Statistical Analysis Plan

NCT Number: NCT05526391

Title: A Randomized, Double-blind, Placebo-Controlled, Phase 2 Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous TAK-341 in Subjects With Multiple System Atrophy

Study Number: TAK-341-2001

Document Version and Date: Version 4, 05 Aug 2025

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

STATISTICAL ANALYSIS PLAN

STUDY NUMBER: TAK-341-2001

A Randomized, Double-blind, Placebo-Controlled, Phase 2 Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous TAK-341 in Subjects With Multiple System Atrophy

Phase 2 Study of TAK-341 for Multiple System Atrophy

PHASE 2

Version: 4

Date: 5 August 2025

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Based on:

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3.0 LIST OF ABBREVIATIONS

αSYN	α-synuclein
ADA	antidrug antibody
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUCτ	area under the concentration-time curve during a dosing interval
BLQ	below the limit of quantification
BMI	body mass index
BP	blood pressure
[redacted]	[redacted]
CGI-S	Clinician Global Impression of Severity
CI	confidence interval
Cmax	maximum observed plasma concentration
CSF	cerebrospinal fluid
C-SSRS	Columbia - Suicide Severity Rating Scale
Ctrough	trough plasma concentration at steady state (observed concentration at the end of a dosing interval)
DAMP	Data Access Management Plan
DBP	diastolic blood pressure
ECG	Electrocardiogram
eCRF	Electronic case report form
GGT	g-glutamyl transferase
HR	heart rate
IA	interim analysis
ITT	intent-to-treat
IV	intravenous
LLN	lower limit of normal
MAR	missing at random
MAV	markedly abnormal value
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance image, magnetic resonance imaging
MSA	multiple system atrophy
[redacted]	[redacted]
N	number of subjects

[redacted]	[redacted]
PD	pharmacodynamics
PK	pharmacokinetic(s)
PT	preferred term
Q4W	every 4 weeks
[redacted]	[redacted]
SBP	Systolic blood pressure
SCOPA-AUT	Scales for Outcomes in Parkinson's Disease - Autonomic Dysfunction
SD	standard deviation
SE	standard error
SOC	system organ class
TEAE	treatment-emergent adverse event
t_{max}	time to reach C_{max}
TS	total score
ULN	upper limit of normal
UMSARS	Unified Multiple System Atrophy Rating Scale

4.0 OBJECTIVES

4.1 Primary Objective

To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on Unified Multiple System Atrophy Rating Scale (UMSARS) Part I, minus the sexual function item, with collapse of the normal and mild ratings on each item.

4.2 Secondary Objectives

- To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on the 11-item UMSARS specified by (Palma et al. 2021).
- To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on the total UMSARS (UMSARS Part I + Part II).
- To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on the Part I UMSARS minus the sexual function item (without collapse of ratings of scale items).
- To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on the Part II UMSARS.
- To evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on the Clinical Global Impression-Severity (CGI-S) scale.
- To evaluate the efficacy of TAK-341 versus placebo on the Scales for Outcomes in Parkinson's Disease - Autonomic Dysfunction (SCOPA-AUT).
- To evaluate the efficacy of TAK-341 versus placebo, as measured by overall survival at 52 weeks.
- To evaluate the effect of TAK-341 versus placebo on levels of CSF free αSYN, as measured by the change from baseline to week 52.
- To assess the serum PK and CSF concentrations of TAK-341 in subjects with MSA.

4.3 Safety Objectives

- To assess the safety and tolerability of TAK-341 in subjects with MSA.
- To assess the immunogenicity of TAK-341 in subjects with MSA.

4.4 Exploratory Objectives

[redacted]

[redacted]

4.5 Study Design

This study is a multicenter, randomized, double-blind, placebo-controlled phase 2 study to assess the efficacy, safety, tolerability, PK, and pharmacodynamics of TAK-341 administered as multiple IV infusions Q4W over 52 weeks in male and female subjects with possible or probable MSA, aged at least 40 years.

The study comprises a screening period of up to 42 days (6 weeks), a 52-week double-blind treatment period, and a follow-up safety visit 90 days after the final infusion. Each subject will receive a total of 13 IV infusions of TAK-341 of approximately 60 minutes each or placebo during the double-blind treatment period, with approximately 4 weeks between infusions (i.e., Q4W dosing). An early PK cohort consisting of the first approximately [redacted] subjects will be randomized [redacted] to receive either TAK-341 or placebo. Dosing in subjects in the early PK cohort will be initiated at 2400 mg Q4W. [redacted] After the early PK cohort is fully enrolled, further subjects will be enrolled into the main cohort. Subjects in the main cohort will receive 2000 mg TAK-341 [redacted]

[redacted]

The overall study duration (screening/enrollment, treatment period, and follow-up) will be up to approximately 67 weeks per subject.

The total UMSARS will be administered to all subjects. The primary objective is to evaluate the effect on a modified UMSARS consisting of the UMSARS Part I, minus the sexual function item, with the 0 (normal) and 1 (mild) rating on each item collapsed during the analysis process.

