STATISTICAL ANALYSIS PLAN

TAK-681 (Glucagon-like peptide-2 (GLP-2) analog-Fc fusion protein)
PHASE 1

A Randomized, Double-blind, Placebo-controlled, Phase 1 Study to Assess the Safety, Tolerability, and Pharmacokinetics of Ascending, Subcutaneous, Single and Multiple Doses of TAK-681 (GLP-2 analog-Fc fusion) in Healthy Adult Subjects

PROTOCOL IDENTIFIER: TAK-681-101

Study Sponsor(s): Shire Human Genetic Therapies, Inc. (Shire)

300 Shire Way, Lexington, MA 02421 USA

Author: [redacted]

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STATISTICAL ANALYSIS PLAN SIGNATURE PAGE - AUTHORS

	Name	Signature	Date
Author:	[redacted]	[redacted]	06Feb2020
Position:	[redacted]		
Company:	IQVIA		
Author:	[redacted]		
Position:	[redacted]		
Company:	IQVIA		

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE - APPROVAL

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

	Name	Signature	Date
Approved By:	[redacted]	[redacted]	
Position:	[redacted]		
Company:	Takeda Pharmaceutical Company Limited		
Approved By:	[redacted]		
Position:	[redacted]		
Company:	Takeda Pharmaceutical Company Limited		
Approved By:	[redacted]		
Position:	[redacted]		
Company:	Takeda Pharmaceutical Company Limited		
Approved By:	[redacted]		
Position:	[redacted]		
Company:	Takeda Pharmaceutical Company Limited		

REVISION HISTORY

Version	Issue Date	Summary of Changes
1.0	11 Jun 2019	New Document
2.0	29 Jan 2020	Update based on Protocol Amendments 3 and 4 [redacted] Minor editorial changes and corrections to typographical errors

TABLE OF CONTENTS

ABBREVIATIONS

AE	adverse event
ADA	anti-drug antibody
ATC	anatomical therapeutic chemical
AUC	area under the plasma concentration-time curve
AUC_{extra}	area under the plasma concentration-time curve extrapolated from time t to infinity as a percentage of $AUC_{0-\infty}$
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity
AUC_{0-last}	area under the plasma concentration-time curve from time zero to the last sampling time at which the concentration is at or above LLOQ
$AUC_{o-\tau}$	area under the curve for the defined dose interval tau
BLQ	below limit of quantitation
C_{max}	maximum observed concentration
CL/F	apparent total body clearance
CL_{ss}	apparent total body clearance at steady-state
C_{trough}	observed concentration at the end of 1st, 2nd, 3rd, 4^{th}, and 5^{th} dosing interval (immediately before next dose)
ECG	electrocardiogram
eCRF	electronic case report form
λ_z	first order rate constant, lambda_z
LLOQ	lower limit of quantitation
MAD	multiple ascending dose
NCA	noncompartmental analysis
PCI	potentially clinically important
PD	pharmacodynamic
PK	pharmacokinetic
PT	preferred term
SAD	single ascending dose
SAP	statistical analysis plan
SC	subcutaneous
SD	standard deviation
SOC	system organ class
TEAE	treatment-emergent adverse event
$t_{1/2}$	apparent terminal half-life
t_{last}	time of the last measurable concentration
V_z/F	apparent volume of distribution
V_{ss}	apparent volume of distribution at steady state

1. INTRODUCTION

This statistical analysis plan (SAP) provides a technical and detailed elaboration of the statistical analyses of safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) data as described in the study protocol amendment 4 dated 19 Nov 2019. Specifications for tables, figures, and listings are contained in a separate document.

2. OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

The primary objective of this study is to assess the safety and tolerability of single and multiple, ascending, subcutaneous (SC) doses of TAK-681 (referred as SHP681 in the study protocol) in healthy adult subjects.

2.1.2 Secondary Objective

The secondary objective of this study is to characterize the PK of TAK-681 following single and multiple, ascending SC doses in healthy adult subjects.

2.1.3 Exploratory Objective

[redacted]

2.2 Endpoints

2.2.1 Safety Endpoints

For both the single ascending dose (SAD) and MAD portions, the safety and tolerability of escalating SC doses of TAK-681 will be evaluated by examining for each cohort: number, severity, seriousness and causality of all treatment-emergent adverse events (TEAEs), including injection site and hypersensitivity reactions, changes in vital signs, electrocardiograms (ECGs), abdominal ultrasounds, and clinical laboratory results (hematology, chemistry, and urinalysis) from baseline to post-baseline timepoints, incidence of anti-drug antibodies (ADA) to TAK-681.

2.2.2 Pharmacokinetic Endpoints

Pharmacokinetic parameters, including exposure parameters, will be calculated for individual subjects using plasma TAK-681 concentration-time data and

noncompartmental analysis (NCA) for both SAD and MAD parts. All calculations will be based on actual sampling times. Effect of ADA on PK will be evaluated.

2.2.3 Exploratory Endpoints

[redacted]

3. STUDY DESIGN

3.1 General Description

This study is a randomized, double-blind, placebo-controlled, Phase 1 study to assess the safety and tolerability, and PK of TAK-681 following single and multiple, ascending, SC doses in healthy adult subjects. This study will be conducted at a single center and 5 dose levels are planned for both the SAD and MAD parts of this study. A total of 102 subjects (30 for the SAD portion and 72 subjects for the MAD portion) are planned to be enrolled for this study.

In the SAD, dose escalation will proceed sequentially to assess the following single SC doses of TAK-681: 0.2 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, and 4 mg/kg.

In the MAD, dose escalation for the first 5 cohorts will proceed sequentially to include the following SC doses: 0.2 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, and 4 mg/kg. Each cohort will receive TAK-681 (or placebo) once weekly for 5 weeks.

A 6th MAD cohort will receive 4 mg/kg TAK-681 (or placebo) every 2 weeks over a 6-week period (3 doses). The dose level of the last MAD cohort was determined based on the results of an interim analysis of the first 3 MAD cohorts.

During dose escalation, protocol defined dose levels may be reduced, repeated, discontinued, or an intermediate increase may be added depending on the outcome of the blinded safety data review between dose escalations.

The study design is shown in the study schema in Figure 1 and

Figure 2.

