, sponsor-unblinded, placebo-controlled clinical study progressively assessing independent cohorts of healthy subjects to continue evaluation of the safety, tolerability, *PK, and PD* of escalating single doses (SD) and multiple doses (MD) of TAK-831…

Revised Text:

The study is an investigator and subject blinded, sponsor-unblinded, placebo-controlled clinical study progressively assessing independent cohorts of healthy subjects to continue evaluation of the safety, tolerability **and PK** of escalating single doses (SD) and multiple doses (MD) of TAK-831…

Rational for Amendment:

Based on a few changes in the protocol amendment which was for clarification and correction.

Page 8-9, Section 5.4 Study Design:

Existing Text:

… In *all CSF cohorts*, *serial lumbar* CSF samples will be collected through an indwelling temporary catheter on 2 separate occasions: *the first from Day 1 through Day 2*, and the second *from Day 16 through Day 17, for 24 hours each*.

Revised Text:

In **CSF cohorts**, **lumbar** CSF samples will be collected through **either a single lumbar puncture or** an indwelling temporary catheter on 2 separate occasions. **In CSF cohorts that use an indwelling temporary catheter on both occasions,** the first **collection will occur on** Day 1,

and the second **on** Day 16. **In CSF cohorts where the predose CSF sample is collected through a single lumbar puncture, the first CSF sample will be collected on Day -1 prior to the start of** CCI **monitoring and the second collection will be obtained through use of an indwelling temporary catheter on Day 16. If an indwelling temporary catheter is being used, it will collect serial CSF samples for 24 hours.**

Rational for Amendment:

Allow combination of CSF measurements at baseline and CCI monitoring, clarifications, and corrections/editorial changes.

Page 9 Section 5.4 Study Design:

Existing Text:

The total confinement period in non-CSF cohorts will be 19 days (Day -2 to Day 17) and 20 days in CSF cohorts *(Day -1 to Day 19)*.

Revised Text:

The total confinement period in non-CSF cohorts will be 19 days (Day -2 to Day 17), 20 days in CSF cohorts **where an indwelling temporary catheter is being used on Day 1 and Day 16 (confinement from Day -1 to Day 19); and 21 days in CSF cohorts where CSF is collected through a single predose lumbar puncture on Day -1 prior to the start of** CCI **measurements and through an indwelling catheter on Day 16 (confinement from Day -2 to Day 19)**.

Rational for Amendment:

Allow combination of CSF measurements at baseline and CCI monitoring, clarifications, and corrections/editorial changes.

Page 9, Figure 5.a Schematics of Study Design

Existing Text:

Cohorts (up to 6 as needed)	**Pretreatment Period**		**Treatment and Assessments (a)**				
			SD Part			**Study Exit**	**Follow-up (b)**
	Screening	**Check-in/ Baseline**	**Treatment**	**Evaluation**	**MD Part**		
Non-CSF	Days -28 to -3	Day -2	Day 1	Days 1 to 2	Days 3 to 16	Day 17	Day 30 (±2)
CSF	Days -28 to -2	Day -1	Day 1	Days 1 to 2	Days 3 to 16	Day 19	Day 30 (±2)
		←	**Confinement**			→	

CSF=cerebrospinal fluid, MD=multiple dose, PD=pharmacodynamic, PK=pharmacodynamic, SD=single dose.
(a) TAK-831 dosing will be on Day 1 for SD and Days 3 through 16 for MD.
(b) The Follow-up Visit will occur by telephone unless abnormal, clinically significant findings are observed upon discharge. In these cases, subjects may then be brought back to the clinic for re-evaluation per investigator's discretion.

Revised Text:

Cohorts (up to 6 as needed)	Pretreatment Period		Treatment and Assessments (a)				
			SD Part				
	Screening	Check-in/ Baseline	Treatment	Evaluation	MD Part	Study Exit	Follow-up (b)
Non-CSF (or CSF cohorts that collect Day -1 CSF samples via a single predose lumbar puncture)	Days -28 to -3	Day -2	Day 1	Days 1 to 2	Days 3 to 16	Day 17 (non-CSF cohort) / Day 19 (CSF cohort where Day -1 CSF is collected via a single predose lumbar puncture)	Day 30 (±2)
CSF	Days -28 to -2	Day -1	Day 1	Days 1 to 2	Days 3 to 16	Day 19	Day 30 (±2)
		←		Confinement		→	

CSF=cerebrospinal fluid, MD=multiple dose, PD=pharmacodynamic, PK=pharmacodynamic, SD=single dose.
(a) TAK-831 dosing will be on Day 1 for SD and Days 3 through 16 for MD.
(b) The Follow-up Visit will occur by telephone unless abnormal, clinically significant findings are observed upon discharge. In these cases, subjects may then be brought back to the clinic for re-evaluation per investigator's discretion.