Early PK Cohort

Approximately [redacted] subjects in this cohort will receive 13 infusions over approximately 52 weeks At Visit 2 (Day 1), eligible subjects will be randomized [redacted] to receive 2400 mg TAK-341 or placebo (IV saline) in double-blinded fashion. [redacted]

[redacted] Subjects will continue to receive Q4W infusions after the collection of early PK data, for a total of 13 infusions.

PK, safety, immunogenicity, and tolerability data from the early PK cohort from baseline through Day 85 will be evaluated once PK data from Visit 5 has been collected in all subjects in the early PK cohort. [redacted]

Main Cohort

Approximately [redacted] subjects in this cohort will receive 13 infusions over approximately 52 weeks. Enrollment and dosing of subjects in the main cohort will continue while PK data from the early PK cohort are being analyzed. At Visit 2 (Day 1), eligible subjects will be randomized [redacted] to receive 2000 mg TAK-341 or placebo (IV saline) in double-blinded fashion until the final dose was selected. After the final dose was selected, eligible subjects will be randomized [redacted] to receive TAK-341 2400 mg or placebo (IV saline) in double-blinded fashion.

[redacted]

[redacted]

All Subjects

All subjects will be treated with either TAK-341 or placebo Q4W for a total of approximately 52 weeks up to Visit 14 (Day 337; Infusion #13). For all subjects, sparse PK sampling (of blood) will be performed at Visit 2 (Day 1; Infusion #1), Visit 5 (Day 85; Infusion #4), Visit 8 (Day 169; Infusion #7), and Visit 14 (Day 337; Infusion #13) except for subjects in the early PK cohort at Visit 5 (Day 85) who will undergo intensive PK sampling on that day. All subjects will undergo Predose and end-of-infusion PK sampling on Visit 3 (Day 29; Infusion #2) and Visit 11 (Day 253; Infusion #10). A single PK sample will be collected at the follow-up safety visit (Visit

16; Day 427) or the early termination visit. [redacted] All subjects will undergo lumbar puncture on Visit 2 (Day 1, Predose) and Visit 15 (Day 365, trough). ADA data will be monitored on an ongoing basis.

MSA Study Population

- Possible or probable MSA per Gilman 2008 criteria (Gilman et al. 2008).
- UMSARS Part I score ≤21 (excluding Item #11, sexual function) with a score ≤2 on Items #2 (swallowing), #7 (walking), and #8 (falling).
- UMSARS Part IV disability score ≤3.
- Sufficiently intact cognition to follow instructions and complete study assessments throughout the duration of the study, per investigator judgment.
- Informed consent understood and signed by the subject (or, when applicable, the subject's legally acceptable representative).

A schematic of the study design is included as Figure 1.a.

[redacted]

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoint

- Change from baseline to Week 52 on UMSARS Part I, minus the sexual function item, with collapse of the normal and mild ratings on each item with TAK-341 compared with placebo.

5.2 Secondary Endpoints

- Change from baseline to Week 52 on the 11-item UMSARS specified by Palma et al (Palma et al. 2021) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS total score (UMSARS Part I + Part II) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS Part I score minus the sexual function item (without collapse of ratings of scale items) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS Part II score with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the CGI-S score with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the SCOPA-AUT total score with TAK-341 compared with placebo.
- Overall survival at 52 weeks with TAK-341 compared with placebo.
- Change from baseline to Week 52 on levels of CSF free αSYN with TAK-341 compared with placebo.
- Serum PK parameters, if feasible, will include but not be limited to the following:
  - C_{max}.
  - t_{max}.
  - Area under the serum concentration-time curve during a dosing interval (AUC_{τ}).
- CSF concentrations of TAK-341.

5.3 Safety Endpoints

- Incidence of treatment-emergent adverse events (TEAEs).
- Incidence of clinically significant abnormal values for clinical laboratory evaluations, vital signs, ECG parameters, and the Columbia-Suicide Severity Rating Scale (CSSRS).
- Findings from clinical laboratory evaluations, vital signs, ECG, C-SSRS, physical examination, neurological examination, [redacted]
- Incidence of ADAs.

5.4 Exploratory Endpoints

[redacted]

6.0 DETERMINATION OF SAMPLE SIZE

A sample size of approximately 138 randomized subjects with MSA would be sufficient to detect an approximately [redacted] difference between TAK-341 and placebo in mean change from baseline on a modified UMSARS score (UMSARS Part I minus the sexual function item, with collapsing of 'normal and mild' ratings during analysis). [redacted]

7.0 METHODS OF ANALYSIS AND PRESENTATION

7.1 General Principles

Unless otherwise described, general principles, definitions, and summaries described in Section 7.1 to 7.12 will be applied to each part of the study.

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 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. When presenting summary statistics by treatment groups, the treatment groups include placebo and TAK-341 or TAK-341 specific doses where appropriate.

