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Protocol Title: A Phase 2a, multicenter, randomized, double-blind, placebo-controlled study to evaluate safety, tolerability, pharmacodynamic markers, and pharmacokinetics of AP-101 in patients with familial amyotrophic lateral sclerosis (fALS) and sporadic amyotrophic lateral sclerosis (sALS)

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Study Phase: Phase 2a

Short Title: A Phase 2a study to evaluate safety, tolerability, pharmacodynamic markers, and pharmacokinetics of AP-101 in patients with amyotrophic lateral sclerosis (ALS)

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1. Protocol Summary

1.1. Synopsis

Protocol Title: A Phase 2a, multicenter, randomized, double-blind, placebo-controlled study to evaluate safety, tolerability, pharmacodynamic markers, and pharmacokinetics of AP-101 in patients with familial amyotrophic lateral sclerosis (fALS) and sporadic amyotrophic lateral sclerosis (sALS)

Short Title: A Phase 2a study to evaluate safety, tolerability, pharmacodynamic markers, and pharmacokinetics of AP-101 in patients with amyotrophic lateral sclerosis (ALS)

Rationale:

The pathobiology of ALS remains incompletely defined. In some patients who develop familial ALS (fALS) it has been demonstrated that mutations in the superoxide dismutase 1 (SOD1) protein result in a toxic gain of function with subsequent misfolding of the SOD1 protein. This results in precipitation of SOD1 aggregates in the cytosol of motor neurons and ultimately results in cell death and the loss of motor neuron function. Directed SOD1 mutagenesis in animal models results in a similar clinical syndrome to that seen in patients. Non-genetic perturbations such as metal depletion, disruption of the quaternary structure, and oxidation, can induce wildtype SOD1 to misfold and to adopt a toxic conformation that is similar to familial ALS-linked mutant SOD1 variants and is found in sALS (Rotunno and Bosco 2013). This is supported by the detection of misfolded wild-type SOD1 within human post-mortem sporadic ALS spinal cord samples (Li and Cashman 2014; Bosco et al. 2010; Forsberg et al. 2011; Forsberg et al. 2010).

Treatment approaches that target pathogenic versions of SOD1 (e.g., mutated, aggregated, oxidized, or misfolded) have the potential to delay progression of the disease in patients. To date, no SOD1 targeting therapeutics have been approved.

The following therapies are approved for use in patients with ALS:

- Riluzole is approved for use in North America, the European Union, and Asia.
- Edaravone is approved for use in North America and Asia; however, it is not approved for use in the European Union.
- The combination of sodium phenylbutyrate and taurursodiol is approved in the United States (Relyvrio™) and Canada (Albrioza™).
- Tofersen received accelerated approval for use in patients with a confirmed mutation in the SOD1 gene in the United States on 25 April 2023.

AP-101 was granted orphan drug designation status by regulatory bodies in 3 regions; EMA (2017; EMA/OD/030/17), Swissmedic (2018; #102577296), and the FDA (2017; #17-5964) supporting the need for innovative treatments.

Objectives and Endpoints – Double-blind Phase

Objectives	Endpoints
Primary	
To determine the safety and tolerability of AP-101 after multiple IV doses over 6 months of treatment	- Incidence of AEs and SAEs including immunogenicity - Incidence of abnormalities in vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms, and changes in weight
Secondary	
To determine the PK profile of AP-101 after multiple IV dose administrations	The primary PK parameters are AUC, concentration at end of infusion and $t_{1/2}$
To determine CSF penetration of AP-101 after multiple IV dosing	AP-101 levels in the CSF
To determine effects of AP-101 on pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101	Measurement of pNfH and NfL levels in the CSF and plasma after multiple IV doses of AP-101
Exploratory	
To determine the effect of AP-101 on disease progression after multiple IV doses over 6 months of treatment	Measurement of the clinical effect of AP-101 on the rate of functional decline as measured by the ALSFRS-R
To determine effects on alternate measures of disease progression after multiple IV doses of AP-101	Measurement of the clinical effect of AP-101 on lung function (SVC) and pulse oximetry, muscle strength (HHD) and disease staging (King's staging tool)
To determine effects on AP-101 on overall patient survival and additional measures of outcome for AP-101 after multiple IV dosing	- Composite time to event measurement comprising time to death or non-invasive ventilation use for 22 to 24 hours per day - Difference in mean rank between treatment arm and control arm in the

Objectives	Endpoints
	joint model of survival and ALSFRS-R progression via the CAFS
Effects on quality of life after multiple IV dosing of AP-101 over 6 months of therapy	Quality of life by ALSSQOL-SF
Identify non-clinical and exploratory biomarkers of disease activity that change over time	- Measurement of total SOD1 and misfolded SOD1 in the CSF after multiple IV doses of AP-101 - Measurement of other biological markers of disease activity including creatinine and transcriptional markers

Abbreviations: AE = adverse event; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; AUC = area under the concentration versus time curve; CAFS = combined assessment of function and survival; CSF = cerebrospinal fluid; HHD = hand-held dynamometry; IV = intravenous; NfL = neurofilament light chain; PK = pharmacokinetic; pNfH = phospho-neurofilament heavy chain; SAE = serious adverse event; SOD1 = superoxide dismutase 1; SVC = slow vital capacity; $t_{1/2}$ = elimination half-life; TEAE = treatment emergent adverse event.

Objectives and Endpoints – Open-label Extension

Objectives	Endpoints
Primary	
To determine the safety and tolerability of AP-101 after multiple IV doses	- Incidence of AEs and SAEs including immunogenicity - Incidence of abnormalities in vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms and changes in weight
Secondary	
To determine the PK profile of AP-101 after multiple IV dose administrations	The primary PK parameters are AUC, concentration at end of infusion and $t_{1/2}$
To determine effects of AP-101 on pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101	Measurement of pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101

Objectives	Endpoints
Exploratory	
To determine the effect of AP-101 on disease progression after multiple IV doses at the end of the open label period	Measurement of the clinical effect of AP-101 on the rate of functional decline as measured by the ALSFRS-R
To determine effects on alternate measures of disease progression after multiple IV doses of AP-101	Measurement of the clinical effect of AP-101 on lung function (SVC) and pulse oximetry and disease staging (King's staging tool)
To determine effects on AP-101 on overall patient survival and additional measures of outcome for AP-101 after multiple IV dosing	- Composite time to event measurement comprising time to death or non-invasive ventilation use for 22 to 24 hours per day - Difference in mean rank between treatment arm and control arm in the joint model of survival and ALSFRS-R progression via the CAFS
Effects on quality of life after multiple IV dosing of AP-101 at the end of the open label period	Quality of life by ALSSQOL-SF
Identify non-clinical and exploratory biomarkers of disease activity that change over time	Measurement of other biological markers of disease activity including creatinine and transcriptional markers
To determine CSF penetration of AP-101 after multiple IV dosing	AP-101 levels in the CSF

Abbreviations: AE = adverse event; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; AUC = area under the concentration versus time curve; CAFS = combined assessment of function and survival; CSF = cerebrospinal fluid; IV = intravenous; NfL = neurofilament light chain; PK = pharmacokinetic; pNfH = phospho-neurofilament heavy chain; SAE = serious adverse event; SVC = slow vital capacity; $t_{1/2}$ = elimination half-life; TEAE = treatment emergent adverse event.

Overall Design

Study AP101-02 is a Phase 2a, multicenter, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of AP-101 in familial and sporadic ALS.

Two participant cohorts will participate in this study: fALS patients with confirmed mutations in SOD1 and patients with sALS. Patients in each participant cohort will be randomly assigned in a 2:1 ratio to either AP-101 treatment or to placebo. Patients will be allowed to continue on stable

doses of medications approved for the treatment of ALS in their country/region, with the exception of tofersen, per the inclusion/exclusion criteria for the duration of the study. Participants will receive a 500 mg AP-101 loading dose on Day 1, and then regularly scheduled doses of 2500 mg will begin on Day 2. After Day 2, dosing will take place once every 3 weeks for a 6-month period starting on Day 22 and following the Schedule of Activities (SoA). After the final dose or following early termination for any reason at any point in the study, participants will be followed for safety and PK for 4 months (approximately 5 half-lives) after their final dose of study treatment.

Safety/tolerability, PK, and exploratory assessments will be performed at prescribed time points during the study. Cerebrospinal fluid (CSF) and other biological samples for biomarker analyses will also be collected from each participant according to the SoA. Efficacy measures (amyotrophic lateral sclerosis functional rating scale – revised [ALSFRS-R], slow vital capacity [SVC], pulse oximetry, hand-held dynamometry [HHD]) and King's staging tool will be evaluated according to the SoA.

Following completion of 6 months of treatment, participants will be invited to join a 6-month open-label extension (OLE) that will continue to evaluate long-term safety for AP-101. If participating in the OLE, patients will complete the planned safety follow-ups and serum PK sampling after receiving their final dose of AP-101.

Disclosure Statement: This is a parallel group, dual-cohort treatment study with 2 arms (AP-101: placebo) that are participant, sponsor, and investigator blinded.

Number of Participants:

Approximately 63 participants (21 with fALS and 42 ± 20 % with sALS) will be randomly assigned to study intervention such that approximately 18 evaluable participants with fALS and 36 evaluable participants with sALS will complete the study. Once all sALS participants have completed the 6-month double blind treatment period, an interim analysis will be performed on sALS participant data. The fALS participants will continue in the study and an additional database lock and analysis will be performed once all fALS patients complete the 6-month double-blind treatment period.

Participants who withdraw from the study before completion of all study activities may be replaced at the discretion of the sponsor.

A participant completes the study when Week 24 dosing and all scheduled procedures for the 21-day follow-up visit as shown in the SoA have been finished.

Intervention Groups and Duration:

It is anticipated that the total study duration for participants will be approximately 10 months to include screening, a 6-month treatment period, and a 4-month safety follow-up period. For participants continuing to the 6-month OLE, the 4-month safety follow-up period will occur at the end of the OLE or after early termination from the study for any reason.

Participants will be screened within 28 days prior to enrollment for each group and will be randomly assigned to either AP-101 2500 mg IV or placebo, administered according to the SoA during the 6-month treatment period. After completing the treatment period, participants may

continue in the study as part of the OLE if they have completed all study visits during the treatment period. All study treatment will be given at the study sites.

