Title: Statistical Analysis Plan for AUT022201

Compound Name/Number: AUT00201

Effective Date: [24 May 2024]

Study Title: A Randomized, Double-blind, Placebo-controlled, Crossover Study of the Effects of Single Doses of AUT00201 in Patients with Myoclonus Epilepsy and Ataxia due to Potassium (K^+) Channel Mutation (MEAK)

Subject: MEAK, AUT00201, Phase 1b study

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ABBREVIATIONS

AE	Adverse Event
AED	Antiepileptic Drug
BB	Bicep brachii
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CSP	Clinical Study Protocol
CI	Confidence Interval
C-SSRS	Columbia-Suicide Severity Rating Scale
CV	Coefficient of Variation
eCRF	electronic Case Report Form
EDC	Extensor digitorum communis
EEG	Electroencephalogram
EMG	Electromyography
GABA	γ-aminobutyric acid
IP	Investigational Product
LBD	Lafora body disease
LICI	Long Interval Cortical Inhibition
MEAK	Myoclonus Epilepsy and Ataxia due to Potassium (K^+) Channel Mutation
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myoclonus Index
MSO	Maximal Stimulator Output
n	number of observations
NC	Not Calculable
OAE	Otoacoustic Emissions
PD	Pharmacodynamic
PK	Pharmacokinetic
PROM	Patient-Reported Outcome Measurement
PT	Preferred Term
rMT	Resting Motor Threshold
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SICI	Short Interval Cortical Inhibition
SNR	Signal-to-Noise Ratio
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TMS	Transcranial Magnetic Stimulation
ULD	Unverricht-Lundborg disease
UMRS	Unified Myoclonus Rating Scale
WHODD	World Health Organization Drug Dictionary

WIN	Words-in-Noise

TRADEMARK INFORMATION

SAS SAS (Statistical Analysis Software) is a registered trademark of SAS Institute Inc.

REVISION HISTORY

Version	Date	Summary of revisions
1.0	[24 April 2024]	Initial version

2.0	[24 May 2024]	• Section 2.3.3 – Exploratory endpoint wording clarified as negative myoclonus during UMRS Sections 4 and 5 is collected as one measurement, rather than two separate ones.
		• Section 8.3 – Baseline definition updated to clarify that only scheduled measurements are considered.
		• Section 9.2.2.2 – Speech test parameters selected to investigate the secondary endpoint have been changed. Full rationale is included within the text.
		• Section 9.2.2.3 – Updates made to EMG and accelerometer parameters due to clarification of the data being recorded.
		• Section 9.2.2.6 – Updates to the list of quantitative EEG parameters due to a clarification of the data being recorded.
		• Section 9.3.1 – Updates on how to handle BLQ measurements in the summarization of PK concentrations.
		• Section 10.2.1 – Updates to Table 4 to align with updates discussed above for Sections 9.2.2.2 and 9.2.2.3.
		• Section 10.2.2 – Updates to Table 5
		  ○ Updates to Tier 1 and 2 with regards to speech test measures.
		  ○ Updates to Tier 2 with regards to quantitative EEG.
		  ○ Added column indicating the anticipated direction of change with improvement.
		• Section 10.2.2 – Update to false positive rate following changes to Tier 1 and Tier 2 criteria, and knowledge of missing participant data.
		• Section 10.4.1 – Updates to which speech test parameters are to be plotted against AUT00201 C_{max}.

1. INTRODUCTION

This is a randomized, double-blind, placebo-controlled, Phase 1b crossover study of the effects of single doses of AUT00201 in patients with myoclonus epilepsy and ataxia due to potassium (K^+) channel mutation (MEAK).

This Statistical Analysis Plan (SAP) provides details of the summaries and analyses to be performed to report the findings of the study. It should be read in conjunction with the Clinical Study Protocol (CSP); AUT022201, Version 4.0, 30 October 2023.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 STUDY OBJECTIVES

2.1.1 PRIMARY OBJECTIVES

The primary objective is:

- To evaluate the safety and tolerability of single doses of AUT00201 in patients with MEAK.

2.1.2 SECONDARY OBJECTIVES

The secondary objectives are:

- To evaluate the effects of single doses of AUT00201 on short interval cortical inhibition (SICI) as measured by paired-pulse transcranial magnetic stimulation (TMS)
- To evaluate the effects of single doses of AUT00201 on dysarthria as measured by automated speech and vocal assessments
- To evaluate the effects of single doses of AUT00201 on myoclonus index (MI) by capturing the amount and time periods with positive myoclonus during a prespecified time period as measured by electromyography (EMG) and accelerometer
- To assess the pharmacokinetic (PK) profile of AUT00201 after single doses of AUT00201 in patients with MEAK
- To evaluate correlations between exposure to AUT00201 and changes in these biomarkers.

2.1.3 EXPLORATORY OBJECTIVES

The exploratory objectives are:

- To evaluate the effects of single doses of AUT00201 on long interval cortical inhibition (LICI) as measured by paired-pulse TMS
- To evaluate the effects of single doses of AUT00201 on myoclonus as assessed by Sections 4 and 5 of the Unified Myoclonus Rating Scale (UMRS) and sub-sections D and E of Section 4
- To evaluate the effects of single doses of AUT00201 on the amount of negative myoclonus (short periods of loss of muscle tone) during a prespecified time period as measured by EMG and accelerometer

- To evaluate the effects of single doses of AUT00201 on quantitative electroencephalogram (EEG) and on EEG frequency responses to chirp stimuli
- To evaluate the effects of single doses of AUT00201 on measures of early visual processing
- To evaluate the effects of single doses of AUT00201 on auditory measures
- To evaluate correlations between exposure to AUT00201 and changes in these biomarkers.

2.2 STUDY ESTIMANDS

No primary estimand will be defined as the primary objective of this study is to evaluate the safety and tolerability of single doses of AUT00201 in patients with MEAK. Refer to Section 10.3 for details on the reporting of safety outcomes.

Whilst estimand strategies have not been explicitly defined in the study protocol, the analysis of pharmacodynamic (PD) outcomes will include all data collected for a participant belonging to the analysis set under investigation (modified pharmacodynamic population) and reported under the treatment which the participant received at the date of assessment.

2.3 STUDY ENDPOINTS

2.3.1 PRIMARY ENDPOINTS

The primary endpoints are:

- Safety and tolerability of single doses of AUT00201 in patients with MEAK:
  - Change from screening/baseline in laboratory assessments (routine hematology, biochemistry, and urinalysis)
  - Incidence of clinically significant laboratory findings
  - Change from baseline in vital signs
  - Change from baseline physical examination findings approximately 24 hours after investigational product (IP) administration
  - Change from screening in 12-lead ECG findings
  - Incidence of adverse events (AEs)

2.3.2 SECONDARY ENDPOINTS

The secondary endpoints are:

- Change from baseline in cortical inhibition as assessed with paired-pulse TMS EMG:
  - SICI as measured by paired-pulse TMS EMG
- Change from baseline in:
  - Measures of dysarthria as assessed by automated standardized speech and vocal tests
  - Myoclonus Index (MI; a measure of positive myoclonus) evaluated with EMG and accelerometer
- PK parameters following single doses of AUT00201: maximum (peak) plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to 24 hours (AUC_{24}) of AUT00201.

2.3.3 EXPLORATORY ENDPOINTS

The exploratory endpoints are:

- Change from baseline in cortical inhibition as assessed with paired-pulse TMS EMG:
  - LICI as measured by paired-pulse TMS EMG
- Change from baseline in:
  - Amount of negative myoclonus evaluated with EMG and accelerometer over time and during each UMRS action myoclonus Section 4: D and E and UMRS Sections 4 and 5
  - UMRS subscale scores: Sections 4 and 5
  - UMRS action myoclonus scores Section 4: D and E
  - Quantitative EEG measures (absolute and relative power of predefined frequency bands)
  - EEG power and phase locking factor induced by chirp stimulus in predefined frequency bands
  - Measures of early visual processing as assessed by:
    - Binocular rivalry
    - An internal noise estimation
  - Measures of auditory processing as assessed by:
    - Otoacoustic Emissions (OAEs)
    - Words-in-Noise (WIN) test performance (signal-to-noise ratio [SNR])

2.4 STATISTICAL HYPOTHESES

No formal hypothesis testing will be performed as the primary objective of this study is to evaluate the safety and tolerability of single doses of AUT00201 in patients with MEAK.

3. STUDY DESIGN

This is a Phase 1b study to investigate the safety, tolerability, PK, and PD effects on biomarkers of AUT00201 in patients with MEAK.

The study will be conducted at 1 hospital research center. It was planned that approximately 6 to 10 patients aged 18 years or older, diagnosed with MEAK, based on documented genetic evidence of the presence of the KCNC1 variant would participate in the study.

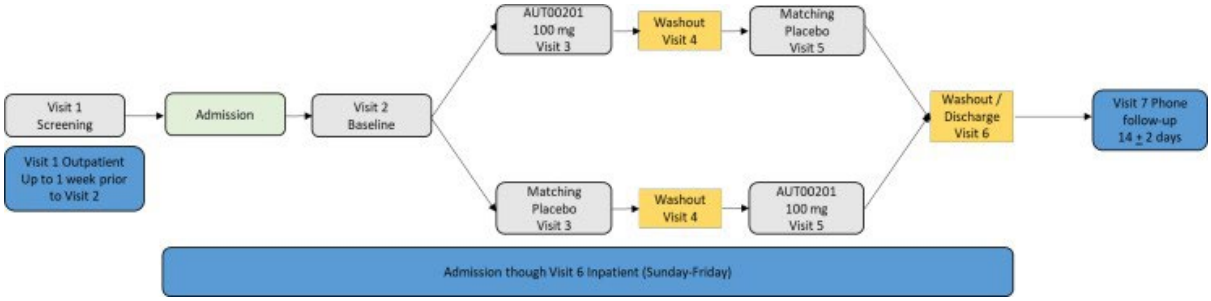
The study is a double-blind, randomized, placebo-controlled treatment in which participants will be administered a single dose of 100 mg AUT00201 and matching placebo in a crossover design. There is an initial outpatient screening and procedure orientation which can be spread over 2 days, followed by a 5-day inpatient stay at a clinical research unit.