All p-values will be rounded to 3 decimal points (e.g., 0.123) and two-sided.

For efficacy assessment based on questionnaires, and CSSR-S, baseline value is defined as the last observed value from no later than the day of the first dose of study medication. For all other endpoints, baseline value is defined as the last observed value before the first dose of study medication.

All data analyses and figures will be generated using SAS System® Version 9.4 or higher and PK analysis will be done using Phoenix® WinNonlin® software version 8.0 or higher (Certara, Princeton, NJ, USA).

7.2 Definition of Study Days and Study Visit Windows

Study day will be calculated relative to the date of the first dose of the study drug for each subject. Study days prior to the first dose of study drug 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).

For each visit, a window will be defined; this window will establish a time interval around which data will be considered for the analysis of the scheduled visit pertaining to that window. Unless it is specifically defined below, as a rule, the lower and upper bounds of each window are the approximate midpoints between the scheduled days for the current visit and its adjacent scheduled visits. The value used in analysis for by-visit summaries is the value within the specified window that is the closest to the scheduled study day. If two observations are equidistant from the scheduled visit date, the observation with a later date/time will be used. The visit windows and applicable study day ranges are presented below (Table 7.a through Table 7.j). Cut-off days for inclusion in the window (number of days following the date of the last dose of double-blind study drug) are provided. If data will be collected outside of the predefined windows it will be up to the clinician to decide if the outcomes are valid. The decision should be made before the unblinding.

Table 7.a Visit Windows for endpoints that are collected every 12 weeks and no follow-up visit (UMSARS)

Visit	Scheduled Day	Range-min	Range-max
Week 12	85	43	126
Week 24	169	127	210
Week 36	253	211	308
Week 52	365	309	Last dosing day + 100

Table 7.b Visit Windows for endpoints that are collected every 12 weeks and includes a follow-up visit (safety laboratory tests)

Visit	Scheduled Day	Range-min	Range-max
Week 12	85	43	126
Week 24	169	127	210
Week 36	253	211	308
Week 52	365	309	395
Follow-up	427	396	Last dosing day + 100

Table 7.c Visit Windows for endpoints that are collected every 24 weeks and no follow-up visit ([redacted], CGI-S, [redacted] SCOPA-AUT, [redacted], [redacted])

Visit	Scheduled Day	Range-min	Range-max
Week 24	169	135	266
Week 52	365	267	Last dosing day + 100

Table 7.d Visit Windows for Endpoints that are collected every 4 weeks with a follow-up visit (vital sign, orthostatic vital sign, C-SSRS, [redacted])

Visit	Scheduled Day	Range-min	Range-max
Week 4	29	15	42
Week 8	57	43	70
Week 12	85	71	98
Week 16	113	99	126
Week 20	141	127	154
Week 24	169	155	182
Week 28	197	183	210
Week 32	225	211	238

Table 7.d Visit Windows for Endpoints that are collected every 4 weeks with a follow-up visit (vital sign, orthostatic vital sign, C-SSRS, [redacted])

Visit	Scheduled Day	Range-min	Range-max
Week 36	253	239	266
Week 40	281	267	294
Week 44	309	295	322
Week 48	337	323	350
Week 52	365	351	395
Follow-up	427	396	Last dosing day + 100

Table 7.e [redacted]

[redacted]

Table 7.f Visit Windows for 12-Lead ECG

Visit	Scheduled Day	Range-min	Range-max
Week 4	29	15	42
Week 8	57	43	70
Week 12	85	71	126
Week 24	169	127	210
Week 36	253	211	308
Week 52	365	309	395
Follow-up	427	396	Last dosing day + 100

Table 7.g Visit Windows for Antidrug antibodies (ADA)

Visit	Scheduled Day	Range-min	Range-max
Week 4	29	15	56
Week 12	85	57	126
Week 24	169	127	210
Week 36	253	211	294

Table 7.g Visit Windows for Antidrug antibodies (ADA)

Visit	Scheduled Day	Range-min	Range-max
Week 48	337	295	350
Week 52	365	351	395
Follow-up	427	396	Last dosing day + 100

Table 7.h [redacted]

[redacted]

Table 7.i Visit Windows for CSF free alpha-synuclein [redacted]

Visit	Scheduled Day	Range-min	Range-max
Week 12	85	43	127
Week 52	365	183	Last dosing day + 100

Table 7.j [redacted]

[redacted]

For efficacy and safety data that are included in Table 7.a through Table 7.j, data that are obtained more than 100 days (approximately 5 half-lives of TAK-341) after the last dose of study medication (start date – last dose date >100) will be listed but excluded from summaries and analyses. Adverse events that start more than 100 days after the last dose of study medication (start date – last dose date >100) will be listed but excluded from the summaries and analyses.

7.2.1 Conventions for Missing Adverse Event Dates

Adverse events with start dates that are completely or partially missing will be imputed as follows:

If month and year are known but day is missing.