Figure 1 Study Design Flow Chart for the SAD portion

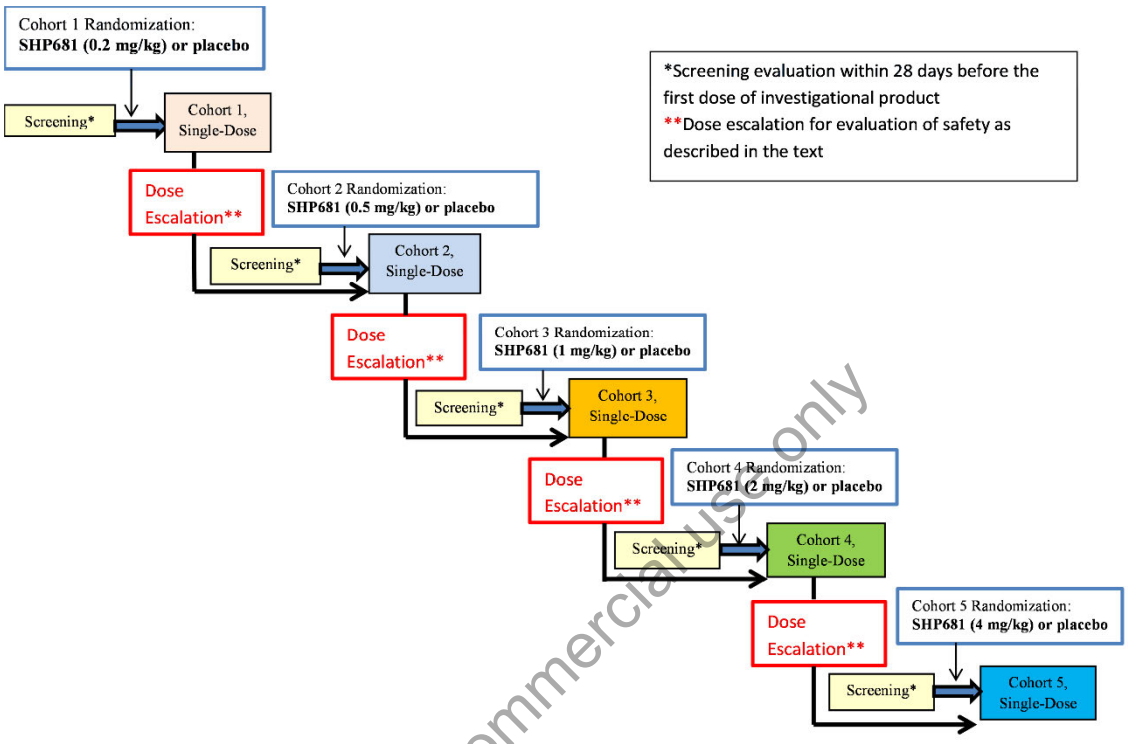
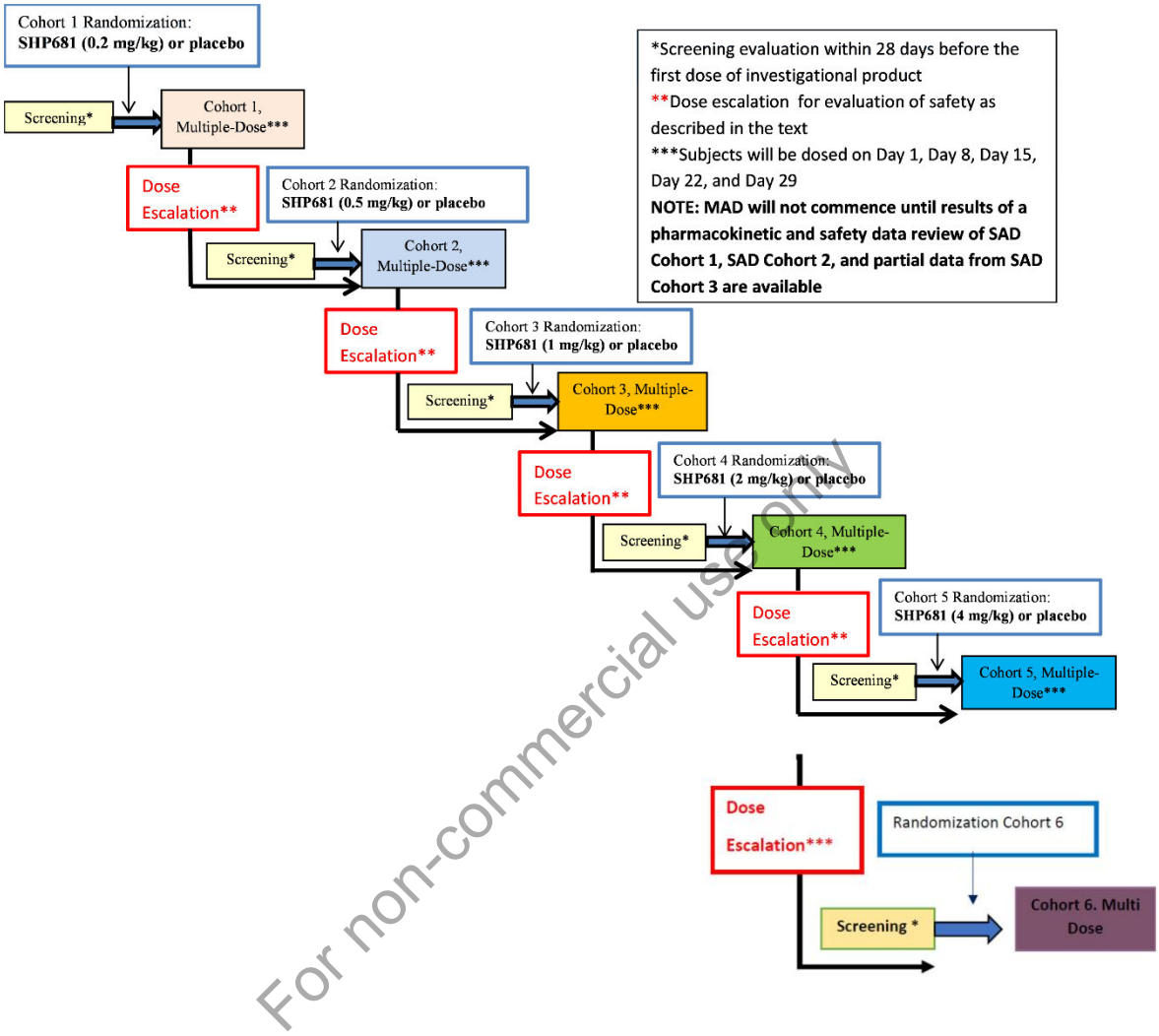


Figure 2 Study Design Flow Chart for the MAD portion



3.2 Randomization

The plan is to enroll in the SAD portion of the study 30 subjects in 5 cohorts. Each cohort will include 6 subjects. Subjects will be randomized within each cohort such that 5 subjects receive TAK-681 and 1 subject receives placebo.