Assessments will be conducted throughout the study as detailed in Appendix A – Schedule of assessments.

Figure 1 provides an illustration of the study design. Outpatient screening and procedure orientation (Visit 1) will occur up to 1 week prior to Visit 2 when participants will be admitted to the clinic for a 5-day inpatient stay. Baseline assessments (Visit 2) will be completed on the first inpatient day. Participants will then be administered a single dose of 100 mg AUT00201 or matching placebo on the second inpatient day (Visit 3). The third

inpatient day (Visit 4) will be a washout day for participants. On the fourth inpatient day (Visit 5), participants will be administered the crossover treatment. On the fifth inpatient day (Visit 6), participants will be discharged from the unit. Participants will be followed up by phone 14 days ±2 days after discharge (Visit 7).

Figure 1: Study Design



It is not possible to replace participants in this study, due to the rarity of the disorder. However, if any technical issues or important protocol deviations occur during a participant's participation that are likely to make the primary, secondary, or exploratory endpoint data unevaluable or impact their validity, the participant may be invited back to participate in the study again, if the sponsor and Investigator are in agreement. If a participant is invited back to participate a second time, they will be given a new screening number and treated as a new participant, including consideration for inclusion and exclusion criteria. Data from the first participation, with the exception of safety and PK data, will not be included in the statistical analysis. All data will be listed, with clear indication if a participant is a repeat participant.

Participants will be assigned a unique code number (randomization number) at Visit 3. The randomization number will encode the participant's assignment to a treatment sequence, according to the randomization schedule generated prior to the study by Fortrea.

For reporting, Study Day 1 is the first day of study treatment. Therefore, visits are linked to study days as follows:

- Visit 1 – Screening
- Visit 2 – Admission on Study Day -1 (day before first dose)
- Visit 3 – Study Day 1 (first treatment received)
- Visit 4 – Study Day 2 (washout)
- Visit 5 – Study Day 3 (second treatment received)
- Visit 6 – Study Day 4 (washout/discharge)
- Visit 7 – phone follow-up

Participants will receive one of the following sequences of treatment:

- A single 100 mg dose of AUT00201, oral capsule, on Study Day 1 and then matching placebo, oral capsule, on Study Day 3
- A matching placebo, oral capsule, on Study Day 1 and then a single 100 mg dose of AUT00201, oral capsule, on Study Day 3.

Their single doses on Study Day 1 (Visit 3) and Study Day 3 (Visit 5) will be taken approximately 30 minutes after completion of a standard meal provided by the clinical site.

Total duration of study participation per participant is up to 3.5 weeks. More specifically, duration can be described as follow:

- Total outpatient participation: Up to 1 week
- Total inpatient participation: Approximately 5 days
- Planned follow-up: 14 days ±2 days after discharge

A participant is considered to have completed the study if he/she has completed all visits of the study. The end of the study is defined as the date of final follow-up (planned as a telephone call) by the last participant in the study.

4. TIMING OF PLANNED ANALYSES

4.1 INTERIM ANALYSES

No interim analysis is planned.

4.2 FINAL ANALYSIS

The final analysis is planned for after study completion (see Section 3 for the definition of end of study), database lock and unblinding of the study randomization.

5. SAMPLE SIZE CONSIDERATIONS

MEAK is an ultra-rare disorder. This study will aim to include all adult patients with MEAK known to the Principal Investigator and sub-investigator(s). It was hoped that approximately 6 to 10 patients could be recruited.

At the time of writing this SAP, a total of 5 participants will be enrolled in the study (one of which was rescreened and participated twice in the study). Recruitment was lower than anticipated as contact could not be made with all patients, and not all patients contacted agreed to participate. The SAP has been written with this lower recruitment taken into account.

6. ANALYSIS POPULATIONS

This section outlines the analysis populations that will be included for this study. In all populations, participants will be analyzed according to treatment actually received during the specific treatment period (before and after the crossover) regardless of the treatment to which they were randomized.

Please note the addition of the modified pharmacodynamic population to the SAP, to handle subjects who participate in the study twice appropriately in PD analyses.

6.1 SCREEN POPULATION

All participants who signed the informed consent and are screened for participation in this study. This population will only be used for the purposes of describing participant disposition.

6.2 SAFETY POPULATION

All participants who received at least 1 dose of AUT00201 or placebo. Unless otherwise specified, this analysis population will be used for all safety analyses and listings.

6.3 PHARMACOKINETIC CONCENTRATION (PKC) POPULATION

All participants who received at least 1 dose of AUT00201 with at least 1 evaluable *(i.e., not impacted by any important protocol deviations or other events)* PK concentration *(even if below the limit of quantification).*

6.4 PHARMACOKINETIC PARAMETER (PKP) POPULATION

All participants in the PK concentration population for whom at least one PK parameter can be derived.

6.5 PHARMACODYNAMIC (PD) POPULATION

All participants in the safety population who have an evaluable baseline and at least 1 evaluable postbaseline measurement for at least 1 PD parameter. A PD parameter includes any parameter outlined in Section 9.2.2.

6.6 MODIFIED PHARMACODYNAMIC (MPD) POPULATION

All participants in the pharmacodynamic population with the following stipulation: for re-screened participants, data collected under a previous participant number will be excluded from this analysis population. Unless otherwise specified, this analysis population will be used for all PD analyses.

7. PROTOCOL DEVIATIONS

The Protocol Deviation Plan describes how protocol deviations are collected and handled. Notably, deviations that impact data relating to the secondary endpoints will be classified as "important". Deviations will be reviewed on a monthly basis.

A per-protocol analysis excluding participants with specific important protocol deviations is not planned.

8. GENERAL CONSIDERATIONS FOR DATA ANALYSES

8.1 STANDARD SUMMARY STATISTICS

To fit better with the lower than anticipated recruitment, the protocol text on standard summary statistics has been modified, as follows.

Continuous data will be summarized using the summary statistics: number of observations (n), mean, standard deviation, minimum, median and maximum. Geometric mean and coefficient of variation will also be presented for appropriate PK parameters. Categorical data

will be summarized using the count of participants and/or events. "Missing" categories (corresponding to participants with missing data for the variable being summarized) may be added as the last category in the list of categories being summarized.

The minimum and maximum values will be presented to the same number of decimal places as the raw data collected on the electronic case report form (eCRF) (or to 3 significant figures for derived parameters). The mean, standard deviation and median will be presented to one additional decimal place.

8.2 TREATMENT DEFINITION

"Treatment" is used in this SAP to refer to either the AUT00201 or placebo treatment.

Treatment sequence refers to the sequence in which participants received treatment. There are two planned sequences participants can follow:

- "AUT00201/Placebo" for participants receiving AUT00201 on Study Day 1, and placebo on Study Day 3
- "Placebo/AUT00201" for participant s receiving placebo on Study Day 1, and AUT00201 on Study Day 3

Summaries will be presented by treatment, unless otherwise specified.

Observations made on or after Study Day 1 treatment administration up to Study Day 3 treatment administration will be summarized under the **treatment received** at Study Day 1. Similarly, observations made on or after Study Day 3 treatment administration up to follow-up will be summarized under the **treatment received** at Study Day 3.

8.3 BASELINE DEFINITION

In general, baseline is defined as the last scheduled measurement obtained before the first dose of study treatment. For pharmacodynamic data, the measurement to be used as baseline is defined more precisely in Section 9.2.2.

8.4 STUDY DAY DEFINITION

Study Day is derived based on the date of first treatment (Visit 3). If an assessment/event date is before the date of first treatment, then Study Day is defined as:

(Assessment/event date) – (Date of first treatment).

If an assessment/event date is on or after the date of first treatment, then Study Day is defined as:

(Assessment/event date) – (Date of first treatment) + 1.

8.5 STRATA AND COVARIATES

Given the small sample size, no formal stratification was used for the randomization.

A crossover design is used to reduce the influence of confounding covariates since each participant serves as their own control. As there will be no statistical modelling of the data the use of covariates for analyses is not relevant.

8.6 STANDARD COMPARISON METHODS

Primary, secondary and exploratory endpoints will be summarized descriptively by treatment and, where specified in sections below, differences between treatments will be summarized.

8.7 STATISTICAL SIGNIFICANCE

Not Applicable, see Section 2.4.

8.8 EXAMINATION OF SUBGROUPS

Given the small sample size, no subgroups will be investigated.

9. DATA HANDLING CONVENTIONS

9.1 PREMATURE WITHDRAWAL AND MISSING DATA

Unless specified, all missing or incomplete data, including dates and times, will be treated as missing. Missing test results or assessments will not be imputed.

9.2 DERIVED AND TRANSFORMED DATA

9.2.1 STUDY POPULATION

9.2.1.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics are recorded at Visit 1 (screening).

If required, the following conversions will be used:

- Height collected in inches will be converted into cm using the formula:

$$\text{Height (cm)} = \text{Height (inches)} / 2.54$$

- Weight collected in pounds will be converted into kg using the formula:

$$\text{Weight (kg)} = \text{Weight (pounds)} / 2.205$$

9.2.1.2 MEDICAL HISTORY

The participant's relevant medical history will be collected and coded using version 26.1 of the Medical Dictionary for Regulatory Activities (MedDRA). The participants will be asked specifically regarding their history of seizures, headaches/migraines, and episodes of dizziness. They will also be asked regarding their history of fever and if they/their caregiver had any impression of an improvement of MEAK symptoms during the fever.

9.2.1.3 PRIOR AND CONCOMITANT MEDICATIONS

Any medication or vaccine that the participant is receiving at Visit 1 (screening) or receives during the study through Visit 7 (follow-up) will be collected on the eCRF. Details for medication taken before screening may also be recorded. All medications will be coded according to version September 2022 of the World Health Organization Drug Dictionary (WHODD).

A prior medication is defined as any medication taken prior to the first dose of treatment. A concomitant medication is defined as any medication continued or newly received on or after

the first dose of treatment. Partial or missing start and stop dates will not be imputed as outlined in Section 9.1.