- If month and year are the same as month and year of first dose date, then impute to first dose date.
- If month and year are not the same as of the first dose, impute first day of the month.

If year is known but day and month are missing.

- If year is same as year of 1st dose date, then 1st dose date will be used instead.
- If year is different than year of 1st dose date, then 1st of January of the year will be used.

If year and day are known but month is missing.

- If year and day are the same as year and day of first dose date, then impute to first dose month.
- If year is the same but the day is different from the first dose date then if day is after the first dose date impute first dose month, otherwise, impute month next after the first dose month.
- If year is different, then impute January as a month.

If year, month, and day are unknown then impute date of the first dose.

Imputing missing AE start date is mandatory. After the imputation, all imputed dates are checked against the start dates to ensure the stop date does not occur before start date.

Adverse events with stop dates that are completely or partially missing will be imputed as follows:

If "ongoing" is checked, no imputation is necessary.

If month and year are known but day is missing, the last day of the month will be imputed.

If year is known, but day and month are missing.

- If YYYY ≤ year of last dose, then 31st of December will be imputed.
- If YYYY > year of last dose, then the imputed end date should be set to the earliest of the (treatment follow up period date, 31DECYYYY, date of death).

If all are missing, no imputation is necessary. The event will be considered "ongoing."

If an AE is ongoing, AE stop date could be missing. Otherwise, AE stop date could be imputed per above rules. If a subject dies during the study and AE stop date is missing, then the death date will be used for AE stop date. After the imputation, all the imputed stop dates are compared with the start dates to ensure the stop date does not occur before the start date. If the imputed stop date occurs prior to the start date, then the imputed stop date will be the same as the start date.

The imputed dates will not be populated on the data listing, they are used for derivation purpose only. The actual and incomplete date will be on the data listing.

7.2.2 Conventions for Missing Concomitant Medication Dates

Concomitant medications with start date that are completely or partially missing will be analyzed as follows:

If month and year are known, but day is missing, then impute day to first of the month.

If year is known, but day and month are missing, then 1st of January of the year will be imputed.

If year and day are known, but month is missing, then January will be imputed.

Concomitant medications with stop dates that are completely or partially missing will be analyzed as follows:

If "ongoing" is checked, no imputation is necessary.

If month and year are known but day is missing, the last day of the month will be imputed.

If year is known, but day and month are missing then 31st of December will be imputed.

If year and day are known, but month is missing then December will be imputed.

If all are missing, no imputation is necessary. Concomitant medication will be considered as "ongoing".

If a concomitant medication is ongoing, the stop date could be missing. Otherwise, concomitant medications stop date could be imputed per above rules. If a subject dies during the study and the concomitant medication stop date is missing, then the death date will be used for the stop date. After the imputation, all the imputed stop dates are compared with the start dates to ensure the stop date does not occur before the start date. If the imputed stop date occurs prior to the start date, then the imputed stop date will be the same as the start date.

The imputed dates will not be populated on the data listing, they are used for derivation purpose only. The actual and incomplete date will be on the data listing.

7.2.3 Conventions for Missing PK Data

Serum and CSF concentrations of TAK-341 that are below the limit of quantification (BLQ) will be treated as zero in the summarization of concentration values. Missing concentrations will be excluded from summaries. Concentration reported as BLQ will be displayed as they are in the data listings.

7.2.4 Conventions for Missing Efficacy Data

The efficacy analyses will be performed according to ITT principle where treatment comparisons are based on the randomized treatment assignment instead of what are received. Descriptive analysis of efficacy data will be presented as observed. Missing data will be assumed to be missing at random (MAR) and hence no imputation will be performed before the mixed model analysis or the survival analysis.

7.3 Analysis Sets

7.3.1 Full Analysis Set (FAS)

The full analysis set will include all randomized subjects. Subjects in this analysis set will be used for disposition, demographics, baseline characteristics summaries and all efficacy analyses.

7.3.2 Safety Analysis Set

The safety analysis set will include all randomized subjects who received at least 1 dose of the study drug. Subjects in this analysis set will be used for medical history, concurrent medical conditions, prior and concomitant medications, study drug exposure and compliance, and all safety summaries.

7.3.3 Pharmacokinetic Analysis Set

The PK analysis set will consist of subjects who are randomized and receive at least 1 dose of study drug and who have at least 1 measurable serum or CSF (as applicable) concentration of TAK-341. Subjects in this analysis set will be used for all PK analysis.

7.3.4 Pharmacodynamic(PD) Analysis Set

The pharmacodynamic analysis set will consist of subjects who are randomized and receive at least 1 dose of study drug and who have at least 1 measurable plasma or CSF (as applicable) concentration of αSYN. Subjects in this analysis set will be used for all PD analysis.

7.4 Disposition of Subjects

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

- All subjects who signed informed consent form.
- All subjects who were randomized.
- All subjects who were not randomized.

The primary reasons for subjects who were not randomized will be summarized.

