# Summary of Amended Protocol Changes

**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)**

Protocol Amendment Status: Final
Original Protocol Date: 03 June 2022
Protocol Amendment 01 (Version 2.0) Date: 21 September 2022
Protocol Amendment 02 (Version 3.0) Date: 24 January 2023
Protocol Amendment 03 (Version 4.0) Date: 30 October 2023

Investigational Product: AUT00201

Sponsor Reference Number: AUT022201
[redacted] Study: 000000233834
IND Number: 160082

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**Primary Changes:**

**The protocol has undergone revisions to study design, objectives, and endpoints based on internal and external review. The primary changes in this amendment, along with the rationale for the/each change as appropriate, are:**

1. [redacted]

2. Secondary and exploratory objectives and endpoints are reorganized based on their relevance and weight with regard to potential effects of AUT00201; these sections (Sections 2.1 and 2.2) as well as Secondary Analysis and Exploratory Analysis sections (Sections 8.8 and 8.10, respectively) are revised as follows:

    - For the assessment of cortical inhibition by paired-pulse transcranial magnetic stimulation (TMS), the short interval cortical inhibition has been kept as a secondary objective and endpoint, while the long interval cortical inhibition has been moved as an exploratory objective and endpoint.
    - The assessments of clinical symptoms (dysarthria and positive myoclonus) have been moved from exploratory to secondary objectives and endpoints. The Secondary Analysis section has also been updated to include that descriptive statistics will be provided for measures of dysarthria and myoclonus index.
    - The evaluation of correlations between exposure to AUT00201 and changes in these biomarkers has been added as a secondary objective and endpoint.
    - Specifications of where certain assessments data are coming from have been updated to accurately reflect the data sources.
    - Details have been added to the Secondary Analysis and Exploratory Analysis sections to specify that the overall effect of AUT00201 on secondary and selected exploratory outcome measures (signs and symptoms of MEAK) will be evaluated with the help of a predefined response matrix, which should guard against 'cherry picking' of results but ensure the detection of a clinically meaningful signal.

3. A Replacement Patients section (Section 3.2) has been added and the Replacement Procedures section (Section 4.4.4) has been updated with details to allow patients who have experienced important protocol deviations or technical issues that are likely to make the primary, secondary, or exploratory endpoint data unevaluable or impact their validity to be invited to take part in the study again if the sponsor and Investigator are in agreement. As the patient cannot be replaced given the rarity of the disease and the known patient population, this option limits impact to the dataset with no anticipated concerns for patients' safety and data integrity.

4. The Background and Benefit-risk Assessment sections (Sections 1.2 and 1.3, respectively) have been updated to reflect the study conditions, to reflect changes made to protocol, and to provide better clarity.
5. The number of patients anticipated to be enrolled has been updated to approximately 6 to 10 patients instead of 8 to 10 patients reflecting more realistic recruitment estimates.
6. The duration of patient participation has been updated throughout the protocol from approximately 1.5 days to up to 2 days for total outpatient participation, and from approximately 4.5 to 5 days for inpatient stay for consistency and clarity.
7. The Discussion of Study Design (Section 3.3) Section has been updated to include the details that patient's history of fever and any perceived improvement of symptoms will be captured as part of their medical history. A corresponding Medical History section (Section 7.6.1) has been added with detailed guidance to ensure proper collection of medical history.
8. The Discontinuation of Study Treatment section (Section 4.4.2) and the Follow-up of Patients Prematurely Discontinued from the Study Treatment Regimen or Withdrawn from Study section (Section 4.4.5) have been updated so that other than safety assessments, a PK sample may also be collected at the discretion of the Investigator, as it would be good to have a PK sample to assess PK/tolerability relationship if the subject withdraws due to an adverse event postdose.
9. The Prohibited Medications section (Section 6.2) has been updated to specify that acetaminophen for pain relief may be taken without prior consent from the Investigator.
10. The Study Assessments and Procedures section (Section 7) has been updated to remove the statement that the detailed procedure to be followed for assessments, which involves patients' activity or measurements based on physical activities, will be described in the study manual.
11. In the Vital Signs, Physical Examination, and Other Safety Evaluations section (Section 7.3) and the Benefit-risk Assessment section (Section 1.3), the position for blood pressure and other vital sign measurements has been updated from supine to sitting to reduce patient burden and accommodate site logistics.
12. The Changes in Myoclonus as Assessed by electromyography (EMG) and Accelerometer Data section (Section 7.5.2) has been updated to reflect the presence of an EMG and accelerometer manual.
13. In the Unified Myoclonus Rating Scale section (Section 7.5.4), an additional assessment of the non-dominant arm has been added to the standard Unified Myoclonus Rating Scale Section 5 to obtain a more complete data set.
14. The Measure of Ataxic Dysarthria: Speech and Vocal Tests section (Section 7.5.5) has been updated to include the details that the patient's native language, including country of origin (eg, English – USA) will be captured as part of patient's demographics. The Demographic Data section (Section 7.6) has been updated accordingly to reflect the change and to provide clarity.

15. The pharmacodynamic (PD) population in the Analysis Populations section (Section 8.2) has been updated to include all patients in the Safety Population with an evaluable baseline and at least 1 evaluable postbaseline measurement for at least 1 PD parameter. The postbaseline measurement can now be any PD parameter instead of paired-pulse TMS measurement only.
16. The baseline definition in the General Considerations section (Section 8.3) has been updated to allow the use of the average across Visits 2 and 4 as baseline if review of blinded data indicates a stability of the measurement on days when patients do not receive treatment.
17. A Study Design/Study Schema (Figure 1) has been updated to provide greater clarity.
18. In the Schedule of Assessments (Appendix 4), the predose pharmacokinetic (PK) sampling timing at Visits 3 and 5 has been revised as follows because there is no scientific rationale why predose PK sampling needs to be collected 1 hour predose ±10 minutes as previously specified.
    - For Visit 3: predose PK can be any time in the morning of dosing as long as it is predose.
    - For Visit 5: predose PK can be any time after 47 hours post Visit 3 dosing and prior to Visit 5 dosing.
19. In the Schedule of Assessments (Appendix 4), the allowable time window to collect non-predose PK sample has been specified (ie, the time window only applies to PK sampling timepoints other than predose) and updated from 10 minutes to 15 minutes. The additional 5-minute window allows flexibility if a pre-sample assessment is taking longer to conduct than anticipated, so it is not interrupted by the sampling requirement. For the PK modeling, the most important data is having the exact time the PK sample was drawn documented.
20. In the Schedule of Assessments (Appendix 4), the time to take off the EMG and accelerometer device on Visit 4 has been corrected from 13:00 to 14:00 (ie, 1 hour longer), as the planned analysis of the EMG and accelerometer data requires a longer data capture and comparable time windows (now with the same time window as Visit 6).
21. In the Schedule of Assessments (Appendix 4), the time to insert a cannula has been updated from 8:00 on Visit 3 to "on admission" prior to Visit 3 based on local procedure. The local procedure is to insert the cannula on admission, which allows for any required rescue medications to be administered in an expedited fashion. A footnote has been added to provide clarity.

**Minor changes:**

1. The synopsis has been updated according to the changes in the protocol body, as applicable.
2. The amendment/version number and date have been updated throughout the protocol.
3. Typographical errors and formatting errors have been corrected, as necessary.

4. Editorial changes have been made, as appropriate.
5. Administrative changes have been made (eg, [redacted]), as appropriate.
6. Abbreviations have been added and removed, as appropriate.

**A detailed summary of changes is presented below:**

The detailed changes can be reviewed in the track changes version of the protocol.

**TITLE PAGE**

# Protocol

## 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)

Protocol Status: Final
Protocol Date: 30 OCT 2023
Protocol Version: 4.0

Investigational Product: AUT00201

Protocol Reference Number: AUT022201
IND Number: 160082

Sponsor:
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Sponsor Signatory:
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## SPONSOR APPROVAL

I have read the following and approve it:

[redacted]

06 Nov 2023

[redacted]
Chief Medical Officer

Date

# INVESTIGATOR AGREEMENT

**Declaration of Principal Investigator**

Protocol 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)

I have read this protocol and agree that it includes all essential information to be able to conduct the study. By signing my name, I agree to conduct the study in compliance with this protocol, the Declaration of Helsinki, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and the national and international regulations governing the conduct of this clinical study.

I will submit this protocol and all other important study-related information to the staff members and responsible Investigators who participate in this study so that they can conduct the study correctly. I am aware of my responsibilities to continuously keep the staff members and responsible Investigators who work with this study informed and trained.

I am aware that quality control of this study will be performed in the form of monitoring and potential audit and/or inspection.

**Principal Investigator:**

[redacted]

07 Nov 2023

Name, Qualification(s)
Principal Investigator

Date

## SYNOPSIS

**Title of study:** 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)

**Indication:** MEAK

**Number of Investigators and study centers:** 1 site

**Development phase:** Phase 1b

**Objectives:**

The objectives are:

Primary

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

Secondary

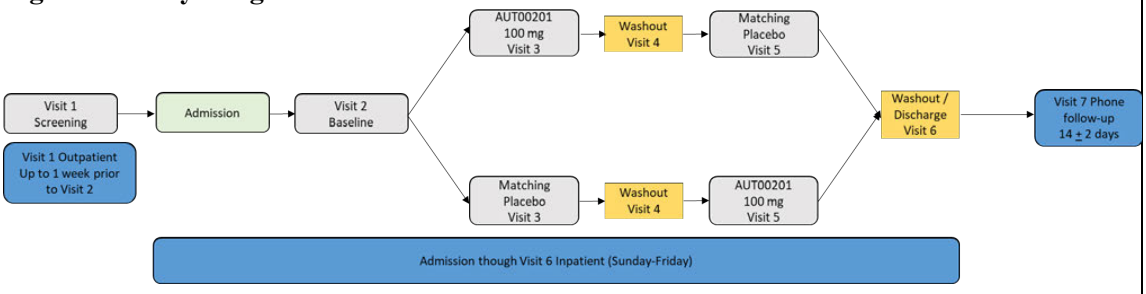
- To evaluate the effects of single doses of AUT00201 on short interval cortical inhibition (SICI) as measured by paired-pulse transcranial magnetic stimulation (TMS)
- To evaluate the effects of single doses of AUT00201 on dysarthria as measured by automated speech and vocal assessments
- To evaluate 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.

Exploratory

- 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.

**Methodology/study design:**
This is a Phase 1b study to investigate the safety, tolerability, PK, and pharmacodynamic (PD) effects on biomarkers of AUT00201 in patients with MEAK. 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 will participate in the study.
The study is a double-blind, randomized, placebo-controlled treatment in which patients 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.
After screening/orientation (Visit 1) and baseline assessments (Visit 2), patients will be administered a single dose of 100 mg AUT00201 or matching placebo the morning of Visit 3. PK assessments will be done at Visits 3, 4, 5, and 6 from predose and up to 27 hours postdose. Visit 4 will be a washout day for patients. The washout period of 48 hours is deemed appropriate based on the half-life observed in the first-in-human (FIH) study. At Visit 5, patients will be administered the crossover treatment. At Visit 6, patients will be discharged from the unit. Safety and tolerability assessments will be conducted throughout. PD parameters will also be assessed.
Follow-up will be conducted over the phone 14 days ±2 days after discharge (Visit 7).
Safety, tolerability, PK, and biomarker assessments will be conducted throughout the study as detailed in the Schedule of Assessments. Figure 1 provides a study schema.

**Figure 1. Study design**




**Number of patients:** It is anticipated that approximately 6 to 10 patients will be enrolled in this study.

**Diagnosis and criteria for inclusion and exclusion:**
The following are the inclusion criteria at Screening Visit (Visit 1) unless otherwise stated:

1. Male or female patients aged 18 years or older at the time of consenting
2. Diagnosed with MEAK, based on documented genetic evidence of the presence of the KCNC1 (c.959G>A; p.Arg320His) variant
3. If taking anticonvulsants, must be on a stable anticonvulsant regimen for at least 30 days prior to Visit 1 and anticipated to remain stable throughout the study, or if not on an anticonvulsant regimen, must be stable in regards to seizures for at least 30 days prior to Visit 1 and anticipated to remain stable throughout the study
4. Must be able to participate and willing to give written informed consent. If patient is unable to provide written informed consent, a legally authorized representative can sign on their behalf.
5. Must be willing to perform study assessments and comply with the study protocol
6. If the patient is dependent on a caregiver and/or will need assistance either travelling to the site, whilst attending clinic visits and/or helping to document study assessment

responses provided by the patient (eg, questionnaires administered on a tablet device), they must have an identified caregiver, considered reliable by the Investigator, to provide support to the patient for the duration of the study. The caregiver must be willing and able to provide support to the patient and, if required, stay for the duration of the study.

7. Medically stable based on Investigator's judgment for at least 90 days prior to Visit 1
8. Women of childbearing potential must have a negative urine pregnancy test on Visit 2 (Baseline Visit)
9. If a vagal nerve stimulator is used, it must be implanted at least 150 days before Visit 1, and parameters must be stable for at least 30 days before Visit 1 and be expected to remain stable throughout the study
10. If a ketogenic diet is followed, it must be stable for at least 30 days before Visit 1 and be expected to remain stable throughout the study
11. Willing to comply with contraceptive requirements. See Section 4.
12. Able to speak, read and understand spoken English at a fluent level.