In the prior and concomitant medication listing, medications will be flagged as either prior, concomitant or both, using the flags P, C and PC, respectively. If there is insufficient information from the start and end dates/times to attribute a medication to one of these categories no flag will be presented.

9.2.1.4 TREATMENT ADMINISTRATION

Participants will receive a single dose of 100 mg AUT00201 or matching placebo (one 100 mg capsule) on Study Days 1 and 3, approximately 30 minutes after completion of a standard meal provided by the clinical site. Date and time of administration, time meal was completed, and reason for not taking a dose if applicable will be captured in the eCRF.

9.2.2 PHARMACODYNAMIC TESTS

9.2.2.1 PAIRED-PULSE TMS EMG

Paired-pulse TMS allows for measurement of cortical inhibition. The two key outcome measures are short interval cortical inhibition (SICI) and long interval cortical inhibition (LICI).

SICI has been shown to be reduced in other myoclonic epilepsies (Unverricht-Lundborg disease (ULD) and Lafora body disease [LBD]; (Canafoglia, 2010)). It is postulated that MEAK patients will show a similar deficit. A defective in LICI was found in LBD but not ULD patients; it is unknown whether MEAK patients will have a LICI deficit.

Initially, the resting motor threshold will be recorded, which is defined as the lowest stimulus intensity (expressed as a percentage of maximal stimulator output, %MSO) required to induce motor evoked potentials of 50μV.

SICI will be elicited with a conditioning stimulus of 70% of resting motor threshold (0.7*rMT) and interstimulus interval of 1, 2.5, and 3 ms; LICI will be elicited with a conditioning stimulus of 120% the resting motor threshold (1.2*rMT) that precedes the test stimulus by 100 and 200 ms.

SICI 1ms is considered to be mediated by tonic γ-aminobutyric acid (GABA). SICI 2.5 and 3 ms are mediated by synaptic $GABA_A$, as evidenced that $GABA_A$ agonists enhance SICI (Ziemann, 2004). It is proposed that LICI is mediated by activation of inhibitory post-synaptic $GABA_B$ receptors (Ziemann, 2004).

At each SICI/LICI interval, an observation will be reported in %-inhibition.

The initial assessment will be timed to coincide with the anticipated AUT00201 t_{max}, between 2 to 4 hours postdose. The assessment will take place between 11am and 1pm on Study Days -1, 1, 2, 3 and 4, as the participant will take their study treatment at 9am on Study Days 1 and 3. A second assessment will be conducted approximately 6 hours postdose, on Study Days 1 and 3. The data collected at the second timepoint will be listed and may be used to inform the PK/PD relationship.

The measurements collected on Admission (Study Day -1) will be the baseline. At each assessment, values for each of the following will be collected:

- Resting motor threshold
- SICI 1ms
- SICI 2.5ms
- SICI 3ms
- LICI 100ms
- LICI 200ms

The average of the SICI 2.5ms and 3ms data will be derived for the assessments 2 to 4 hours postdose on each day and 6 hours postdose on Study Days 1 and 3.

The secondary endpoint

- Change from baseline in cortical inhibition as assessed with paired-pulse TMS EMG:
  - SICI as measured by paired-pulse TMS EMG

will be investigated using the changes from baseline of the averaged SICI 2.5 and 3ms measurements from the 2 to 4 hour postdose assessment. SICI is expected to increase with successful treatment.

Additionally, the following SICI measurements are considered as exploratory endpoints:

- SICI 1, 2.5 and 3ms measurements, at each timepoint
- Average of the SICI 2.5 and 3ms measurements from the 6 hour timepoint

The exploratory endpoint

- Change from baseline in cortical inhibition as assessed with paired-pulse TMS EMG:
  - LICI as measured by paired-pulse TMS EMG

will be investigated using the changes from baseline in LICI 100ms and 200ms measurements at each timepoint. LICI is expected to increase with successful treatment.

9.2.2.2 SPEECH AND VOCAL TESTS

Speech analysis can pick up subtle signs of dysarthria. A battery of speech tests will be utilized to assess participant's speech and derive specific measures of dysarthria. In total, 11 speech metrics will be assessed, as part of four tests, as shown in Table 1.

Table 1: Speech Tests

Metric Name	Metric Description	Unit	Feasible Values
Diadochokinetic Rate (DDK) (<1 minute)			
Buttercup Count	Number of times that the prompted word was repeated	buttercups	>0
Picture Description (1 minute)			
Description Duration	Duration of the response to a picture; averaged across all picture descriptions	seconds	0 to 60

Monotonicity (Picture)	Average pitch variation across all picture descriptions	Hz	0 to 100
Search Time	Mean duration of the response to a picture; averaged across all picture descriptions	seconds	>0
Sentence Reading (2 minutes)			
Articulation Rate	Articulation rate (speed of individual speech sounds) averaged across all sentences in the sentence reading task	syllables/second	>0
Articulatory Precision	A measure of how precisely words are pronounced for all sentences in the sentence reading task. Expected range of possible values is around -10 to 0 where -10 is the most impaired and 0 is the most precise, but values lower than -10 are possible	ratio	-10 to 0
Monotonicity (Sentences)	Pitch variation, averaged across all sentences in a sentence reading task	Hz	0 to 100
Speaking Rate	A measure of how quickly speech is produced, on average, across all sentences in the sentence reading task	syllables/second	>0
Sustained Phonation (<1 minute)			
Breathy Vocal Quality	Vocal quality score, averaged across sustained phonation attempts	dB	0 - 40
Maximum Phonation Time	Longest duration of vowel phonation across all sustained phonation attempts	seconds	>0
Pitch Instability	Instability in fundamental frequency (a measure of variability in pitch; used to measure vocal control through fluctuation in the pitch pattern), averaged across all sustained phonation tasks	Hz	>0

Articulatory Precision was originally identified as the most relevant measure of dysarthria for this population; as it is a measure of motor coordination and cerebellar control. Physicians have reported patients as having 'scanning' dysarthria due to their ataxia. However, only 2 participants had valid articulatory precision data from both Visits 3 and Visit 5 (Study Day 1 and Day 3). The Speaking Rate has been selected as another relevant measure as this is also linked to motor control and the Buttercup Count is another measure of interest, as this requires high levels of coordination of different muscle groups to pronounce. Other measures were considered less sensitive and/or linked to muscle weakness which is unknown in this population.

Speech tests will be conducted on Admission (Study Day -1) and Study Days 1, 2, 3, and 4. The measurements collected on Admission (Study Day -1) will be the baseline.

The secondary endpoint

- Change from baseline in:

- Measures of dysarthria as assessed by automated standardized speech and vocal tests

will be investigated using the changes from baseline in speaking rate and buttercup count. Both are expected to increase with successful treatment. The other metrics are considered as exploratory endpoints.

9.2.2.3 EMG AND ACCELEROMETER

Surface EMG and accelerometry can provide valuable information about the nature and the source of symptoms (e.g., tremor and myoclonus) in movement disorders.

Participants will wear EMG and Accelerometer devices on their dominant arm (bicep brachii [BB] muscle) and non-dominant arm (extensor digitorum communis [EDC] muscle on the forearm) from Visit 2 (Study Day -1) through Visit 6 (Study Day 4), the duration of the inpatient portion of the study. These devices will take measurements to assess positive and negative myoclonus.

Participants will have 'action myoclonus' assessments on Study Days -1, 1, 2, 3 and 4 where they complete sub-sections 4D and 4E of the Unified Myoclonus Rating Scale (UMRS) assessment. The UMRS tasks are described below in Section 9.2.2.4. On Study Days -1, 1 and 3, the assessments will be at specified timepoints from 8am till 5pm (see Appendix A – Schedule of assessments, relating to 1 hour predose until 8 hours postdose on Study Days 1 and 3). On Study Days 2 and 4, the assessments will be at specified timepoints from 8am until 1pm (see Appendix A – Schedule of assessments). Note the 9am timepoint on Study Days 1 and 3 is postdose. At each timepoint participants will be asked to complete a set of movements of their arms in accordance with the UMRS Section 4: Parts D and E. At 11am (relating to 2 hours postdose) on Study Days -1, 1 and 3, UMRS Sections 4 and 5 will be performed. The positive myoclonus index and negative myoclonus for each arm will be calculated during the 20 minutes that the participants complete the UMRS Section 4 and 5 assessments; they will also be calculated during the specific sub-sections 4D and 4E at this timepoint separately.

The measurements collected on Admission (Study Day -1) will be used to derive the baseline. If an endpoint involves averaging measurements across arms or across timepoints, then the baseline derivation will follow the same approach.

Positive myoclonus, assessed using the Myoclonus Index (MI)

Positive myoclonus means an involuntary and quick jerk of muscles that may occur spontaneously. The positive myoclonus will be quantified using the myoclonus index (MI), for each arm, over the duration of each action myoclonus assessment. MI is calculated (Rissanen, 2021) as a score >0, expressed as a decimal.

The most interesting period of assessment is up to 4 hours postdose (anticipated to cover t_{max}). To investigate this period, at each timepoint from 9am until 1pm, measurements across action myoclonus assessments from both arms will be averaged to calculate a single MI. For the 11am timepoint, there will be one UMRS Section 4 and 5 MI measurement (UMRS 4 and 5 is combined) for each arm as they complete both tasks, the MI from the two arms will be averaged to provide the 2 hour postdose MI. These single MI measurements from the 9am to

1pm timepoints will then be averaged. The calculation of MI during the sub-sections 4D and 4E at 11am will not be used in any analyses.

The secondary endpoint

- Change from baseline in:
  - MI (positive myoclonus) evaluated with EMG and accelerometer

will be investigated using the changes from baseline in the average MI across each action myoclonus/UMRS assessment from both arms, from 9am until 1pm (using the UMRS 4 and 5 score at 11am). Positive myoclonus is expected to decrease with successful treatment.

The MI measured for each arm (dominant and non-dominant, rather than left and right) separately and averaged across arms during each action myoclonus assessment, during UMRS Section 4 and during UMRS Section 5 are considered exploratory endpoints.