The MAD portion of this study may initiate upon completion of a PK and safety data review of SAD Cohort 1, SAD Cohort 2, and partial data from SAD Cohort 3. The plan is to enroll 72 subjects within 6 MAD cohorts. Each cohort will include 12 subjects. Subjects will be randomized within each cohort such that 10 subjects receive TAK-681 and 2 subjects receive placebo.

3.3 Blinding

This is a randomized, double-blind, placebo-controlled study.

3.4 Sample Size and Power Considerations

SAD (Part 1)

It is planned that dose escalation will proceed for up to 5 dose levels. Each cohort will include 5 subjects treated with TAK-681 and 1 subject treated with placebo for a planned total of 30 subjects. However, if dose levels are repeated, modified, or not conducted or if additional dose levels are studied, the total number of subjects may change. The number of subjects is expected to provide reasonable information on initial testing of TAK-681 for safety and PK while exposing as few subjects as possible to investigational drug materials. The number of subjects in this study is not based on statistical hypothesis testing and power considerations as this is the first in human study. The statistical analyses are primarily descriptive, and no hypothesis testing is specified for this portion.

MAD (Part 2)

It is planned that the MAD portion of the study will include 6 cohorts. Each cohort will include 10 subjects treated with TAK-681 and 2 subjects treated with placebo for a planned total of 72 subjects. The number of subjects is expected to provide reasonable information on initial testing of TAK-681 for safety and PK while exposing as few subjects as possible to investigational drug materials. The number of subjects in this study is not based on statistical power considerations as this is the first in human study. The statistical analyses are primarily descriptive, and no hypothesis testing is specified in the study.

4. STATISTICAL ANALYSIS SETS

Subject populations are separately defined to analyze the data in the SAD portion and the MAD portion of the study.

4.1 Safety Analysis Set

The Safety Analysis Set includes subjects who have received at least 1 dose of TAK-681 or placebo. Analysis will be performed according to the treatment actually received regardless of the randomized treatment.

4.2 Pharmacokinetic Analysis Set

The PK Analysis Set consists of subjects who have received at least 1 dose of TAK-681 and have at least 1 post-dose PK concentration value which was evaluable and interpretable.

Examples of situations that may exclude a subject from the PK analysis set include, but may not be limited to:

- Sample processing errors that lead to inaccurate bioanalytical results
- Inaccurate dosing on the day of PK sampling.

In the case of an important protocol deviation, PK data collected during the affected treatment will be excluded from the study results.

5. STUDY SUBJECTS

5.1 Disposition of Subjects

The number of subjects in the Safety and PK Analysis Sets will be summarized by a pooled placebo group (Safety Analysis Set only), each dose cohort of TAK-681, and TAK-681 overall.

The number and percentage of subjects who completed and prematurely discontinued will be presented for a pooled placebo group, each dose cohort of TAK-681, and TAK-681 overall for the Safety Analysis Set. Reasons for premature discontinuation as recorded on the study completion/termination page of the electronic case report form (eCRF) will be similarly summarized (number and percentage) and listed as well.

5.2 Demographic and Other Baseline Characteristics

Descriptive summaries of demographic and baseline characteristics will be presented by a pooled placebo group, each dose cohort of TAK-681, and TAK-681 overall for the Safety Analysis Set.

The following demographic characteristics will be summarized in the following order in the tables: age (years), sex, ethnicity, race, weight (kg), height (cm), and body mass index (kg/m^2).

5.3 Medical History

Medical history will be collected at the Screening Visit and will be coded using MedDRA Version 22.0 or newer. A listing will be provided using the Safety Analysis Set.

5.4 Prior Medications

Prior medications will be coded using the WHO Drug Dictionary Global (Enhanced w/WHO Herbal Dictionary) Version March2019 or newer.

Prior medication is defined as any medication with the start date and end date prior to the date of the first dose of investigational product.

All prior medications will be listed for the Safety Analysis Set.

5.5 Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary Global (Enhanced w/WHO Herbal Dictionary) Version March2019 or newer.

Concomitant medication is defined as any medication with a start date prior to the date of the first dose of investigational product and continuing after the first dose of investigational product or with a start date on or after the date of the first dose of investigational product.

Concomitant medication usage will be summarized by the number and proportion of subjects receiving each medication by therapeutic class (Anatomical Therapeutic Chemical [ATC] Level 1) and preferred term (PT) for the Safety Analysis Set.

All concomitant medications will be listed for the Safety Analysis Set.

5.6 Exposure to Investigational Product

A listing will be created by subject number and study day giving the date and time of dose administration.

5.7 Measurements of Treatment Compliance

Compliance will be assessed by observation of dosing by the investigator or designee. No listing or summary of compliance will be presented.

5.8 Protocol Deviations

Protocol deviations will be recorded by the site separately from the clinical database. Protocol deviations, including a classification of minor or major, will be listed for the Safety Analysis Set.

6. PHARMACODYNAMIC ANALYSIS

[redacted]

7. SAFETY ANALYSIS

The safety summary will be performed using the Safety Analysis Set. Safety variables include AEs, clinical laboratory variables, vital signs, and ECG variables. For each safety variable, the last value collected before the first dose of investigational product, including unscheduled when applicable, will be used as baseline for all analyses of that safety variable.

All safety analyses will be conducted according to the treatment the subject actually received.

7.1 Adverse Events

Adverse events will be coded using MedDRA Version 22.0 or newer.

An AE (classified by PT) that occurs during the Double-blind Evaluation Phase will be considered a TEAE if it has a start date on or after the first dose of double-blind investigational product or placebo.

An overall summary of the number of subjects with TEAEs as well as the number of events will be presented, including the number and percentage of subjects with any TEAEs, serious TEAEs, TEAEs related to investigational product and TEAEs leading to discontinuation.

The number and percentage of subjects reporting TEAEs, as well as the number of events (where applicable), in each treatment group and TAK-681 overall will be tabulated by system organ class (SOC) and PT; by SOC, PT, and severity. TEAEs considered related to investigational product will also be summarized by SOC and PT. If more than 1 AE occurs with the same PT and the same severity for the same subject, then the subject will be counted only once for that PT and that severity for the summarization by severity. Presentation by SOC and PT will present SOC sorted alphabetically and PT within SOC by descending incidence.

Serious TEAEs, TEAEs leading to discontinuation of investigational product will be summarized by SOC, PT, and treatment group and listed. Serious TEAEs leading to death will be listed.

7.1.1 Adverse Events of Special Interest

Separate summaries will be provided for the TEAEs of injection site reactions as identified in the eCRF.