The following are the exclusion criteria at Screening Visit (Visit 1) unless otherwise stated:

1. Known pathogenic mutation in another gene that causes epilepsy or a different mutation in the KCNC1 gene than the c.959G>A variant
2. Clinically significant metabolic, hepatic, hematological, pulmonary, cardiovascular, gastrointestinal, or urological disorder
3. Clinically significant abnormal vital signs or laboratory test results
4. Hypersensitivity to AUT00201 or any of the excipients
5. Any medical condition or other factors, as judged by the Investigator, which may interfere with the patient's participation in this study and/or compromise the patient's ability to safely complete the study
6. Known to abuse drugs or those who test positive on urine screen for drugs of abuse will be excluded based on Investigator's judgment
7. Positive hepatitis B surface antigen or hepatitis C antibody
8. Clinically significant abnormality on the 12-lead electrocardiogram (ECG)
9. Having received an investigational product (IP) 90 days prior to Visit 1
10. Currently using felbamate <1 year prior to Visit 1, or any evidence of ongoing hepatic or bone marrow dysfunction associated with current/prior felbamate treatment. Patients who are currently using felbamate for >1 year prior to Visit 1 and have no evidence of ongoing hepatic or bone marrow dysfunction associated with felbamate treatment are allowed
11. Currently using vigabatrin and having received vigabatrin for <2 years prior to Visit 1
12. Suicidal ideation with some intent to act within 6 months prior to Visit 1 based upon a response in the Columbia-Suicide Severity Rating Scale positive response to questions 4 or 5 of the suicidal ideation section) and as judged by the Investigator as having a significant impact on trial participation or patient safety. History of suicidal behavior within 1 year prior to Visit 1.

**Test products, dose, and mode of administration:**
Patients will receive one of the following sequences of treatment:

- A single 100 mg dose of AUT00201, oral capsule then matching placebo, oral capsule
- A matching placebo, oral capsule then a single 100 mg dose of AUT00201, oral capsule.

**Reference therapy, dose, dose form, and mode of administration:**
Patients will receive matching placebo capsules in a crossover design. There is no active comparator product in the study.

**Duration of patient participation in study:**
Total outpatient participation: Up to 2 days
Total inpatient participation: Approximately 5 days
Planned follow-up: 14 days ±2 days after discharge
**Total duration of study participation:** Up to 3.5 weeks

**Study populations:**

- Safety Population: All patients who received at least 1 dose of IP
- PK Concentration Population: All patients who received at least 1 dose of IP with at least 1 quantifiable PK concentration at any scheduled PK timepoint
- PK Parameter Population: All patients in the PK Concentration Population for whom PK parameters can be derived
- PD Population: All patients in the Safety Population who have an evaluable baseline and at least 1 evaluable postbaseline measurement for at least 1 PD parameter.

In all populations, patients 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.

**Evaluation: Safety, Tolerability, PK, and PD**
The endpoints are:
Primary Endpoint:

- 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 IP administration
  - Change from screening in 12-lead ECG findings
  - Incidence of adverse events (AEs).

Secondary Endpoints:

- 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
  - MI (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.

Exploratory Endpoints:

- 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 each 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]).
  - Changes in biomarkers as compared to exposure to AUT00201.

## Statistical methods:

All statistical analysis will conform to the relevant International Council for Harmonisation (ICH) requirements, including but not limited to those set out in ICH documents E3 and E9.
Statistical Analysis Software (SAS) version 9.2 or later will be used for the analysis.
A detailed Statistical Analysis Plan (SAP) will be finalized prior to database lock and data unblinding. The SAP will document all analyses procedures.

**Sample Size Determination**

MEAK is an ultra-rare disorder. The global prevalence is estimated at <1 in 1 000 000 (ORPHA: 435438). There are only 10 patients known to the Epilepsy Neurogenetics Initiative at the Children's Hospital of Philadelphia which keeps registry of patients who have KCNC1-related disorders in North America.
It is hoped that approximately 6 to 10 patients can be recruited.
Therefore, a formal power analysis to estimate a desired sample size is not useful. Primary endpoint is safety and tolerability of AUT00201. Nonetheless, a sample size of 8 provides an 80% power to detect an effect size of approximately 0.8 in a paired sample t-test at a one-sided alpha level of 0.1 (as will be used for the primary outcome measure, the composite response score).

**General Considerations for Data Analyses**

Standard summary statistics for continuous baseline and outcome variables are: N, mean, standard deviation (SD), median, quartiles, minimum, maximum, and 95% confidence intervals at baseline and each timepoint at which the measures are obtained. In addition, the same descriptive statistics will be obtained for change from baseline in each treatment period for each timepoint at which the measures are obtained. The standard summary statistics for categorical baseline and outcome variables are: count and proportion (expressed as a percentage). Geometric mean and coefficient of variation will also be presented for PK concentrations and the PK parameters.
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 (or to 3 significant figures for derived parameters). The mean, median, and percentiles (eg, Q1 and Q3) will be presented to one additional decimal place. The SD and standard error will be presented to 2 additional decimal places.
In general, baseline will be defined as the last measurement obtained before IP administration. However, if review of blinded data indicates a stability of the measurement on days when

patients do not receive treatment, baseline may also be defined as the average across Visits 2 and 4. This will be described in the SAP.
All summaries will be presented by treatment group unless otherwise specified. Summary tables and figures will be supported by full patient listings in each treatment period.
A response matrix will be used to assess the overall effect of AUT00201 on signs and symptoms of MEAK. A response matrix will guard against 'cherry picking' of results but ensure the detection of a clinically meaningful signal. Details will be provided in a separate SAP.

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## LIST OF TABLES AND FIGURES

## LIST OF ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| AE | adverse event |
| AED | antiepileptic drug |
| $AUC_{24}$ | area under the plasma concentration-time curve from time zero to 24 hours |
| BID | twice daily |
| BLQ | below the limit of quantification |
| BP | blood pressure |
| CFR | Code of Federal Regulations |
| CI | confidence interval |
| $C_{max}$ | maximum (peak) plasma concentration |
| CNS | central nervous system |
| CRO | Contract Research Organization |
| CS | conditioning stimulus |
| C-SSRS | Columbia-Suicide severity rating scale |
| CYP | cytochrome P450 |
| DDK | diadochokinetic rate |
| ECG | electrocardiogram |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| EEG | electroencephalogram |
| EMG | electromyography |
| EPM7 | progressive myoclonic epilepsy type 7 |
| ETP | end-of-treatment period |
| FIH | first-in-human |
| FMRP | fragile X mental retardation protein |
| GABA | gamma-aminobutyric acid |
| GCP | Good Clinical Practice |
| IB | Investigator's Brochure |
| ICF | informed consent form |
| ICH | International Council for Harmonisation |
| IP | investigational product |
| IRB | Institutional Review Board |
| ISI | interstimulus interval |
| $K^+$ | potassium |
| $K_v$ | voltage-gated potassium channel |
| LICI | long interval cortical inhibition |
| MEAK | myoclonus epilepsy and ataxia due to potassium ($K^+$) channel mutation |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MOC | medial olivocochlear |
| MI | myoclonus index |
| NOAEL | no observed-adverse effect level |
| OAE | Otoacoustic Emission |
| PD | pharmacodynamic |
| PK | pharmacokinetic |
| PME | progressive myoclonic epilepsy |
| p.o. | orally |

| Abbreviation | Definition |
|---|---|
| PROM | patient-reported outcome measurement |
| PSS | Patient Safety Solutions |
| PT | preferred term |
| PTZ | pentylenetetrazole |
| PV | parvalbumin |
| PV+ | parvalbumin-positive |
| QTcB | QT interval corrected for heart rate by Bazett's formula |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SAS | Statistical Analysis Software |
| SD | standard deviation |
| SE | standard error |
| SICI | short interval cortical inhibition |
| SNR | signal-to-noise ratio |
| SOC | system organ class (MedDRA classification) |
| SOP | standard operating procedure |
| TEAE | treatment-emergent adverse event |
| $t_{max}$ | time to reach maximum (peak) plasma concentration |
| TMS | transcranial magnetic stimulation |
| UMRS | Unified Myoclonus Rating Scale |
| WIN | Words-in-Noise test |

# 1. INTRODUCTION

AUT00201 is a small molecule developed as a potent and selective modulator of human voltage-gated potassium ($K^+$) ion channels ($K_v$): $K_v3.1$ and $K_v3.2$. The $K_v3$ family includes 4 members: $K_v3.1$, $K_v3.2$, $K_v3.3$, and $K_v3.4$. $K_v3$ channels are activated by depolarization of the neuronal plasma membrane to potentials typically above -20 mV. These channels open rapidly during the depolarizing phase of the neuronal action potential in order to initiate repolarization and prevent significant sodium channel inactivation. As the neuron begins to repolarize, the channels deactivate quickly and thus do not contribute significantly to the after-hyperpolarization. [1,2] These distinct properties allow the channels to terminate the action potential rapidly without compromising the action potential threshold, rise time, magnitude, or increasing the duration of the refractory period. As a consequence, neurons expressing $K_v3$ channels are able to sustain action potential firing at high frequencies. $K_v3$ channel subtypes are differentially expressed by subclasses of interneuron in corticolimbic brain areas,[3,4,5,6] in the thalamus,[7] and cerebellum.[8] $K_v3.1$ and $K_v3.3$ channels are also expressed at high levels in auditory brainstem nuclei.[9,10] $K_v3.1$ and $K_v3.2$ channels are particularly expressed by fast-spiking, parvalbumin-positive (PV+) interneurons in corticolimbic circuits, including motor and sensorimotor cortex. They are the critical component to enable the accurate, high-frequency firing of these interneurons which give rise to gamma frequency oscillations that underlie attention, sensory processing, and cognition. Genetic deletion of $K_v3.1$, $K_v3.2$, and $K_v3.3$ channels are associated with altered sensitivity to seizure.[11,12]

Pathogenic variants in the KCNC1 gene, which encodes the $K_v3.1$ channel, have been linked recently to a spectrum of epilepsy and intellectual disability phenotypes. A recurrent pathogenic variant of KCNC1 (c.959G>A; p.Arg320His) results in a form of progressive myoclonic epilepsy (PME) called MEAK (myoclonus epilepsy and ataxia due to $K^+$ channel mutation).[13] The loss of $K_v3.1$ channel function is suggested to lead to reduced cortical inhibition, which gives rise to motor and sensorimotor cortex hyperexcitability.[14] This hyperexcitability underlies the susceptibility to rhythmic myoclonic activity in MEAK and similar hyperexcitability caused by the reduced $K_v3$ channel function in cerebellar neurons is likely to give rise to the progressive ataxia observed in these patients.

MEAK is an ultra-rare disorder. Based on a mutational model, which takes into account both the local sequence context of the mutation site and regional factors, Muona et al estimated the incidence to be 1 in 5,700,000 conceptions.[13] Given a birth number of 3,605,201 in 2020 in the US, in 2 years only 1 to 2 people are born in the US with this mutation. Consistently, there are currently 10 patients known to the registry at the Children's Hospital of Philadelphia which was built through the Epilepsy Neurogenetics Initiative.

MEAK is a severe and debilitating condition. On a background of usually normal development, the first symptom in the majority of patients is myoclonus (sometimes reported as tremor), mostly action-induced, at the ages of 6 to 14 years. Progressive ataxia develops in parallel but is usually overshadowed by myoclonus as the major motor impediment although it has presented as an initial symptom in some cases. Despite its designation as myoclonic epilepsy both myoclonic and tonic-clonic seizures are infrequent in all patients. During adolescence myoclonus generally becomes very severe, limiting ambulation, and requiring a walking aid or wheelchair by mid to late teens. There is notable preservation of cognition in the context of severe motor disability.

Learning disability and mild cognitive decline are sometimes noted but mental retardation or profound cognitive decline has not been observed. In cases, where formal neuropsychological tests were done, verbal measures were in the low normal range. Ataxia dysarthria is common leading to diminished quality of speech; but symptoms commonly noted with other causes of PME, such as hearing loss, retinal abnormalities, and sensory impairment, have not been reported.[13,14,15,16] For further description in Section 1.2.

Based on the observed nonclinical profile of AUT00201, which positively modulates human recombinant $K_v3.1$ and $K_v3.2$ channels and rescues the seizure susceptibility and ataxia phenotype in a genetic mouse model of MEAK, it is hypothesized that the drug could be an effective orally administered treatment for MEAK and potentially other neurological disorders associated with cortical hyperexcitability caused by reduced function of PV+ interneurons.