Negative myoclonus

Negative myoclonus means a sudden and brief loss of muscular tone that may lead, for example, to the loss of posture or the dropping of objects from hands. Negative myoclonus is recorded as a count, for each arm, which reflects the frequency of negative myoclonus over the duration of each action myoclonus assessment (Rissanen, 2021).

As with positive myoclonus, to investigate the up to 4 hours postdose period, at each timepoint from 9am until 1pm, measurements across action myoclonus assessments from both arms will be averaged to calculate a single negative myoclonus. As above, for the 11am timepoint, UMRS Section 4 and 5 measurements from the two arms will be averaged and will be used as the 2 hour postdose negative myoclonus. These single MI measurements from the 9am to 1pm timepoints will then be averaged.

The exploratory endpoint

- Change from baseline in:
  - Amount of negative myoclonus evaluated with EMG and accelerometer over time and during each UMRS action myoclonus Section 4: D and E and each UMRS Sections 4 and 5.

will be investigated using the same methods as described for positive myoclonus. Negative myoclonus is expected to decrease with successful treatment.

9.2.2.4 UMRS Sections 4 and 5 (Clinical Rating)

The UMRS is a quantitative 73-item clinical rating instrument developed to evaluate myoclonus and response, of patients with myoclonus, to antimyoclonic therapy. The scale consists of 8 sections. The UMRS is the most widely used standardized assessment of myoclonus and is appropriate in this patient population as a measure of the action induced myoclonus symptom associated with MEAK.

In this study, only UMRS Sections 4 and 5 will be evaluated on Admission (Study Day -1), and Study Days 1 and 3 at 11am (i.e., relating to 2 hours postdose on Study Days 1 and 3). The measurements collected on Admission (Study Day -1) will be the baseline. Additional

assessment (two items) of the non-dominant arm has been added to the standard UMRS Section 5 to obtain a more complete data set.

Section 4 – severity of myoclonus with action (frequency and amplitude, 10 items)

- Items are: eyelids (A), neck (B), trunk (C), right arm (D), left arm (E), right leg (F), left leg (G), arising (H), standing (I), walking (J)

Section 5 – assess performance on functional tests (7 items)

- Items are: writing (A), right hand spiral (B), left hand spiral (C), pouring water (dominant hand) (D1), pouring water (non-dominant hand) (D2), soup spoon (dominant hand) (E1), soup spoon (non-dominant hand) (E2)

Participants will be videoed performing the assessment, and the videos are submitted for scoring by a single centralized scorer. The scorer is blinded to the order of the videos and no cues are included in the video as to the timepoint of the assessment.

Following the scoring instruction provided in the original paper (Frucht, 2002), Section 4 includes ratings of frequency and amplitude of myoclonic jerks each on a scale of 0 to 4 affecting each body region. Total score for Section 4 is calculated by multiplying frequency by amplitude scores for each body region, and then adding, with a maximum score of 160. Section 5 includes ratings on a scale of 0 to 4, each to assess performance on different functional tests. Total score for Section 5 of the UMRS is calculated by simple addition, with a maximum score of 28.

The exploratory endpoint

- Change from baseline in:
    - UMRS subscale clinical scores: Sections 4 and 5

will be investigated by using the changes from baseline in the Section 4 and Section 5 total scores. UMRS scores are expected to decrease with successful treatment, however it is not expected that a clinical rating will be sensitive enough to capture a drug effect after a single dose treatment.

9.2.2.5 UMRS SECTION 4: PARTS D AND E

A predominant symptom for patients is an action-induced myoclonus. To ensure assessment of this symptom over the duration of the study, the participants will need to be active at regular intervals, more than the assessments for UMRS Sections 4 and 5 alone.

At the specified assessments, participants will be asked to complete a set of movements of their arms in accordance with the UMRS Section 4: Parts D (for the right arm) and E (for the left arm). As in Section 9.2.2.4, participants are videoed, and the video is scored by a single centralized scorer. Items D and E are rated on a scale of 0 to 4 for amplitude and frequency and the frequency and amplitude scores are multiplied to produce a maximum total myoclonus clinical score of 16 for each item. When analyzing these data, the Part D and E scores will be mapped to the dominant and non-dominant arms.

The measurements collected on Admission (Study Day -1) will be used as baselines for the corresponding timepoints.

The exploratory endpoint

- Change from baseline in:
  - UMRS action myoclonus clinical scores: Section 4: D and E

will be investigated using the changes from baseline in the dominant and non-dominant arm scores.

9.2.2.6 ELECTROENCEPHALOGRAMS (EEGS)

An EEG is a test that measures electrical activity in the brain. The EEG will be recorded in the resting state, with eyes open and eyes closed, on Admission (Study Day -1) and Study Days 1 and 3, at 1pm (relating to 4 hours postdose on Study Days 1 and 3). The measurements collected on Admission (Study Day -1) will be the baseline for the calculation of changes from baseline.

Quantitative EEG measures

During a resting state, at electrodes Fz and Pz, absolute power ($\mu V^2/Hz$) in the following canonical frequency bands will be collected: θ, δ, α, β, γ_1 (low-gamma), γ_2 (high gamma). In addition to absolute power in the above frequency bands, absolute total power ($\mu V^2/Hz$) will also be collected at electrodes Fz and Pz. Relative power (%) is calculated for each of the frequency bands selected for reporting, by calculating the absolute power as a percentage of absolute total power at the electrode at which it is measured.

The most appropriate frequencies, electrodes and states were selected for reporting:

- absolute γ_1 (low gamma) power at Fz during eyes-closed
- relative γ_1 (low gamma) power at Fz during eyes-closed
- absolute γ_1 (low gamma) power at Fz during eyes-open
- relative γ_1 (low gamma) power at Fz during eyes-open

- absolute γ_2 (high gamma) power at Fz during eyes-closed
- relative γ_2 (high gamma power at Fz during eyes-closed
- absolute γ_2 (high gamma power at Fz during eyes-open
- relative γ_2 (high gamma power at Fz during eyes-open

- absolute α (alpha) power at Pz during eyes-closed
- relative α (alpha) power at Pz during eyes-closed
- absolute α (alpha) power at Pz during eyes-open
- relative α (alpha) power at Pz during eyes-open

- α (alpha) spectral centroid at Pz during eyes-closed
- α (alpha) spectral centroid at Pz during eyes-open

- absolute total power at Fz during eyes-closed
- absolute total power at Fz during eyes-open
- absolute total power at Pz during eyes-closed
- absolute total power at Pz during eyes-open

The exploratory endpoint

- Change from baseline in
  - Quantitative EEG measures (absolute and relative power of predefined frequency bands)

will be investigated using the changes from baseline in absolute and relative power, for all frequency bands above. The primary focus will be γ_1 (low gamma) absolute power at the Fz electrode in the eyes open state. It is expected that patients with MEAK will show increased power in resting state gamma (γ_1). Successful treatment is expected to normalize such abnormalities.

The alpha spectral centroid indicates the frequency within the alpha band that shows the highest power. It is speculated that patients with MEAK show a left shift (to lower frequencies) of the alpha spectral centroid. Successful treatment is expected to shift it to the right (to higher frequencies).

EEG Power and Phase Synchrony Induced by Chirp Stimulus

An EEG will also be recorded during the presentation of an auditory chirp stimulus. About 300 chirp stimuli will be presented separated by an intertrial interval randomly jittered between 1500 and 2000 ms. Induced and evoked power of gamma oscillations, and phase synchrony, will be evaluated in 8 predefined frequency bands ranging from 20 to 84 Hz (in steps of 8 Hz), with the lowest frequency band being 20-28 Hz. Power and phase synchrony will be measured at both electrodes Fz and Cz.

The exploratory endpoint

- Change from baseline in:
  - EEG power and phase locking factor induced by chirp stimulus in predefined frequency bands

will be investigated using the changes from baseline in absolute power and phase synchrony at electrode Fz, for the 8 frequency bands defined above. The primary focus will be absolute power and phase synchrony at electrode Fz at 40 Hz (low gamma), which corresponds to the 36-44 Hz frequency band. It is expected that patients with MEAK will show decreases both in power of evoked and induced gamma frequency and in phase synchrony during the presentation of the chirp stimulus. Successful treatment is expected to normalize such abnormalities.

9.2.2.7 EARLY VISUAL PROCESSING TESTS

The measures of early visual processing will include tests of binocular rivalry (10 minutes) and internal noise estimation (15 minutes). All responses from the participant will be verbal. These will be conducted on Admission (Study Day -1) and following dosing on Study Days 1 and 3. The measurements collected on Admission (Study Day -1) will be the baseline for the calculation of changes from baseline.

Binocular rivalry is made up of two components, whilst internal noise estimation is made up of four components, as shown in Table 2.

Table 2: Measures of Early Visual Processing

Name	Description	Unit
Binocular rivalry		
Alternation Rate	how quickly the perception switches in the rivalry task	switches/second
Median Dominance Duration	inverse of the Alternation Rate (shows typical percept duration)	seconds
Internal noise estimation		
Percent Correct	percentage of trials answered correctly (a measure of task performance)	percent
D-Prime	also measure of task performance just on a different scale than percent correct (also corrects for possible response biases)	N/A
Consistency	percentage of second pass trials answered exactly the same on the first pass (each trial is tested twice)	percent
Internal Noise Estimate	estimate of internal noise where consistency is corrected for percent correct (high consistency = less noise, but the exact value depends on percent correct)	A.U.

The exploratory endpoint

- Change from baseline in:
  - Measures of early visual processing as assess by:
    - Binocular rivalry
    - An internal noise estimation

will be investigated using the changes from baseline in the median dominance duration (binocular rivalry) and internal noise estimate (internal noise estimation). Median dominance duration is expected to increase, while internal noise estimate is expected to decrease with successful treatment.

9.2.2.8 AUDIOLOGY TESTS

Auditory processing will be assessed using two methods: otoacoustic emissions (OAEs) and words-in-noise (WIN) tests.

Otoacoustic Emissions Test

The OAEs are recordable sounds generated by the inner ear when tones are played to the ears of the participant. The OAE test will be repeated at frequencies of 1, 1.5, 2, 3 and 4 kHz, and will measure a signal (dBs) and noise (dBs). The test will be rerun if a reproducibility measurement of <85% recorded.