Disposition of randomized subjects (FAS) will be tabulated as below by corresponding treatment groups (randomized arm, placebo andTAK-341) and overall:

- All subjects who randomized but did not receive any study drug.
- All subjects who received at least one dose of study drug.
- Subjects who completed the study drug.
- Subjects who prematurely discontinued study drug.
- Subjects who completed all study visits.

- Subjects who prematurely discontinued study visits.

Primary reasons for discontinuation of study drug/visits, as entered on the electronic case report form (eCRF), will be tabulated. The primary reasons for premature discontinuation of study drug/study visit will be presented for each subject in listings.

Significant protocol deviations will be listed and summarized using the FAS by corresponding treatment groups and overall.

7.5 Demographic and Other Baseline Characteristics

Demographics of the screening failures collected on the eCRF will be summarized.

For subjects in the FAS, demographics and other baseline characteristics will be summarized by treatment groups and overall. Descriptive statistics will be used to summarize data for continuous variables like age and weight (number of subjects [N], mean, median, SD, minimum, and maximum) and for categorical variables like sex, ethnicity, and race.

All individual demographic and baseline characteristics will be listed by treatment and subject number. The demographic data listing will include subject identifier, treatment, date of informed consent, date of birth, age at date of informed consent, sex, race, height, baseline weight and baseline BMI and other demographic and baseline information collected in the eCRF.

7.6 Medical History and Concurrent Medical Conditions

For subjects in the safety analysis set, medical history includes any significant conditions or diseases relevant to the disease under study that stopped at or prior to signing of informed consent. Concurrent medical conditions are those significant ongoing conditions or diseases present at signing of informed consent. Medical history and concurrent medical conditions will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA, version 23 or higher) coding system.

All medical history and concurrent medical condition data will be listed by subject. The listing will contain cohort, 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.

7.7 Prior and Concomitant Medications

For subjects in the safety analysis set, medication history information obtained includes any medication stopped at or within 30 days prior to signing of informed consent. Medications used from signing of informed consent through the end of study will be considered as concomitant medications.

No summary statistics for prior and concomitant medications will be provided. All medication history and concomitant medications data will be listed by subject.

7.8 Study Drug Exposure and Compliance

The date and time of each dose for each subject will be reported in the data listing.

The summary of study drug exposure and compliance will be based on the safety analysis set.

Study drug exposure and compliance will be calculated in two ways:

1) Number of infusions each subject had.
2) Compliance calculated as the number of infusions each subject had / expected number of infusions for each subject *100%.

For each treatment, study medication compliance will be summarized as the number of subjects and the frequency in each compliance category (<50%, 50 to 70%, and ≥70%). The number of infusion and study medication compliance will also be summarized as continuous variables using descriptive statistics (n, mean, SD, median, minimum, and maximum) for each treatment group.

All study drug administration and accountability data will be listed by subject ID, site ID, age/gender, treatment group, dose dates, dose level, medication identification number, and compliance percentages.

7.9 Efficacy Analysis

Efficacy analysis will be based on FAS and subjects will be analyzed according to the treatment group to which they were randomized. Control of Type-1 Error will be maintained for primary analysis only.

7.9.1 Modified UMSARS Part I Total Score Analysis

The primary objective is to evaluate the efficacy of TAK-341 versus placebo, as measured by the change from baseline to Week 52 on modified Unified Multiple System Atrophy Rating Scale (UMSARS) (Wenning et al. 2004) Part I total score (excluding the sexual function item, and combining the normal and mild ratings on each item), and is labelled as modified UMSARS Part I total score (mUMSARS).

Summary statistics of observed value and change from baseline at each visit of mUMSARS will be tabulated by treatment group and visits. Mean (SD) change from baseline of mUMSARS vs. visit will be plotted by treatment group.

The linear mixed effect model will be used to evaluate the effect of TAK-341 on the change from baseline in the mUMSARS score. Baseline mUMSARS score and diagnostic certainty (possible vs. probable) will be included as the covariates in the model. [redacted]

Unstructured Variance Covariance Metrics will be assumed for the model. If the procedure does not converge, we will use a compound symmetry (cs) variance covariance matrix. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. The least squares mean of change from baseline in mUMSARS score for each treatment and visit and the associated SE and 95% CI will be calculated for TAK-341 and placebo, along with difference

between TAK-341 and placebo and associated SEs, 95% CIs, and p-values. The Least squares mean and SE vs. visit will be plotted.

[redacted]

7.9.2 Secondary UMSARS Analysis

The following endpoints will be analyzed in the same way as the primary endpoint.

- Change from baseline to Week 52 on the 11-item UMSARS specified by Palma et al (Palma et al. 2021) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS total score (Part I + II) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS Part I score minus the sexual function item (without collapse of ratings of scale items) with TAK-341 compared with placebo.
- Change from baseline to Week 52 on the UMSARS Part II score with TAK-341 compared with placebo.