### 1.1. Study Rationale

Treatment with AUT00201 provides an opportunity to test a targeted therapy in the MEAK patient population, who have a known mutation in one allele of their KCNC1 genes which encodes $K_v3.1$ channels. This precision medicine approach is supported by nonclinical data, which shows that AUT00201 positively modulates $K_v3.1$ current, even in the presence of the mutant channel, and thus has the potential to rescue the impact of the MEAK mutation. A genetic mouse model of MEAK, namely Kcnc1-p.R320H/+ (also referred to as 'Het', 'H/+', or 'MEAK mice') has been developed at the Children's Hospital of Philadelphia. The model recapitulates key elements of the human MEAK syndrome, notably increased vulnerability to seizure and progressive ataxia. In order to explore this phenotype and the ability of AUT00201 to rescue the phenotype, repeated administration of the chemoconvulsant, pentylenetetrazole (PTZ) was used to induce progressively more severe seizures ("kindling epileptogenesis") in the mice. The MEAK mice showed a greater sensitivity to PTZ-induced seizures and a more rapid kindling compared to wild-type mice. AUT00201 (5 mg/kg and 20 mg/kg orally [p.o.]) significantly prolonged survival and reduced the rate of kindling of the MEAK mice administered PTZ. AUT00201 also had a statistically significant effect on the rate of PTZ kindling in wild-type mice at both the 5 mg/kg and 20 mg/kg p.o. doses, suggesting an anticonvulsant effect of the drug.

The ataxia phenotype of Kcnc1-p.R320H/+ mutant mice was determined from their impaired performance on an accelerating Rotarod. The MEAK mice showed a significant impairment in performance on the Rotarod compared to the wild-type mice. However, the difference in performance between wild-type and MEAK mice on the Rotarod was completely and significantly rescued by AUT00201 at 5 mg/kg and 20 mg/kg p.o. [redacted]

The planned study in MEAK patients will provide the safety and tolerability of single doses of AUT00201 in patients with MEAK. The study includes a range of biomarker assessments in

order to capture signals of efficacy in this population and will provide the first proof-of-mechanism that this class of drugs can be effective in patients with a PME syndrome.

Another important aim of this study is the characterization of the cortical pathophysiology and biomarker profile of MEAK patients. A better understanding of the pathophysiology of these patients may help to optimize their treatment and will also allow comparison to other forms of PME where AUT00201 could have potential to improve symptoms. Such a characterization may also support the use of these biomarkers in future studies in other indications in which dysfunction of PV+ interneurons has been implicated. These biomarkers will include paired-pulse transcranial magnetic stimulation (TMS) to probe motor cortex excitability and measures of auditory and visual information processing that have been associated with dysfunction of PV+ interneurons that express $K_v3$ channels. We predict that we will observe evidence for reduced cortical inhibition in the MEAK patients and subtle but consistent abnormalities in early auditory and visual information processing. Previous studies have shown reduced cortical inhibition in other forms of myoclonic epilepsy, such as Unverricht-Lundborg syndrome.[17,18,19] On this basis, there would be a reasonable rationale to explore the therapeutic potential of AUT00201 in these other epilepsy syndromes.

Prior to the development of AUT00201, the sponsor has conducted clinical studies with 2 other $K_v3$ modulators (AUT00063; IND 122711), and AUT00206 (EudraCT numbers: 2015-002364-16, 2016-000216-14, 2016-002704-63, 2021-003211-26), the latter of which is being progressed for certain neuropsychiatric indications, such as Fragile X Syndrome. This study plans to enroll patients aged 18 years and older diagnosed with MEAK (see Section 4.1). The safety and tolerability of AUT00201 at exposures exceeding those proposed to be used in the current study has been established in a first-in-human (FIH) study in healthy volunteers (see Section 3.5).

## 1.2. Background

Pathogenic variants in the KCNC1 gene, which encodes the $K_v3.1$ channel, have been linked recently to a spectrum of epilepsy and intellectual disability phenotypes.[13,14,20,21,22] A pathogenic variant of KCNC1 results in a form of PME called MEAK, this is a severe and debilitating, ultra-rare disease which has been described in approximately 40 individuals in the medical literature.

This recurrent de novo mutation in KCNC1 leading to PME was first uncovered in a study evaluating whole exome sequencing in 2 unrelated cohorts of patients with PME of unknown etiology. From these 2 cohorts, 16 cases (13 unrelated) were identified with a KCNC1 c.959G>A; p.Arg320His mutation.[13] The expression of $K_v3.1$ is mainly limited to the central nervous system (CNS) and a specific subset of gamma-aminobutyric acid (GABA)ergic inhibitory interneurons known as PV+ fast-spiking interneurons, which are critical components of cortical circuits, including motor and sensorimotor cortex. $K_v3.1$ channels are also particularly expressed in the cerebellum and auditory brainstem. Thus, p.Arg320His substitution in $K_v3.1$ may result in cortical disinhibition due to the impaired firing of fast-spiking GABAergic interneurons, leading to myoclonus and seizures as well as ataxia and motor impairments that may arise from dysfunction of cerebellar neurons. Alterations of auditory and visual function have not been reported in patients with MEAK, although appropriate tests to discern such impairments most likely have not been applied. Small molecule positive modulators that

selectively target $K_v3.1$ channels have been developed by Autifony Therapeutics and have the potential to be used in treatment of KCNC1-related disorders, including MEAK. Nonclinical studies in cellular systems and transgenic mice expressing MEAK mutant $K_v3.1$ channels support this potential.

Twenty patients with the recurrent KCNC1 c.959G>A; p.Arg320His mutation were described in the original study. [14] Of these, 14 were sporadic (unrelated) cases, 4 were from 1 family, and 2 were a mother-child pair). The patients were 17 to 63 years of age when they were studied. Onset of neurologic symptoms was at a median of 9.5 years (range 3 to 15 years). In the majority, the first symptom was myoclonus (brief jerks). Three patients presented with ataxia (with or without myoclonus) and 3 presented with generalized tonic-clonic seizures between the ages of 9 and 14 years without prior myoclonus. All of these patients were described as having progressively severe and frequent (daily) myoclonus which was action-induced and often resulted in falls, ataxia primary factor limiting ambulation. About half of the patients became wheelchair-dependent, with severe myoclonus noted as the predominant reason. Myoclonus is also reported as being so severe as to interfere with daily activities such as being able to eat independently and over half of the 20 patients needed assistance with all functions of daily living, reported as fully or mostly dependent.

A diurnal pattern was noted – myoclonus was worse in the morning. Other exacerbating factors included stress, anxiety, startle, or menses. Generalized tonic-clonic seizures were rare, occurring most often during teenage years. At the time of the report, all of the patients were being treated with 1 or more anti-seizure medications, with variable efficacy. In this cohort of 20 patients, the progression of the disorder appeared to stabilize in adulthood and did not appear to result in early death. The description of these findings was based on retrospective review of information collected for clinical purposes and did not utilize standardized instruments, thus limiting quantitative data which could be followed over time.[23]

As discussed by Oliver et al, the loss of $K_v3.1$ channel function is suggested to lead to reduced cortical inhibition, which gives rise to motor and sensorimotor cortical hyperexcitability.[14] This hyperexcitability underlies the susceptibility to rhythmic myoclonic activity in MEAK as well as in a broader range of PMEs due to other genetic causes. The synchronized discharges of pyramidal neurons transferred through the pyramidal tract to the peripheral motor system results in synchronous motor unit discharges that lead to rhythmic myoclonic jerks, rather than normal integrated movements.

Cortical excitability has been investigated with the help of TMS. In particular, paired-pulse TMS allows measurement of the function of GABA-dependent cortical interneuron circuits which is especially relevant in the context of genetic epilepsies like MEAK in which abnormal interneuron function in strongly implicated.[24] In other PMEs, decreased cortical inhibition has been demonstrated using this approach.[25] The measures of interest include short interval cortical inhibition (SICI) and long interval cortical inhibition (LICI).

Reduced inhibitory control in MEAK is consistent with the high expression of $K_v3.1$ channels on PV+ GABA interneurons where they enable these neurons to fire rapidly and accurately. [22] Loss of $K_v3.1$ function is therefore likely to result in reduced activity of this important class of interneuron. Studies have demonstrated that normal functioning of PV+ interneurons is critical

for the generation of highly synchronized gamma oscillations which are important for enhancing signal transmission in the neocortex by reducing circuit noise and amplifying circuit signals. [23] Dysfunction of PV+ interneurons is associated with reduced phase synchrony of evoked gamma oscillations associated with increased power of resting state gamma oscillations. [26] This has been observed in other indications where PV+ interneuron dysfunction has been implicated; for example, studies in fragile X mental retardation protein (FMRP) knock-out mice and patients with Fragile X Syndrome show identical electroencephalogram (EEG) abnormalities, strongly suggesting that desynchronized but increased resting state gamma oscillations represent a translational biomarker of PV+ interneuron dysfunction.[27] In patients with MEAK, resting state gamma oscillations have not been investigated, but it is plausible to assume that similar abnormalities should be observed. Thus, measures of evoked gamma synchrony and resting state gamma frequency power using EEG may serve as a further biomarker for the underlying pathophysiology in MEAK.

Finally, $K_v3.1$ channels play a key role in the auditory brainstem, where they contribute to the accurate firing of auditory projection neurons. In a related ataxia disorder caused by mutation in the KCNC3 gene that codes for $K_v3.3$, alterations in inter-aural sound discrimination could be detected.[26] Although specific measures of central auditory processing have not been investigated in patients with MEAK, we can speculate that some dysfunction in these processes could be present due to reduced $K_v3.1$ channel function in auditory neurons.

Dysfunction of inhibitory interneurons and GABAergic transmission in primary and secondary visual cortices has also been implicated in subtle abnormalities in early visual perceptual processes like contour integration, contrast sensitivity, visual orientation discrimination, and binocular rivalry.[28,29] These visual processes have not been investigated in patients with MEAK. Their evaluation will complement the assessment of auditory functioning and provide a comprehensive characterization of potential biomarker abnormalities associated with reduced $K_v3.1$ channel function.

The study will investigate safety, tolerability, PK, and pharmacodynamic (PD) outcomes of a $K_v3.1/K_v3.2$ modulator, AUT00201 in MEAK patients following a single dose of AUT00201. It will investigate biomarkers of cortical excitability, and PV+ interneuron function in patients with MEAK. Exploration of measures of speech, central auditory, and visual function will also be included where this is feasible without overburdening the patients.

The study will aim to validate and extend the use of biomarkers of PV+ interneuron dysfunction and cortical hyperexcitability, respectively, in the MEAK population. Cortical excitability using TMS has already been explored in patients with other forms of PME. [27,30] It is expected that patients with MEAK will demonstrate EEG abnormalities mentioned earlier, as well as the paired-pulse TMS abnormalities observed in other PMEs. In addition, subtle abnormalities in auditory and visual information processing and perception may be observed. If indeed these abnormalities can be demonstrated and prove to be susceptible to treatment with AUT00201, then they could be used in the future to identify patients with PME and possibly other neuropsychiatric disorders in which PV+ interneuron dysfunction and cortical hyperexcitability play a prominent role or have been implicated.

## 1.3. Benefit-risk Assessment

AUT00201 is the third orally administered positive modulator of $K_v3$ channels to enter human evaluation. The first was AUT00063, which was evaluated in 1 Phase 1a and 3 Phase 2a studies. The second was AUT00206, which was evaluated in 2 Phase 1a and 2 Phase 1b studies. The nonclinical and Phase 1 clinical safety of AUT00201 appears similar to both AUT00063 and AUT00206 at the same stage of development. No deaths or suspected unexpected serious adverse events (SAEs) have occurred following administration of any of these compounds.

The prior FIH study with AUT00201 (AUT011201) established the safety and tolerability of the drug in healthy volunteers for single oral doses of up to 320 mg once daily and 200 mg twice daily (BID), and repeat doses of up to 100 mg BID for 14 days. All reported adverse events (AEs) were mild to moderate and there was no notable relationship between AUT00201 dose and the frequency or severity of reported AEs compared to placebo. There were no deaths or SAEs reported in patients. No adverse cardiac signals were observed in the completed Phase 1 study of AUT011201, nor in the completed or ongoing clinical studies with the structurally-related $K_v3$ modulator AUT00206.

Based upon its structure, the available nonclinical and clinical safety data, and clinical data from the mechanistic and structurally-related compound AUT00206, AUT00201 is considered as a Low Risk Molecule.

In this study, AUT00201 will be administered to MEAK patients. MEAK is caused by a recurrent de novo heterozygous mutation in the KCNC1 gene which encodes for the Kv3.1 protein. Treatment with AUT00201 provides an opportunity to test a targeted therapy in patients with a known dysfunction in the Kv3.1 channel. This precision medicine approach is supported by the nonclinical data, which shows that AUT00201 can positively modulate Kv3.1 current, even in the presence of the MEAK mutation in vitro, and completely reverse the observed seizure sensitivity and ataxia phenotype in the mice in vivo.