The signal and noise measurements will allow for the calculation of a signal-to-noise ratio (SNR) in dB, using the following:

SNR at X kHz = Signal at X kHz – Noise at X kHz.

The SNR will be calculated for the following:

- right ear (independent)
- left ear (independent)
- right ear (no noise)
- left ear (no noise)
- right ear (noise presented to left)
- left ear (noise presented to right)
- right ear (contralateral noise suppression)
- left ear (contralateral noise suppression)

Right ear contralateral noise suppression will be calculated using the following equation:

right ear (contralateral noise suppression) = right ear (no noise) – right ear (noise presented to left)

The result is analogous for left ear (contralateral noise suppression).

The OAEs will be assessed on Admission (Study Day -1) and Study Days 1 and 3 at 2pm (relating to 5 hours post-dose on Study Days 1 and 3). The measurements collected on Admission (Study Day -1) will be the baseline.

The exploratory endpoint

- Change from baseline in:
    - Measures of auditory processing as assessed by:
        - Otoacoustic Emissions (OAE)

will be investigated using the changes from baseline in the SNRs for all parameters above, but the primary focus will be with the right and left ear (independent) responses. SNRs for the independent responses are expected to be larger with successful treatment.

Words-In-Noise (WIN) Test

The WIN test was developed as an instrument to quantify the ability of listeners to recognize monosyllabic words in background noise. The WIN test involves the presentation of target words in a background of multi-talker babble set at SNRs from 24 to 0 dB in 4 dB decrements. The participant will have to repeat the word that they hear back to the trained study staff. The outcome measure is the SNR at which 50% of words can be correctly identified as calculated using the Spearman-Kärber equation, which is written as

$$T_{50\%} = 24 + (0.5*4) – (4*r/5)$$

where r is the correct number of responses.

The WIN test will be assessed on Admission (Study Day -1) and Study Days 1 and 3 at 2pm (relating to 5 hours post-dose on Study Days 1 and 3). On each occasion, the test will be performed at both 70-dB HL and 40-dB HL binaurally. The measurements collected on Admission (Study Day -1) will be the baseline.

The exploratory endpoint

- Change from baseline in:
    - Measures of auditory processing as assessed by:

- Words-in-noise (WIN) test performance (signal-to-noise ratio [SNR])

will be investigated using the changes from baseline in the SNRs from the 70-dB HL and 40-dB HL tests separately. SNRs are expected to be smaller with successful treatment.

9.2.3 SAFETY DERIVATIONS

9.2.3.1 ADVERSE EVENTS

Adverse events, based on clinical signs and symptoms and laboratory measurements, will be monitored throughout the study and will be collected from the time the informed consent is signed until their follow-up (end of study) phone call. The term AE is used to include both serious and non-serious AEs.

A treatment-emergent adverse event (TEAE) is defined as any AE that begins, or any pre-existing AE that worsens in severity or frequency, on or after the first dose of treatment, but no later than the 48 hours after the participant's last treatment. Any pre-existing AE that worsens in severity or frequency will be recorded as a new AE in the eCRF.

An adverse event will be regarded as treatment-emergent unless there is clear evidence to indicate it started before dosing on Study Day 1:

- there is sufficient information from the partial end date/time to indicate the event ended before the participant's first dose (Study Day 1).
- there is sufficient information from the partial start date/time to indicate the event started before the participant's first dose (Study Day 1).

TEAEs before study dosing on Study Day 3 will be summarized under the **treatment received** on Study Day 1. TEAEs on or after dosing on Study Day 3 treatment administration, but no later than 48 hours after dosing, will be summarized under the **treatment received** on Study Day 3. A TEAE will be summarized under both treatments unless there is clear evidence to indicate it either started before or after dosing on Study Day 3:

- there is sufficient information from the partial end date/time to indicate the event ended before the participant's second dose (Study Day 3).
- there is sufficient information from the partial start date/time to indicate the event started after the participant's second dose (Study Day 3).

AEs recorded between the screening visit and dosing on Study Day 1 will be reported as pre-treatment AEs and those from 48 hours after the participant's last treatment will be recorded as follow-up AEs. Listings will identify whether an AE is pre-treatment, treatment-emergent or during follow-up.

The following variables will be collected on the eCRF for each AE:

- AE term (verbatim)
- Date/time when the AE started and stopped
- Severity (Grade 1 - mild, Grade 2 - moderate, Grade 3 - severe, Grade 4 – life threatening, Grade 5 – death)

- Seriousness (yes or no)
- Relationship to study treatment (not related, unlikely related, possibly related, related)
- Action taken with study treatment (dose not changed, drug withdrawn, not applicable, unknown)
- Outcome (fatal, not recovered/not resolved, recovered/resolved, recovered/resolved with sequelae, recovering/resolving, unknown)
- Did the AE cause the participant to be discontinued from the study (yes or no)

Adverse events will be coded using version 26.1 of the MedDRA.

TEAEs with missing severity and/or causality will be treated as severe and possibly related, respectively, for tabulations. The information will be presented as missing in the listing.

For TEAE summaries, TEAEs summarized as treatment-related are defined as those classified as either "possibly related" or "related" to treatment by the Investigator in the eCRF.

The duration of an AE is calculated as follows:

Duration (hours)= (End datetime of AE – Start datetime of AE)

If start or end datetime is partially or completely missing, then duration will be set to missing.

9.2.3.2 CLINICAL LABORATORY EVALUATIONS

Samples for clinical chemistry, hematology, urinalysis, hormones and serology will be assessed during the study at the visits as defined in Appendix A – Schedule of assessments. Additional safety samples may be collected if clinically indicated at the discretion of the Investigator. Units will be standardized if samples are assayed using different laboratories.

Change from baseline (Screening Visit 1 measurement) will be calculated for each continuous laboratory parameter.

9.2.3.3 VITAL SIGNS

Sitting and standing (where feasible) blood pressure, sitting pulse rate, respiratory rate, and body temperature will be assessed at the timepoints indicated in Appendix A – Schedule of assessments. Unscheduled measurement of vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required. Body temperature, blood pressure, respiratory rate, and pulse rate will be measured singly and repeated once if outside the relevant clinical reference ranges.

Body temperature collected in degrees Fahrenheit (°F) will be converted into degrees Celsius (°C) using the formula:

Temperature (°C) = (Temperature (°F) - 32) × 5/9

Change from baseline (Study Day -1 measurement) will be calculated for each vital sign.

9.2.3.4 PHYSICAL EXAMINATION

A full physical examination will be performed at screening (Visit 1) and admission on Day -1 (Visit 2) and a brief or targeted physical examination will be performed approximately 24 hours after each treatment (i.e., Study Days 2 and 4).

The Study Day -1 measurement will be used as baseline when assessing changes from baseline.

9.2.3.5 ECGS

The 12-lead ECGs will be obtained at the timepoints specified in Appendix A – Schedule of assessments.

Change from baseline (Screening Visit 1 measurement) will be calculated for each ECG parameter.

9.2.3.6 COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

The C-SSRS will be administered at the timepoints specified in Appendix A – Schedule of assessments. The C-SSRS is a clinician-rated instrument that captures the occurrence, severity, and frequency of suicidal ideation and/or behavior during the assessment period.

During study, the C-SSRS interview format will be administered by trained site staff. The C-SSRS will monitor for any changes in suicide ideation and/or behavior in patients participating in this trial. The "screening" C-SSRS will be used at Visit 1 to capture suicidal ideation in the past 6 months and suicidal behavior in the past 1 year, and the "since last visit" C-SSRS will be used at all other timepoints.

9.2.4 PATIENT REPORTED MEASURES

Patient-Reported Outcome Measurement (PROM) – ataxia

The PROM of ataxia is a valid and reliable measure of motor ataxia, quality of life, and mental health. It is a 70-item questionnaire completed on a 5-point Likert scale (scores range from 0-4, with a maximum possible total score of 280), and will be evaluated at screening (Visit 1). The questionnaire is comprised of the following subsections: physical, activities of daily living, mental 1 (symptoms of depression and anxiety) and mental 2 (cognition).

Patient/clinician impression of treatment

The participant and clinician will be asked (separately) if they believe the participant received AUT00201 or placebo 24 hours ±2 hours after each dose (i.e., Study Days 1 and 3) and if they perceived any change in their condition.

Patient top 3 concerns

Participants will be asked to give their top 3 concerns (responses as free text) regarding their condition and functioning in their daily life, at screening (Visit 1). They will then be asked to score the severity of the concern on a 7-point Likert scale (scores range from 1-7).

9.3 CLINICAL PHARMACOLOGY

9.3.1 DERIVATION OF PHARMACOKINETIC PARAMETERS

Blood samples for the determination of AUT00201 plasma concentrations will be collected at the timepoints specified in Appendix A – Schedule of assessments.

The PK parameters of AUT00201 (in Table 3) will be derived using noncompartmental analysis methods.