7.2 Clinical Laboratory Data

Descriptive statistics for clinical laboratory values and changes from baseline at each assessment timepoint as well as shift tables from baseline to each visit for quantitative variables will be presented by treatment group for the following clinical laboratory variables:

Hematology Hemoglobin, hematocrit, red blood cells, platelet count, white blood cell count – total and differential, total neutrophils (absolute), eosinophils (absolute), monocytes (absolute), basophils (absolute), lymphocytes (absolute)

Biochemistry Sodium, potassium, glucose, blood urea nitrogen, creatinine, amylase, calcium, chloride, thyroid stimulating hormone, uric acid, phosphorus, total protein, total CO_2 (bicarbonate), albumin, aspartate transaminase, lipase, alanine transaminase, gamma glutamyl transferase, alkaline phosphatase, total bilirubin

Urinalysis pH, glucose, protein, blood, ketones, bilirubin, nitrites, leukocyte esterase, specific gravity, erythrocytes, leukocytes, casts, bacteria

Clinical laboratory test values/changes are potentially clinically important (PCI) if they meet either the low or high PCI criteria listed in Appendix 15.2 Table 1. The number and percentage of subjects with post-baseline PCI values will be tabulated by treatment group. The percentages will be calculated relative to the number of subjects with available baseline values and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 post-baseline PCI value. A supportive listing of subjects with PCI values will be provided.

All laboratory data will be listed for the Safety Analysis Set.

7.3 Vital Signs

Descriptive statistics for vital signs (systolic and diastolic blood pressure, pulse rate, temperature, and body weight) and their changes from baseline at each post-baseline visit and at the end of study will be presented by treatment group.

Vital sign values will be considered PCI if they meet both the observed value criteria and the change from baseline criteria listed in Appendix 15.2 Table 2. The number and percentage of subjects with PCI post-baseline values will be tabulated by treatment group. The percentages will be calculated relative to the number of subjects with baseline and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 PCI post-baseline vital sign value. A supportive listing of subjects with PCI values will be provided.

All vital signs data will be listed for the Safety Analysis Set.

7.4 Electrocardiogram

The average (for continuous data) or worst assessment (for interpretation) from the triplicate ECG measurements collected at each nominal timepoint will be used for analysis. Descriptive statistics for ECG variables (heart rate, PR, RR, QRS, and QT intervals) and their changes from baseline at each assessment timepoint will be presented by treatment group. QTc interval will be calculated using both Bazett ($QTcB=QT/(RR)^{1/2}$) and Fridericia ($QTcF=QT/(RR)^{1/3}$) corrections; and if RR is not available, QTc will not be calculated. Electrocardiogram interpretation will be summarized by visit. A shift table from baseline to each visit for qualitative ECG results will be presented.

Electrocardiogram variable values will be considered PCI if they meet or exceed the upper limit values listed in Appendix 15.2 Table 3. The number and percentage of subjects with post-baseline PCI values will be tabulated by treatment group. The percentages will be calculated relative to the number of subjects with available non-PCI baseline and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 PCI post-baseline ECG value. A listing of all subjects with PCI value will be provided.

All ECG data will be listed for the Safety Analysis Set.

7.5 Immunogenicity

SAD (Part1)

Blood draws (6 ml in K2EDTA tubes for each applicable timepoint) for determination of ADAs will be collected prior to dose on Day 1 and at 672 hours post-dose. Subjects with detectable ADAs in their plasma might require additional visits and blood collection for follow-up assessments of antibody titers up to a year post-dose.

MAD (Part 2)

Blood draws for determination of ADAs will be collected (6 mL in K2EDTA tubes for each applicable timepoint) prior to each dose, 7 days after the last dose (Day 36), and 28 days after the last dose (Day 57) in each cohort. Subjects with detectable ADAs in their plasma on Day 57 might require additional visits and blood collection for follow-up assessments of antibody titers up to a year post-dose.

Immunogenicity in each dose cohort will be analyzed by number and percent of subjects testing positive for ADA by predose and post-dose study visits. Overall, a subject will be categorized as ADA positive if at least one confirmatory positive result at any timepoint is reported, otherwise ADA negative if all results negative.

Immunogenicity data will be listed for the Safety Analysis Set.

7.6 Ultrasound

The results of abdominal ultrasound tests for MAD Cohort 6 will be listed.

8. PHARMACOKINETIC ANALYSIS

The PK analysis will be based on the PK analysis set (Section 4.2).

Pharmacokinetic parameters will be calculated from plasma TAK-681 concentration-time data using noncompartmental methods and all calculations will be based on actual sampling times.

The PK analysis of parameter estimates will be conducted by a contract research organization for the Clinical Pharmacology and Pharmacokinetics Department of Shire using Phoenix WinNonlin Version 8.0 or higher (Certara, L.P., Princeton, New Jersey, USA).

8.1 Drug Concentration

The plasma sample analysis for specified analyte concentrations will be performed according to the relevant Standard Operating Procedures at the contract bioanalytical lab. Plasma concentrations will be measured using the most current validated bioanalytical method.

In addition, selected plasma samples may be used to investigate incurred sample reproducibility (full details will be described in the bioanalytical study plan). The presence of other metabolites or artifacts may be monitored or quantified as appropriate.

All TAK-681 PK concentration data will be summarized by part, cohort, and/or scheduled timepoint, as appropriate. Repeated and unscheduled measurements are included in the listings but not used for statistical analysis or summary tables, unless the repeated measurement was performed due to unreliable values/technical reasons, e.g., clotted samples.

8.2 Single Ascending Dose

The PK analysis will be based on the PK analysis set for Part 1 SAD.

Individual subject's raw concentrations of TAK-681 and actual sampling time will be listed by nominal sampling timepoint and summarized by dose cohort with descriptive statistics such as number of subjects, arithmetic mean, standard deviation (SD), coefficient of variation, minimum, median, maximum, geometric mean, and geometric coefficient of variation.

Individual subject's concentration-time curves will be presented in linear/linear and log/linear scale using lattice plots, with one panel for each subject grouped by dose cohort. Figures showing the mean (with ±SD as error bar) as well as the median (with 25th to 75th quantiles as error bar) concentration time profiles will be presented in linear/linear and log/linear scale using lattice plots, with one panel for each dose cohort. Mean (±SD) concentrations after first dose and just prior to next dose (C_{trough}) will be summarized by each cohort and presented in a single figure containing all cohorts (without SD) for a visual assessment of attainment of steady-state.

8.3 Multiple Ascending Dose

The PK analysis will be based on the PK analysis set for Part 2 MAD.

Individual raw concentration data and actual sampling time will be listed by nominal sampling timepoint.