Currently, there is no specific treatment for MEAK. Therapy focuses on symptomatic relief, using primarily broad spectrum antiepileptics.[14,15,31] However, while these treatments may target the rare occurrence of tonic-clonic seizures they have no or only very limited efficacy against MEAK patients' primary symptoms of action-induced myoclonus and ataxia. There is no treatment for action-induced myoclonus. Clonazepam is the only drug approved by the US Food and Drug Administration as monotherapy for the treatment of myoclonic seizures, but these are not the key disabling symptoms of these patients. In addition, clonazepam is associated with significant side effects such as sedation and dependence. The efficacy of AUT00201 in humans with MEAK has not been evaluated to date. It is anticipated that continued exposure to AUT00201 will act on $K_v3.1$ channels on specific populations of neurons in the cortex and cerebellum, restoring their ability to sustain rapid firing required for normal function. It is postulated that this will reverse the reduced cortical inhibition and will in turn normalize motor and sensorimotor cortical hyperexcitability. This hyperexcitability underlies the susceptibility to rhythmic myoclonic activity in MEAK; thus AUT00201 may be an effective treatment for myoclonus. Furthermore, improved function of cerebellar neurons with AUT00201 treatment may reduce symptoms of ataxia. Thus, AUT00201 has the potential to provide therapeutic

benefit to patients with MEAK where there are currently no effective treatments for their action myoclonus and ataxia.

The study is designed in a way to manage the risks identified in the previous nonclinical studies and the potential risks based on the mechanism of action. The following provides a list of identified risks and mitigation strategies from a safety perspective:

**Safety Assessments**: The FIH study reported no SAEs nor any apparent relationship between dose or exposure and treatment-emergent adverse events (TEAEs) at doses that achieved AUT00201 exposures above those proposed in this new study in MEAK patients (the proposed exposure for this study is based on the exposures associated with efficacy in the equivalent mouse genetic model of MEAK) – see Section 1.1.

All MEAK patients will be evaluated prior to dosing at Visit 1 and Visit 2 using the standard battery of safety assessments; laboratory assessments (routine hematology, biochemistry, urinalysis), physical examinations, 12-lead electrocardiograms (ECGs), vital signs (sitting and standing), Columbia-Suicide Severity Rating Scale (C-SSRS). Adverse events will be monitored frequently inpatient and until the patient's follow-up visit.

[redacted]

Patients will be inpatient in a clinical research unit, with strong experience in CNS research, for approximately 5 days and for at least 24 hours postdose. Neurological checks will be completed every 12 hours whilst inpatient consistent with local requirements. Paired-pulse TMS

assessments will be scheduled to be conducted at around the anticipated time to reach maximum (peak) plasma concentration ($t_{max}$) and therefore the patients will be under direct supervision during this time.

For safety reporting purposes, all SAEs will be considered unexpected.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of AUT00201 may be found in the IB.

# 2. OBJECTIVES AND ENDPOINTS

## 2.1. Study Objectives

The primary objective is:

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

The secondary objectives are:

- To evaluate the effects of single doses of AUT00201 on SICI as measured by paired-pulse 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 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.

The exploratory objectives are:

- To evaluate the effects of single doses of AUT00201 on 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 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. 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 AEs.

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
  - MI (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.

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 each 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])
- Changes in biomarkers as compared to exposure to AUT00201.

# 3. INVESTIGATION PLAN

## 3.1. Overall Study Design and Plan Description

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. 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 will participate in the study.

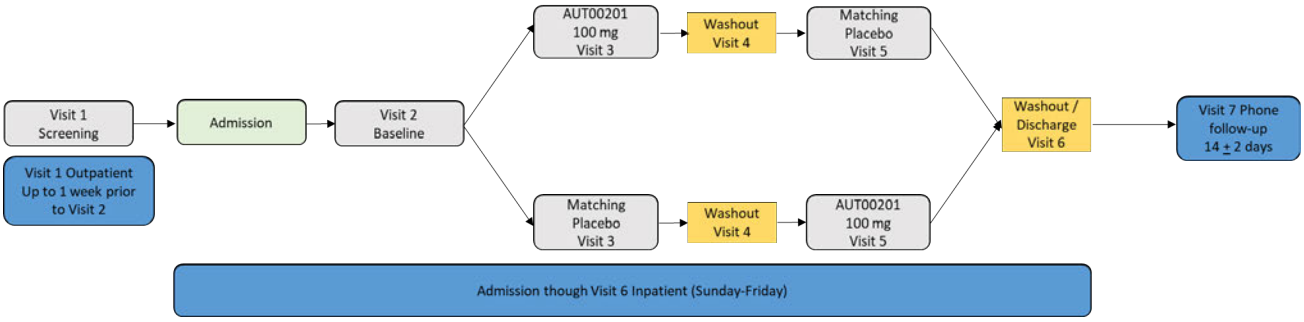
The study is a double-blind, randomized, placebo-controlled treatment in which patients will be administered a single dose of 100 mg AUT00201 and matching placebo in a crossover design. There is an initial outpatient screening and procedure orientation which can be spread over 2 days, followed by a 5-day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

Once a patient has signed consent, a unique screening number will be assigned. Outpatient screening and procedure orientation (Visit 1) will occur up to 1 week prior to Visit 2. Patients will be admitted to the clinic for a 5-day inpatient stay. Baseline assessments (Visit 2) will be completed on the first inpatient day. Patients will then be administered a single dose of 100 mg AUT00201 or matching placebo on the second inpatient day (Visit 3). PK assessments will be done at specified timepoints (Visits 3, 4, 5, and 6) from predose and up to 27 hours postdose. The third inpatient day (Visit 4) will be a washout day for patients. On the fourth inpatient day (Visit 5), the patients will be administered the crossover treatment. On the fifth inpatient day (Visit 6), patients will be discharged from the unit. Safety and tolerability assessments will be conducted throughout. PD parameters will also be assessed. Patients will be followed up by phone 14 days ±2 days after discharge (Visit 7).

The 48 hours washout period is deemed appropriate based on the half-life observed in the FIH data. [redacted] Thus, it is anticipated that a dose of 100 mg fed will result in negligible plasma concentrations. This washout period has also been chosen to minimize patient burden, by limiting the number of times the patients will have to travel to site.

Safety, tolerability, PK, and biomarker assessments will be conducted throughout the study as detailed in the Schedule of Assessments (Appendix 4). Figure 1 provides an illustration of the study design.

**Figure 1: Study Design**




## 3.2. Replacement Patients

It is not possible to replace patients in this study given the rarity of MEAK and the known patient population. However, if any technical issues or important protocol deviations occur during a patient's participation that are likely to make the primary, secondary, or exploratory endpoint data unevaluable or impact their validity, the patient may be invited back to participate in the study again, if the sponsor and Investigator are in agreement.

If a patient is invited back to participate a second time, they will be given a new screening number and treated as a new patient, including consideration for inclusion and exclusion criteria. There will therefore be a minimum of 90 days between the last dose of IP in the first particpation and the first dose of IP in the second partcipation. Their data from the first particpation, with the exception of safety and PK data, will not be included in the statistical analysis. All data will be listed, with clear indictaion if a patient is a repeat participant.

There are no safety concerns for rechallenging the patients. Participants in the FIH study received up to 4 single doses separated by 10-day washout periods; they received escalating doses, the highest of which was 160 mg to 200 mg BID (400 mg total dose).

There is no impact on the data integrity by rechallenging patients. The assessments have been selected for the crossover design of the study and no learning effect is expected.

## 3.3. Discussion of Study Design

The study will be double-blind to avoid bias in the collection and evaluation of data during its conduct. The Investigator and sponsor will remain blinded to the treatment randomization code until after the database has been locked. Placebo has been chosen as the control to assess whether any observed effects are treatment-related or simply reflect the study conditions. A crossover design is used to increase the power of the study and reduce the influence of confounding covariates since each patient serves as its own control.

It is reported that patients have noted transient improvement in gait and myoclonus with high fever. [14] This typically lasted just hours or a few days while the patient was febrile, but was noticeable. This has been attributed to the temperature-dependent leftward shift in activation of wild-type Kv3.1 subunit–containing channels, which would counter the loss of function observed for mutant channels. Therefore, it is postulated that improvements following a relatively short

treatment may be captured by the biomarkers used or even be evident in clinical assessments. Patient's history of fever and any perceived improvement of symptoms will be captured as part of their medical history.

The planned safety assessments that will be performed during the study are considered acceptable measure for ensuring the safety of patients during a clinical study.

### 3.4. End of Study Definition

A patient is considered to have completed the study if he/she has completed all visits of the study including all the required procedures scheduled as shown in the Schedule of Assessments (Appendix 4).

The end of the study is defined as the date of final Follow-up (planned as a telephone call) by the last patient in the study.

### 3.5. Selection of Doses in the Study

Selection of doses of AUT00201 for this study was based on completed nonclinical testing and the FIH study (AUT011201).

The FIH study assessed single ascending (Part A) and repeated doses (Part B) of AUT00201 in separate groups. The starting single dose was 3 mg and was escalated to 200 mg BID in Part A (Table 1) in healthy male subjects.

**Table 1: First-in-Human Study (AUT011201) Part A1 Single Doses of AUT00201**

| Groups | Session 1 | Session 2 | Session 3 | Session 4 |
|---|---|---|---|---|
| 1 | 3 mg | 6 mg | 12 mg | 20 mg |
| 2 | 20 mg | 60 mg | 160 mg | 300 mg |
| 3 | 160 mg | 320 mg | 2 × 160 mg | 2 × 200 mg |

Note: Doses were given in the fasted state in Groups 1 and 2 and in the fed state in Group 3.

In Part B of the FIH study, 2 groups of healthy males received oral doses of AUT00201 or placebo BID, for 14 days. In each group, 6 subjects received active treatment and 2 subjects received placebo (3:1 ratio). The first group received 100 mg BID and the second group received 40 mg BID.

There were no deaths, severe AEs, or other SAEs reported in the FIH study (AUT011201). The reported AEs were mild or moderate in severity and no patient was withdrawn from the study due to an AE. Thus, a single dose of 200 mg BID and repeated 100 mg BID dosing for a period of 14 days were considered as safe and well tolerated.

This study will generate safety and tolerability data and explore the PK and PD effects of a single oral dose of 100 mg AUT00201 in MEAK patients.

[redacted]

[redacted]

The PK data from the FIH study showed that the highest $C_{max}$ value after a single dose of 320 mg of AUT00201 to healthy volunteers was 1620 ng/mL, and the corresponding maximum $AUC_{24}$ was 13160 ng.h/mL. Furthermore, even higher exposures were observed after repeat doses, with the maximum exposure in the FIH study observed after 14 days with 100 mg BID dosing: the highest $C_{max}$ value was 2060 ng/mL, and the highest $AUC_{24}$ was 30,128 ng.h/mL. These exposures were not associated with any significant AEs in healthy subjects and there was no notable relationship between AEs and dose or exposure. All AEs seen in the FIH study were mild or moderate.

[redacted]

In the proposed study, MEAK patients will have outpatient and inpatient visits in a hospital clinical research unit and will be discharged at Visit 6. The patients will be administered paired-pulse TMS over the anticipated $t_{max}$ and therefore will be under direct supervision during this time. Patients will also have 12-hourly neurological checks and standard clinical safety

assessments. The PK of AUT00201 is expected to be similar in healthy volunteers and MEAK patients. AUT00201 is not an inhibitor of cytochrome P450 (CYP) enzymes and is metabolized by multiple CYP isoforms (predominantly CYP3A4 and CYP2C9, and possibly CYP2C19), therefore the likelihood of a significant PK interaction with any of the expected anticonvulsant concomitant medications that the MEAK patient will be taking is considered low.

Based upon the clinical experience already available on AUT00201, patients will be monitored within the clinical inpatient unit for the duration of dosing, and the expected duration of exposure to the IP the sponsor considers a single dose of 100 mg AUT00201 to be acceptable for this study.

As the effect of drug in pregnant women is not known, AUT00201 will be discontinued in the patients who may conceive or are tested positive for pregnancy during the study. From safety perspective, study treatment will be discontinued per criteria listed in Section 4.4.2. Further, the stopping rules for study treatment are provided in Section 4.5.

# 4. SELECTION OF STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

There are only 10 patients known to the Epilepsy Neurogenetics Initiative at the Children's Hospital of Philadelphia. It is hoped approximately 6 to 10 patients who are over the age of 18 years will participate.

## 4.1. Inclusion Criteria

Patients must satisfy all the following criteria at Screening Visit (Visit 1) unless otherwise stated:

1. Male or female patients aged 18 years or older at the time of consenting
2. Diagnosed with MEAK, based on documented genetic evidence of the presence of the KCNC1 (c.959G>A; p.Arg320His) variant
3. If taking anticonvulsants, must be on a stable anticonvulsant regimen for at least 30 days prior to Visit 1 and anticipated to remain stable throughout the study, or if not on an anticonvulsant regimen, must be stable in regards to seizures for at least 30 days prior to Visit 1 and anticipated to remain stable throughout the study
4. Must be able to participate and willing to give written informed consent. If patient is unable to provide written informed consent, a legally authorized representative can sign on their behalf
5. Must be willing to perform study assessments and comply with the study protocol
6. If the patient is dependent on a caregiver and/or will need assistance either travelling to the site, whilst attending clinical visits and/or helping to document study assessment responses provided by the patient (eg, questionnaires administered on a tablet device) they must have an identified caregiver, considered reliable by the Investigator, to provide support to the patient for the duration of the study. The caregiver must be willing and able to provide support to the patient and, if required, stay for the duration of the study.
7. Medically stable based on Investigator's judgment for at least 90 days prior to Visit 1
8. Women of childbearing potential must have a negative urine pregnancy test on Visit 2 (Baseline Visit)
9. If a vagal nerve stimulator is used, it must be implanted at least 150 days before Visit 1, and parameters must be stable for at least 30 days before Visit 1 and be expected to remain stable throughout the study
10. If a ketogenic diet is followed, it must be stable for at least 30 days before Visit 1 and be expected to remain stable throughout the study
11. Willing to comply with contraceptive requirements (see below).
12. Able to speak, read and understand spoken English at a fluent level.