Table 3: Pharmacokinetic Parameters

Abbreviation	Definition	Calculation
Concentrations and times		
C_{max}	Maximum (peak) plasma concentration	The maximum (peak) plasma concentration will be obtained directly from the concentration-time data
t_{max}	Time to reach maximum (peak) plasma concentration	The first time of maximum (peak) plasma concentration will be obtained directly from the concentration-time data
Half-life		
λ_z	Terminal rate constant	The apparent terminal phase rate constant (λ_z) will be estimated by linear regression of logarithmically transformed concentration versus time data.
$t_{½}$	Terminal half-life	The terminal half-life calculated from the terminal slope of the log concentration-time curve, as follows: $t_{½} = \log_e(2) / \lambda_z$
Areas under the curve		
AUC_t	Area under the plasma concentration-time curve from time zero to 24 hours	The area under the concentration-time curve from zero time (predose) to the last observed quantifiable concentration (C_t) will be calculated using the log-linear trapezoidal method
AUC_∞	Area under the plasma concentration-time curve from time zero to infinity	The area under the concentration-time curve will be calculated using the log-linear trapezoidal method for the interval 0 to t (time t is the time at which the last nonzero level was recorded), plus the area under the exponential curve from t to

		infinity, calculated as follows: $AUC_{t-\infty} = \hat{C}_t / \lambda_z$ where $\hat{C}_t$ is the predicted value of the concentration at t. The percentage of AUC_∞ obtained by extrapolation ($\%AUC_{ex}$) will be calculated as follows: $\%AUC_{ex} = 100*(AUC_\infty - AUC_t) / AUC_\infty$
Clearance, volume of distribution, and mean residence time		
CL/F	Apparent total clearance from plasma after oral administration	Apparent total clearance from plasma will be calculated using the following formula: $CL/F = Dose / AUC_\infty$
V_z/F	Apparent volume of distribution during terminal phase after non-intravenous administration	Apparent volume of distribution will be calculated using the following formula: $V_z/F = Dose / \lambda_z \bullet AUC_\infty$
MRT	Mean Residence Time	The mean residence time will be calculated using: $MRT = AUMC / AUC_\infty$

Actual times will be used to derive PK parameters. For calculation of all PK parameters, plasma concentrations below the limit of quantification of the assay (BLQ) will be treated as follows:

- Values that occur before t_{max} will be taken as zero; all other values will be taken as missing.

For calculation of plasma concentration summary statistics, BLQ values will be taken as zero.

The AUC_∞ values with a percentage of extrapolation >20% will be presented in a separate table and not included in the summary table of the PK results.

The apparent terminal phase rate constant (λ_z) estimated by linear regression of logarithmically transformed concentration versus time data and the associated AUC_∞ will be presented in a separate table and not included in the summary table of results if the estimated R^2 value will be < 0.8.

9.4 ASSESSMENT TIME WINDOWS

Due to the nature of this trial, there will be no visit windowing applied other than the identification of baseline as per definitions given in Section 8.3.

Data collected at unscheduled visits will be excluded from summaries but will be included in listings.

10. STATISTICAL ANALYSES AND METHODOLOGY

10.1 STUDY POPULATION

10.1.1 DISPOSITION OF PARTICIPANTS

The number of participants will be tabulated for the following:

1. Screened (including rescreened participants)
2. Rescreened
3. Screen failure (with reasons)
4. Dosed on Study Day 1
5. Dosed on Study Day 3
6. Completed Period 1
7. Completed Period 2
8. Completed the Study (all visits including phone follow-up)
9. Discontinued the Study (with reasons)

Rows (1) - (9) will be tabulated over all participants in the screened population. The number of participants in each analysis population will also be tabulated over all participants in the screened population. Participant disposition will be listed.

10.1.2 PROTOCOL DEVIATIONS

A listing of all participants with one or more important/non important protocol deviations will be presented for the screened population. The listing will identify the last treatment received prior to the deviation; no treatment will be listed if the deviation is prior to any treatment.

10.1.3 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

The following analyses and listings will apply to the safety population.

Age, sex, race, ethnicity, height, weight and body mass index (BMI) will be summarized. All demographic data will be listed.

Medical history (including MedDRA system organ class [SOC] and preferred term [PT]), history of fever and whether an improvement of MEAK symptoms during fever was observed will be listed.

Prior and concomitant medications will be listed with each medication being identified as either prior, concomitant or both (P, C or PC, respectively). Study day and treatment sequence will be included to help identify when the medication was taken in relation to study treatment.

Regular concomitant medication administration dates and times through Visits 3 to 6 will also be listed.

10.1.4 TREATMENT ADMINISTRATION

Dates and times of treatment administration, time meal was completed prior to this, and reason for not taking a dose if applicable will be listed.

10.1.5 PATIENT REPORTED MEASURES

The following patient reported measures will be listed:

- PROM – ataxia
- Patient/clinician impression of treatment
- Patient top 3 concerns

10.2 PHARMACODYNAMIC ANALYSES

10.2.1 PHARMACODYNAMIC ENDPOINTS

Table 4 shows the timepoints at which each of the PD assessments are collected, and outlines each of the analyses that will be performed for the secondary and exploratory endpoints. Analyses will include summary statistics for the change from baseline for participants in the mPD population. To produce placebo-controlled PD outcomes measures, within-participant treatment differences for each PD endpoint will also be calculated.

Table 4: Summary of PD Endpoints

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
Paired-Pulse TMS EMG	-1, 1, 2, 3 and 4	➢ 2 to 4 hours postdose ➢ 6 hours postdose	1. Resting Motor Threshold 2. SICI 1ms 3. SICI 2.5ms 4. SICI 3ms 5. LICI 100ms 6. LICI 200ms	1. SICI: Average of the 2.5ms and 3ms at 2 to 4 hours postdose [a]	1. Resting Motor Threshold 2. SICI: 1, 2.5 and 3ms, at 2 to 4 and 6 hours postdose 3. SICI: Average of the 2.5ms and 3ms at 6 hours postdose [a] 4. LICI: 100 and 200ms at 2 to 4 and 6 hours postdose
Speech and Vocal Tests	-1, 1, 2, 3 and 4	➢ 1 hour postdose	1. Buttercup Count 2. Description Duration 3. Monotonicity (Picture) 4. Search Time 5. Articulation Rate 6. Articulatory Precision 7. Monotonicity (Sentences) 8. Speaking Rate 9. Breathy Vocal Quality 10. Maximum Phonation Time 11. Pitch Instability	1. Speaking Rate 2. Buttercup Count	1. Description Duration 2. Monotonicity (Picture) 3. Search Time 4. Articulation Rate 5. Articulatory Precision 6. Monotonicity (Sentences) 7. Breathy Vocal Quality 8. Maximum Phonation Time 9. Pitch Instability

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
Myoclonus index	-1, 1, 2, 3 and 4	➢ -1, 0, 1, 3, 4 hours postdose (all Days) and 5, 6, 7 and 8 hours postdose (Days -1, 1 and 3 only), for each arm ➢ At 2 hours postdose UMRS Sections 4 and 5, for each arm	1. Measurement at each timepoint, for each arm 2. Measurement during Section 4 and 5 (2 hours postdose), for each arm	1. Average MI from 0 to 4 hours postdose, using MI averaged across arms at each timepoint [a]	1. MI measured for each arm (dominant and non-dominant) separately during: • Each action myoclonus assessment • UMRS Section 4 and 5 2. MI averaged across arms [a] during: • Each action myoclonus assessment • UMRS Section 4 and 5
Negative myoclonus	-1, 1, 2, 3 and 4	➢ -1, 0, 1, 3, 4 hours postdose (all Days) and 5, 6, 7 and 8 hours postdose (Days -1, 1	1. Measurement at each timepoint, for each arm 2. Measurement during Section 4 and 5 (2 hours postdose), for each arm		1. Average negative myoclonus from 0 to 4 hours postdose, using negative myoclonus averaged across arms at each timepoint ([a] 2. Negative myoclonus measured for each arm (dominant and non-dominant) separately during:

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
		and 3 only), for each arm ➢ At 2 hours postdose UMRS Sections 4 and 5, for each arm			• Each action myoclonus assessment • UMRS Section 4 and 5 3. Negative myoclonus averaged across arms [a] during: • Each action myoclonus assessment • UMRS Section 4 and 5
UMRS Section 4 and 5 Clinical Ratings	-1, 1 and 3	➢ 2 hours postdose	1. Section 4 Total Score 2. Section 5 Total Score		1. Section 4 Total Score [a] 2. Section 5 Total Score [a]
UMRS Section 4: Parts D and E Clinical Ratings	-1, 1, 2, 3 and 4	➢ -1, 0, 1, 3, 4 hours and 5, 6, 7 and 8 hours (Days -1, 1 and 3 only)	1. Section 4: Part D Score (Right Arm) 2. Section 4: Part E Score (Left Arm)		1. Dominant arm score 2. Non-dominant arm score
EEG absolute and relative power	-1, 1 and 3	➢ 4 hours postdose	1. Absolute and relative power in the α, γ_1 and γ_2 (low and high gamma) canonical frequency		1. Absolute and relative power in the α, γ_1 and γ_2 canonical frequency bands in eyes open and eyes closed states

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
			bands in eyes open and eyes closed states. Fz and Pz electrodes will be used for the γ and α powers, respectively		
EEG	-1, 1 and 3	➢ 4 hours postdose	1. Alpha spectral centroid, from the Pz electrode, in eyes open and eyes closed states		1. Alpha spectral centroid, from the Pz electrode, in eyes open and eyes closed states
EEG Power and Phase Synchrony Induced by Chirp Stimulus	-1, 1 and 3	➢ 4 hours postdose	1. Power and phase synchrony in discrete frequency bands from 20 Hz to 84 Hz (in steps of 8 Hz) covering low and high gamma frequencies measures at electrode Fz		1. Power and phase synchrony in the 40 Hz frequency band (36-44 Hz; low gamma) measured at electrode Fz 2. Power and phase synchrony in the other discrete frequency bands from 20 Hz to 84 Hz (in steps of 8 Hz) covering low and high gamma frequencies measured at electrode Fz
Early Visual Processing:	-1, 1 and 3	➢ 0 hours postdose	1. Alternation Rate		1. Median Dominance Duration

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
Binocular Rivalry			2. Median Dominance Duration		
Early Visual Processing: Internal Noise Estimation	-1, 1 and 3	➢ 0 hours postdose	1. Percent Correct 2. D-Prime 3. Consistency 4. Internal Noise Estimate		1. Internal Noise Estimate
Otoacoustic Emissions	-1, 1 and 3	➢ 5 hours postdose	SNR (for the parameters below) at frequencies of 1, 1.5, 2, 3 and 4 kHz: • right ear (independent) • left ear (independent) • right ear (no noise) • left ear (no noise) • right ear (noise presented to left) • left ear (noise presented to right) • right ear (contralateral noise suppression)		SNR (for the parameters below) at frequencies of 1, 1.5, 2, 3 and 4 kHz: • right ear (independent) • left ear (independent) • right ear (no noise) • left ear (no noise) • right ear (noise presented to left) • left ear (noise presented to right) • right ear (contralateral noise suppression) • left ear (contralateral noise suppression)

PD Test	Study Days	Timepoints	Assessment Parameters	Secondary Endpoints	Exploratory Endpoints
			• left ear (contralateral noise suppression)		
Words-in-noise	-1, 1 and 3	➢ 5 hours postdose	1. SNR at 70-dB HL 2. SNR at 40-dB HL		1. SNR at 70-dB HL 2. SNR at 40-dB HL

[a] To be derived for analyses/listings.