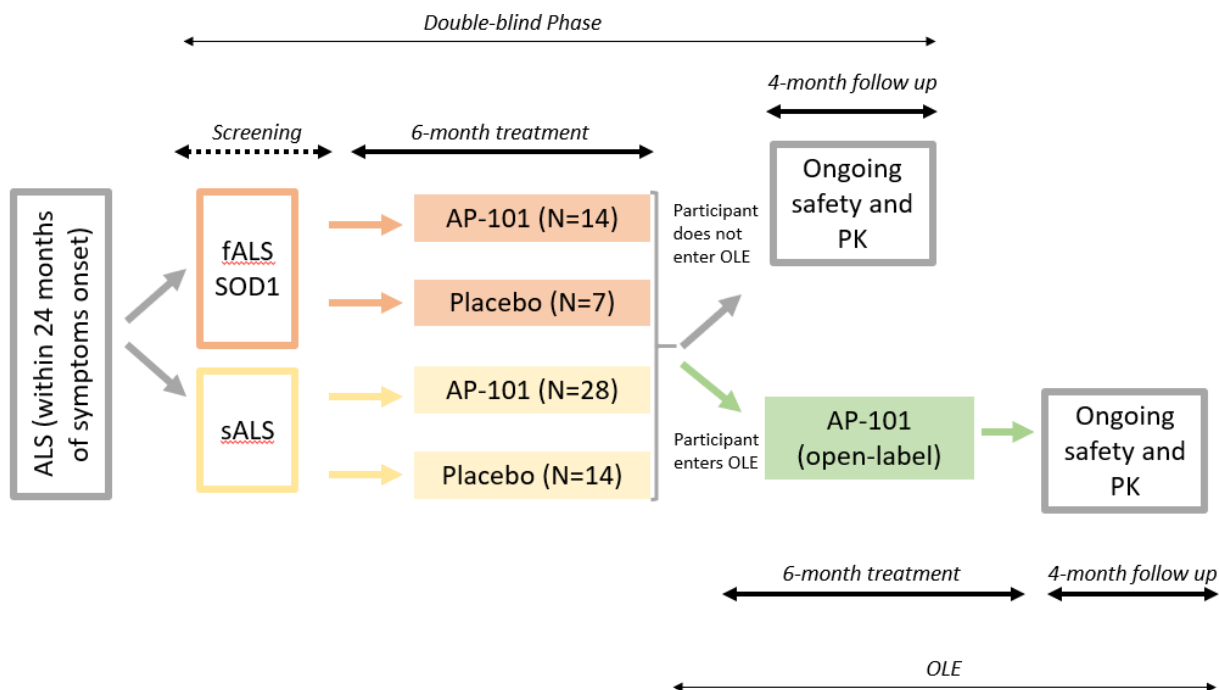
This is the first multiple-dose study for AP-101 and therefore safety data would be the primary criteria for any dose modification. Safety data, in particular adverse events (AEs), serious adverse events (SAEs), and adverse laboratory abnormalities, will be independently assessed by the investigator, sponsor, and SRC and will be considered related to the study drug unless there is clear evidence that the event is not related. PK results (exposure) and total body clearance of drug calculated after intravenous (IV) administration [CL_T] will be used as supporting data for any dose modifications, if available. After review of these data, any dose modifications need to be agreed upon by the investigator, sponsor, and SRC.

Safety Data Monitoring:

AL-S Pharma has created a Safety Review Committee (SRC) with independent physician experts in ALS to objectively evaluate and monitor participants during the trial. For additional information on the remit and operations of the SRC, refer to the Safety Review Committee Charter.

An interim safety review was performed after 10 participants had been randomized to receive AP-101 and followed for at least 30 days. Enrollment was paused briefly while the analysis was performed. The SRC met in early January 2023 to review the data and concluded that the study should continue without modification. This recommendation was communicated to investigators, ERBs, and regulatory health authorities.

1.2. Schema



Abbreviations: ALS = amyotrophic lateral sclerosis; fALS = familial amyotrophic lateral sclerosis;
OLE = open-label extension; PK= pharmacokinetics; sALS = sporadic amyotrophic lateral sclerosis;
SOD1 = superoxide dismutase.

1.3. Schedule of Activities (SoA) – Double Blind Phase

Visit/Week	Screening	Treatment Period											Safety/ Survival Follow-Up or Early Termination (ET)		
		Base-line/ Dose 1 ^a	Dose 2	Phone call ^b	W3	W6	W9	W12	W15	W18	W21	W24	21 days post last dose or ET	8 weeks post last dose or ET	16 weeks post last dose or ET
Day	-28 to -1	1	2	3	22 ± 3 days	43 ± 3 days	64 ± 3 days	85 ± 3 days	106 ± 3 days	127 ± 3 days	148 ± 3 days	169 ± 3 days	± 3 days	-3 to + 14 days	-3 to + 14 days
Informed consent	X														
Medical history	X														
ALS history	X														
Eligibility criteria	X	X													
Demographics	X														
HBsAg & HCV	X														
Randomization		X													
IV dose		X ^a	X ^a		X	X	X	X	X	X	X	X			
ALSFRS-R	X	X						X				X	X	X	X
SVC	X	X						X				X	X	X	X
King's staging	X	X						X				X	X	X	X
Muscle strength (HHD)		X						X				X	X	X	X
Pulse oximetry ^c		X	X		X	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X	X		X			X				X	X	X	X
Neurological exam	X	X	X		X			X				X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CSSR-S	X	X			X	X	X	X	X	X	X	X	X	X	X
ALSSQOL-SF	X	X						X				X	X	X	X
Survival endpoints ^d					X	X	X	X	X	X	X	X	X	X	X

Visit/Week	Screening	Treatment Period											Safety/ Survival Follow-Up or Early Termination (ET)		
		Base-line/ Dose 1 ^a	Dose 2	Phone call ^b	W3	W6	W9	W12	W15	W18	W21	W24	21 days post last dose or ET	8 weeks post last dose or ET	16 weeks post last dose or ET
Day	-28 to -1	1	2	3	22 ± 3 days	43 ± 3 days	64 ± 3 days	85 ± 3 days	106 ± 3 days	127 ± 3 days	148 ± 3 days	169 ± 3 days	± 3 days	-3 to + 14 days	-3 to + 14 days
Vital signs ^h	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Height	X														
Weight	X	X	X		X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG ^c	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety laboratory measurements	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Immunogenicity		X	X		X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test (local test)		X	X		X	X	X	X	X	X	X	X	X	X	X
Serum pregnancy test	X														
Urine drug screening	X														
Pharmacogenetic testing (whole blood)	X														
PK sampling ^f		X	X		X	X	X	X	X	X	X	X	X	X	X
CSF PK and biomarkers ^g	X							X				X			
Coagulation testing	X						X				X				
Serum biomarkers		X	X		X	X	X	X	X	X	X	X	X	X	X

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; CSF = cerebrospinal fluid; CSSR S = Columbia Suicide Severity Rating Scale; ECG = electrocardiogram; HBsAg = surface antigen of the hepatitis B virus; HCV = hepatitis C virus; HHD = hand-held dynamometry; IV = intravenous; PK = pharmacokinetic; SVC = slow vital capacity; W = week.

Participants discontinuing from the study at any point (end of treatment period, end of 6-month OLE or early termination for any reason) should return to the site 21 days after their last dose of treatment and undergo all assessments scheduled for the 21-day post-last dose visit, and then return to the site 8 weeks and 16 weeks after their last dose of treatment and undergo all assessments scheduled per the SoA.

Visit windows of ± 3 days allow for flexibility in scheduling (i.e., if dosing takes place on a Friday, the next visit can be scheduled for a Monday).

Note: If multiple procedures take place at same time point, the following order of procedures should be used: ECG, vital signs, and venipuncture. Unless otherwise specified, pre-dose sample may be taken at any time prior to dosing, based on study site activity schedule. At Screening, lumbar puncture (LP) should be the last procedure performed.

Note: All baseline values measured on Day 1 must still pass the eligibility criteria pre-dosing the participant, with the exception of the safety labs as the results will not be available on the baseline day.

- ^a After randomization, all participants will receive either placebo or AP-101 according to the schedule of activities. On Day 1, participants will receive 500 mg of AP-101 or placebo, and on Day 2, participants will receive 2500 mg of AP-101 or placebo. Thereafter, the dose schedule will follow the SoA with dosing with 2500 mg of AP-101 or placebo every 3 weeks starting at Day 22. The observation period after IV infusion is 1 hour, or can be extended to 3 to 6 hours, as per local site procedures, if an infusion-related reaction occurs.
- ^b Phone call will take place approximately 24 hours after the second baseline dose.
- ^c Pulse oximetry can be done at any time prior to dose and can be performed between other assessments.
- ^d Survival endpoints will include time to event measurements comprising time to death, or non-invasive ventilation use for 22 to 24 h per day.
- ^e Single ECG will be collected at screening. Triplicate ECGs will be collected at all other time points. A minimum of 1 min can be observed in between each ECGs.
- ^f A single serum PK sample will be collected pre-dose and immediately following the end of the infusion during all visits except for Week 24, when PK sampling will be performed pre-dose and at 4 (± 2) hours post-dose. For participants who do not enter into the OLE, a single PK sample will be taken at 21D, 8W and 16W post final dose, this may be done at any time during the visit. Times of PK sampling are intended as a guide and can be modified to accommodate clinical procedures. Actual date and time of sample must be collected. For participants who discontinue early, a sample should be obtained at the ET visit.
- ^g CSF samples for PK and biomarkers: LP at Week 12 and Week 24 may be done 1-3 days before dosing.
- ^h Orthostatic blood pressure will be recorded at Baseline Dose 1 and Dose 2.

1.4. Schedule of Activities (SoA) – Open-label Extension

Visit/Week	Baseline	Treatment Period										Safety/ Survival Follow-Up or Early Termination (ET)		
	Data Collections at W24 of Double-blind Phase	W3 Dose 1 ^a	Dose 2	Phone call ^b	W6	W9	W12	W15	W18	W21	W24	21 days post last dose or ET	8 weeks post last dose or ET	16 weeks post last dose or ET
Day	1	22	23	24	43 ± 3 days	64 ± 3 days	85 ± 3 days	106 ± 3 days	127 ± 3 days	148 ± 3 days	169 ± 3 days	± 3 days	-3 to + 14 days	-3 to + 14 days
Informed consent	X													
Eligibility criteria ⁱ	X	X												
IV dose	X ^a	X ^a	X ^a		X	X	X	X	X	X	X			
ALSFRS-R	X	X					X				X	X	X	X
SVC	X	X					X				X	X	X	X
King's staging	X	X					X				X	X	X	X
Muscle strength (HHD)	X	X												
Pulse oximetry ^c	X	X	X		X	X	X	X	X	X	X	X	X	X
Physical exam	X	X	X		X		X				X	X	X	X
Neurological exam	X	X	X		X		X				X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CSSR-S	X	X			X		X				X	X	X	X
ALSSQOL-SF	X	X									X	X	X	X
Survival endpoints ^d	X	X	X		X	X	X	X	X	X	X	X	X	X
Vital signs ^h	X	X	X		X	X	X	X	X	X	X	X	X	X
Weight	X	X	X		X	X	X	X	X	X	X	X	X	X
12-Lead ECG ^e	X	X			X		X				X	X	X	X

Visit/Week	Baseline	Treatment Period										Safety/ Survival Follow-Up or Early Termination (ET)		
	Data Collections at W24 of Double-blind Phase	W3 Dose 1 ^a	Dose 2	Phone call ^b	W6	W9	W12	W15	W18	W21	W24	21 days post last dose or ET	8 weeks post last dose or ET	16 weeks post last dose or ET
Day	1	22	23	24	43 ± 3 days	64 ± 3 days	85 ± 3 days	106 ± 3 days	127 ± 3 days	148 ± 3 days	169 ± 3 days	± 3 days	-3 to + 14 days	-3 to + 14 days
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety laboratory measurements	X	X	X		X	X	X	X	X	X	X	X	X	X
Immunogenicity	X	X			X	X	X	X	X	X	X	X	X	X
Urine pregnancy test (local test)	X	X	X		X	X	X	X	X	X	X	X	X	X
PK sampling ^f	X		X									X	X	X
CSF PK and biomarkers ^g	X										X			
Coagulation testing										X				
Serum biomarkers	X	X			X	X	X	X	X	X	X	X	X	X

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised;

ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; CSF = cerebrospinal fluid; CSSR-S = Columbia Suicide Severity Rating Scale; ECG = electrocardiogram; HBsAg = surface antigen of the hepatitis B virus; HCV = hepatitis C virus; HHD = hand-held dynamometry; IV = intravenous; PK = pharmacokinetic; SVC = slow vital capacity; W = week.