**Contraceptive requirements: Men**

Male patients must not plan to father a child or donate sperm, during the study and for 90 days after their follow-up visit.

Male patients must not have sex without using a condom, during the study and for 90 days after their follow-up visit, if their partner is a woman of childbearing potential. They do not need to use any contraception if: they had a vasectomy and surgical success has been confirmed by medical assessment, or their partner is not of childbearing potential. Partners who are not of childbearing potential are defined as: men; postmenopausal women (no menstrual periods for at least 365 days); or women who have no uterus, ovaries, or fallopian tubes.

During the study, patients must not have sex with a woman who is pregnant or breastfeeding, without using a condom.

**Contraceptive requirements: Women**

Women of childbearing potential must use a highly effective method of contraception with low-user dependency during the study. They must have been using that method for at least 28 days before Visit 1 and continue to use it for 180 days (6 months) after their last dose of study medication. Highly effective methods of contraception include:

- intrauterine device
- intrauterine hormone-releasing system
- established use of oral, injected, or implanted hormonal methods of contraception
- bilateral tubal occlusion
- vasectomized partner (with surgical success confirmed by medical assessment).

A woman is considered to be of non-childbearing potential if she meets one of the following criteria:

- is postmenopausal (the last menstrual period was at least 365 days ago, and follicle-stimulating hormone at Screening confirms postmenopausal status)
- has no uterus, ovaries, or fallopian tubes.

Patients who practice true abstinence or who only have same-sex relationships need not use contraception, provided it is in line with their preferred and usual lifestyle (Note: periodic abstinence [eg, calendar, ovulation, symptothermal, and postovulation methods] and withdrawal are not acceptable methods of contraception). Should any such patient stop practicing true abstinence, they must use contraception as described above.

## 4.2. Exclusion Criteria

Patients will be excluded from the study if they satisfy any of the following criteria at Screening Visit (Visit 1) unless otherwise stated:

1. Known pathogenic mutation in another gene that causes epilepsy or a different mutation in the KCNC1 gene than the c.959G>A variant
2. Clinically significant metabolic, hepatic, hematological, pulmonary, cardiovascular, gastrointestinal, or urological disorder
3. Clinically significant abnormal vital signs or laboratory test results
4. Hypersensitivity to AUT00201 or any of the excipients
5. Any medical condition or other factors, as judged by the Investigator, which may interfere with the patient's participation in this study and/or compromise the patient's ability to safely complete the study
6. Known to abuse drugs or those who test positive on urine screen for drugs of abuse will be excluded based on Investigator's judgment
7. Positive hepatitis B surface antigen or hepatitis C antibody
8. Clinically significant abnormality on the 12-lead ECG
9. Having received an IP 90 days prior to Visit 1
10. Currently using felbamate for <1 year prior to Visit 1 or any evidence of ongoing hepatic or bone marrow dysfunction associated with current/prior felbamate treatment. Patients who are currently using felbamate for >1 year prior to Visit 1 and have no evidence of ongoing hepatic or bone marrow dysfunction associated with felbamate treatment are allowed
11. Currently using vigabatrin and having received vigabatrin for <2 years prior to Visit 1
12. Suicidal ideation with some intent to act within 6 months prior to Visit 1 based upon a response in the C-SSRS (positive response to questions 4 or 5 of the suicidal ideation section) and as judged by the Investigator as having a significant impact on trial participation or patient safety. History of suicidal behavior within 1 year prior to Visit 1.

### 4.3. Lifestyle Restrictions

AUT00201 is predominately metabolized by CYP3A4 and CYP2C9. Accordingly, the consumption of dietary supplements (eg, St. John's wort), foods, and forms of fruit juice (eg, Seville oranges, grapefruit juice) that may affect the expression or function of CYP3A4 or CYP2C9 will not be allowed beginning 7 days before Visit 1 and continuing whilst inpatient.

No alcohol will be allowed during the period from 24 hours before Screening until the end of the inpatient stay. Patients who regularly drink caffeinated drinks will be asked to maintain their routine, but nonregular drinkers will be asked to avoid all caffeine. Restrictions on smoking whilst inpatient will be discussed with the patients at Screening.

## 4.4. Discontinuation Criteria

### 4.4.1. Screen Failures

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAEs with concomitant medication details.

When 1 or more correctable inclusion or exclusion criteria are found at Screening, after appropriate management, the patient can be rescreened at the discretion of the Investigator and if approved by the sponsor or sponsor designee in writing.

### 4.4.2. Discontinuation of Study Treatment

Patients may withdraw from the study at any time at his/her own request or legal guardian's request (if applicable), or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. Additionally, patients may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records. A patient that withdraws from the study may be asked to stay in the clinic for a further day in order to complete safety assessments such as ECG, vital signs, and safety laboratory tests. These assessments will be at the discretion of the Investigator in order to ensure safety can be reviewed prior to the patient's discharge. A PK sample may also be collected at the discretion of the Investigator. Refer to the Schedule of Assessments (Appendix 4) for potential data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Additional reasons for discontinuation can include, but are not limited to:

- Administration of treatments not allowed in the protocol
- AE
- Lack of efficacy
- Pregnancy
- Sponsor determination
- Investigator's decision
- Death.

### 4.4.3. Lost to Follow-up

A patient will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible, counsel the patient or legal guardian on the importance of maintaining the assigned visit schedule, and ascertain whether or not the patient wishes to and/or should continue in the study.
- In cases in which the patient is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the patient or legal guardian (if applicable) (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's medical record.
- Should the patient or legal guardian (if applicable) continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

### 4.4.4. Replacement Procedures

Patients who withdraw from the study or who are withdrawn by the Investigator for safety reasons will not be replaced. However, if a patient discontinues the study for technical issues or important protocol deviations that are likely to make the primary, secondary, or exploratory endpoint data unevaluable or impact their validity, the patient may be invited back to participate in the study again, if the sponsor and Investigator are in agreement.

If a patient is invited back to participate a second time, they will be treated as a new patient, including consideration for inclusion and exclusion criteria. There will therefore be a minimum of 90 days between the last dose of IP in the first participation and the first dose of IP in the second partcipation. Their data from the first particpation, with the exception of safety and PK data, will not be included in the statistical analysis. All data will be listed, with clear indictaion if a patient is a repeat participant.

There are no safety concerns for rechallenging the patients. Participants in the FIH study received up to 4 single doses separated by 10-day washout periods; they received escalating doses, the highest of which was 160 mg to 200 mg BID (400 mg total dose).

There is no impact on the data integrity by rechallenging patients. The assessments have been selected for the crossover design of the study and no learning effect is expected.

### 4.4.5. Follow-up of Patients Prematurely Discontinued from the Study Treatment Regimen or Withdrawn from Study

Due to the continued scientific importance of patient data even if study treatment is discontinued early, patients who withdraw from the study may be asked to stay in the clinic for a further day in order to complete safety assessments such as ECG, vital signs, and safety laboratory tests. These assessments will be at the discretion of the Investigator in order to ensure safety can be reviewed prior to the patient's discharge. A PK sample may also be collected at the discretion of the Investigator. Refer to the Schedule of Assessments (Appendix 4) for potential data to be

collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed. Patients who are no longer hospitalized due to early IP discontinuation may complete their assessments by telephone or via telemedicine or other means of remote communication. Laboratory assessment will be done at on-site visits.

All treatment-related (S)AEs that are ongoing at the time of discontinuation, or that develop before the protocol-specified follow-up visit, will be followed for 30 days, or until resolution or stabilization.

### 4.5. Stopping Rules

Dosing of additional patients may be paused and a safety review meeting, including the Investigator, sponsor, and medical monitor will be convened if any of the following criteria are met:

- If 2 or more patients experience a similar severe (Grade 3) AE or SAE
- A life-threatening or fatal (Grade 4 or 5) SAE in any patient.

### 4.6. Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. The study site will be closed upon study completion. The study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed. Reasons for study termination may include, but are not limited to:

- AEs unknown to date (ie, not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)
- Increased frequency and/or severity and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined as baseline signs and symptoms)
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of patients
- Cancellation of drug development.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study site by the sponsor or Investigator may include, but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the Institutional Review Board (IRB) or local health authorities, the sponsor's procedures, or Good Clinical Practice guidelines
- Inadequate recruitment of participants by the Investigator.

# 5. STUDY TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study patient according to the study protocol.

## 5.1. Treatments Administered

Each patient will receive oral doses of AUT00201 or matching placebo as capsules. AUT00201 will be administered as capsules containing 100 mg (size 0) or matching placebo capsules (Table 2).

Patients will receive a single dose of 100 mg AUT00201 or matching placebo (one 100 mg capsule) at Visits 3 and 5, approximately 30 minutes after completion of a standard meal provided by the clinical site.

**Table 2: Description of Study Treatment and Placebo**

| **Study Treatment Name:** | **AUT00201** | **Placebo** |
|---|---|---|
| **Dosage Formulation:** | Swedish orange-colored, hard gelatin capsules of size 0 | Matching placebo capsules (Swedish orange-colored, hard gelatin capsules of size 0) |
| **Unit Dose Strengths:** | 100 mg (size 0) | To match active |
| **Route of Administration:** | Oral | Oral |
| **Dosing Instructions:** | Randomized, double-blind: 1 × 100 mg capsules (size 0), 30 minutes after a standard meal[a] | As for active |
| **Packaging and Labeling:** | AUT00201 capsules will be provided as blinded medication in HDPE bottles. Each bottle will be labeled in a manner that is indistinguishable from the placebo. | Placebo capsules will be provided as blinded medication in HDPE bottles. Each placebo bottle will be labeled in a manner that is indistinguishable from the active study treatment. |

Abbreviations: HDPE = high-density polyethylene.
[a] As provided by the clinical site.

## 5.2. Preparation, Storage, Handling, and Accountability

The study drug will be supplied by the sponsor (or designee), along with the batch/lot numbers and Certificates of Analysis. The study treatment will be provided in opaque white, high-density polyethylene bottles with child-resistant closures. The recommended storage conditions, and expiry date (wherever required), will be stated on the product label.

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatments received and any discrepancies are reported and resolved before use of the study treatment.

All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

Only patients enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment while patient is inpatient. The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatment are provided in the Pharmacy Manual.

### 5.3. Method of Treatment Assignment

Patients will be assigned a unique code number (randomization number) at Visit 3. The patient number will encode the patient's assignment either to study treatment or to placebo arm of the study, according to the randomization schedule generated prior to the study by [redacted] Each patient will be dispensed blinded study treatment or placebo, labeled with his/her unique patient number, and traceable to the batch number of the medication.

### 5.4. Blinding

The study will be a double-blind, crossover, placebo-controlled, where the patients will receive blinded single doses of AUT00201 or matching placebo treatment that is identical in appearance and similar in taste and smell.

The specific blinded treatment to be taken by a patient will be assigned using randomization schedule. The study treatment will be dispensed per the randomization schedule by the pharmacy. Active and placebo treatments will be labeled such that it is not possible to distinguish between them. Unblinding should occur only in the event of medical emergency, given the knowledge of the study drug is essential for clinical management or welfare of the patient. In the event of emergency unblinding by the Investigator, the sponsor must be informed as soon as possible. The date and reason that the blind was broken must be recorded in the source documentation and electronic case report form (eCRF), as applicable. Once the study is complete, all envelopes (sealed and opened) must be inventoried and returned to the sponsor.

### 5.5. Treatment Compliance

Records shall be maintained of the delivery of the study treatment to the study site, the inventory at the study site, the use for each patient, and the return to the sponsor or destruction.

These records shall include dates, quantities, expiry dates, and the unique code numbers assigned to the study treatment and study patients.

The Investigator shall be responsible for ensuring that the records adequately document that the patients were provided the doses specified in the protocol and that all study treatment received

from the sponsor is reconciled; dates and times of dosing will be recorded and entered in patient's eCRF.

# 6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

## 6.1. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives during the study must be recorded in the eCRF along with:

- reason for use
- dates of administration including start and end dates
- dosage information including dose and frequency.

Patients must be instructed not to take any new medications or change their current regimen, including over-the-counter products, without first consulting with the Investigator. The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. Medications that are necessary for the patient's safety and well-being are permitted at the discretion of the Investigator. It is not recommended that patients are withdrawn from any of their currently prescribed medications for the study. Data on all concomitant drugs administered, doses and duration of concomitant use, and the specific timing of administration relative to the administration of AUT00201 or placebo will be captured in the source documents and eCRF throughout the study.