7.9.3 CGI Analysis

Number and percentage of subjects in each categories for both CGI-S [redacted] (Guy 1976) will be provided by treatment and visits. In addition, for change in CGI-S, The CGI-S responses will be mapped to 1 to 7, starting with 1 = Normal and continuing in order, and subjects will be divided into 5 response categories depending on their level of change:

- Moderate Improvement: ≥ 2-point decrease from baseline

- Minimal Improvement: 1-point decrease
- No change: equal to baseline
- Minimal Worsening: 1-point increase
- Moderate Worsening: ≥ 2-point increase

Number and percentage of subjects in each category of change will be provided by treatment and visits.

[redacted]

The CGI-S score will also be treated as continuous variable, summary statistics of observed value and change from baseline at each visit of CGI-S score will be tabulated by treatment group and visits. The linear mixed effect model will be used to evaluate the effect of TAK-341 on the change from baseline in the CGI-S score. Baseline CGI-S score will be included as the covariates in the model. Treatment, visit and treatment by visit interaction will be included as fixed effect and subject as a random effect. Unstructured Variance Covariance Metrics will be assumed for the model. If the procedure does not converge, compound symmetry (cs) variance covariance matrix will be used. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. The least squares mean of change from baseline in CGI-S score for each treatment and visit and the associated SE and 95% CI will be calculated for TAK-341 and placebo, along with difference between TAK-341 and placebo and associated SEs, 95% CIs, and p-values.

7.9.4 SCOPA-AUT Analysis

SCOPA-AUT (Visser et al. 2004) has total of 23 questions which is summarized as six domains (gastrointestinal, urinary, cardiovascular, thermoregulatory, pupillomotor and sexual) scores and one total score. The total score ranges from 0-69. The gastrointestinal domain includes seven questions (swallowing/ choking, saliva dribble, food stuck, feel full, constipation, strain to defecate, fecal incontinence) and ranges from 0-21. The urinary domain includes six questions (urgency, urinary incontinence, incomplete emptying, weak urine stream, frequency, urine at night) and ranges from 0-18. The cardiovascular domain includes three questions (lightheaded standing up, lightheaded standing for some time, fainted) and ranges from 0-9. The thermoregulatory domain includes four questions (excess sweat day, excess sweat night, cold intolerance, heat intolerance) and ranges from 0-12. The pupillomotor has one question and ranges from 0-3. The sexual domain has two different questions for either sex and ranges from 0-6.

Summary statistics of observed value and change from baseline at each visit of SCOPA-AUT domain and total scores will be tabulated. The linear mixed effect model will be used to evaluate the effect of TAK-341 on the change from baseline in the SCOPA-AUT total score. Baseline SCOPA-AUT total score will be included as the covariate in the model. [redacted]
Unstructured Variance Covariance Metrics will be assumed for the model. The least squares mean of change from baseline in SCOPA-AUT total score for each treatment and visit and the associated SE and 95% CI will be calculated for TAK-341 and placebo, along with difference between TAK-341 and placebo and associated SEs, 95% CIs, and p-values. The domain scores will be analyzed similarly as the total score.

7.9.5 Overall Survival Analysis

The overall survival of the treatment groups will be estimated with Kaplan-Meier survival estimates, along with their 95% CI and compared with a log-rank test. A cox proportional hazards model will be fitted to model the survival probability with treatment as the predictor. The hazard ratio estimates of TAK-341 vs. placebo and its associated SE, 95% CI, and p-value will be presented.

7.10 [redacted]

[redacted]

7.10.1 [redacted]

[redacted]

[redacted]

[redacted]

- [redacted]

[redacted]

- [redacted]

[redacted]

[redacted]

7.10.2 [redacted]

[redacted]

[redacted]

7.10.3 [redacted]

[redacted]

7.10.4 [redacted]

[redacted]

7.10.5 [redacted]

[redacted]

7.10.6 [redacted]

[redacted]

[redacted]

7.10.7 [redacted]

[redacted]

7.10.8 [redacted]

[redacted]

7.11 Pharmacokinetic Analysis

PK analysis will be based on the PK analysis set.

7.11.1 Serum and CSF Concentration

TAK-341 serum and CSF concentrations will be tabulated and summarized at each scheduled time point using descriptive statistics (including number of observations (n), mean, median, SD, coefficient of variation [CV], minimum, maximum, geometric mean, geometric SD and

geometric CV). Any values that are reported as below the LLOQ will be set as zero when calculating the summary statistics but will be listed as reported in the listing. Geometric mean, geometric SD and geometric CV will not be calculated when there is "0" value and will be displayed as "NC" for "Not calculated".

Individual subject serum and CSF concentration data and actual sampling times will be listed.

7.11.2 Plasma Pharmacokinetic Parameters

The serum PK parameters of TAK-341 will be determined from the concentration-time profiles for all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. [redacted]

The serum PK parameter estimates will be tabulated and summarized by using descriptive statistics (n, mean, median, SD, CV, minimum, maximum, geometric mean and geometric CV). Individual PK parameters will be presented in a data listing for each subject.