Participants discontinuing from the study at any point (end of 6-month OLE or early termination for any reason) should return to the site 21 days after their last dose of treatment and undergo all assessments scheduled for the 21-day post-last dose visit, and then return to the site 8 weeks and 16 weeks after their last dose of treatment and undergo all assessments scheduled per the SoA.

Visit windows of ±3 days allow for flexibility in scheduling (i.e., if dosing takes place on a Friday, the next visit can be scheduled for a Monday).

Note: If multiple procedures take place at same time point, the following order of procedures should be used: ECG, vital signs, and venipuncture. Unless otherwise specified, pre-dose sample may be taken at any time prior to dosing, based on study site activity schedule.

Note: All baseline values measured at Week 3 must still pass the eligibility criteria (Inclusion Criteria 6, 10, 11, 12, 13, 14 and 15 and Exclusion Criteria 6, 9, 10 and 13) pre-dosing the participant, with the exception of the safety labs as the results will not be available on the baseline day. If a participant joins the OLE, the participant will complete the scheduled 21 day follow up visit of the double blind phase at Week 3 of the OLE.

- ^a Participants will receive either 2500 mg of AP-101 or placebo at the baseline visit (Week 24 of the double-blind phase), as per their randomized treatment from the double-blind phase. To protect the blinding of the double-blind phase, participants prior treatment information will remain blinded; therefore, after entry into the OLE, all participants who received placebo in the double-blind phase of the study will receive blinded 500 mg of AP-101 on Day 22 and 2500 mg of AP-101 on Day 23. After entry into the OLE, all participants who received active treatment in the double-blind phase of the study will receive blinded placebo (same infusion volume of 500 mg AP-101) on Day 22 and 2500 mg of AP-101 on Day 23. Thereafter, the dose schedule will follow the SoA with dosing with open-label 2500 mg of AP-101 every 3 weeks starting at Day 22. Consent is to be obtained Day 1 of the OLE which would coincide with Week 24 of the double-blind period. At Day 22/Week 3 of the OLE prior to administration of study drug, it will be confirmed that approval from the coordinating center has been granted, that the open-label period informed consent has been obtained, and that all the Week 24 assessments have been completed. The observation period after IV infusion is 1 hour, or can be extended to 3 to 6 hours, as per local site procedures, if an infusion-related reaction occurs.
- ^b Phone call will take place approximately 24 hours after the second baseline dose.
- ^c Pulse oximetry can be done at any time prior to dose and can be performed between other assessments.
- ^d Survival endpoints will include time to event measurements comprising time to death, or non-invasive ventilation use for 22 to 24 h per day.
- ^e Triplicate ECGs will be collected at baseline and Week 3. A minimum of 1 min can be observed in between each ECGs. Single ECG will be collected at all other time points.
- ^f At Week 24 of the double-blind phase (Week 1 of the OLE) and at Dose 2 of Week 3 of the OLE, PK sampling will be performed pre-dose and at 4 (+/-2) hours post-dose. A single PK sample will be taken at 21D, 8W and 16W post final dose, this may be done at any time during the visit. Times of PK sampling are intended as a guide and can be modified to accommodate clinical procedures. Actual date and time of sample must be collected. For participants who discontinue early, a sample should be obtained at the ET visit.
- ^g CSF samples for PK and biomarkers. LP at Week 24 may be done 1-3 days before dosing.
- ^h Orthostatic blood pressure will be recorded at Baseline Dose 1 and Dose 2.
- ⁱ Participants who join the OLE, are eligible for inclusion only if they meet Inclusion Criteria 6, 10, 11, 12, 13, 14 and 15 and are to be excluded from the OLE if any of the following criteria apply, including Exclusion Criteria 6, 9, 10 and 13 as assessed.

2. Introduction

2.1. Background

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease resulting in loss of the motor neurons in the motor cortex, brainstem and spinal cord. It currently affects 3 of every 100,000 people in the US. The majority of patients are between 40 and 70 years of age (ALS Association (ALS Association resource page [WWW])). Rapid progression of symptoms directly results from degeneration of motor neurons, ultimately leading to loss of motor neuron function, including speaking, swallowing and ultimately respiratory function. The primary cause of death in ALS is respiratory failure, with approximately 10% of ALS patients surviving for more than 10 years (Tateno et al. 2014).

The exact pathological mechanisms that cause ALS are still somewhat unclear. Up to 90% of cases are reported to be sALS, although new evidence suggests that significant numbers of patients without a family history may have ALS caused by genetic mutations in one of a number of genes. What is understood is that there are both genetic and non-genetic drivers of the disease. For example, in some fALS patients, genetic mutations in SOD1 result in protein misfolding. It is widely accepted that this ultimately confers a toxic gain of function and the formation of misfolded SOD1 protein aggregates (Bosco et al. 2010). In fact, mutations in SOD1 were the first confirmed genetic drivers of ALS. Further evidence that these mutations are disease causing is supported by data demonstrating that these same mutations lead to ALS-like disease in animal models (Maier et al. 2018).

Up to 30% of patients with fALS have mutations in the SOD1 gene. Overall, SOD1 genetic mutations occur in approximately 6-10% of all ALS patients, regardless of classification as sALS or fALS (Forsberg et al, 2011). Since the discovery of the SOD1 mutation in 1993, more than 180 different mutations within the gene have been discovered (ALS online database [WWW]). Survival from certain mutations can be less than 15 months from diagnosis.

The exact pathological mechanisms that drive disease onset and progression in ALS are still under investigation. However, in patients with a SOD1 mutation, research has conclusively shown that these genetic changes result in protein misfolding and ultimately confer a toxic gain of function resulting in intracellular cytosolic inclusions of misfolded SOD1 protein aggregate. Accumulation of these aggregates results in neuronal cell death. Further evidence suggests that disease progression at a cellular level may be driven by spatiotemporal transmission of misfolded SOD1 in a prion-like manner (Li and Cashman 2014; Gomes et al. 2008; Munch et al. 2011; Polazzi et al. 2013; Urushitani et al. 2006).

A number of non-genetic perturbations such as metal depletion, disruption of the quaternary structure, and oxidation, have also been shown to cause wildtype non-mutant SOD1 to misfold. This misfolding also results in a toxic conformation change in SOD1 that is structurally similar to that seen with familial ALS-linked mutant SOD1 variants (Rotunno and Bosco 2013). This suggests that both genetic and non-genetic drivers of SOD1 misfolding result in formation of toxic conformers that initiate and drive ALS pathogenesis.

Importantly and supporting this hypothesis, recent evidence suggests that misfolded SOD1 aggregates can also be detected in the spinal cord of sALS patients, suggesting common elements in the pathology of disease between familial and sporadic ALS (Li and Cashman 2014; Bosco et al. 2010; Forsberg et al. 2011; Forsberg et al. 2010). Therefore, a monoclonal antibody directed toward a misfolded or aggregated SOD1 protein is an appropriate and potentially important therapeutic strategy, potentially effective in both SOD1 mutation-positive ALS as well as sporadic ALS, where similar aggregation or misfolding of SOD1 is present.

There is currently no cure for ALS. There are limited treatment options available worldwide.

- Riluzole is approved for use in North America, the European Union, and Asia (Miller et al. 2012).
- Edaravone is only approved for use in North America and Asia (Miller et al. 2012). Mitsubishi Tanabe Pharma GmbH withdrew its EU marketing authorisation application for edaravone in May 2019 (EMA 2019).
- The combination of sodium phenylbutyrate and taurursodiol was approved in the United States (September 2022, Relyvrio™ [Relyvrio product labelling 2022]) and Canada (June 2022, Albrioza™ [Albrioza Product Labelling 2023]).
- Tofersen received accelerated approval for use in patients with a confirmed mutation in the SOD1 gene in the United States on 25 April 2023 (Tofersen Product Labelling 2023).

These therapies have a limited impact on survival and disease progression, demonstrating a modest slowing of decline in the ALS functional rating scale (ALSFRS) in a subset of patients. Tofersen shows promise, but has only been approved in the US on the basis of a surrogate endpoint (neurofilament) in a subset of ALS patients (Tofersen Approval Package 2023).

AP-101 (α -miSOD1) is a fully human IgG1m3 allotype antibody with selective high affinity binding to misfolded SOD1 protein. AP-101 is produced by expression of recombinant DNA in clonally derived Chinese hamster ovary cells. There is no assigned generic or trade name for this IMP. The naming convention used by AL-S Pharma for investigational purposes is AP-101. The drug product name used in pre-clinical studies was α -miSOD1.

Various neurodegenerative disorders, including Alzheimer's disease (amyloid and tau) and Parkinson's disease (alpha synuclein), have programs in development with misfolded proteins as the target, and several of these have shown promising results in early clinical development (ALS Association resource page [WWW]). Given that both sALS and fALS patients exhibit toxic misfolding, aggregation and accumulation of SOD1, a similar strategy with AP-101 is rational. The focal initiation and contiguous spreading of neuropathology through the three-dimensional anatomy of the upper and lower motor neuron system in ALS suggest an actively propagating process.

Support for this hypothesis has been demonstrated with preclinical data in transgenic mouse models suggest that AP-101 targeting of misfolded SOD1 can be beneficial in reducing signs of motor neuron disease and prolonging survival in in both SOD1 G93A and G37R (Maier et al. 2018) transgenic mouse models of fALS.

2.2. Study Rationale

The current Phase 2a study will evaluate the safety and tolerability of AP-101 after multiple doses in patients with SOD1 mutant fALS and sALS. In addition, the study will evaluate AP-101 PK after multiple dose administration. PD markers that may change in response to modulation of mSOD1 after multiple doses of AP-101 in fALS and sALS patients will also be examined.

AP-101 is proposed as a treatment for patients with ALS. At this stage in development, ALS Pharma intends to initiate a 2-arm study to examine the effects of AP-101 on disease related biomarkers in both SOD1 mutation positive fALS patients and sALS patients.

While the exact pathological mechanisms that cause ALS are still somewhat unclear, there are both genetic and non-genetic drivers of the disease. In SOD1 mutant fALS patients, genetic mutations in SOD1 result in protein misfolding and lead to toxic gain of function and the formation of misfolded SOD1 protein aggregates. Accumulation of these aggregates results in neuronal cell death (Parakh and Atkin 2016). Further evidence that these mutations are disease causing is supported by data demonstrating that these same mutations lead to ALS-like disease in animal models.

A number of non-genetic perturbations such as metal depletion, disruption of the quaternary structure, and oxidation, have also been shown to cause wildtype non-mutant SOD1 to misfold. This misfolding also results in a toxic conformation change in SOD1 that is structurally similar to that seen with familial ALS-linked mutant SOD1 variants (Rotunno and Bosco 2013). This suggests that both genetic and non-genetic drivers of SOD1 misfolding result in formation of toxic conformers that initiate and drive ALS pathogenesis.

Supporting this hypothesis, recent evidence suggests that misfolded SOD1 aggregates can also be detected in the spinal cord and cerebrospinal fluid (CSF) of sALS patients, suggesting common elements in the pathology of disease between familial and sporadic ALS (Li and Cashman 2014; Bosco et al. 2010; Forsberg et al. 2011; Forsberg et al. 2010; Meier et al. 2020).