In addition to the summary tables, individual profiles over time will be plotted for selected endpoints in Table 4 with all participants on the same plot per endpoint, and Study Day presented on the x-axis, based on the mPD population. Each participant will be presented as a separate line, with different symbols indicating the different days:

- Study Days -1, 2 and 4: black circle symbol
- Study Day 1 or 3 (AUT00201): blue dot symbol
- Study Day 1 or 3 (Placebo): red dot symbol

Individual profiles of MI (averaged across both arms) over time will also be plotted for each participant with different lines for each study day, with time of test on the x-axis. For this plot different line colours and symbols will be used for each study day.

- Study Day -1: black circle symbol and black line
- Study Day 2: black square symbol and black line
- Study Day 4; black triangle symbol and black line
- Study Day 1 or 3 (AUT00201): blue dot symbol and blue line
- Study Day 1 or 3 (Placebo): red dot symbol and red line

All PD data collected will be listed for the PD population, including both absolute values and changes from baseline.

10.2.2 DETERMINATION OF A POSITIVE IMPACT WITH AUT00201

The primary objective of the study is to evaluate the safety and tolerability of single doses of AUT00201 and the study was not powered with respect to the PD endpoints. Although the study has a small number of participants, it has a large number of PD assessments. Given the number of PD assessments and resulting endpoints an attempt has been made to prioritize the PD endpoints and to identify criteria for concluding a positive signal from the study with respect to PD assessment. This approach has been taken to avoid "cherry picking" when the study is unblinded and to give some indication of the probability of a false positive conclusion. For these purposes the PD assessments have been assigned to tiers of importance as detailed in Table 5.

Table 5: PD Tiers

Level	Criteria for Tier Assignment	PD Outcome Measure	Relevance	Direction of change indicating an improvement
Tier 1	Measures of motor control and cortical inhibition, reflecting symptomatology and critical pathophysiology	(1) Average SICI at 2.5ms and 3ms (2-4 hours postdose) (2) Speaking rate (1 hour postdose) (3) MI (average of the action myoclonus assessments 0-4 hours postdose)	• Critical • Signal required to declare trial positive	(1) Increase (2) Increase (3) Decrease
Tier 2	Measures with strong evidence for association with functioning of parvalbumin interneurons or Measures assumed to be moderately affected by deficient motor coordination	(1) Absolute power of low gamma oscillations in resting, eyes open state EEG (4 hours postdose) measured at electrode Fz (2) Absolute power of low gamma oscillations (40 Hz) during auditory stimulation ('Chirp') (4 hours postdose) measured at electrode Fz (3) Phase synchrony of low gamma oscillations (40 Hz) during auditory stimulation ('Chirp')	• Supportive • Providing important biomarker information	(1) Decrease (2) Increase (3) Increase (4) Increase

		(4 hours postdose) measured at electrode Fz (4) Buttercup count (1 hour postdose)		
Tier 3	Measures with hypothesized association with functioning of parvalbumin interneurons	(1) Independent Otoacoustic emissions (OAEs) from the left and right ears (5 hours postdose) (2) Words-in-noise (WIN) SNR from the 40-dB HL test (5 hours postdose) (3) Median dominance duration (binocular rivalry) (approx. 30 minutes post dose) (4) Internal Noise Estimate (approx. 30 minutes post dose)	• "Academic" • Potential validation of additional biomarkers	(1) Increase (2) Decrease (3) Increase (4) Decrease

For each of the outcome measures in Table 5, a positive outcome for a participant is identified as their response being 'better' following AUT00201 than following placebo. A positive signal for the study will be concluded if one of the following criteria in Table 6 are met.

Table 6: Criteria for determining a positive outcome

Criterion 1:	≥2 Tier 1 outcome measures have positive outcomes for ≥4 participants
Criterion 2:	≥3 Tier 1 outcome measures have positive outcomes from ≥3 participants

Criterion 3:	≥2 Tier 1 outcome measures have positive outcomes from ≥3 participants AND ≥3 Tier 2 outcome measures have positive outcomes from ≥ 3 participants. *Please note that it could be a different set of participants fulfilling the Tier 1 and Tier 2 components of this criteria.*

The difference between the outcome measures on the treatment days (AUT00201 - Placebo) will be calculated for each participant and tabulated as '+' (i.e., positive outcome, AUT00201 better than placebo), '=' (i.e., equivalent outcome) or '–' (i.e., negative outcome, placebo better than AUT00201).

Within each tier, the total numbers of participants with '+' outcomes will then be tabulated for each outcome measure and the total number of outcome measures with '+' outcomes will be tabulated for each participant.

To assess the false positive rates of each criterion simulations were performed. These simulations took into account the knowledge that 5 participants will be included in the mPD population; however, one participant will only have treatment comparison data for one Tier 1 outcome measure and three Tier 2 outcome measures, and another participant will only have treatment comparison data for two Tier 1 outcome measures and three Tier 2 outcome measures.

The false positive rates for criteria (1) - (3) were calculated as:

- Criteria 1: 1.17%
- Criteria 2: 1.95%
- Criteria 3: 3.76%

Overall, the false positive rate is calculated to be 6.22%. However, these calculations assume independence of the outcome measures. This is unlikely to be the case, but there is insufficient information to take this into account in the calculations.

10.2.3 ASSESSING CLINICAL MEANINGFULNESS

While the analysis outlined in Section 10.2.2 assesses if the pattern of observed results indicates a positive effect of AUT00201 it does not assess the clinical meaningfulness of the observed results. The clinical meaningfulness will be assessed as follows for each outcome measure:

1. The standard deviation (SD) and mean will be calculated for Study Days -1, 2 and 4, (i.e., when participants did not receive any study treatment). Note SD and mean will only be calculated for Study Day 1 for endpoints that do not collect data on Study Days 2 and 4.
2. The median SD will be determined from the SDs calculated in (1).
3. The changes from baseline will be calculated for both treatments for each participant.
4. The difference between treatments (AUT00201 – placebo) will be calculated for each participant.
5. The changes from baseline [calculated in (3)] and the difference between treatments [calculated in (4)] will then be divided by the median SD [calculated in (2)], so the

changes and differences are pressed as multiples of the 'off treatment' between participant standard deviation.

In general, an effect size (multiple of a baseline SD) of 0.2 is considered small, of 0.5 moderate and of ≥0.8 large (Cohen, 1988). Of note, an effect size of 0.5 is commonly used to represent the minimally important differences in health-related quality of life instruments (Norman, 2003).

The clinical meaningfulness of the study results will be evaluated using these 'guideposts'.

A summary table will present the frequency of participants with changes from baseline for each treatment and differences between treatments in the following categories:

- Worsen
- No change
- < 0.1 SD improvement
- 0.1 to <0.2 SD improvement
- 0.2 to <0.3 SD improvement
- and so forth in 0.1 SD increments until all changes/differences are accounted for.

The median SD calculated in (2) above assumes that there will be not carryover of treatment on Study Day 1 to Study Day 2, or from treatment on Study Day 3 to Study Day 4 (i.e., that values have re-baselined on Study Days 2 and 4). The table described above will also include a summary of the changes from baseline for Study Days 2 and 4.

10.3 SAFETY ANALYSES

All safety analyses and listings will be based on the safety population.

10.3.1 ADVERSE EVENTS

The number of participants, and number of events, in the following categories will be presented by treatment:

- Any TEAE
- Any TEAE related to treatment (IP)
- Any TEAE leading to discontinuation of the study
- Any serious TEAE
- Any serious TEAE related to treatment (IP)
- Any TEAE with severity grade of 3 or higher
- Any TEAE with severity grade of 3 or higher, related to treatment (IP)

The number of participants with at least one TEAE will be summarized by SOC and PT, by treatment. The total number of TEAEs observed within each SOC and PT will also be reported.

All AEs will be listed. In addition to the AE details collected in the eCRF, the listing will include:

- Whether the AE was pre-treatment, treatment-emergent or during the follow-up
- Treatment sequence

- Onset study day and time relative to the last dose
- End study day and duration of the AE if this can be calculated (see Section 9.2.3.1).

10.3.2 CLINICAL LABORATORY EVALUATIONS

All hematology, chemistry, urinalysis, urinalysis microscopic, hormones and serology data collected will be listed separately. Records outside of the normal range and clinically significant values will be flagged in the listings. Low values with respect to the laboratory reference range (i.e., below the lower limit) and high values (i.e., above the upper limit) will be flagged as 'L' and 'H', respectively, in the listing. Absolute values and changes from baseline will be included for continuous data. Study day and treatment sequence will be provided to help identify the last treatment received before the sample was taken. The listing will be sorted by participant, parameter and then visit/date/time.

Urine pregnancy tests and results will be listed separately.

10.3.3 VITAL SIGNS

All vital signs data collected will be listed. Study day and treatment sequence will be provided to help identify the last treatment received before the measurement was taken. Absolute values and changes from baseline will be included. The listing will be sorted by participant, parameter and then visit/date/time.

10.3.4 PHYSICAL EXAMINATION

All physical examination data collected will be listed, by participant, examination and then visit/date/time. Any clinically significant values will be flagged in the listings.

10.3.5 ECGS

All ECG data collected will be listed. Study day and treatment sequence will be provided to help identify the last treatment received before the measurement was taken. Absolute values and changes from baseline will be included. The listing will be sorted by participant, parameter and then visit/date/time.

An overall interpretation of findings will be given, and any clinically significant values will be flagged in the listing.

10.3.6 C-SSRS

C-SSRS data will be listed. If the answers to *'1. Wish to be dead'* and *'2. Non-Specific Active Suicidal Thoughts'* are both "no", only the suicidal behavior data at that visit is listed.