[redacted] Details of the population PK analysis may be documented and reported separately. [redacted]

7.12 Pharmacodynamic Analysis

7.12.1 [redacted] CSF Concentration

[redacted] CSF concentrations of αSYN will be tabulated and summarized at each scheduled time point using descriptive statistics (including number of observations (n), mean, median, SD, CV, geometric mean, geometric CV, minimum, and maximum). Any values that are reported as below the LLOQ will be set as zero (0) when calculating the summary statistics but will be listed as reported in the listing. Geometric mean and geometric CV will not be calculated when there is "0" value and will be displayed as "NC" for "Not calculated".

Any values that are above the hemolysis threshold and reported as "NR" are excluded from calculating the summary statistics.

Change and percent change from baseline for free αSYN in CSF will be calculated, tabulated and summarized at each scheduled time point using descriptive statistics (including number of observations (n), mean, median, SD, CV, minimum, and maximum).

[redacted]

Two sample t-test will be used to test whether changes or percent changes from baseline at week 52 of free αSYN in CSF [redacted] are different between the two treatment arms.

Individual subject [redacted] CSF concentrations of αSYN will be listed.

7.13 Safety Analysis

7.13.1 Adverse Events

The Treatment-Emergent Adverse Events (TEAE) will be defined as an AE observed after starting administration of the study drug and up to 90 days after the last dose of the study drug. The TEAE summary tables will include numbers and percentages of subjects experiencing at least one TEAE by SOC and PT and will be tabulated by treatment. The TEAEs will also be summarized for all subjects in the overview assessment. The following is a list of TEAE summary tables to be generated:

- Overview of Treatment-Emergent Adverse Events. Overall column will be presented in this table only.
- 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.
- Most Frequent (≥3% based on total number of safety set subjects in either one of the treatment groups) Treatment-Emergent Adverse Events by Preferred Term.
- Most Frequent (≥3% based on total number of safety set subjects in either one of the treatment groups) Non-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.
- Intensity of Drug-Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term.

In addition, data listings will be provided for all AEs including: TEAEs, AEs leading to death, AEs leading to study drug or study visit discontinuation, and SAEs.

7.13.1.1 Treatment Emergent Adverse Events of Special Interest (AESIs)

TEAEs related to either 1) Ophthalmological AESIs or 2) Infusion reactions / hypersensitivity reactions will be summarized by using SOC = “Eye Disorders” and the broad SMQ = “Hypersensitivity” respectively and the associated preferred terms. The AESI summary tables

will include numbers and percentages of subjects experiencing at least one TEAE and will be tabulated by treatment.

The following is a list of AESI summary tables to be generated:

- Treatment-Emergent Adverse Events of Special Interests
- Serious Treatment-Emergent Adverse Events of Special Interests
- Drug-Related Treatment-Emergent Adverse Events of Special Interests

In addition, data listings will be provided for all AESIs including the severity and relatedness.

7.13.2 Clinical Laboratory Evaluations

Clinical safety laboratory tests include clinical chemistry, hematology, and urinalysis. A list of all the clinical laboratory evaluations can be found in Protocol Section 9.1.12.

Descriptive statistics (N, mean, SD, median, minimum, and maximum) of clinical laboratory variables will be summarized for baseline and post-dose values, as well as change from baseline to post dose values by study visits and treatment arm the subjects are randomized to.

Individual results for clinical laboratory tests will be evaluated against the Takeda predefined laboratory markedly abnormal values (MAV) criteria (Appendix A) using the result and criteria in SI units. All subjects with at least 1 post-dose laboratory result that meets the MAV criteria will be presented in a data listing.

The number and percentage of subjects with at least one post-dose markedly abnormal laboratory test result will also be summarized by treatment. Subjects who meet the MAV criteria will be mapped to their respective qualifying laboratory result. All post-dose clinical lab MAV results, including scheduled and unscheduled measurements, will be included in the MAV summaries, and summarized by treatment arm and visit.

Listings of all clinical safety laboratory data will be provided in the listings and will be presented in SI units. Laboratory data outside of the normal reference range will be indicated in the listings.

7.13.3 Vital Signs

7.13.3.1 Sitting Position

Vital sign measurements include blood pressure (SBP and DBP), heart rate, respiratory rate, and body temperature taken at the sitting position will be summarized (N, mean, SD, median, minimum, and maximum) for baseline, post-baseline, and change from baseline by treatment. Both the scheduled and unscheduled measurements will be included in the summary.

All individual vital signs that meet Takeda's predefined criteria for MAVs (Appendix B) will be listed. The number and percentage of subjects with at least one post-dose markedly abnormal vital sign measurement will be summarized by treatment. Subjects who meet the MAV criteria will be mapped to their respective qualifying vital sign result. All post-dose MAV vital signs,

including both scheduled and unscheduled measurements, will be included in the MAV summaries. Listings of all vital signs data will be provided in the listings, and vital sign MAVs will be flagged.