## 6.2. Prohibited Medications

Patients will refrain from use of any new prescription or nonprescription medications/products during the study until the follow-up unless the Investigator (or designee) has given their prior consent (with the sole exception for acetaminophen for pain relief).

The use of any other investigational agents is not allowed during the study.

# 7. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in the Schedule of Assessments (Appendix 4). Protocol waivers or exemptions are not allowed, with the exception of mitigating immediate safety concerns, if immediate safety concerns are identified, these should be discussed with the sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue the study. Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure (Section 4.4.1), as applicable. Procedures conducted as part of the patient's routine clinical management (eg, blood count) and obtained before signing the informed consent form (ICF) may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the timeframe defined in the Schedule of Assessments.

With the sponsor's approval, additional blood sampling timepoints may be introduced, and changes to timepoints may be made if there is a scientific reason to believe that the additional blood sampling might improve the quality of the data (for example, the scenario, where an important effect of the IP is being observed, but no measurements are scheduled for that timepoint), or if extra procedures are needed in the interest of the patients' safety.

Overall, the maximum amount of blood collected from each patient over the duration of the study, including any extra assessments that may be required (for the reasons stated above), will not exceed the blood volume collection limitations established by local IRB. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

A prescreening telephone questionnaire, approved by the IRB, will be developed to collect basic information on the patients before patients visit the site (eg, has their current medication regimen been stable for last 90 days?). This will help to reduce the risk of screen failure and also patient burden of travelling to the site.

A pre-study ICF may be used to obtain basic patient identifiers so that patient travel may be scheduled prior to the Screening Visit.

The study-related questionnaires are required to be filled by the patient within the timeframe defined in the Schedule of Assessments. Alternatively, where the patient is unable to complete due to physical disabilities, caregiver can assist these patients to complete the questionnaires by helping to document the responses provided by the patient.

## 7.1. Safety and Tolerability Assessments

### 7.1.1. Adverse Events

Adverse event definitions and assignment of severity and causality are detailed in Appendix 1.

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 ICF is signed until follow-up (end of study) visit.

Adverse events will be elicited from the patient (or when appropriate, the patient's legally authorized representative) by the study site staff using a nonleading question such as "How are you feeling today?" or "Have you had any health concerns since your last visit?" Patients will also be encouraged to spontaneously report AEs occurring at any other time during the study.

The Investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the patient to discontinue the study drug (see Section 4.4.2).

### 7.1.2. Reporting Serious Adverse Events

All SAEs, occurring after the signing of the ICF until end of study and regardless of study drug relationship, must be entered into the clinical database and the Investigator or delegate must report the SAE(s) to the sponsor or designee within 24 hours of obtaining knowledge of the event. The SAE should be reported by completing the paper SAE Form, provided in the Investigator/delegate site file. The completed, signed, and dated paper SAE Form must, within 24 hours, be scanned and emailed to: [redacted]. The study site should notify the site monitor via phone or email about the submission of the SAE report.

The SAE Form will collect data surrounding the event (eg, the nature of the symptom[s], time of onset in relation to initiation of therapy, duration, intensity, and whether or not therapy was interrupted or discontinued). The Investigator's assessment of the probable cause of the event will also be included. In addition, relevant medical history, concomitant medications, laboratory and diagnostic tests reports, and procedures as well as all pertinent medical information related to the event will also be collected.

[redacted] Patient Safety Solutions (PSS) will forward SAE queries requesting incomplete or missing information directly to the Investigator. It is the Investigator's responsibility to be diligent in providing this information back to [redacted] PSS as soon as it is available.

The site will follow their institutional requirements for submission of SAEs to their IRB. Additional reporting requirements are noted in the Safety Management Plan, if applicable.

**Follow-up of Adverse Events/Serious Adverse Events**

Adverse events/SAEs will be followed up to 30 days or until resolution or stabilization of the event, completion of the patient's participation, or study termination, or the Investigator or designee and sponsor agree that follow-up is no longer necessary, whichever occurs first.

Assessments should be made each day (or more frequently, if necessary) of any changes in the severity, the relationship to the study drug intervention required to treat it, and the outcome.

Side effects known for AUT00201 are provided in the IB. All AEs will be immediately recorded in the source documents.

### 7.1.3. Pregnancy

For female patients of childbearing potential, a urine pregnancy test will be performed at Visit 2 (at time of admission). A patient who tests positive at any time should be immediately discontinued from the study. Appropriate contraceptive measures must be adhered to for the duration of the study and 180 days (6 months) after their last dose of study medication.

Although not considered an AE, it is the responsibility of the Investigator or their designee to report any pregnancy in a patient or the patient's sexual partner that occurs during the study. All patients who become pregnant must immediately discontinue the study drug and be withdrawn from the study. The patient will be followed to completion/termination of the pregnancy. This information is important for both drug safety and public health concerns. If a patient or the patient's sexual partner is found to be pregnant after the study treatment was administered, the Investigator should report this to the [redacted] and sponsor immediately and document the pregnancy on the Pregnancy Form in the eCRF.

The Investigator must make every effort to follow the patient or the patient's sexual partner until completion of pregnancy. If the events during the pregnancy and/or outcome of pregnancy (ie, complications regarding mother/fetus) meet the criteria for classification of an SAE, the Investigator must follow the procedures for reporting SAEs.

## 7.2. Clinical Laboratory Evaluations

Laboratory tests during the study will be performed by a local laboratory. Blood and urine samples will be collected for clinical laboratory evaluations at the timepoints indicated in the Schedule of Assessments (Appendix 4). Clinical laboratory evaluations are listed in Appendix 2. Urine will be tested for drugs of abuse according to the laboratory's standard operating procedure (SOP). Tests will include amphetamines, cocaine, opiates, cannabis, barbiturates, benzodiazepines. Patients known to abuse drugs or those who test positive at Visit 1 may be excluded from the study based on Investigator's judgment. Additional clinical laboratory evaluations will 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 clinical laboratory safety evaluations is required.

An Investigator (or designee) will perform a clinical assessment of all clinical laboratory data. Abnormal/out of range clinically significant laboratory findings (based on Investigator's judgment) will be recorded as AEs and will be flagged for out of reference ranges per site standards.

## 7.3. Vital Signs, Physical Examination, and Other Safety Evaluations

Sitting and standing blood pressure (BP), sitting pulse rate, respiratory rate, and body temperature will be assessed at the timepoints indicated in the Schedule of Assessments (Appendix 4). 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, BP, respiratory rate, and pulse rate will be measured singly and repeated once if outside the relevant clinical reference ranges. Patients must be sitting for at least 5 minutes before BP and pulse rate measurements.

A full physical examination will be performed at Visits 1 and 2 and a brief or targeted physical examination will be performed at Visits 4 and 6 (approximately 24 hours after each IP administration) as specified in the Schedule of Assessments (Appendix 4).

### 7.3.1. Height and Weight

Height and weight will be obtained at the timepoints specified in the Schedule of Assessments (Appendix 4). 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. This procedure will be described in the study manual.

### 7.3.2. Electrocardiogram

The 12-lead ECGs will be obtained at the timepoints specified in the Schedule of Assessments (Appendix 4).

### 7.3.3. Columbia-Suicide Severity Rating Scale

The C-SSRS will be administered at the timepoints specified in the Schedule of Assessments (Appendix 4). 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. The scale includes suggested questions to solicit the type of information needed to determine if suicidal ideation and/or behavior occurred. 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 of a centrally-acting drug. Patients that exhibit suicide ideations and/or behaviors as assessed by C-SSRS or otherwise clinically should be referred for immediate evaluation and treatment per investigational site procedures. The screening C-SSRS will be used at Visit 1, and the since last visit C-SSRS will be used at all other timepoints.

## 7.4. Pharmacokinetic Analysis

A blood sample for the determination of AUT00201 plasma concentrations will be collected at the timepoints specified in the Schedule of Assessments (Appendix 4).

A central laboratory will measure the plasma concentrations of AUT00201. Derivation of PK parameters will be performed at the end of study. Actual times will be used to derive PK parameters. Missing data will not be imputed. For calculation of all PK parameters, and for individual concentration-time plots, 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, unless they fall between 2 quantifiable concentrations, in which case they will be treated as missing.

Following PK parameters (Table 3) of AUT00201 will be derived using noncompartmental analysis methods:

**Table 3: Pharmacokinetic Parameters**

| Text Symbol/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 |
| $C_{trough}$ | Trough plasma concentration | Trough plasma concentration (measured concentration taken directly before next administration) obtained directly from the concentration-time data |
| **Half-life** | | |
| $\lambda_z$ | Terminal rate constant | The apparent terminal phase rate constant ($\lambda_z$) will be estimated by linear regression of logarithmically transformed concentration versus time data. |
| $t_{1/2}$ | Terminal half-life | The terminal half-life calculated from the terminal slope of the log concentration-time curve, as follows: $t_{½} = \frac{\log_e 2}{l_z}$ |
| **Areas under the curve** | | |
| $AUC_{24}$ | 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 24 hours will be calculated using the log-linear trapezoidal method |
| $AUC_{\infty}$ | 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 $t_{last}$ (time $t_{last}$ is the time at which the last nonzero level was recorded), plus the area under the exponential curve from $t_{last}$ to infinity, calculated as follows: $AUC_{t-\infty} = \frac{\hat{C}_t}{\lambda_z}$ where $\hat{C}_t$ is the predicted value of the concentration at $t_{last}$. |
| **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: $CL/F = \frac{Dose}{AUC_{\infty}}$ |
| $V_z$/F | Apparent volume of distribution during terminal phase after non-intravenous administration | Apparent volume of distribution will be calculated using the following formula: $V_z/F = \frac{Dose}{\lambda_z \bullet AUC_{\infty}}$ |

| Text Symbol/Abbreviation | Definition | Calculation |
|---|---|---|
| MRT | Mean Residence Time | The mean residence time will be calculated using: $MRT = \frac{AUMC}{AUC_{\infty}}$ |

Instructions for collection, handling, processing, storage, and shipment of PK samples will be included in the laboratory manual.

Concentrations of antiepileptic drugs (AEDs) may be determined, from the samples collected: predose/postdose on Visit 3 and Visit 5. The testing will be performed, if there is a commercially available analytical test procedure (anticipated current AEDs include: valproate, topiramate, zonisamide, clonazepam, levetiracetam, acetazolamide, perampanel, brivaracetam).

## 7.5. Biomarker Assessments

### 7.5.1. Cortical Inhibition as Assessed with Paired-Pulse TMS EMG

- SICI
- LICI

Paired-pulse TMS allows measurement of cortical inhibitory circuit functions. In paired-pulse TMS protocols, 2 consecutive pulses are delivered to the hand motor region at a fixed interstimulus interval (ISI) such that the motor-evoked potential, captured by surface EMG sensors, resultant from the second (test) stimulus is modulated by an antecedent (conditioning) stimulus. Depending on stimulus intensity and the ISI, paired-pulse TMS can reveal the magnitude of regional inhibitory signaling strength. A tracking threshold approach will be used to assess intracortical inhibition as it has been shown to have better reliability than the classical approach.

SICI will be elicited with a conditioning stimulus (CS) and interstimulus interval of 1, 2.5, and 3 ms; LICI will be elicited with a CS that precedes the test stimulus by 100 or 200 ms. SICI at 1 ms is considered to reflect "ambient" GABAergic, that is inhibitory tone, SICI at 2 to 3 ms is considered to reflect $GABA_A$-mediated inhibition whereas LICI is thought to reflect $GABA_B$-mediated inhibition. It is expected that patients with MEAK will show reduced SICI at all ISIs and possibly also alterations in LICI.

The exact duration and timings of the paired-pulse TMS assessments will be described in a study manual. The initial assessment will be timed to coincide with the anticipated $t_{max}$, between 2 to 4 hours postdose, and will be conducted at Visits 2, 3, 4, 5, and 6 at corresponding times. A second assessment will be conducted approximately 6 hours postdose, at Visits 3 and 5. TMS is a noninvasive procedure. The risks associated with paired-pulse TMS are very low.[32]

### 7.5.2. Changes in Myoclonus as Assessed by EMG and Accelerometer Data

Surface EMG and accelerometry can provide valuable information about the nature and the source of symptoms (eg, tremor and myoclonus) in movement disorders. Surface EMG shows the pattern of a single myoclonus discharge, the rhythmicity of consecutive myoclonus

discharges, and the spread of myoclonus among different muscles. Accelerometry measures the amplitude and frequency of myoclonus. At rest, positive myoclonus occurs in EMG as a burst of brief (duration of around 100 ms) myoclonic potentials that may occur synchronously in agonist and antagonist muscles. During muscle contraction, it occurs as a brief (duration 50 to 100 ms) suppression of muscle activity.[33] This is referred to as 'positive myoclonus'. However, patients can also experience short periods of loss of muscle tone. This is referred to as a 'negative myoclonus'.