10.4 CLINICAL PHARMACOLOGY

10.4.1 AUT00201 PHARMACOKINETICS

These analyses will be based on the PK concentration population and PK parameter population, as relevant.

Plasma concentrations and PK parameters will be summarized (using the summary statistics from Section 8.1) and listed. The concentration listing will include the derived time of

sampling relative to dosing and the deviation (in minutes) from the scheduled time. In cases where a planned PK parameter could not be calculated (e.g., all concentrations are non-measurable), the result will be listed as "Not Calculable" (NC).

The individual concentration profiles (presented on a linear scale) against actual time postdose will be plotted with all participants on the same plot. The pre-dose timepoint will be set to time=0.

Additional plots will be generated to assess the relationship between PD assessment differences from placebo and AUT00201 exposure. The difference between treatments (AUT00201 – placebo) will be plotted against the AUT00201 C_{max} (Study Day 1 or 3, as applicable) for the following:

1. SICI - the average of the SICI 2.5ms and 3ms data derived from the assessments 2 to 4 hours postdose on Study Days 1 and 3.
2. MI - the average of the action myoclonus assessments from 9am to 1pm, i.e., 0-4 hours postdose on Study Days 1 and 3.
3. Speaking rate 1 hour postdose on Study Days 1 and 3.
4. Buttercup count 1 hour postdose on Study Days 1 and 3.

For each of the above, all participants will be presented on the same plot.

The difference between treatments (AUT00201 – placebo) will also be plotted for MI against time, with the plasma concentration curve overlaid (Study Day 1 or 3, as applicable). Each participant will be displayed on separate plots.

10.4.2 ANTIEPILEPTIC PHARMACOKINETICS

Plasma concentrations for antiepileptic drugs (AEDs), for which assays are commercially available to the clinical site, will be listed, for the safety population.

11. CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSIS

The following planned changes are listed below:

- Removed the final exploratory endpoint "Change from baseline in: Changes in biomarkers as compared to exposure to AUT00201." In hindsight it was felt that this covered a set of analyses, rather than being an endpoint. Outputs comparing exposure (via PK parameters) to biomarkers are described in Section 10.4.1.
- Added the modified pharmacodynamic population to the SAP, to handle subjects who participate in the study twice appropriately in PD analyses.
- Minor changes to the presentation of summary statistics (including PK analyses) have been made to account for anticipated smaller sample size.
- No reference to the mean (±SD) plasma concentration-time profiles discussed in the protocol. Individual profiles will be presented.
- Table 3 of the Protocol refers to Ctrough and AUC24; these have been changed to Cpredose for a more accurate term for the single-dose design of the study and AUCt to account for the 27-hour PK sample timepoint.

12. BIBLIOGRAPHY

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13. APPENDIX A – SCHEDULE OF ASSESSMENTS

All timings are provided as a guide only for the order of assessments. However, it is important to note:

- At times where pharmacokinetics is assessed, it should be drawn immediately after the action myoclonus assessment (or UMRS Sections 4 and 5 assessment) is performed
- TMS should be performed at consistent times throughout
- UMRS (Sections 4 and 5) should be performed prior to TMS.

Visit Number/ Status	Visit 1 / Outpatient						
Visit Name / Day	Screening Visit / Friday [a]						
Time	09:00	10:00	11:00	12:00	13:00	14:00	15:00
Informed Consent	X						
Review Eligibility Criteria		X					
Demographics		X					
Medical History		X					
Physical Examination				X			
Vital Signs				X			
Height and Weight[c]				X			
12-Lead Electrocardiogram					X		
Columbia-Suicide Severity Rating Scale			X				
Biochemistry and Hematology			X				
Urinalysis (Dipstick)			X				
Urine Drug Screen			X				
TSH, T3, T4, FSH			X				
HBsAg, HCAb			X				
Patient Concerns						X	
PROM-Ataxia						X	
Review test equipment with patient (EEG cap, TMS, VR headset, audiology headphones)	X						
Adverse Events	X						
Prior or Concomitant Medications	X						

Visit Number/ Status	Visit 2 / Inpatient									
Visit Name / Day	Baseline / Monday									
Time	08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00
Patient Admission	X									
Cannula In[h]										
Physical Examination	X									
Urinalysis (Dipstick)	X									
Urine Pregnancy Test	X									
Action Myoclonus (Section 4: D and E)	X	X	X		X	X	X	X	X	X
Vital Signs		X								
Measure of Early Visual Processing		X								
Speech Tests			X							
UMRS (Section 4 and 5)				X						
Paired-pulse TMS					X					
EEG Resting State and Presentation of Chirp Stimuli						X				
OAEs							X			
WIN Test							X			
EMG and Accelerometer	X[f]									
Adverse Events	X[f]									
Prior or Concomitant Medications	X[f]									

Visit Number/ Status	Visit 3 / Inpatient									
Visit Name / Day	Treatment 1 / Tuesday									
Time	08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00
Time Relative to Dose (Hour)	-1	0	+1	+2	+3	+4	+5	+6	+7	+8
Action Myoclonus (Section 4: D and E)	X	X	X		X	X	X	X	X	X
IP Administration (AM) (30 minutes after completion of a standard meal)		X								
Vital Signs		X								
Measure of Early Visual Processing		X								
Speech Tests			X							
PK Sampling[e]	X[i]		X	X	X	X		X		X
Biochemistry and Hematology			X							
AED Concentration[d]	X			X						
Urinalysis (Dipstick)			X							
12-Lead Electrocardiogram			X							
UMRS (Sections 4 and 5)				X						
Paired-pulse TMS					X			X		
EEG Resting State and Presentation of Chirp Stimuli						X				
OAEs							X			
WIN Test							X			
EMG and Accelerometer	X[f]									
Adverse Events	X[f]									
Prior or Concomitant Medications	X[f]									

Visit Number / Status	Visit 4 / Inpatient						
Visit Name / Day	Washout / Wednesday						
Time	08:00	09:00	10:00	11:00	12:00	13:00	14:00
Action Myoclonus	X	X	X	X	X	X	
Vital Signs		X					
Physical Examination[b]		X					
Physician Impression[g]			X				
Patient Impression[g]			X				
Speech Tests			X				
PK Sampling[e]		X			X		
Biochemistry and Hematology		X					
Paired-pulse TMS				X			
EMG and Accelerometer	X[f]						
Adverse Events	X[f]						
Prior or Concomitant Medications	X[f]						

Visit Number/ Status	Visit 5 / Inpatient									
Visit Name / Day	Treatment 2 / Thursday									
Time	08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00
Time Relative to Dose (Hour)	-1	0	+1	+2	+3	+4	+5	+6	+7	+8
Action Myoclonus (Section 4: D and E)	X	X	X		X	X	X	X	X	X
IP Administration (AM) (30 minutes after completion of a standard meal)		X								
Vital Signs		X								
Measure of Early Visual Processing		X								
Speech Tests			X							
PK Sampling[e]	X[j]		X	X	X	X		X		X
Biochemistry and Hematology			X							
AED Concentration[d]	X			X						
Urinalysis (Dipstick)			X							
12-Lead Electrocardiogram			X							
UMRS (Sections 4 and 5)				X						
Paired-pulse TMS					X			X		
EEG Resting State and Presentation of Chirp Stimuli						X				
OAEs							X			
WIN Test							X			
EMG and Accelerometer	X[f]									
Adverse Events	X[f]									
Prior or Concomitant Medications	X[f]									

Visit Number / Status	Visit 6 / Inpatient							Visit 7
Visit Name / Day	Washout / Discharge / Friday							Phone Follow-up/ 14 days after Visit 6 ±2 days
Time	08:00	09:00	10:00	11:00	12:00	13:00	14:00	
Urinalysis (Dipstick)			X					
Action Myoclonus (Section 4: D and E)	X	X	X	X	X	X		
Vital Signs		X						
Physical Examination[b]		X						
Physician Impression[g]			X					
Patient Impression[g]			X					
Speech Tests			X					
Cannula Out						X		
PK Sampling[e]		X			X			
Biochemistry and Hematology		X						
Paired-pulse TMS					X			
Columbia-Suicide Severity Rating Scale						X		
EMG and Accelerometer	X[f]							
Adverse Events	X[f]							X
Prior or Concomitant Medications	X[f]							X
Patient discharge							X	

Abbreviations: AED = antiepileptic drug; EEG= electroencephalogram ; EMG= electromyography; FSH = follicle-stimulating hormone; HBsAg = hepatitis B surface antigen; HCAb = hepatitis C antibody; OAE = otoacoustic emissions; PK = pharmacokinetic; PROM = Patient-reported Outcome Measurement; T3 = triiodothyronine; T4 = thyroxine; TMS = transcranial magnetic stimulation; TSH = thyroid-stimulating hormone; UMRS = Unified Myoclonus Rating Scale; WIN = Words-in-Noise.

[a] Screening Visit over 1 or 2 days up to 1 week prior to the baseline (Visit 2) to accommodate patient travel and availability; Thursday/Friday is a suggested timeframe.
[b] Brief or targeted physical examination at Visits 4 and 6.
[c] If height cannot be reliably obtained, the measure length of forearm (ulna) from the point of the elbow (olecranon process) and the midpoint of the prominent bone of the wrist (styloid process) will be used to predict height.
[d] Concentrations of AED will be analyzed, if there is a commercially available test; samples will be taken pre-IP-dose and postdose on Visits 3 and 5.
[e] The non-predose PK samples should be taken at the specified time with an allowable ±15 minutes' deviation. See superscript "i" and "j" for further details on predose PK samples for Visit 3 and Visit 5, respectively.
[f] For duration of inpatient stay.
[g] The clinician and the patient will be asked (separately) if they believe they received active or placebo treatment after each dose and if they perceived any change in their condition.

[h] The cannula will be inserted on admission to the hospital, as required by local procedure. The cannula will be inserted prior to Visit 3.
[i] For Visit 3: predose PK can be any time in the morning of dosing as long as it is predose.
[j] For Visit 5: predose PK can be any time after 47 hours post Visit 3 dosing and prior to Visit 5 dosing.