Raw concentrations for each nominal sampling timepoint for post first dose, between first and last dose, and post last dose will be summarized by dose cohort with descriptive statistics such as number of subjects, arithmetic mean, SD, coefficient of variation, minimum, median, maximum, geometric mean, and geometric coefficient of variation.

Individual subject's Day 1 concentration-time profile up to 24 hours after the first dose will be visualized with correspondingly superimposed concentration-time profile up to 24 hours following the last dose in linear/linear and log/linear scale using lattice plots, with one panel for each subject grouped by dose cohort.

Mean (±SD) concentrations after first dose and just prior to next dose (C_{trough}) will be summarized by each cohort and presented in a single figure containing all cohorts (without SD) for a visual assessment of attainment of steady-state.

Individual concentration-time curves following the last dose will be presented in linear/linear and log/linear scale using lattice plots, with one panel for each subject grouped by dose cohort. Mean (with ±SD as error bar) as well as the median (with 25th to 75th quantiles as error bar) concentration time profiles will be presented in linear/linear and log/linear scale using lattice plots, with one panel for each dose cohort. Mean and median concentration time profiles for all dose cohorts will be superimposed in one plot.

8.4 Handling Below Limit of Quantitation (BLQ) Values

Values below the lower limit of quantitation (LLOQ) will be replaced as zero for descriptive statistics of PK concentrations.

For the PK analysis, predose sample concentrations that are BLQ will be assigned a numerical value of zero for the calculation of area under the plasma concentration-time curve (AUC). Samples not collected will be identified as missing. Any anomalous concentration values observed at predose will be identified in the study report and used for the computation of PK parameters, even if the anomalous value is greater than 5% of the maximum observed concentration (C_{max}). Plasma concentrations of BLQ before the last quantifiable data point will be taken as zero for calculating the AUC (i.e., embedded BLQ values will be set to zero). Plasma concentrations of BLQ after the last quantifiable data point will be set to 'zero' and will not be considered for the determination of lambda_z (λ_z).

8.5 Pharmacokinetic Parameters

The PK analysis will be based on the PK analysis set for each study part.

Pharmacokinetic parameters will be evaluated and listed for all volunteers who provide sufficient concentration time data. Noncompartmental computation of PK parameters will be performed.

Individual PK parameters will be calculated using unrounded actual sampling times (or using scheduled time if actual time is not available). The predose sample will be considered as if it had been taken simultaneously with the administration of study drug.

Pharmacokinetic parameters will be calculated from plasma TAK-681 concentration-time data based on actual sampling times. Pharmacokinetic parameters will include the following:

Part 1 SAD:

$AUC_{0-\infty}$	Area under the concentration-time curve in serum from time zero (predose) extrapolated to infinity, calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration (C_t) divided by the terminal rate constant, λ_z [ie, $AUC_{0-last} + C_t/\lambda_z$].
AUC_{0-last}	Area under the plasma concentration-time curve from time zero to the last sampling time at which the concentration is at or above LLOQ, calculated according to the mixed log linear trapezoidal rule (i.e., linear up/log down).
AUC_{extra}	AUC extrapolated from time t_{last} to infinity obtained by extrapolation (%). Equation: $(1 - [AUC_{0-last} / AUC_{0-\infty}]) \times 100$.
C_{max}	Maximum observed concentration obtained directly from the concentration-time profile after a single dose.
$C_{avg,0-24}$	Average concentration from time zero to 24 hours postdose
CL/F	Apparent total body clearance for extravascular

	administration divided by the fraction of dose absorbed calculated as dose divided by $AUC_{0-\infty}$.
λ_z	First order rate constant, lambda_z, associated with the terminal (log-linear) portion of the terminal data points of the curve. A minimum of 3 points will be included in the regression analysis.
$t_{1/2}$	Apparent terminal half-life calculated as $\ln 2/\lambda_z$.
t_{last}	Time of the last measurable concentration.
t_{max}	Minimum time to reach the C_{max} concentration after a single dose.
V_z/F	Apparent volume of distribution following extravascular administration divided by the fraction of dose absorbed calculated as CL/F divided by λ_z.

Part 2 MAD (Post first dose):

$C_{avg,0-24}$	Average concentration from time zero to 24 hours postdose

MAD (Between first dose and last dose:

C_{trough}	Observed concentration at the end of each dosing interval (immediately before next dose for the first 5 cohorts and immediately before 2nd and 3rd dose of the 6th MAD cohort)

Part 2 MAD (Post last dose):

$AUC_{0-\infty}$	Area under the concentration-time curve in serum from time zero (predose) extrapolated to infinity, calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration (C_t) divided by the terminal rate constant, λ_z [i.e., $AUC_{0-last} + C_t/\lambda_z$].
$C_{avg,0-24}$	Average concentration from time zero to 24 hours postdose

$AUC_{0\text{-last}}$	Area under the plasma concentration-time curve from time zero (for the last dose) to t_{last}, calculated according to the mixed log linear trapezoidal rule (i.e., linear up/log down).
$AUC_{0\text{-tau}}$	Area under the concentration versus time curve over a dosing interval (tau). Only calculated if interpretable i.e., last sample timepoint for dose interval is present, and sample at t_{max} is present.
C_{max}	Maximum observed concentration obtained directly from the concentration-time profile.
CL/F	Apparent total body clearance for extravascular administration divided by the fraction of dose absorbed calculated as dose divided by $AUC_{0\text{-tau}}$.
λ_z	First order rate constant, lambda_z, associated with the terminal (log-linear) portion of the terminal data points of the curve. A minimum of 3 points will be included in the regression analysis.
$t_{1/2}$	Apparent terminal half-life calculated as $\ln 2/\lambda_z$.
t_{last}	Time of the last measurable concentration.
t_{max}	Minimum time to reach the C_{max} concentration.
V_z/F	Apparent volume of distribution following extravascular administration divided by the fraction of dose absorbed, calculated as CL/F divided by λ_z.

No dose adjustment will be made for parameters that require dose for calculation.

The following PK parameters will be calculated for diagnostic purposes and provided in a separate table for each study part, but will not be summarized:

- The time interval (h) of the log-linear regression to determine λ_z ($t_{½}$, Interval).
- Number of data points ($t_{½}$, N) included in the log-linear regression analysis to determine λ_z.
- Goodness-of-fit statistic (Rsq_adj) for calculation of λ_z, if <0.800 then parameters using λ_z will be flagged for exclusion in summary tables.

- If AUC_{extra} is > 20.0% then $AUC_{0-\infty}$ will be flagged for exclusion in summary tables.

Any anomalous concentration values observed at predose will be identified. Predose TAK-681 plasma concentrations that are greater than 5% of the maximum concentration of the individual's profile will exclude that individual (concentrations and PK parameters) from the PK analysis set.