Therefore, a monoclonal antibody directed toward a misfolded or aggregated SOD1 protein is an appropriate and potentially important therapeutic strategy, potentially effective in both SOD1 mutation positive ALS as well as sporadic ALS where similar aggregation or misfolding of SOD1 is present. After administration of loading doses of placebo or AP-101, a total of 2500 mg of the drug product AP-101 or matching placebo will be administered once every 3 weeks as an IV solution to participating subjects. The proposed dose was selected based on a dose-finding first-in-human study in ALS patients (NCT03981536). Human PK data from the study allowed a compartmental model of pharmacokinetics to be developed. Optimal exposures were simulated suggesting that a 500-mg loading dose the first day and 2500 mg the second day would enable us to rapidly reach steady state given the long half-life.

As well as safety and tolerability, the goal of the current study is to determine the impact of AP-101 on measurable levels of several ALS associated biomarkers. The revised ALSFRS-R is the accepted clinical endpoint for functional impact on disease progression in ALS (FDA 2019; EMA 2015). One of the ongoing challenges for both the diagnosis and treatment of ALS is the lack of additional validated diagnostic and prognostic biomarkers. Recent data suggests that several neurofilament proteins may have clinical utility in ALS prognosis and response to therapy. Preclinical models originally suggested that phospho-neurofilament heavy chain (pNfH)

and neurofilament light chain (NfL) could serve as biomarkers for monitoring axonal degeneration during ALS (Brettschneider et al. 2006b; Boylan et al. 2009; Gaiottino et al. 2013). These studies have been supported more recently in publications describing increases in neurofilament markers with disease onset and correlations between levels of these proteins and the overall rate of progression in ALS (Benatar et al. 2020). Further, there is now emerging evidence that neurofilament levels in serum and CSF have potential as early markers of therapeutic efficacy (Miller et al. 2020). Based on these lines of evidence, the current study will look at the effects of AP-101 on multiple potential biomarkers of disease including SOD1, misfolded SOD1, pNfH and NfL. These studies will support future trials designed to examine disease progression and patient survival endpoints as primary outcomes.

2.3. Benefit/Risk Assessment

AP-101 has been administered to humans in a single ascending dose study and was found to be safe and tolerable up to doses including 2500 mg (NCT03981536). Based on these results, to date, any identified risks are considered to be monitorable and manageable at the planned dose of 2500 mg in ALS patients. These data support this multi-dose study for AP-101.

AL-S Pharma has created a Safety Review Committee (SRC) with independent physician experts in ALS to objectively evaluate and monitor participants. The remit and operations of the SRC will be outlined in the SRC charter.

Together with these evaluations, the proposed dosing as outlined in this protocol, the proposed clinical monitoring, and the SRC charter, this multiple dose protocol provides for a comprehensive safety risk minimization to address any uncertainties with the administration of AP-101.

This is the first multiple dose study for AP-101 and therefore safety data would be the primary criteria for any dose modification. Safety data, in particular AEs, SAEs, and adverse laboratory abnormalities, will be independently assessed by the investigator, sponsor, and SRC and will be considered related to the study drug unless there is clear evidence that the event is not related. PK results (exposure) and total body CL calculated after IV administration will be used as supporting data for any dose modifications. After review of these data, an agreement on the appropriate dose modification will be made by the investigator, sponsor and SRC.

Hypersensitivity reaction may occur after administration of any monoclonal antibody.

Immediate/acute hypersensitivity reactions, including infusion reactions or anaphylaxis, may occur during or within hours of the infusion. Subjects should be monitored for hypotension, fever, chills, bronchospasm, angioedema, and other symptoms or signs of anaphylaxis. Infusion should be slowed or stopped, and reactions managed as indicated in study protocols.

Delayed hypersensitivity reaction can develop days or weeks after infusion. Subjects should be monitored for fever, rash, arthralgia, myalgia, hematuria, or changes in hematology, liver, or renal tests. Delayed hypersensitivity reactions can be managed symptomatically and treated with corticosteroids.

Subjects should not receive additional doses of AP-101 after any confirmed/suspected case of delayed hypersensitivity reaction to AP-101.

Risk related to COVID-19 was also assessed. AP-101 is not known to alter immune function, however, there may be additional risk to participants due to exposure to COVID-19 during study related visits (dependent on the country/region conditions). Additionally, the sponsor will monitor country conditions and prepare contingency plans for COVID-19 related restrictions that may prevent site visits (Appendix 8, Section 10.8). To minimize risk for COVID-19 infection, participants should follow local guidance and Institutional Review Board (IRB) recommendations.

More detailed information about the supporting preclinical data, known, and expected benefits, risks, SAEs and reasonably anticipated AEs of AP-101 may be found in the Investigator's Brochure, Participant Information Leaflet and Development Safety Update Reports (DSUR).

3. Objectives and Endpoints

3.1. Double-blind Phase Objectives and Endpoints

Objectives	Endpoints
Primary	
To determine the safety and tolerability of AP-101 after multiple IV doses over 6 months of treatment	- Incidence of AEs and SAEs including immunogenicity - Incidence of abnormalities in vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms and changes in weight
Secondary	
To determine the pharmacokinetic (PK) profile of AP-101 after multiple IV dose administrations	The primary PK parameters are AUC, concentration at the end of infusion and $t_{1/2}$
To determine CSF penetration of AP-101 after multiple IV dosing	AP-101 levels in the CSF
To determine effects of AP-101 on pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101	Measurement of pNfH and NfL levels in the CSF and plasma after multiple IV doses of AP-101
Exploratory	
To determine the effect of AP-101 on disease progression after multiple IV doses over 6 months of treatment	Measurement of the clinical effect of AP-101 on the rate of functional decline as measured by the ALSFRS-R
To determine effects on alternate measures of disease progression after multiple IV doses of AP-101	Measurement of the clinical effect of AP-101 on lung function (SVC) and pulse oximetry, muscle strength (HHD), and disease staging (King's staging tool)
To determine effects on AP-101 on overall patient survival and additional measures of outcome for AP-101 after multiple IV dosing	- Composite time to event measurement comprising time to death or non-invasive ventilation use for 22 to 24 hours per day - Difference in mean rank between treatment arm and control arm in the

Objectives	Endpoints
	joint model of survival and ALSFRS-R progression via the CAFS
Effects on quality of life after multiple IV dosing of AP-101 over 6 months of therapy	Quality of life by ALSSQOL-SF
Identify non-clinical and exploratory biomarkers of disease activity that change over time	- Measurement of total SOD1 and misfolded SOD1 in the CSF after multiple IV doses of AP-101 - Measurement of other biological markers of disease activity including creatinine and transcriptional markers

Abbreviations: AE = adverse event; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; AUC = area under the concentration versus time curve; CAFS = combined assessment of function and survival; CSF = cerebrospinal fluid; HHD = hand-held dynamometry; IV = intravenous; NfL = neurofilament light chain; PK = pharmacokinetic; pNfH = phospho-neurofilament heavy chain; SAE = serious adverse event; SOD1= superoxide dismutase 1; SVC = slow vital capacity; $t_{1/2}$ = elimination half-life; TEAE = treatment emergent adverse event.

3.2. Open-label Extension Objectives and Endpoints

Objectives	Endpoints
Primary	
To determine the safety and tolerability of AP-101 after multiple IV doses	- Incidence of AEs and SAEs including immunogenicity - Incidence of abnormalities in vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms and changes in weight
Secondary	
To determine the PK profile of AP-101 after multiple IV dose administrations	The primary PK parameters are AUC, concentration at end of infusion and $t_{1/2}$
To determine effects of AP-101 on pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101	Measurement of pNfH and NfL levels in CSF and plasma after multiple IV doses of AP-101

Objectives	Endpoints
Exploratory	
To determine the effect of AP-101 on disease progression after multiple IV doses at the end of the open label period	Measurement of the clinical effect of AP-101 on the rate of functional decline as measured by the ALSFRS-R
To determine effects on alternate measures of disease progression after multiple IV doses of AP-101	Measurement of the clinical effect of AP-101 on lung function (SVC) and pulse oximetry and disease staging (King's staging tool)
To determine effects on AP-101 on overall patient survival and additional measures of outcome for AP-101 after multiple IV dosing	- Composite time to event measurement comprising time to death or non-invasive ventilation use for 22 to 24 hours per day - Difference in mean rank between treatment arm and control arm in the joint model of survival and ALSFRS-R progression via the CAFS
Effects on quality of life after multiple IV dosing of AP-101 at the end of the open label period	Quality of life by ALSSQOL-SF
Identify non-clinical and exploratory biomarkers of disease activity that change over time	Measurement of other biological markers of disease activity including creatinine and transcriptional markers
To determine CSF penetration of AP-101 after multiple IV dosing	AP-101 levels in the CSF

Abbreviations: AE = adverse event; ALSFRS R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ALSSQOL-SF = Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Short Form; AUC = area under the concentration versus time curve; CAFS = combined assessment of function and survival; CSF = cerebrospinal fluid; IV = intravenous; NfL = neurofilament light chain; PK = pharmacokinetic; pNfH = phospho-neurofilament heavy chain; SAE = serious adverse event; SVC = slow vital capacity; $t_{1/2}$ = elimination half-life; TEAE = treatment emergent adverse event.

4. Study Design

4.1. Overall Design

AP101-02 is a Phase 2a, multicenter, randomized, double-blind, placebo-controlled study to evaluate efficacy safety, tolerability, PK, and PD of AP-101 in patients with fALS and sALS.

Following completion of 6 months of treatment, participants will be invited to join a 6-month open-label extension (OLE) that will continue to evaluate long-term safety for AP-101.

A schematic representation of the study design is shown in Section 1.2. The full visit schedule for the double-blind phase is outlined in the double-blind phase SoA (Section 1.3). A second full visit schedule for the OLE is outlined in the OLE SoA (Section 1.4).

To minimize risk for COVID-19 infection, participants should follow local guidances and Institutional Review Board (IRB) recommendations.

4.1.1. Screening

Potential participants with an onset of symptoms within 24 months of screening will be screened in the 28 days prior to baseline (Day 1). Study eligibility for each participant will be reviewed based on all enrollment criteria (Section 5). Screening procedures are displayed in the double-blind phase SoA (Section 1.3).

4.1.2. Treatment Period

Eligibility criteria will be re-reviewed at baseline. Participants who meet all criteria will have treatment allocated by interactive web-response system (IWRS) and begin their treatment according to the SoA. Baseline procedures are displayed in the double-blind phase SoA (Section 1.3).

After randomization on Day 1, participants will receive a dose of either 500 mg AP-101 or placebo, and, on Day 2, a dose of either 2500 mg AP-101 or placebo. Thereafter, dosing with AP-101 2500 mg or matching placebo will take place every 3 weeks for 6 months as specified in the SoA. Patients will be allowed to continue on stable doses of medications approved for the treatment of ALS in their country/region, with the exception of tofersen, per the inclusion/exclusion criteria for the duration of the study.

A telephone call to participants to assess AEs and record concomitant medications will take place approximately 24 hours after the second dose.

Dosing will then continue every 3 weeks through Week 24, with the above assessments performed as indicated in the double-blind phase SoA (Section 1.3).

Dose levels, sampling schedule, timing of procedures (e.g., time of PK sample collection, electrocardiograms [ECGs]) may be adjusted in view of emerging safety or PK data during the study. Any changes will be made in consultation with and approval by SRC, investigator and sponsor.