7.13.3.2 Orthostatic Blood Pressure and Orthostatic Heart Rate

Orthostatic blood pressure and orthostatic heart rate will be derived from the vital signs that are measured at the supine and standing positions.

Blood pressure (SBP and DBP) and heart rate measurements of the supine or standing position, or the difference between supine and standing will be summarized (N, mean, SD, median, minimum, and maximum) for baseline, post-baseline, and change from baseline by treatment.

Orthostatic hypotension is defined as the incidence that meets the following criteria: supine-standing SBP >= 20 mmHg or supine-standing DBP>=10 mmHg. The number and percentage of subjects who met this criterion at each visit will be summarized by treatment.

Both the scheduled and unscheduled measurements will be included in the summary.

7.13.4 12-Lead ECGs

A standard 12-lead ECG will be recorded. The investigator (or a qualified observer at the investigational site) will manually interpret the ECG using one of the following categories: within normal limits, abnormal but not clinically significant, or abnormal clinically significant.

ECGs will also be interpreted by a central reader using one of the following two categories: normal and abnormal.

ECG results will be tabulated by treatment group and visit as normal and abnormal based on the central reads only. If there are multiple ECG results within the same visit window, the worst result will be selected.

The following parameters will be calculated automatically by the ECG machine: heart rate, PR interval, QT interval, QRS interval, and QT interval with Fridericia correction method (QTcF).

Descriptive statistics of the continuous ECG parameters will be summarized for baseline, post-dose, and change from baseline at each post-dose time point by treatment group. Both the scheduled and unscheduled measurements will be included in the summary.

No statistical tests will be performed for the observed ECG parameters.

All individual ECGs that meet Takeda's predefined criteria for MAVs (Appendix C) will be listed. The number and percentage of subjects with at least one post-dose markedly abnormal ECG measurement will be summarized by treatment. Subjects who meet the MAV criteria will be mapped to their respective qualifying ECG result. All post-dose MAV ECG parameters, including both scheduled and unscheduled measurements, will be included in the MAV summaries.

Individual subject ECGs (both local reads and central reads) will be presented in a data listing by visit.

7.13.5 Other Observations

Findings on physical exams, neurological exams, [redacted] anti-drug antibody levels (if not considered part of clinical laboratory evaluations) and C-SSRS results will be presented as either summary statistics or frequency tables and listings.

7.14 Unblinded Data Reviews

Unblinded data safety review will be performed by Takeda team and the process is described in detail in the Data Access Management Plan (DAMP).

7.15 [redacted]

[redacted].

7.15.1 [redacted]

[redacted]

7.15.2 [redacted]

[redacted]

7.15.3 [redacted]

[redacted]

7.15.4 [redacted]

[redacted]

[redacted]

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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	--	>1.5x ULN
	SI	--	>1.5x ULN
Albumin	Conventional	<2.5 g/dL	--
	SI	<25 g/L	--
Total protein	Both	<0.8x LLN	>1.2x ULN
Creatinine	Conventional	--	>1.5x ULN
	SI		>1.5x ULN
Blood urea nitrogen	Conventional		>40 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	>5.3 mEq/L
	SI	<3.0 mmol/L	>5.3 mmol/L
CK	Both	--	>3x ULN
Glucose	Conventional	<50 mg/dL	>300 mg/dL
	SI	<2.8 mmol/L	>19.4 mmol/L
Calcium	Conventional	<7.7 mg/dL	>11.1 mg/dL
	SI	<1.92 mmol/L	>2.77 mmol/L

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

Additional Serum Chemistry—Criteria for Markedly Abnormal Values

Parameter	Unit	Low Abnormal	High Abnormal
Triglycerides	Conventional	--	>2.5x ULN
	SI		>2.5x ULN
Phosphorus	Conventional	<1.6 mg/dL	>6.2 mg/dL
	SI	<0.52mmol/L	>2.000 mmol/L
Total Cholesterol	Conventional	<200 mg/dL	>300 mg/dL
	SI	<5.18 mmol/L	>7.72 mmol/L

ULN=upper limit of normal.

Appendix B Criteria for Markedly Abnormal Values for Vital Signs

Parameter	Unit	Lower Criteria	Upper Criteria
Pulse	bpm	<40	>115
Systolic blood pressure	mm Hg	<90	≥160
Diastolic blood pressure	mm Hg	<50	≥100
Systolic blood pressure change	mm Hg		>20, >30
Diastolic blood pressure change	mm Hg		>20, >30
Body temperature	°C		>38.5
Respiratory Rate	Breath/min		>21

Appendix C Criteria for Markedly Abnormal Values for Electrocardiograms

Parameter	Lower Criteria	Upper Criteria
Heart rate	<40 beats per minute	>115 beats per minute
PR	≤80 milliseconds	≥200 milliseconds
QTcF Interval	≤300 milliseconds	>500 milliseconds OR ≥30 milliseconds change from baseline and >450 milliseconds
QRS	≤80 milliseconds	≥180 milliseconds

Appendix D [redacted]

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