Predose TAK-681 plasma concentrations below the level of quantitation (BLQ) and BLQ plasma values prior to quantifiable concentrations will be set to zero for both descriptive statistics estimations and PK concentration tables. If the actual time is missing (i.e. not recorded) then the scheduled sampling time will be used for the estimation of the PK parameters, unless otherwise warranted by the data.

Individual subject's PK parameters will be listed by part, dose cohort, and applicable dose (Part 2, MAD). Diagnostic parameters such as the number of timepoints of the terminal log-linear phase used to estimate the terminal rate constant and percentage of extrapolated AUC will be listed similarly.

PK parameters will be summarized by part, dose cohort, and applicable dose (Part 2, MAD) with descriptive statistics such as number of subjects, arithmetic mean, SD, coefficient of variation, minimum, median, maximum, geometric mean, and geometric coefficient of variation. The parameters, t_{max} and t_{last}, will be summarized by median, minimum, and maximum grouped by dose cohort.

Pharmacokinetic parameters of $AUC_{0-\infty}$, AUC_{0-tau}, AUC_{0-24}, and C_{max} will be presented in box plots by part when appropriate for each dose cohort.

8.6 Statistical Analysis of Pharmacokinetic Data

The statistical analyses are primarily descriptive, and no hypothesis testing is specified for this portion.

8.7 Assessment of Immunogenicity on Pharmacokinetic Parameters

Subjects that test positive for immunogenicity markers ADA will have PK parameters summarized by part and cohort using descriptive statistics. Only subjects with both immunogenicity and PK parameter data will be included.

9. EXPLORATORY PHARMACODYNAMIC ANALYSES

[redacted]

10. INTERIM ANALYSIS/DATA MONITORING COMMITTEE

To support an initiation of the MAD phase and confirm the dosing interval, an interim analysis will be required. Blinded plasma concentrations at nominal collection times obtained after dosing in Cohort 1, Cohort 2, and part of Cohort 3 in the SAD portion will be evaluated and justified for this purpose. To support additional development decisions, blinded plasma concentrations at nominal collection times obtained after SAD, and 3 cohort MAD portions will be evaluated and justified for this purpose. All of this work will be done separately from the final NCA analyses and reported separately.

An interim analysis will be performed after the last patient from the 3rd MAD cohort has completed the Day 39 visit, to determine the dose level of the 6th MAD cohort, and to assess any safety, tolerability, or PK questions based on the safety review. The data for this analysis will be source data verified and cleaned according to the Interim Snapshot Plan. This analysis will unblind all completed SAD cohorts and the first 3 MAD cohorts

for the Sponsor and external statistical groups. MAD cohorts 4, 5 and 6 will not be unblinded to any parties due to this interim analysis.

No adaptive design or data monitoring committee is planned, all applicable data will be reviewed by the Clinician and Clinical PK (Sponsor).

11. DATA HANDLING CONVENTIONS

11.1 General Data Reporting Conventions

Continuous variables will be summarized using the following descriptive statistics: the number of subjects (n), mean, median, SD, minimum, and maximum. Categorical and count variables will be summarized by the number of subjects and the percent of subjects in each category. Additional summary statistics will be provided for PK endpoints and are indicated in Section 8.

11.2 Definition of Baseline

Baseline is defined as the last non-missing value observed prior to dosing with TAK-681 or placebo.

11.3 Repeated or Unscheduled Assessments of Safety Parameters

If a subject has repeated assessments before the start of investigational product, then the results from the final assessment made prior to the start of investigational product will be used as baseline. If end-of-study assessments are repeated or unscheduled, the last post-baseline assessment will be used as the end of study assessment for generating descriptive statistics. However, all post-baseline assessments will be used for PCI value determination and all assessments will be presented in the data listings.

11.4 Handling of Missing, Unused, and Spurious Data

Missing safety data will not be imputed.

Missing PK concentrations will be handled as described in Section 8.4.

11.4.1 Missing Date of Investigational Product

When the date of the last dose of investigational product is missing for a subject in the Safety Analysis Set, all efforts should be made to obtain the date from the investigator.

11.4.2 Missing Date Information for Prior or Concomitant Medications (Therapies/Procedures)

For prior or concomitant medications, incomplete (i.e., partially missing) start date and/or stop date will be imputed. When the start date and the stop date are both incomplete for a subject, impute the start date first.

11.4.2.1 Incomplete Start Date

The following rules will be applied to impute the missing numerical fields. If the stop date is complete and the imputed start date is after the stop date, then the start date will be imputed using the stop date.

11.4.2.1.1 Missing Day and Month

- If the year of the incomplete start date is the same as the year of the date of the first dose of investigational product, then the day and month of the date of the first dose of investigational product will be assigned to the missing fields
- If the year of the incomplete start date is before the year of the date of the first dose of investigational product, then December 31 will be assigned to the missing fields
- If the year of the incomplete start date is after the year of the date of the first dose of investigational product, then 01 January will be assigned to the missing fields.

11.4.2.1.2 Missing Month Only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

11.4.2.1.3 Missing Day Only

- If the month and year of the incomplete start date are the same as the month and year of the date of the first dose of investigational product, then the day of the date of the first dose of investigational product will be assigned to the missing day
- If either the year is before the year of the date of the first dose of investigational product or if both years are the same but the month is before the month of the date of the first dose of investigational product, then the last day of the month will be assigned to the missing day
- If either the year is after the year of the date of the first dose of investigational product or if both years are the same but the month is after the month of the date of the first dose of investigational product, then the first day of the month will be assigned to the missing day.

11.4.2.2 Incomplete Stop Date

The following rules will be applied to impute the missing numerical fields. If the date of the last dose of investigational product is missing, then replace it with the last visit date.

If the imputed stop date is before the start date (imputed or non-imputed start date), then the imputed stop date will be equal to the start date.

11.4.2.2.1 Missing Day and Month

- If the year of the incomplete stop date is the same as the year as of the date of the last dose of investigational product, then the day and month of the date of the last dose of investigational product will be assigned to the missing fields
- If the year of the incomplete stop date is before the year of the date of the last dose of investigational product, then 31 December will be assigned to the missing fields
- If the year of the incomplete stop date is after the year of the date of the last dose of investigational product, then 01 January will be assigned to the missing fields.