The successful development of wearable sensors has enabled continuous objective measurement of physiological signals, movement, and physical activity during daily activities in patients suffering from both positive and negative myoclonus. The specific method used in this study has been validated in patients with progressive myoclonic epilepsy type 1 (EPM1, Unverricht–Lundborg disease; caused by a mutation in the CSTB gene).[33]

Patients will wear the wireless device(s) whilst inpatient; the exact number, placement of the devices, timing, and duration will be described in the EMG and accelerometer manual but it is anticipated that 1 sensor will be worn on each arm from Visit 2 through Visit 6. The devices will remain on the patient for the duration of the inpatient portion of the study, except when they are charged each evening; patients will be able to complete all daily activities and the devices can be removed during showering. The long-term data capture will enable evaluation of the fluctuation of myoclonus over the day.

The patients will also wear the devices whilst completing the UMRS Sections 4 and 5 assessments and UMRS Section 4: D and E action- induced myoclonus/arm movement assessments (see Section 7.5.3). The EMG and accelerometer recordings are noninvasive and are not associated with any risks.

### 7.5.3. Action-induced Myoclonus

The patients' predominant symptom is an action-induced myoclonus ("positive myoclonus"). To ensure assessment of this symptom over the duration of the study, the patients will need to be active at regular intervals. At specified timepoints, as per the Schedule of Assessments (Appendix 4) and the study manual, the patient will be asked to complete a set of movements of their arms in accordance with the UMRS action myoclonus Section 4: Parts D and E. The study staff will provide instructions to the patients. The time and duration of the test will be noted. These assessments will also be videoed for centralized clinical assessment.

### 7.5.4. Unified Myoclonus Rating Scale

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.[34]

- Section 1 – patient questionnaire completed by the patient (help to document the answer may be provided by caregiver) (11 items)
- Section 2 – myoclonus at rest (frequency and amplitude, 16 items)

- Section 3 – assesses stimulus sensitivity of myoclonus (17 items)
- Section 4 – severity of myoclonus with action (frequency and amplitude, 20 items)
- Section 5 – assess performance on functional tests (5 items)
- Section 6 – physician rating of patient's global disability (1 item)
- Section 7 – presence of negative myoclonus (1 item)
- Section 8 – severity of negative myoclonus (1 item).

Each item is rated on a scale of 0 to 4, except for section 3 (where stimulus sensitivity is either present [score 1] or absent [score 0]), section 7 (negative myoclonus present [score 1] or absent [score 0]), and section 8 (scores: 0 to 3).

Total scores for sections 1, 3, 5, 6, 7, and 8 of the UMRS are calculated by simple addition. The maximum scores for these sections are 44, 17, 28, 4, 1, and 3, respectively. Sections 2 and 4 include ratings of frequency and amplitude of myoclonic jerks affecting each body region. The scores of these sections are calculated by multiplying frequency by amplitude scores for each body region. The maximum score for sections 2 and 4 are 128 and 160, respectively.

In this study, only the UMRS Sections 4 and 5 will be evaluated at Visit 2 (baseline) and Visits 3 and 5. Change from baseline in UMRS subscore Sections 4 and 5 will be an exploratory endpoint. Additional assessment of the non-dominant arm has been added to the standard UMRS Section 5 to obtain a more complete data set. 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. It is unknown whether the sensitivity of this measure will allow for an effect to be detected in an acute dose study, but completion of the UMRS will allow for a robust and standardized assessment to complete with the EMG and accelerometer device recoding myoclonus and allow for characterizing data to be collected in anticipation of repeat dose studies in this population.

The UMRS will be conducted while the patients are wearing the EMG- accelerometer device. This provides a structured set of activities that will produce action-induced myoclonus and ataxia that can be measured clinically via UMRS scoring and objectively using the EMG and accelerometer data. The UMRS assessment will be videoed by trained study staff, as per the videography instructions provided in the original paper by Frucht et al. The videos will not be blurred to obscure patients' faces as this will compromise the ability for centralized/independent rating of the videoed assessment.

### 7.5.5. Measure of Ataxic Dysarthria: Speech and Vocal Tests

Speech analysis has been clinically and technically validated to assess motor, respiratory, and cognitive symptomatology associated with a number of neuropsychiatric disorders. In particular, speech analysis can pick up subtle signs of dysarthria – a common sign in patients with MEAK who show ataxic dysarthria due to cerebellar dysfunction and characterized by an abnormality in speech articulation and prosody (also referred to as cerebellar 'scanning' speech).

A battery of speech tests will be utilized to assess patients' speech and derive specific measures of dysarthria. These tests will include: Sentence Reading (2 minutes), Diadochokinetic Rate (DDK) (<1 minute), Picture Description (1 minute), and Sustained Phonation (<1 minute). A full description of the assessments will be included in the manual. These assessments are not associated with any risks.

Speech tests will be conducted at Visit 2 (baseline) and Visits 3, 4, 5, and 6. The patient's native language, including country of origin (eg, English – USA), will be captured as part of patient's demographics.

### 7.5.6. Measures of Early Visual Processing

The measures of early visual processing will include tests of binocular rivalry (10 minutes) and internal noise estimation (15 minutes). The performance of these tasks is assumed to depend on proper inhibitory interneuron functioning in visual cortex.

Measures of visual processing will be conducted on Visit 2 (baseline) and Visits 3 and 5. Full details will be included in a separate manual. The tests will be administered through a virtual reality headset. All responses from the patient will be verbal and will be entered on a laptop by trained study staff. It is possible that patients may experience some motion sickness while using the virtual reality headset, the likelihood is considered low but patients can stop the test at any time if it is not tolerated.

### 7.5.7. Measures of Auditory Processing: Audiology Tests

#### Otoacoustic Emissions

The OAEs are recordable sounds generated by the inner ear when tones are played to the ears of the patient. OAEs assess the functioning of the medial olivocochlear (MOC) efferent system and bilateral connections between the 2 ears which rely on fast neuronal relays that express $K_v3.1$ and $K_v3.3$ channels. The MOC branch of the auditory efferent system consists of fibers that predominately project from the medial superior olive to synapse on the outer hair cells of the opposite cochlea. This auditory efferent system modifies peripheral hearing function to improve sound detection in noise and to protect the periphery from acoustic trauma.

When OAEs are recorded, no response from the patient is required and they will sit quietly while tones are played to their ears. The OAEs will be collected for each ear.[35] There are no known risks for OAEs and the process is well tolerated.

The OAEs will be assessed on Visit 2 (baseline) and Visits 3 and 5.

#### Words-in-Noise (WIN) Test Performance

The WIN test was developed as an instrument to quantify the ability of listeners to recognize monosyllabic words in background noise[36] It provides a quantified measurement of auditory processing by determining the SNR needed for speech perception and is a measure of the ability of cortical circuits to separate relevant from irrelevant information. There is evidence that inhibitory interneurons play a critical part in this function.

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 patient 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.

In a previous study, treatment with a related compound (AUT00206) led to an improved ability of patients with schizophrenia to perform this task; that is, they were able to recognize words at more difficult listening levels (ie, lower SNRs).

The WIN test will be assessed during Visit 2 (baseline) and Visits 3 and 5.

### 7.5.8. Electroencephalogram during Resting State and Presentation of Chirp Stimuli

The EEG will be recorded at resting state and 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 and intertrial phase synchrony will be evaluated over all frequency bands. Nonclinical studies in rodents and pharmacological challenge studies in healthy volunteers have provided convincing evidence that dysfunction of PV interneurons affects both resting state as well as stimulus induced and evoked gamma oscillations (power and phase synchrony) with an increase of power of resting state gamma but a decrease of power in the stimulus induced and evoked gamma oscillations and reduced phase synchrony.

It is expected that patients with MEAK will show such changes, ie, increased power in resting state gamma but deficits in evoked and induced gamma frequency during the presentation of the chirp stimulus in both power and phase synchrony. Treatment with AUT00201 is expected to normalize such abnormalities.

EEG recordings are noninvasive and are not associated with any risks.

A resting state EEG recoding will be conducted on Visit 2 (baseline) and Visits 3 and 5.

### 7.5.9. Clinician and Patient Impressions of Treatment

The clinician and the patient will be asked (separately) if they believe they received active or placebo treatment at Visits 4 and 6 (24 hours ±2 hours after each dose) and if they perceived any change in their condition.

## 7.6. Demographic Data

The following demographic data will be collected: year of birth and age (in years) at baseline, gender, race, ethnicity, height, weight, body mass index, dominant arm, whether they have a vagal nerve stimulator, whether they follow a ketogenic diet, native language (specified by country of origin [eg, English – USA]), and for females only, start date of last menstrual cycle and normal cycle length (in days).

### 7.6.1. Medical History

The patient's relevant medical history will be collected. The patient 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.

### 7.6.2. Patient-reported Outcome Measurement - Ataxia

The patient-reported outcome measurement (PROM) of ataxia is a valid and reliable measure of motor ataxia, quality of life, and mental health.[36] It is a 70-item questionnaire completed on a 5-point Likert scale. It will be evaluated on Visit 1 (Screening).

### 7.6.3. Patient Top 3 Concerns

Patients will be asked at Visit 1 (Screening) to give their top 3 concerns regarding their condition and functioning in their daily life. They will be then asked to score the severity of the concern on a 7-point Likert scale.

# 8. SAMPLE SIZE AND DATA ANALYSES

All statistical analysis will conform to the relevant International Council for Harmonisation (ICH) requirements, including but not limited to those set out in ICH documents E3 and E9. Statistical Analysis Software (SAS) version 9.2 or later will be used for the analysis.

A detailed Statistical Analysis Plan (SAP) will be finalized prior to database lock and data unblinding. The SAP will document all analyses procedures.

## 8.1. Sample Size Determination

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 is hoped that approximately 6 to 10 patients can be recruited.

Therefore, a formal power analysis to estimate a desired sample size is not useful. Primary endpoint is safety and tolerability of AUT00201. Nonetheless, a sample size of 8 provides an 80% power to detect an effect size of approximately 0.8 in a paired sample t-test at a one-sided alpha level of 0.1 (as will be used for the primary outcome measure, the composite response score).

## 8.2. Analysis Populations

The following analysis populations will be included for this study:

**Safety Population:** All patients who received at least 1 dose of IP.

**Pharmacokinetic Concentration Population**: All patients who received at least 1 dose of IP with at least 1 quantifiable PK concentration at any scheduled PK timepoint.

**Pharmacokinetic Parameter Population**: All patients in the PK Concentration Population for whom PK parameters can be derived. Further details will be provided in the SAP.

**Pharmacodynamic Population:** All patients in the Safety Population who have an evaluable baseline and at least 1 evaluable postbaseline measurement for at least 1 PD parameter.

In all populations, patients 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.

## 8.3. General Considerations

Standard summary statistics for continuous baseline and outcome variables are: N, mean, standard deviation (SD), median, quartiles, minimum, and maximum, and 95% confidence intervals (CIs) at baseline and each timepoint at which the measures are obtained. In addition, the same descriptive statistics will be obtained for change from baseline for each timepoint at which the measures are obtained. The standard summary statistics for categorical baseline and outcome

variables are: count and proportion (expressed as percentage). Geometric mean and coefficient of variation will also be presented for PK concentrations and the PK parameters.

The minimum and maximum values will be presented to the same number of decimal places as the raw data collected on the eCRF (or to 3 significant figures for derived parameters). The mean, median, and percentiles (eg, Q1 and Q3) will be presented to one additional decimal place. The SD and standard error will be presented to 2 additional decimal places.

In general, baseline will be defined as the last measurement obtained before IP administration. However, if review of blinded data indicates a stability of the measurement on days when patients do not receive treatment, baseline may also be defined as the average across Visits 2 and 4. This will be described in the SAP.

All summaries will be presented by treatment group unless otherwise specified. Summary tables and figures will be supported by full patient listings in each treatment period.

## 8.4. Disposition of Patients

The disposition of all patients in the safety population will be summarized including: number of patients randomized, number completing the study and by treatment, and number discontinued from the study.

## 8.5. Demographic and Baseline Characteristics

Demographic and other baseline characteristics will be presented overall using descriptive statistics for continuous and categorical data.

## 8.6. Medical History and Prior/Concomitant Medication

Medical history records will be summarized overall using descriptive statistics by system organ class (SOC) and preferred term (PT).

Prior or concomitant medications will be summarized separately using descriptive statistics for continuous and categorical data.

## 8.7. Safety Analysis

Summaries and listings of safety data will use the safety population.

### 8.7.1. Adverse Events

Adverse events will be coded using the version of the Medical Dictionary for Regulatory Activities (MedDRA) current at the time of database lock. All AEs will be listed. The number of patients with at least 1 TEAE will be tabulated by actual treatment and MedDRA SOC and PT.

For each of the following, the number of patients with AEs will be summarized by treatment as follows:

- TEAEs by SOC and PT
- Drug-related TEAEs by SOC and PT.

Patients with one or more TEAE will be counted only once, at the maximum causality, for each SOC and PT. AEs with missing severity and/or causality will be treated as severe and possibly related, respectively. Adverse events leading to withdrawal, deaths, and SAEs will be listed separately (fatal events will be listed separately from nonfatal events).