Pharmacokinetic results (exposure) and total body clearance of drug calculated after IV administration (CL) will be used as supporting data for any dose modifications. After review of

these data, an agreement on the appropriate dose modification will be made by the investigator, sponsor and SRC.

An interim safety review was performed after 10 participants had been randomized to receive AP-101 and followed for at least 30 days. Enrollment was paused briefly while the analysis was performed. The SRC met in early January 2023 to review the data and concluded that the study should continue without modification. This recommendation was communicated to investigators, ERBs, and regulatory health authorities.

4.1.3. Safety/Survival Follow-up or Early Termination

If the participant elects not to join the OLE, following the double-blind phase treatment period will be a 4-month safety/survival follow-up period, with visits at 21 days, 8 weeks, and 16 weeks after the last infusion of AP-101 or placebo. Assessments are displayed in the double-blind phase SoA (Section 1.3). If the participant joins the OLE, the assessments of the first double-blind phase safety/survival follow-up visit (i.e., 21 days after the last infusion) will be performed at Week 3 of the OLE. A 4-month safety/survival follow-up period, with visits at 21 days, 8 weeks, and 16 weeks after the last infusion of AP-101, will be completed following the OLE. Assessments are displayed in the OLE SoA (Section 1.4).

The same assessments will be performed at the early termination visit for participants who discontinue from the study.

4.1.4. Open-Label Extension

Following completion of the 6-month treatment period, eligible participants (see Section 5) will be invited to join a 6-month OLE that will continue to evaluate long-term safety for AP-101.

For eligible participants who consent to join the 6-month OLE, the Week 24 visit of the double-blind phase will be considered the baseline visit for the OLE. Applicable eligibility criteria (see Section 5) will be reviewed at baseline and Week 3 before dosing. Baseline procedures are displayed in the OLE SoA (Section 1.4).

To protect the blinding of the double-blind phase, participant's prior treatment information will remain blinded; therefore, after entry into the OLE, all participants who received placebo in the double-blind phase will receive blinded 500 mg of AP-101 on Day 22 and 2500 mg of AP-101 on Day 23. After entry into the OLE, all participants who received active treatment in the double-blind phase of the study will receive placebo (same infusion volume of 500 mg AP-101) on Day 22 and 2500 mg of AP-101 on Day 23. Thereafter, dosing with open-label AP-101 2500 mg will take place every 3 weeks for 6 months as specified in the OLE SoA (Section 1.4). Patients will be allowed to continue on medications approved for the treatment of ALS in their country/region, with the exception of tofersen, per the inclusion/ exclusion criteria for the duration of the study. Allowed ALS treatments can be started in the OLE after Week 3 if needed based on investigator opinion. Doses can be adjusted as appropriate at any time during the OLE.

A telephone call to participants to assess AEs and record concomitant medications will take place approximately 24 hours after the second dose.

Dosing will then continue every 3 weeks through Week 24, with assessments performed as indicated in the OLE SoA (Section 1.4).

4.2. Scientific Rationale for Study Design

The exact pathological mechanisms that cause ALS are still somewhat unclear. What is understood is that there are both genetic and non-genetic drivers of the disease. For example, in some fALS patients, genetic mutations in SOD1 result in protein misfolding. This ultimately confers a toxic gain of function and the formation of misfolded SOD1 protein aggregates. In fact, mutations in SOD1 were the first confirmed genetic drivers of ALS. Further evidence that these mutations are disease causing is supported by data demonstrating that these same mutations lead to ALS-like disease in animal models.

A number of non-genetic perturbations such as metal depletion, disruption of the quaternary structure, and oxidation, have been shown to cause wildtype non-mutant SOD1 to misfold. This misfolding also results in a toxic conformation change in SOD1 that is structurally similar to that seen with familial ALS-linked mutant SOD1 variants (Rotunno and Bosco 2013). This suggests that both genetic and non-genetic drivers of SOD1 misfolding result in formation of toxic conformers that initiate and drive ALS pathogenesis.

This hypothesis has been supported in a number of studies examining biological samples from both fALS and sALS patients including CSF and post-mortem spinal cord (Li and Cashman 2014; Bosco et al. 2010; Forsberg et al. 2011; Forsberg et al. 2010; Meier et al. 2020). Based on these data treatment approaches which target or reduce pathogenic misfolded SOD1 in both fALS and sALS would have the potential to positively impact the course of, or delay progression of the disease, and therefore provide significant clinical benefit for both fALS SOD1 and sALS patients. This trial will be a randomized and placebo-controlled trial per current guidance from the FDA and EMA on design for trials in ALS (FDA 2019; EMA 2015).

One of the ongoing challenges for both the diagnosis and treatment of ALS is the lack of validated diagnostic and prognostic biomarkers. Further, the accepted functional clinical endpoint ALSFRS-R is variable and subjective, the impact of which is a need for relatively long studies with quite high numbers of patients required to validate effects of a therapeutic agent.

Preclinical models of disease have shown that soluble neurofilament proteins are undetectable in the sera of normal animals but are readily detectable in the sera of adult rats following various types of experimental spinal cord injury and traumatic brain injury (Shaw et al. 2005; Anderson et al. 2008). While not specific to ALS, but useful as diagnostic biomarkers, neurofilament levels in serum and CSF do appear to correlate with axonal damage and in some cases can be modulated by therapeutic treatment. This is perhaps most striking in SMA, where early administration of a therapeutic soon after diagnosis results in normalization of multiple developmental endpoints and also lowers the associated NF levels (De Vivo et al. 2019).

Preclinical models suggested that pNfH and NfL could serve as biomarkers for monitoring axonal degeneration during ALS and thereby have potential as biomarkers of disease progression (Brettschneider et al. 2006; Boylan et al. 2009; Gaiottino et al. 2013). These studies have been supported by more recently in publications describing increases in neurofilament markers with disease onset and correlations between levels of these proteins and the overall rate of progression in ALS (Benatar et al. 2020).

There is also now emerging evidence that neurofilament levels in serum and CSF have potential as early markers of therapeutic efficacy (Miller et al. 2020). Based on these lines of evidence, as

well as safety and tolerability, the current study will look at the effects of AP-101 on multiple potential biomarkers of disease including SOD1, misfolded SOD1, pNFH, and NFL. These studies will support future trials designed to examine disease progression and patient survival endpoints as primary outcomes.

Currently approved therapies are described in Section 2.1. On 25 April 2023, tofersen received accelerated approval from the US FDA on the basis of the surrogate endpoint neurofilament. This is the first specific SOD1 targeting therapeutic approved by any regulator worldwide; however, its use is limited to patients with a confirmed mutation in the SOD1 gene and full approval requires clinical confirmation in a subsequent trial.

All patients in the trial, including those receiving placebo, will continue on any approved standard of care therapy (for their country/region, except for tofersen) for the duration of the trial per the inclusion/exclusion criteria. The intent of the placebo arm is to enable study blinding and establish control data for key outcome measures.

AP-101 (α -miSOD1) is a fully human IgG1m3 allotype antibody with selective high affinity binding to misfolded SOD1 protein. This study will test the safety, tolerability, efficacy, PD and PK of AP-101 in familial and sporadic ALS. Of note is that AP-101 was granted orphan drug designation status by regulatory bodies in 3 regions; EMA (2017; EMA/OD/030/17), Swissmedic (2018; #102577296), and the FDA (2017; #17-5964) supporting the need for innovative treatments.

The OLE will allow all participants to have the opportunity to receive active AP-101 treatment. The OLE will determine safety and tolerability over a longer than 6 months period of time and the long-term PK profile of AP-101 after multiple dose administration will be assessed at the end of the OLE.

4.3. Justification for Dose

The proposed dose of 2500 mg AP-101 was selected based on a dose finding first-in-human (FIH) study in ALS patients (NCT03981536). This dose level has been confirmed as having an acceptable safety and tolerability profile after a single IV administration. The current study is designed with 2 loading doses on consecutive days, followed by repeat dosing once every 3 weeks for 6 months. Based on in silico modeling of PK data obtained from the FIH study, the dose schedule has been designed to enable patients to reach steady state as quickly as possible. The dose frequency is based on maintaining steady state throughout the treatment phase of the study.

Based on the preclinical, toxicology and clinical data available, a loading dose of 500 mg and 2500 mg given on consecutive days followed by once every 3 weeks dosing of 2500 mg IV is planned. The dose level is based on preclinical efficacy studies performed in a mouse transgenic model of ALS which suggested that weekly intraperitoneal dosing of a mouse-human chimeric α -miSOD1 at 3, 10, or 30 mg/kg (180-1800 mg dose equivalent, FDA 2005) showed efficacy in specific endpoints. Further, no overt toxicity (body weight loss) was observed in these animals. For additional details on the characterization of the transgenic models and the preclinical efficacy studies conducted in them, including dose ranges and route of administration, please refer to the Investigator's Brochure.

The potential for AP-101 to produce toxicity or unwanted effects was evaluated in a 1-month study in cynomolgus monkeys (Study 8384444) which consisted of 2 IV injections separated by 2 weeks, each followed by an approximately 2-week postdosing observation phase. The monkey is a relevant toxicology species because AP-101 binds to human and monkey SOD-1; AP-101 does not bind to rodent SOD-1. No AP-101-related effects on toxicology or safety pharmacology parameters occurred at doses up to and including 400 mg/kg (24,000 mg for a 60-kg person), the highest dose tested. Therefore, 400 mg/kg is considered the no-observed-adverse-effect level (NOAEL).

In this 1-month study, monkeys were given bolus IV injections of 10, 60, or 400 mg/kg AP-101 on Study Days 1 and 15, with subsequent necropsy on Study Day 29. The top dose was selected to provide a multiple of the proposed human doses. Assessment of toxicity was based on mortality, clinical observations, body weight, ophthalmic observations, clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis parameters) and anatomic pathology (organ weights, macroscopic and microscopic pathology). Additional toxicology and safety pharmacology parameters are described in the Investigator's Brochure.

There were no AP-101-related effects observed in any of the study parameters assessed. Serum AP-101 was measured at several timepoints and CSF AP-101 was measured at a single timepoint taken at necropsy, 2 weeks after second administration of AP-101. Serum exposure increased in a dose-proportional manner from 10 to 400 mg/kg and there was no evidence of accumulation with this dose interval. AP-101 was detectable in some 60- and 400-mg/kg monkey CSF samples collected at necropsy (approximately 2 weeks after the second IV dose).

The relevant margins of safety based on the NOAEL from the 1-month monkey study are presented in [Table 4.1](#). Exposure multiples are based on observed single-dose data for both monkey and humans. The proposed clinical dose of 2500 mg is approximately 9.5x lower than the NOAEL established in the 6-month monkey study. This margin of safety together with the clinical study design with appropriate safety monitoring, reinforces that AP-101 has a safety profile that supports continuation of clinical development.

Based on the PK observed in cynomolgus monkeys and humans, PK profiles were utilized for calculation of human exposure multiples compared to the NOAEL in [Table 4.1](#).

Table 4.1. Margin of Safety for Intravenous Administration of AP-101 Based on Administered Dose and Observed Exposure

	Dose (mg/kg)	Dose Multiple ^a	AUC ₀₋₃₃₆ (µg*h/mL)	Exposure Multiple ^a
Human Maximum (2500 mg)	42	9.5x	111,000	8.7x
Monkey NOAEL^b	400		961,000	

Abbreviations: AUC₀₋₃₃₆ = area under the plasma concentration time curve from time 0 to 336 hours;

NOAEL = no-observed-adverse-effect level.