11.4.2.2.2 Missing Month Only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

11.4.2.2.3 Missing Day Only

- If the month and year of the incomplete stop date are the same as the month and year of the date of the last dose of investigational product, then the day of the date of the last dose of investigational product will be assigned to the missing day
- If either the year is before the year of the date of the last dose of investigational product or if both years are the same but the month is before the month of the date of the last dose of investigational product, then the last day of the month will be assigned to the missing day
- If either the year is after the year of the last dose of investigational product or if both years are the same but the month is after the month of the date of the last dose of investigational product, then the first day of the month will be assigned to the missing day.

11.4.3 Missing Date Information for Adverse Events

For AEs with partial start dates, non-missing date parts will be used to determine if the AE is treatment-emergent or not. If a determination cannot be made using the non-missing date parts as to when the AE occurred relative to study drug administration, e.g., AE start year and month are the same as the year and month of the first dose of investigational product, then the AE will be classified as treatment-emergent.

To facilitate categorization of AEs as treatment emergent, imputation of dates can be used. For AEs, the default is to only impute incomplete (i.e., partially missing) start dates. Incomplete stop dates may also be imputed when calculation of the duration of an AE is

required per the protocol. If imputation of an incomplete stop date is required, and both the start date and the stop date are incomplete for a subject, impute the start date first.

11.4.3.1 Incomplete Start Date

[Follow the same rules as in Section 11.4.2.1]

11.4.3.2 Incomplete Stop Date

[Follow the same rules as in Section 11.4.2.2]

11.4.4 Missing Severity Assessment for Adverse Events

If severity is missing for an AE starting prior to the date of the first dose of investigational product, then a severity of "Mild" will be assigned. If the severity is missing for an AE starting on or after the date of the first dose of investigational product, then a severity of "Severe" will be assigned. The imputed values for severity assessment will be used for incidence summaries, while both the actual and the imputed values will be used in data listings.

11.4.5 Missing Relationship to Investigational Product for Adverse Events

If the relationship to investigational product is missing for an AE starting on or after the date of the first dose of investigational product, a causality of "Related" will be assigned. The imputed values for relationship to double-blind investigational product will be used for incidence summaries, while both the actual and the imputed values will be presented in data listings.

11.4.6 Character Values of Clinical Laboratory Variables

If the reported value of a clinical laboratory variable cannot be used in a statistical analysis due to, for example, that a character string is reported for a numerical variable. The appropriately determined coded value, upon discussion and agreement with the study physician, will be used in the statistical analysis. However, the actual values as reported in the database will be presented in data listings.

12. ANALYSIS SOFTWARE

Statistical analyses will be performed using Version 9.4 (or newer) of SAS® on a suitably qualified environment.

The PK analysis will be conducted using Phoenix WinNonlin Version 8.0 or higher (Certara, L.P., Princeton, New Jersey, USA).

13. CHANGES TO ANALYSIS SPECIFIED IN PROTOCOL

. Pharmacokinetic parameter $C_{avg,0-24}$ was added to the following study portions: SAD, MAD post first dose, and MAD post last dose. MAD post first dose parameters of AUC_{0-24}, $C_{max,\ 24}$ and $t_{max,\ 24}$ were removed. Interim PK analysis results showed the MAD post first dose sampling scheme was insufficient to accurately characterize these parameters.

Immunogenicity data for nADA will not be presented as the analysis was not performed for nADA.

14. REFERENCES

There are no references.

15. APPENDICES

15.1 Schedule of Activities

Refer to Tables 1-7 in the protocol.

15.2 Potential Clinical Importance (PCI) Criteria for Laboratory, Vital Signs and ECGs in Individual Healthy Subjects

Table 1

POTENTIALLY CLINICALLY IMPORTANT CHANGES IN LABORATORY TEST RESULTS FOR HEALTHY VOLUNTEERS		
Category Variable	Classification (on CDRs)	Criteria: SI Units (Conventional Units)
Hematology		
RBC count	HIGH	>7.5 $x10^{12}$/L
RBC count	LOW	<3 $x10^{12}$/L
Hematocrit	LOW and DECREASE	≤0.6 x LLN and Decrease of ≥46.0% from baseline value
Hematocrit	HIGH	>1.3 x ULN
Hemoglobin	LOW and DECREASE	< 100 g/L (10g/dL) and Decrease of ≥ 20 g/L (2.0 g/dL) from baseline value
Hemoglobin	HIGH	>200 g/L (20g/dL)
MCH	HIGH	> 34 pg/cell
MCH	LOW	< 26 pg/cell
MCHC	HIGH	> 37 g/dL (370 g/L)
MCHC	LOW	< 31 g/dL (310 g/L)
MCV	HIGH	> 100μm^3 (100 fL)
MCV	LOW	< 80μm^3 (80 fL)
WBC		
WBC count	HIGH	>2xULN OR >16.0 x 10^9/L (16 x $10^3/\mu L$)
WBC count	LOW	< 0.5xLLN OR < 3.0 x 10^9/L (3 x $10^3/\mu L$)
Neutrophils	LOW	< 1.5 x 10^9/L (1.5 x 10^3 µL) OR < 40%
Neutrophils	HIGH	> 6.2 x 10^9/L (6.2 x 10^3 µL) OR > 70 %
Lymphocytes	HIGH	> 4.0 x 10^9/L (4.0 x 10^3 µL) OR > 44 %
Lymphocytes	LOW	< 0.8 x 10^9/L (0.8 x 10^3 µL) OR < 22 %
Monocytes	HIGH	>1.1 x 10^9/L (1.1 x 10^3 µL) OR >11 %
Eosinophils	HIGH	> 0.5 x 10^9/L (> 500/µL) and > 10.0%
Eosinophils	LOW	NA
Basophils	HIGH	>0.2 x 10^9/L (0.2 x 10^3 µL) OR > 2%
Basophils	LOW	NA