Rational for Amendment:

Allow combination of CSF measurements at baseline and CCI monitoring, clarifications, and corrections/editorial changes.

Page 11 Section 6.3 Additional Endpoints

Existing Text:

CCI [redacted]

Revised Text:

CCI [redacted]

CCI

[redacted]

Rational for Amendment:

Allow combination of CSF measurements at baseline and CCI monitoring, clarifications, and corrections/editorial changes.

Page 16 Section 8.9.1 Pharmacokinetic Analysis

Existing Text:

In CSF cohorts, serial CSF samples will be collected On Day 1 *and* Day 16 at predose (within 30 minutes before dosing) and at 1, 4, 8, 12, and 24 hours after the morning dose.

The following pharmacokinetic parameters will be calculated for plasma (Day -1, Day 1 and Day 16) and CSF (Days 1 *and* 16) concentrations of TAK-831

Revised Text:

In CSF cohorts, serial CSF samples will be collected On Day 1 **and/or** Day 16 at predose (within 30 minutes before dosing) and at 1, 4, 8, 12, and 24 hours after the morning dose.

The following pharmacokinetic parameters will be calculated for plasma (Day -1, Day 1 and Day 16) and CSF (Days 1 **and/or** 16) concentrations of TAK-831 **when appropriate**

Rational for Amendment:

Allow combination of CSF measurements at baseline and CCI monitoring, clarifications, and corrections/editorial changes.

Page 17 Section 8.9.2 Pharmacodynamic Analysis

Existing Text:

CCI [redacted]

Revised Text:

CCI [redacted]

Rational for Amendment:

CCI [redacted]

Page 18 Section 8.9.2 Pharmacodynamic Analysis:

Existing Text:

CCI [redacted]

Revised Text:

CCI [redacted]

Rational for Amendment:

Editorial change due to the change in study design.

Page 22 Section 8.11.5 CCI [redacted]

Exiting Text:

CCI [redacted]

CCI

[redacted]

Revised Text:

CCI

[redacted]

CCI

[redacted]

Rational for Amendment:

Based on the recent scientific white paper on concentration-QTc modeling published by Garnett et. al, (published online 5 December 2017)[2], a pre-specified and dedicated modeling analysis is recommended. Therefore, the concentration-QTc analysis will be performed outside of the current SAP and results will be reported separately for regulatory purposes.

Page 23 Section 9.0 References

Existing Text:

1. A Phase 1, Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Safety, Tolerability and Pharmacokinetic Study of Escalating Single and Multiple Doses of TAK-831 in Healthy Subjects, Takeda Development Center Europe, Ltd., Protocol No. TAK-831-1005, Amendment No.1, dated 15 August 2017.

Revised Text:

1. A Phase 1, Randomized, Investigator and Subjects Blinded, Sponsor Unblinded, Placebo-Controlled, Safety, Tolerability and Pharmacokinetic Study of Escalating Single and Multiple Doses of TAK-831 in Healthy Subjects, Takeda Development Center Europe, Ltd., Protocol No. TAK-831-1005, Amendment No.2, dated 19 March 2018.
2. Garnett C, Bonate PL, Dang Q, Ferber G, et.al. (2018) Scientific white paper on concentration-QTc modeling. *J. Pharmacokinet Pharmacodyn*. 45, 383-397.

Rational for Amendment:

Adding more references for the changes.

ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
PPD	Biostatistics Approval	01-Oct-2018 15:23 UTC
	Pharmacovigilance Approval	01-Oct-2018 15:40 UTC
	Clinical Pharmacology Approval	01-Oct-2018 15:47 UTC
	Clinical Science Approval	01-Oct-2018 16:02 UTC