^a Dose multiple is the dose in animals/dose in humans based on mg/kg. Exposure multiple is calculated as the observed AUC₀₋₃₃₆ in animals / observed AUC₀₋₃₃₆ in humans, both after a single dose administration.

^b NOAEL determined in a 6-month repeat dose toxicity study (Study 8384445).

In the 6-month repeat dose study (Study 8384445), monkeys were given IV injections of 60 or 400 mg/kg AP-101 every 2 weeks for 26 weeks (a total of 13 doses). Assessment of toxicity was based on mortality, clinical observations, body weight, ophthalmic observations, clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis parameters) and anatomic pathology (organ weights, macroscopic and microscopic pathology).

There were no AP-101-related effects observed in any of the study parameters assessed, so the NOAEL was considered to be 400 mg/kg.

Consistent with the absence of adverse effects in the 1- and 6-month monkey toxicology studies, there were no drug-related adverse events during the FIH study (AP101-01). For PK modeling and exposure data, refer to the Investigator's Brochure.

The treatment emergent adverse events (TEAEs) that were reported in the 9-patient FIH study are listed fully in the Investigator's Brochure. Those TEAEs occurring in 2 or more patients included fatigue, fall, headache, nausea, contusion, post-lumbar puncture syndrome, arthralgia and muscular weakness.

The primary goal of this study is to determine the safety and tolerability of AP-101 after multiple doses. In the completed Phase 1 dose escalation study (NCT03981536), single doses of AP-101 at up to 2500 mg have shown no clinically significant safety or tolerability concerns supporting the premise that AP-101 is safe and well-tolerated. This study will also examine the impact of AP-101 on measurable levels of several ALS associated biomarkers, including several neurofilament proteins (NfL and pNfH).

4.4. End of Study Definition

A participant is considered to have completed the study if Week 24 dosing and all scheduled procedures for the 21-day follow-up visit have been completed, as shown in the double-blind phase SoA (Section 1.3). If a participant joins the OLE, the participant will complete the scheduled 21-day follow-up visit of the double-blind phase at Week 3 of the OLE. The participant is considered to have completed the study if Week 24 dosing of the OLE and all

scheduled procedures for the OLE 21-day follow-up visit have been completed, as shown in the OLE SoA (Section [1.4](#)).

The end of the study is defined as the date after the last participant completed 6 months of study treatments and completed any applicable follow-up.

5. Study Population

Eligibility of study participants will be based on the results of assessments including screening, medical and disease history, physical examination, vital signs, clinical laboratory tests, Columbia-Suicide Severity Rating Scale (CSSR-S), ALS-Specific Quality of Life-Revised (ALSSQOL-SF), and ECG as described in the double-blind phase SoA (Section 1.3). All baseline values measured pre-dose on Day 1 must still pass the eligibility criteria, with the exception of the safety samples because the results of these tests will not be available before randomizing/dosing and Inclusion Criterion 5 which must be within 24 months of screening.

Participants who successfully complete up to Week 24 of the double-blind phase of the study, who elect to join the OLE and have not developed any systemic issues will be permitted to join the OLE, unless in the opinion of the investigator or sponsor, they are unsuitable for inclusion in the OLE for any reason. Participants who join the OLE are eligible for inclusion only if they meet Inclusion Criteria 6, 10, 11, 12, 13, 14 and 15, and are to be excluded from the OLE if any of the following criteria apply, including Exclusion Criteria 6, 9, 10 and 13, as assessed at the visits and times described in the OLE SoA (Section 1.4). Participants who participated in the FIH trial AP-101-01 are eligible for screening for this trial, AP101-02. These participants must also meet all AP101-02 inclusion and exclusion criteria.

The nature of any conditions present at the time of the physical examination and any preexisting conditions will be documented.

Screening may occur up to 28 days prior to dosing.

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

5.1. Inclusion Criteria

Participants are eligible for inclusion in the study only if they meet all of the following criteria at screening, unless otherwise specified:

1. Male and female participants. Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.
 - a. Male participants who agree to use highly effective/effective methods of contraception may participate in this trial:
 - i. All men should refrain from sperm donation for the duration of the study and for 6 months after the final administered dose of AP-101
 - ii. Male participants with partners of childbearing potential must either remain abstinent (if this is their preferred and usual lifestyle), or must use condoms during intercourse for the duration of the study, and for 6 months after the final administered dose
 - iii. Male participants who are in exclusively same sex relationships are not required to use contraception.

- b. Male participants must adhere to the contraception restrictions specified in Section 10.6.
- c. Female participants of childbearing potential must adhere to contraception restrictions specified in Appendix 6 (Section 10.6). Female participants should refrain from egg donation for the duration of the study and for 6 months following the final administered dose of AP-101.
- d. Female participants are considered women not of childbearing potential if
 - i. they have a congenital anomaly such as Mullerian agenesis,
 - ii. they are infertile due to surgical sterilization, or
 - iii. they are post-menopausal. Postmenopausal female is defined as, women with:
 - 12 months of amenorrhea for women >55, with no need for follicle-stimulating hormone (FSH)
 - 12 months of amenorrhea for women >40 years old with FSH ≥ 40 mIU/mL and no other medical condition such as anorexia nervosa and not taking medications during the amenorrhea (e.g., oral contraceptives, hormones, gonadotropin releasing hormone, anti-estrogens, selective estrogen receptor modulators (SERMs), or chemotherapy that induced amenorrhea).

Examples of surgical sterilization include: hysterectomy, bilateral oophorectomy, tubal ligation.

2. Aged 18 years or over.
3. Have possible, clinically probable, clinically probable-laboratory supported or definite familial or sporadic ALS in accordance with the El-Escorial criteria (Appendix 9, Section 10.9) or who have a diagnosis of ALS as defined by the Gold Coast Criteria; progressive motor impairment documented by history or repeated clinical examination, preceded by normal motor development, and presence of upper and lower motor neuron dysfunction in at least 1 body region or lower motor neuron dysfunction in at least 2 body regions and investigations excluding other conditions.
4. In familial ALS patients, a confirmed pathogenic SOD1 mutation.
5. Onset of symptoms i.e., weakness within past 24 months prior to screening, at the time of obtaining informed consent.
6. Have SVC of $\geq 50\%$ of predicted values. Participants with SVC of $< 50\%$ of predicted values may be permitted to enter the OLE, based on the opinion of the investigator.
7. Absence of bilevel positive airway pressure (BiPAP)/proportional assist ventilation (PAV) >4 hours for symptoms attributable to ALS. Use of a CPAP for pre-existing conditions will be allowed.
8. If on riluzole, must be on a stable dose for at least 30 days prior to baseline (randomization) and remain on a stable dose throughout the study. Riluzole cannot be initiated during the double-blind phase of the study. Riluzole can be initiated during the

OLE after Week 3 if needed based on the opinion of the investigator and the dose may be adjusted as appropriate.

9. If on edaravone, must have completed 2 cycles at baseline (randomization) and are expected to remain on the same dose throughout the study. Edaravone cannot be initiated once the participant is randomized and for the duration of the double-blind phase of the study. Edaravone can be initiated during the OLE after Week 3 if needed based on the opinion of the investigator and the dose may be adjusted as appropriate.
10. If on sodium phenylbutyrate or taurursodiol, patients must be on a stable dose for at least 30 days prior to baseline (randomization) and remain on a stable dose throughout the study. Treatment with sodium phenylbutyrate or taurursodiol cannot be initiated during the double-blind phase of the study. Treatment with sodium phenylbutyrate or taurursodiol can be initiated during the OLE after Week 3 if needed based on the opinion of the investigator and the dose may be adjusted as appropriate.
11. Capable of giving signed informed consent as described in Appendix 1 (Section 10.1.3), which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.
12. Have venous access sufficient to allow for blood sampling as per protocol.
13. Have clinical laboratory test results within normal reference range for the population or study site, or results with acceptable deviations that are judged to be not clinically significant by the investigator.
14. Are able to visit the study site for outpatient treatment.
15. Are willing to make themselves available for the duration of the study and are willing to follow study specific procedures.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Are investigator or site personnel affiliated with this study, or the immediate family of any of these. Immediate family is defined as a spouse, parent, child or sibling, whether biological or legally adopted.
2. Are participating in or have participated in another clinical trial (or have taken an experimental therapy within the context of a clinical trial) within 5 half-lives of baseline (Day 1).
3. Have undergone a tracheostomy for ALS symptoms.
4. Are on nasal intermittent positive pressure ventilation (NIPPV) >4 hours per day for the treatment of ALS related symptoms.
5. Have other causes of neuromuscular weakness.
6. Have severe active psychiatric illness.
7. Have a diagnosis of another neurodegenerative disease (e.g., Parkinson's disease, Alzheimer's disease).

8. Have a significant pulmonary disorder not attributed to ALS or who require treatments that might complicate the evaluation of the effect of ALS on respiratory function.
9. Have cognitive impairment, severe disease in the renal, cardiovascular or hematological system.
10. Pregnant or nursing women.
11. Have been exposed to any antisense treatment targeting SOD1, including tofersen, within 6 months of the baseline visit or have known allergies or reactions to AP-101 or any of its excipients.
12. Have undergone stem cell therapy.
13. In the opinion of the investigator or sponsor, are unsuitable for inclusion in the study for any reason.

5.3. Lifestyle Considerations

Throughout the study, participants may undergo medical assessments and review of compliance with requirements before continuing in the study.

Medically prescribed use of cannabis is allowed, per discretion of the investigator, during the study.

Contraception guidelines are presented in Section [10.6](#).

5.4. Screen Failures

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

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened 1 time with sponsor approval. The time period from screen fail to rescreen will be determined by the investigator. If rescreening is performed, the participant will be assigned a new identification number. Additionally, all screening procedures except lumbar puncture must be conducted at rescreen to ensure all eligibility criteria are met.

At screening and throughout the study, procedure/lab retests may be conducted at the discretion of the investigator and do not constitute a rescreen.

6. Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to/used by a study participant according to the study protocol.

6.1. Study Intervention(s) Administered

In the double-blind phase, after randomization on Day 1, participants will receive a dose of either AP-101 500 mg or placebo, and on Day 2, another dose of either AP-101 2500 mg or placebo. Thereafter at each visit during the treatment period, AP-101 2500 mg or matching placebo will be administered via IV infusion to all participants over at least 60 minutes. Infusion duration may be increased or the infusion may be stopped as deemed necessary based on the standard operation procedures of the study site, or if an infusion reaction is observed (Section 6.6.2). However, IV administration needs to be completed within not more than 2 hours (120 minutes) from start of infusion. During the infusion and throughout the patient visit, the study site must have resuscitation equipment, emergency drugs, and appropriately trained staff available during the infusion period and for up to 6 hours after participants have completed their infusion. The actual start and stop time of the infusion will be recorded in the electronic data capture (EDC).