Coagulation		
Platelet count (thrombocytes)	HIGH	>1.5 x ULN OR > 500 x 10^9/L (100 x 10^3/μL)
Platelet count (thrombocytes)	LOW	0.6 x LLN OR < 100 x 10^9/L (100 x 10^3/μL)
INR	HIGH	>1.2 x ULN
Prothrombin time (PT)	HIGH	> 1.2 x ULN
aPTT	HIGH	> 1.2 x ULN
Blood Chemistry		
Sodium	HIGH	> 5 mmol/L (5 mEq/L) above ULN
	LOW	> 5 mmol/L (5 mEq/L) below LLN
Potassium	HIGH + INCREASE	Above ULN and increase of > 0.5 mmol/L (0.5 mEq/L) from baseline value
	LOW + DECREASE	Below LLN and decrease of > 0.5 mmol/L (0.5 mEq/L) from baseline value
Creatinine	HIGH + INCREASE	> 150μmol/L and increase > 30% from baseline value
BUN	HIGH	> 1.5 x ULN
Glucose (fasting)	HIGH	≥ 6.7 mmol/L
	LOW	≤ 4.2 mmol/L
Calcium	HIGH and INCREASE	Above ULN and Increase of ≥ 0.25 mmol/L (1.0 mg/dL) from baseline value
	LOW and DECREASE	Below LLN and Decrease of ≥ 0.25 mmol/L (1.0 mg/dL) from baseline value
Magnesium	HIGH and INCREASE	Above ULN and Increase of ≥ 0.21 mmol/L (0.5 mg/dL) from baseline value
	LOW and DECREASE	Below LLN and Decrease of ≥ 0.21 mmol/L (0.5 mg/dL) from baseline value
Phosphorus	HIGH	> 0.162 mmol/L (0.5 mg/dL) above ULN
	LOW	> 0.162 mmol/L (0.5 mg/dL) below LLN
Total protein	HIGH and INCREASE	Above ULN and Increase of ≥ 20 g/L (2.0 g/dL) from baseline value
	LOW and DECREASE	Below LLN and Decrease of ≥ 20 g/L (2.0 g/dL) from baseline value
Albumin	HIGH and INCREASE	Above ULN and Increase of ≥ 10 g/L (1.0 g/dL) from baseline value
	LOW and DECREASE	Below LLN and Decrease of ≥ 10 g/L (1.0 g/dL) from baseline value
Uric acid (with normal diet)	HIGH and INCREASE	Above ULN and Increase of > 0.119 mmol/L (2.0 mg/dL) from baseline value
	LOW and DECREASE	Below LLN and Decrease of > 0.119 mmol/L (2.0 mg/dL) from baseline value
Creatinine (CK)	HIGH	> 1.5 x ULN
Total Cholesterol (fasting)	HIGH	≥ 6.18 mmol/L (240 mg/dL)
Triglycerides (fasting)	HIGH	≥ 1.8 mmol/L (160 mg/dL)

Liver Enzymes Tests / Liver Function Tests (LFTs)		
ALT/SGPT	HIGH	> 2 x ULN
AST/SGOT	HIGH	> 2 x ULN
ALP	HIGH	> 1.5 x ULN
GGT	HIGH	> 1.5 x ULN
LDH (total LDH)	HIGH	> 1.5 x ULN
Total bilirubin	HIGH	> 1.5 x ULN
Thyroid Panel		
T4	HIGH	> 140.28 nmol/L
	LOW	< 57.92 nmol/L
T3	HIGH	> 2.765 nmol/L
	LOW	< 0.922 nmol/L
TSH	HIGH	> 5.0 µIU/mL
	LOW	< 0.5 µIU/mL
URINALYSIS		
Glucose	HIGH	≥ 1+
Blood	HIGH	≥ 2+
Bilirubine	HIGH	Positive
Protein	HIGH	≥ 2+
Nitrite	HIGH	Positive
Ketones	HIGH	≥ 2+
RBC	HIGH	≥ 2 / high power field
WBC	HIGH	≥ 2 / high power field
Leukocyte Esterase	HIGH	Positive

Abbreviations: AST/SGOT = Aspartate aminotransferase/serum glutamic oxaloacetic transaminase; ALT/SGPT = alanine aminotransferase/serum glutamic pyruvic transaminase; ALP= Alkaline phosphatase; BUN = blood urea nitrogen; CK = creatine kinase; GGT = γ-glutamyl-transferase; LDH = lactic dehydrogenase; LFT = liver function test; RBC = red blood cells / erythrocytes; WBC=white blood cells, leucocytes;
MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; MCV=Mean Corpuscular Volume.
INR= International Normalized Ratio; aPTT = Activated Partial Thromboplastin time; T4= Thyroxine; T3= Triiodothyronine; TSH=Thyroid-stimulating hormone

Reference: Laboratory Reference Values. New England Journal of Medicine 2004; 351: 1548-1563

Table 2

POTENTIALLY CLINICALLY IMPORTANT CHANGES IN VITAL SIGNS AND BODY WEIGHT		
Variable	Classification (on CDRs)	Criteria
Sitting and Supine		
Systolic BP (mm Hg)	HIGH and INCREASE	≥ 140 and increase of ≥ 20 from baseline value
	LOW and DECREASE	≤ 90 and decrease of ≥ 20 from baseline value
Diastolic BP (mm Hg)	HIGH and INCREASE	≥ 90 and increase of ≥ 15 from baseline value
	LOW and DECREASE	≤ 50 and decrease of ≥ 15 from baseline value
Pulse Rate (bpm)	HIGH and INCREASE	≥ 100 and increase of > 15 from baseline value
	LOW and DECREASE	≤ 45 and decrease of ≥ 15 from baseline value
Orthostatic (Supine to Standing)		
Systolic BP (mm Hg)	DECREASE	Decrease of ≥ 20 from supine value
Diastolic BP (mm Hg)	DECREASE	Decrease of ≥ 20 from supine value
Pulse Rate (bpm)	INCREASE	Increase of ≥ 30 from supine value
Temperature (regardless of method)	HIGH	> 38.3°C or > 100.9°F
	LOW	< 35°C or < 95°F
Respiratory rate (breaths/min)	HIGH	> 25
	LOW	< 10
Weight	HIGH	Increase of ≥ 5% from baseline value
	LOW	Decrease of ≥ 5% from baseline value

Abbreviations: BP = blood pressure: bpm = beats per minute

Table 3

POTENTIALLY CLINICALLY IMPORTANT CHANGES IN ELECTROCARDIOGRAMS		
Variable	Classification (on CDRs)	Criteria
Overall Evaluation	ABNORMAL	Overall Evaluation is ABNORMAL
Rhythm	NON-SINUS RHYTHM	Rhythm is not SINUS
Heart rate (bpm)	HIGH and INCREASE	≥ 100 and increase of > 15 from baseline value
	LOW and DECREASE	≤ 45 and decrease of > 15 from baseline value
PR interval (msec)	HIGH and INCREASE	≥ 200 and increase of ≥ 20 from baseline value
QRS interval (msec)	HIGH	≥ 120
QTc interval (men) (msec)*	HIGH	> 430 and increase from baseline value > 30
QTc interval (women) (msec)*	HIGH	> 450 and increase from baseline value > 30

Abbreviations: BP = blood pressure; bpm = beats per minute; QTc- QT interval corrected.
*Values noted refer to both Bazett's (QTcB) and Fridericia's (QTcF) formulas.

- QTc intervals < 330 ms in males or < 340 ms in females should be considered diagnostic of SQTS
- QTc intervals < 360 ms in males or < 370 ms in females should only be considered diagnostic of SQTS when supported by symptoms or family history