### 8.7.2. Clinical Laboratory Evaluations

Data from hematology and clinical biochemistry will be summarized by treatment. Any laboratory value outside the reference interval for that variable will be flagged per Site's standard process.

### 8.7.3. Other Safety Measures

Vital signs at each planned assessment, and change in vital signs from baseline at each planned postbaseline assessment, will be summarized by treatment. Vital signs of potential clinical importance will be listed separately.

ECG variables will be summarized by treatment. Changes from Screening will be summarized by treatment. QT interval will be corrected using Bazett's (QTcB) formula. QT or QTcB >450 ms and increases in QT or QTcB from Screening (Visit 1) of >30 ms will be considered to be potentially clinically important.

Abnormal physical examination findings considered clinically significant by the Investigator will be listed.

## 8.8. Secondary Analysis

Descriptive statistics will be provided for the paired-pulse TMS outcome measures, measures of dysarthria and MI. If indicated, outcome measures will be evaluated using paired t-tests, repeated measures analysis of covariance, and McNemar tests or an alternative test for non-binary categorical data, if applicable, for change from baseline of continuous and categorical data.

The overall effect of AUT00201 on secondary and selected exploratory outcome measures will be evaluated with the help of a predefined response matrix. This approach should guard against 'cherry picking' as well as missing a weak but potentially relevant treatment effect.

Details will be provided in the SAP.

## 8.9. Pharmacokinetic Analysis

Plasma concentrations and PK parameters will be listed and summarized, by treatment, using descriptive statistics, including: N (number of patients receiving the treatment in the population),

n (number of observations), arithmetic mean, median, minimum, maximum, SD, % coefficient of variation and the 95% CI of the arithmetic mean. For log-transformed variables, all of the above plus the geometric mean, its 95% CI, and the SD of the log-transformed variables will be provided.

Plasma concentrations and PK parameters will be listed and summarized, by treatment, using descriptive statistics. Individual and mean (±SD) plasma concentration-time profiles will be presented graphically. All available data will be used to derive PK parameters in individual patients.

Individual and mean (±SD) plasma concentration-time profiles will be presented graphically. All available plasma concentrations will be used to derive PK parameters in individual patients.

Plasma concentrations for AEDs for which assays are commercially available at the sponsor's chosen bioanalytical laboratory will be listed only.

### 8.10. Exploratory Analysis

Descriptive statistics will be provided for all exploratory measures. If indicated, exploratory outcome measures will be evaluated using paired t-tests, repeated measures analysis of covariance and McNemar tests, or an alternative test for non-binary categorical data, if applicable, for change from baseline of continuous and categorical (ie, nominal data). Details will be provided in the SAP.

A response matrix will be used to assess the overall effect of AUT00201 on signs and symptoms of MEAK. A response matrix will guard against 'cherry picking' of results but ensure the detection of a clinically meaningful signal. Details will be provided in a separate SAP.

### 8.11. Interim Analysis

No planned interim analysis.

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# 10. APPENDICES

## Appendix 1 – Adverse Event Definitions

### Definitions

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. This includes the following:

- Any clinically significant worsening of a pre-existing condition.
- Note: Emergence of a new pathogen associated with a clinical event during therapy at a site other than the initial site of infection will be considered to be an AE.
- Any recurrence of a pre-existing condition.
- An AE occurring from overdose of a sponsor study drug whether accidental or intentional (ie, a dose higher than that prescribed by a health care professional for clinical reasons).
- An AE occurring from abuse of a sponsor study drug (ie, use for nonclinical reasons).
- An AE that has been associated with the discontinuation of the use of a sponsor study drug.

Note: A procedure is not an AE, but the reason for a procedure may be an AE.

A pre-existing condition is a clinical condition (including a condition being treated) that is diagnosed before the patient signs the informed consent form (ICF) and diagnosed prior to or at the latest during screening procedure. These medical occurrences should be documented as part of the patient's medical history.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or frequency will be used to determine whether an event is a treatment-emergent adverse event (TEAE). An AE is considered to be treatment-emergent if (1) it is not present when the active phase of the study begins and is not a chronic condition that is part of the patient's medical history, or (2) it is present at the start of the active phase of the study or as part of the patient's medical history, but the severity or frequency increases during the active phase. The active phase of the study begins at the time of the first dose of the study drug. The active phase of the study ends at the follow-up visit.

### Reporting of Adverse Events

At each visit, the Investigator, or delegate, will determine whether or not any AEs have occurred. Nonleading questions such as "How are you feeling today?" or "Have you had any health concerns since your last visit?" should be used to elicit the patient to report any possible AEs. Independently from the time of the next visit, the patient can revisit the Investigator in case of an AE. If any AEs have occurred, they will be recorded in the AE section of the electronic case report form (eCRF) and in the patient's source documents. If known, the diagnosis should be recorded, in preference to listing the individual signs and symptoms.

Adverse event reporting begins from the time of informed consent and ends at follow-up (end of study) visit.

**Assessment of Severity**

The Investigator will be asked to provide an assessment of the severity of the AE using the following categories:

- **Grade 1**: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- **Grade 2**: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- **Grade 3**: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- **Grade 4:** Life-threatening consequences; urgent intervention indicated
- **Grade 5:** Death related to AE.

**Relationship to Study Treatment**

The Investigator will make a determination of the relationship of the AE to the study drug using a four-category system according to the following guidelines:

- **Not related**: when the AE is definitely caused by the patient's clinical state, or the study procedure/conditions.
- **Unlikely Related**: when the temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly Related**: when the AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the patient's clinical state or the study procedures/conditions.
- **Related**: when the AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesized cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

**Action Taken for Adverse Events**

The Investigator or designee will record the action taken for the AE in the eCRF. Actions taken will include:

- **Patient withdrawn**: The medication schedule was modified through termination of the prescribed regimen of medication and the patient was withdrawn from the study.
- **No action taken.**

**Follow-up of Adverse Events**

All (Serious)AEs that are ongoing at the time of discontinuation, or that develop prior to the final Follow-up Telephone Call, will be followed for 30 days, or until resolution or stabilization.

**Adverse Drug Reactions**

All noxious and unintended responses to an investigational product (IP) (ie, where a causal relationship between an IP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function, is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg, Investigator's Brochure [IB] for an unapproved IP).

**Serious Adverse Events**

An SAE is any AE occurring at any dose that meets 1 or more of the following criteria:

- Results in death
- Is life-threatening (see below)
- Requires patient hospitalization or prolongation of an existing hospitalization (see below)
- Results in a persistent or significant disability or incapacity (see below)
- Results in a congenital anomaly or birth defect
- Results in an important medical event (see below).

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not require hospitalization, or development of drug dependency or drug abuse.

A ***life-threatening adverse event*** is any AE that places the patient at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that might have caused death had it occurred in a more severe form but that did not create an immediate risk of death as it actually occurred. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though drug-induced hepatitis of a more severe nature can be fatal. Hospitalization is to be considered only as an overnight admission.

***Hospitalization*** or prolongation of a hospitalization is a criterion for considering an AE to be serious. In the absence of an AE, the participating Investigator should not report hospitalization or prolongation of hospitalization. This is the case in the following situations:

- Hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol. Day or night survey visits for biopsy or surgery required by the protocol are not considered serious.
- Hospitalization or prolongation of hospitalization is part of a routine procedure followed by the study center (eg, stent removal after surgery). This should be recorded in the study file.
- Hospitalization for survey visits or annual physicals fall in the same category.

In addition, a hospitalization planned before the start of the study for a pre-existing condition that has not worsened does not constitute an SAE (eg, elective hospitalization for a total knee replacement due to a pre-existing condition of osteoarthritis of the knee that has not worsened during the study).

***Disability*** is defined as a substantial disruption in a person's ability to conduct normal life functions (ie, the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the patient's bodily function/structure, physical activities, or quality of life).

Medical and scientific judgment should be exercised in deciding whether a case is serious in those situations where important medical events may not be immediately life-threatening or result in death, hospitalization, disability, or incapacity. These include events that may jeopardize the patient or may require medical intervention to prevent 1 or more outcomes listed in the definition of serious. Such events should usually be considered as serious.

## Appendix 2 – Clinical Laboratory Evaluations

The following clinical laboratory analytes will be assessed during the study at the visits as defined in the Schedule of Assessments (Appendix 4). Instructions on sample collection, processing, storage, packaging, and shipping will be provided in the laboratory manual.

Urine will be tested for drugs of abuse according to the laboratory's SOP. Tests will include amphetamines, cocaine, opiates, cannabis, barbiturate, and benzodiazepines. Patients known to abuse drugs or those who test positive at Screening will be excluded from the study based on Investigator's judgment.

| **Biochemistry:** | **Hematology (CBC):** |
|---|---|
| Albumin | Hematocrit |
| ALP | Hemoglobin |
| ALT | MCH |
| AST | MCHC |
| BUN | MCV |
| Calcium | Platelet count |
| Chloride | RBC count |
| Cholesterol | WBC count |
| Creatinine | WBC differential |
| GGT | (% & Abs): |
| Globulin | Basophils |
| Glucose | Eosinophils |
| LDH | Lymphocytes |
| Phosphate | Monocytes |
| Potassium | Neutrophils |
| Sodium | |
| Total Bilirubin | |
| Total Protein | **For Women Only:** |
| Triglycerides | Urine Pregnancy Test |
| Uric acid | |
| | **Serology (Screening only):** |
| **Complete Urinalysis: Dipstick** | Hepatitis B (hepatitis B surface antigen) |
| Color and appearance | Hepatitis C antibody |
| pH and specific gravity | |
| Bilirubin | |
| Urobilinogen | |
| Glucose | |
| Ketones | **Hormone Panel:** |
| Leukocyte esterase | TSH |
| Nitrite | Free triiodothyronine (T3) |
| Blood | Free thyroxine (T4) |
| Protein | FSH |
| Microscopic[a] | |

Abbreviations: Abs = absolute; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CBC = complete blood count; FSH = follicle-stimulating hormone; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; TSH = thyroid-stimulating hormone; WBC = white blood cell.

[a] Only if dipstick test for protein, blood, leukocyte esterase, or nitrites is abnormal.

## Appendix 3 – Regulatory, Ethical, and Study Oversight Considerations

### Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations.

The protocol, protocol amendments, informed consent form (ICF), Investigator's Brochure, and other relevant documents (eg, advertisements) must be submitted to an Institutional Review Board (IRB) by the Investigator and reviewed and approved by the IRB before the study is initiated.

- Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- In case of nonsubstantial protocol amendments which do not require Competent Authority's and/or IRB approval, the reasoning should be documented, referenced with applicable laws and regulations (if any), and signed by the responsible managers.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB
- Notifying the IRB of serious adverse event (SAEs) or other significant safety findings as required by IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB, and all other applicable local regulations.

### Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

### Informed Consent

Prior to starting participation in the study, each patient or his/her legally authorized representative will be provided with a study-specific ICF giving details of the study drugs, procedures, and potential risks of the study. Patients on his/her legally authorized representative will be instructed that they are free to obtain further information from the Investigator (or

designee) and that their participation is voluntary and they are free to withdraw from the study at any time. Patients or his/her legally authorized representative will be given an opportunity to ask questions about the study prior to providing consent for participation.

Patients or his/her legally authorized representative will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, and the IRB or study center, where applicable. The patient or his/her legally authorized representative will be given a copy of the signed ICF and the original will be maintained with the patient's records.

Patients must be re-consented to the most current version of the ICF(s) during their participation in the study, as appropriate.

**Patient Data Protection**

Patients will be assigned a unique identifier and will not be identified by name in electronic case report forms (eCRFs), study-related forms, study reports, or any related publications. Patient and Investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual patients or Investigators will be redacted according to applicable laws and regulations.

The patient must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient. The patient must also be informed that his/her medical records may be examined by sponsor or Contract Research Organization (CRO) auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

**Disclosure**

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the sponsor.

**Data Quality Assurance**

The following data quality steps will be implemented:

- All patient data relating to the study will be recorded on eCRFs unless directly transmitted to the sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

- The sponsor or designee is responsible for the data management of this study including quality checking of the data. Predefined, agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a Data Management Plan.
- [redacted] will be responsible for clinical monitoring of the study. Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator in accordance with 21 CFR 312.62(c) unless local regulations or institutional policies require a longer retention period, unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

**Investigator Documentation Responsibilities**

All individual, patient-specific study data will be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion. All data generated from external sources (eg, central laboratory of pharmacokinetics, pharmacodynamics) and transmitted to the sponsor or designee electronically will be integrated with the patient's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each patient who signs an ICF and undergoes any pre-screening or screening procedures, according to the eCRF completion instructions. The sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, the data queries, and the site notifications.

**Publications**

All data generated in this study will be the property of Autifony Therapeutics Ltd. Any publication of the study results shall be patient to the prior written approval by Autifony Therapeutics Ltd. Study results may not be published, presented, or referred to, in whole or in part, by the CRO, its affiliates, its permitted third-party vendors or external consultants, or any center or Investigator involved in the performance of the study, without the prior expressed written consent of the sponsor, which it may withhold in its sole discretion.

## Appendix 4 – Schedule of Assessments

### Table 4: 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.