In the OLE, participants will receive either 2500 mg of AP-101 or placebo at the baseline visit (Week 24 of the double-blind phase), as per their randomized treatment from the double-blind phase. To protect the blinding of the double-blind phase, participants prior treatment information will remain blinded; therefore, after entry into the OLE, all participants who received placebo in the double-blind phase of the study will receive blinded 500 mg of AP-101 on Day 22 and 2500 mg of AP-101 on Day 23. After entry into the OLE, all participants who received active treatment in the double-blind phase of the study will receive placebo (same infusion volume of 500 mg AP-101) on Day 22 and 2500 mg of AP-101 on Day 23. Thereafter, the dose schedule will follow the SoA with dosing with open-label 2500 mg of AP-101 every 3 weeks starting at Day 22, administered via IV infusion to all participants over at least 60 minutes. Infusion duration may be increased or the infusion may be stopped as deemed necessary based on the standard operating procedures of the study site, or if an infusion reaction is observed (Section 6.6.2). However, IV administration needs to be completed within not more than 2 hours (120 minutes) from start of infusion. During the infusion and throughout the patient visit, the study site must have resuscitation equipment, emergency drugs, and appropriately trained staff available during the infusion period and for up to 6 hours after participants have completed their infusion. The actual start and stop time of the infusion will be recorded in the EDC.

Section 6.6 details dose modifications.

The AP-101 drug product is a monoclonal antibody formulated for IV administration. AP-101 for injection is supplied for clinical trial use as a solution in a single-use glass vial. The vial is manufactured to provide 100 mg of AP-101 at a 20 mg/mL concentration. In order to ensure complete withdrawal and delivery of the label amount of 100 mg of AP-101, vials contain an approximate 10% volume overfill. The drug product is filled into a single-use Type I glass vial.

6.2. Preparation/Handling/Storage/Accountability

Vials of AP-101 are stable and should be stored under refrigerated conditions (2° to 8°C). Pharmacy personnel will receive training and instructions for preparation of each dose of AP-101 IV solutions.

Further guidance regarding preparation, handling, storage, and accountability is as follows:

1. The investigator or designee must confirm appropriate storage conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.
2. Only participants enrolled in the study may receive study intervention. Only study personnel may supply, prepare, or administer study intervention. All study intervention 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 study personnel.
3. The investigator or authorized study personnel are responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).
4. The investigator or designee is responsible for returning all unused medication to sponsor or its designee at the end of the study. In some cases, sites may destroy the material if, during the investigative site selection, the evaluator has verified and documented that the site has appropriate facilities and written procedures to dispose of clinical materials. Further guidance and information for the final disposition of unused study interventions are provided in the pharmacy manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

This is a double-blind study. On Day 1, all participants will be centrally assigned to randomized study intervention using an IWRS.

Study intervention will be dispensed at the study visits as summarized in the SoA.

If an investigator, site personnel performing assessments, or participant is unblinded, the participant must be discontinued from the study but will be given the opportunity to enroll in the OLE. In cases where there are ethical reasons to have the participant remain in the study, the investigator must obtain specific approval from a sponsor clinical research physician for the participant to continue in the study.

The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participants' intervention assignment is warranted. Participant safety must always be the first consideration in making such a determination. If a participant's intervention assignment is unblinded, the sponsor must be notified immediately after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form (CRF), as applicable.

Participants will be randomly assigned in a 2:1 ratio to receive study intervention. Investigators will remain blinded to each participant's assigned study intervention throughout the course of the study. In order to maintain this blind, an otherwise uninvolved and unblinded 3rd party will be

responsible for the preparation of all blinded study intervention per each participant treatment assignment. The unblinded 3rd party personnel will receive training and instructions for preparation of each dose of study intervention, i.e., AP-101 or matching placebo solution for IV administration. The blinded study intervention will be administered to participant by blinded staff following randomization.

For those participants who join the OLE, the participants prior treatment information will remain blinded to protect the blinding of the double-blind phase; therefore, after entry into the OLE, all participants who received placebo in the main part of the study will receive blinded 500 mg of AP-101 on Day 22 and 2500 mg of AP-101 on Day 23. All participants who received active treatment in the main part of the study will receive placebo (same infusion volume of 500 mg AP-101) on Day 22 and 2500 mg of AP-101 on Day 23.

In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded study intervention records at the site(s) to verify that randomization/dispensing has been done accurately.

6.4. Study Intervention Compliance

Study intervention will be administered under medical supervision by the investigator or designee. Documentation of treatment administration will occur at the site. The date and time of each dose administered will be recorded in the source documents and in the electronic case report form (eCRF).

6.5. Concomitant Therapy

As standard of care agents, treatment with locally approved agents is allowed per the inclusion/exclusion criteria. Participants are required to be at a stable dose for at least 30 days prior to screening and remain on a stable dose throughout study. Allowed ALS treatments cannot be initiated during the placebo-controlled portion of the study; however, they can be initiated in the OLE after Week 3 and the dosage can be changed as deemed appropriate by the Investigator. Experimental therapy, exposure to any antisense treatment targeting SOD1, including tofersen, and stem cell therapy are not allowed for the entire duration of the trial.

In general, concomitant medication should be avoided; however, over-the-counter medications may be administered at the investigator's discretion. (e.g., acetaminophen for treatment of headaches). If the need for concomitant medication (other than over-the-counter medications) arises, inclusion or continuation of the subject may be at the discretion of the investigator after consultation with the sponsor. Any medication used during the course of the study must be documented.

Medically prescribed use of cannabis is allowed, per discretion of the investigator, during the study.

Any SARS-CoV-2 vaccine is allowed; however, it is recommended that study drug not be administered on the same day as a SARS-CoV-2 vaccination.

6.6. Dose Modification

Dose levels, sampling schedule, timing of procedures (e.g., time of PK sample collection, ECGs) may be adjusted in view of emerging safety or PK data during the study. This is the first multiple dose study for AP-101 and therefore safety data would be the primary criteria for any dose modification. Safety data, in particular AEs, SAEs and adverse laboratory abnormalities, will be independently assessed by the investigator, sponsor and SRC and will be considered related to the study drug unless there is clear evidence that the event is not related. PK results (exposure) and total body clearance of drug calculated after IV administration [CL] will be used as supporting data for any dose modifications. After review of these data, an agreement on the appropriate dose modification will be made by the investigator, sponsor and SRC. Any dose modifications will be made only with the agreement of the investigator, sponsor and SRC.

6.6.1. Premedication for Infusions

Premedication for the infusions is not planned. However, if an infusion reaction occurs, appropriate medication may be used as determined by the study investigator(s). If infusion reactions are observed, administration of acetaminophen, 500 to 1000 mg and/or an antihistamine may be administered orally or IV 30 to 60 minutes prior to the start of infusion for subsequent dose administrations.

The decision to implement premedication for specific participants exhibiting infusion reactions will be made by the investigator, the sponsor, and the SRC, and recorded in the study documentation.

Any premedications given will be documented as a concomitant therapy (see Section 6.5).

6.6.2. Management of Infusion Reactions

There is a risk of infusion reaction with any biological agent; therefore, all participants should be monitored closely. Symptoms and signs that may occur as part of an infusion reaction include, but are not limited to: fever, chills, nausea, headache, bronchospasm, hypotension, angioedema, throat irritation, rash, pruritus, myalgia, and dizziness. If a significant infusion reaction occurs, the following guidance should be followed:

- The investigational product infusion should be slowed (for example, reduce infusion rate by 50% [for example, an infusion rate of 12 mL/hr becomes 6 mL/hr or slower]) or stopped, depending on the symptoms/signs present:
 - if slowed, the infusion should be completed at the slower rate, as tolerated
 - if determined by the investigator that the infusion should no longer continue, no further attempts to dose the participant should be made
- Supportive care should be employed in accordance with the symptoms/signs

6.7. Intervention after the End of the Study

Following completion of the 6-month treatment period, eligible participants will be invited to participate in a 6-month OLE.

7. Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal

Discontinuation (closure) of specific sites or of the study are handled as part of Appendix 1, Section 10.1.8 (Study and Site Start and Closure).

Participants discontinuing from the treatment prematurely from the double-blind phase or the OLE for any reason should complete AE and other follow-up procedures per Section 1.3 or Section 1.4, respectively, of this protocol.

7.1. Discontinuation of Study Intervention

If the sponsor or investigator identifies a participant who did not meet enrollment criteria and was inadvertently enrolled, a discussion must occur between the sponsor and the investigator to determine if the participant may continue in the study. If both agree it is medically appropriate to continue, the investigator must obtain documented approval from the sponsor to allow the inadvertently enrolled participant to continue in the study with or without continued treatment with investigational product.

Participants will be discontinued from the study in the following circumstances:

- Enrollment in any other clinical study involving an investigational product or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study
- Participation in the study needs to be stopped for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP)
- If the investigator, after consultation with the sponsor-designated medical monitor, determines that a systemic hypersensitivity reaction has occurred related to study drug administration, the participant should be permanently discontinued from the investigational drug.
- Abnormal liver tests when a patient meets one of the following conditions after consultation with the sponsor designated medical monitor:
 - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5X upper limit of normal (ULN)
 - ALT or AST >3X ULN along with one or more of the following criteria:
 - sustained for more than 2 weeks or
 - total bilirubin (TBL) >2X ULN or
 - prothrombin time >1.5X ULN or
 - the appearance of fatigue, nausea, vomiting, right upper-quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
 - alkaline phosphatase (ALP) >3X ULN
 - ALP >2.5X ULN and TBL >2X ULN
 - ALP >2.5X ULN with the appearance of fatigue, nausea, vomiting, right quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

- Investigator Decision
 - the investigator decides that the participant should be discontinued from the study
 - if the participant, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of the study indication, discontinuation from the study occurs prior to introduction of the new agent
- Participant Decision
 - The participant, or legal representative, requests to be withdrawn from the study. See Section [7.2](#).
 - Termination of the study by the sponsor or regulatory authorities

Participants who withdraw from the study before completion of all study activities may be replaced at the discretion of the sponsor.

If a clinically significant finding is identified (including, but not limited to changes from baseline in QT interval corrected using [Bazett's formula [QTcB] or Fridericia's formula [QTcF]]) after enrollment, the investigator or qualified designee will determine if the participant can continue in the study and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

See the SoA for data to be collected at the time of intervention discontinuation and follow-up and for any further evaluations that need to be completed.

7.1.1. Temporary Discontinuation

Temporary discontinuation is not permitted for the current study.

7.2. Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study:

- at any time at his/her own request
- at the request of his/her designee (for example, parents or legal guardian)
- at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons
- if the participant becomes pregnant during the study (Appendix 6, Section [10.6](#) Female Participants Who Become Pregnant)
- if enrollment in any other clinical study involving an investigational product or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study
- if the participant, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of the study indication, discontinuation from the study occurs prior to introduction of the new agent.

Discontinuation is expected to be uncommon.

At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted, as shown in the SoA. See SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed. The participant will be permanently discontinued both from the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

7.2.1. Discontinuation of Inadvertently Enrolled Participants

If the sponsor or investigator identifies a participant who did not meet enrollment criteria and was inadvertently enrolled, then the participant should be discontinued from study treatment unless there are extenuating circumstances that make it medically necessary for the participant to continue study treatment. If the investigator and the sponsor clinical research physician agree it is medically appropriate to continue, the investigator must obtain documented approval from the sponsor clinical research physician to allow the inadvertently enrolled participant to continue in the study with or without treatment with investigational product. Safety follow-up should be performed as outlined in Section 1.3 (SoA), Section 1.4 (the OLE SoA), Section 8.2 (Safety Assessments), and Section 8.3 (Adverse Events and Serious Adverse Events) of the protocol.

7.3. Discontinuation of the Study: Stopping / Pausing Rules

The sponsor may pause enrolment and/or stop the study at any time at which the sponsor determines the risk to participants in the study outweighs potential study benefits. This decision will be made in consultation with the SRC. Full details are included in the SRC Charter. Communication of study pausing/stopping will be made immediately to all investigators, Ethics Committees and Competent Authorities.

In addition to the individual stopping criteria specific in Sections 7.1 and 7.2, blinded safety data including AEs, SAEs, laboratory abnormalities and ECG abnormalities will be reviewed at regular intervals by the SRC to ensure appropriate participant safety.

If a fatal or life-threatening AE that is possibly-, probably-, or definitely- related to study drug is observed, enrolment will be paused for a review of safety data by the SRC.

As specified in Section 9.5, enrolment will be paused for a review of safety data by the SRC once 10 participants have been randomized to AP-101, received the loading dose and at least one dose of 2500 mg, and been followed for at least 30 days following this initial treatment. For further details please see the SRC Charter.

7.4. Lost to Follow-Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. Site personnel or designee are expected to make diligent attempts to contact participants who fail to return for a scheduled visit or were otherwise unable to be followed up by the site.

Site personnel, or an independent third party, will attempt to collect the vital status of the participant within legal and ethical boundaries for all participants randomized, including those who did not get investigational product. Public sources may be searched for vital status information. If vital status is determined to be deceased, this will be documented, and the participant will not be considered lost to follow-up. Sponsor personnel will not be involved in any attempts to collect vital status information.

8. Study Assessments and Procedures

- Study procedures and their timing are summarized in the SoA.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Appendix 2 (Section 10.2) lists the laboratory tests that will be performed for this study.

Table 8.1 provides a summary of the maximum number and volume of invasive samples for all sampling during the study.

Table 8.1. Protocol AP101-02 Blood Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests ^a	25	1	25
Post-screening clinical laboratory tests ^b	15	13	195
Pharmacokinetics	5	23	115
Serum biomarkers	5	13	65
Total	50	50	400

^a Includes sampling for surface antigen of the hepatitis B virus (hBsAg) & hepatitis C virus (HCV) and clinical safety laboratory measures.

^b Additional samples, including for hypersensitivity analyses, may be drawn if needed for safety purposes.

In addition to the blood sampling volumes noted in Table 8.1, 3 post-screening CSF samples of 5 mL each will be collected, for a total of 15 mL. A screening CSF sample of 5 mL will also be drawn.

Unless otherwise stated in this protocol, all samples collected for specified laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

8.1. Efficacy Assessments

Efficacy in this study will be measured as a decline in disease progression rate as measured by the change from baseline to the last visit in the ALSFRS-R, lung function via SVC and/or pulse oximetry, muscle strength via hand-held dynamometry (HHD), quality of life by ALSSQOL-SF, and disease staging via King's staging tool.

8.1.1. Survival

Survival data may be confounded by the use of ventilation strategies. Therefore, a composite measurement of time to death or permanent ventilator use will be used.

8.1.2. Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised

The ALSFRS-R will be assessed at the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4).

The ALSFRS-R is a validated rating instrument for monitoring the progression of disability in patients with ALS. The ALSFRS-R scores correlate significantly with quality of life as measured by the Sickness Impact Profile, indicated that the quality of function is a strong determinant of quality of life in ALS.

The ALSFRS-R has been demonstrated to predict survival. It measures 4 functional domains, including respiratory, bulbar function, gross motor skills, and fine motor skills. There are 12 questions, each scored 0 to 4 for a possible total score of 48 with higher score denoting better function (Cedarbaum et al. 1999).

8.1.3. Slow Vital Capacity

Upright SVC will be determined by performing 3-5 measures at the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4), in accordance with criteria established by the American Thoracic Society and the European Respiratory Society (Goncalves de Barros et al. 2013).

8.1.4. Pulse Oximetry

SpO₂ stands for peripheral capillary oxygen saturation, an estimate of the amount of oxygen in the blood. Low SPO₂ is associated with worsening respiratory function. SpO₂ measurements will be performed as according to the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4).

8.1.5. Hand-Held Dynamometry

Muscle strength will be assessed through the use of HHD instruments at the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4). Muscle strength is an important determinant of both function and ultimate survival in ALS. Quantitative muscle strength will be evaluated using HHD, which tests isometric strength of multiple muscles using standard positioning. Approximately 8 muscle groups will be examined bilaterally in both upper and lower extremities. A statistical analysis of 4 muscle groups can be used (HHD0) to accurately predict outcome.

8.1.6. King's Staging Tool

Participants' ALS will be staged according to the King's staging tool, measured at the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4) (Roche et al. 2012). This tool uses the following classifications of ALS development:

- Stage 1: symptom onset (involvement of first region)

- Stage 2A: diagnosis
- Stage 2B: involvement of second region
- Stage 3: involvement of third region
- Stage 4A: need for gastrostomy
- Stage 4B: need for non-invasive ventilation

This functional staging system ranks patients based upon their level of independence (Stages 1 and 2) and the need for significant interventions (feeding tube, respiratory support).

8.1.7. The Combined Assessment of Function and Survival (CAFS)

The combined assessment of function and survival (CAFS) ranks participants' clinical outcomes based on survival time and change in the ALSFRS-R score. Each participant's outcome is compared to every other participant's outcome, assigned a score, and the summed scores are ranked. The mean rank score for each treatment group can then be calculated. A higher mean CAFS score indicates a better group outcome (Berry et al. 2013).

8.2. Safety Assessments

Safety assessments will include clinical laboratory evaluations (hematology, clinical chemistry, endocrinology, and urinalysis), coagulation assessments, physical and neurological examinations, ECGs, vital signs, pregnancy tests, CSSR-S, quality of life, AE recording, and AE grading. In the event of a treatment-emergent hepatic abnormality additional selected clinical tests may be obtained and are detailed in Appendix 4 (Section 10.4).

Planned time points for all safety assessments are provided in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4). Adverse events are discussed in Section 8.3.

8.2.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal and neurological systems. Height (screening only) and weight will also be measured and recorded.
- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2. Neurological Examinations

A directed neurological examination will be performed by the investigator or designee. If abnormalities are noted at these time points, additional examinations should be performed until the participant has returned to baseline. The examiner should be familiar with the participant's baseline examination. Mandated elements of the examination include inspection for cranial nerves, tremor, extraocular movements, brachial and patellar deep tendon reflexes, finger-nose pointing, and Romberg sign.

Work up for participants with clinically significant changes in neurological examination should be considered.

8.2.3. Vital Signs

For each participant, vital signs, including blood pressure, pulse rate, and body temperature, measurements will be recorded.

Single measurements will be taken at screening and the baseline visit. Supine triplicate blood pressure and pulse rate will be collected at all other time points. Body temperature to be obtained as single measurement. Pre-dose vital signs should be taken approximately 1 hour prior to the scheduled dosing.

At time points when orthostatic measurements are obtained, participants should be supine for at least 5 minutes and stand for approximately 2 minutes. When triplicate blood pressure or pulse rate measurements precede the orthostatic measurement, the last supine blood pressure or pulse rate measurement will be used for orthostatic calculations. If the participant feels unable to stand, supine vital signs only will be recorded.

Unscheduled orthostatic vital signs should be assessed, if possible, during any AE of dizziness or posture-induced symptoms. Additional vital signs may be measured during each study period if warranted.

8.2.4. Electrocardiograms

For each participant, a 12-lead digital ECG will be collected. Sites are recommended to conduct ECGs prior to any blood sample collection. Participants must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake during ECG collection. Electrocardiograms may be obtained at additional times, when deemed clinically necessary. All ECGs recorded should be stored at the investigational site.

Electrocardiograms will be interpreted by a qualified physician (the investigator or qualified designee) at the site as soon after the time of ECG collection as possible, and ideally while the participant is still present, to determine whether the participant meets entry criteria at the relevant visit(s) and for immediate participant management, should any clinically relevant findings be identified.

If a clinically significant quantitative or qualitative change from baseline is identified after enrolment, the investigator will assess the participant for symptoms (for example, palpitations, near syncope, syncope) to determine whether the participant can continue the study. The investigator or qualified designee is responsible for determining if any changes in participant management is needed and must document his/her review of the ECG printed at the time of evaluation form at least 1 of the replicate ECGs from each point.

The machine-read ECG intervals and heart rate may be used for data analysis and report writing purposes unless a cardiologist overread of the ECGs is conducted prior to completion of the final study report (in which case the overread data would be used).

8.2.5. Clinical Safety Laboratory Assessments

- See Appendix 2 (Section 10.2) for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The investigator must review the laboratory results, document this review, and report any clinically relevant changes occurring during the study as an AE. The laboratory results must be retained with source documents unless a Source Document Agreement or comparable document cites an electronic location that accommodates the expected retention duration. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 16 weeks after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.
 - If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.
 - All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the SoA, standard collection requirements, and laboratory manual.
- If laboratory values from non-protocol specified laboratory assessments performed at an investigator-designated local laboratory require a change in participant management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification), then report the information as an AE.

8.2.5.1. Coagulation

The prothrombin time (PT) w/INR test measures the length of time it takes for a blood clot to form in a sample of blood.

8.2.5.2. Hepatic Monitoring

Close hepatic monitoring

Laboratory tests (Appendix 4, 10.4), including ALT, AST, ALP, TBL, direct bilirubin, gamma glutamyl transferase (GGT), and creatine kinase (CK), should be repeated within 48 to 72 hours to confirm the abnormality and to determine if it is increasing or decreasing, if one or more of these conditions occur:

If a participant with baseline results of ...	develops the following elevations:
ALT or AST <1.5x ULN	ALT or AST ≥ 3 x ULN
ALP <1.5x ULN	ALP ≥ 2 x ULN
TBL <1.5x ULN	TBL ≥ 2 x ULN (except for patients with Gilbert's syndrome)
ALT or AST ≥ 1.5 x ULN	ALT or AST ≥ 2 x baseline
ALP ≥ 1.5 x ULN	ALP ≥ 2 x baseline
TBL ≥ 1.5 x ULN	TBL ≥ 1.5 x baseline (except for patients with Gilbert's syndrome)

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; TBL = total bilirubin; ULN = upper limit of normal.

If the abnormality persists or worsens, clinical and laboratory monitoring, and evaluation for possible causes of abnormal liver tests should be initiated by the investigator in consultation with the Lilly-designated medical monitor. At a minimum, this evaluation should include physical examination and a thorough medical history, including symptoms, recent illnesses (for example, heart failure, systemic infection, hypotension, or seizures), recent travel, history of concomitant medications (including over-the-counter), herbal and dietary supplements, history of alcohol drinking and other substance abuse.

Initially, monitoring of symptoms and hepatic biochemical tests should be done at a frequency of 1 to 3 times weekly, based on the participant's clinical condition and hepatic biochemical tests. Subsequently, the frequency of monitoring may be lowered to once every 1 to 2 weeks, if the participant's clinical condition and lab results stabilize. Monitoring of ALT, AST, ALP, and TBL should continue until levels normalize or return to approximate baseline levels.

Comprehensive hepatic evaluation

A comprehensive evaluation should be performed to search for possible causes of liver injury if one or more of these conditions occur:

If a participant with baseline results of...	develops the following elevations:
ALT or AST <1.5x ULN	ALT or AST ≥ 3 x ULN with hepatic signs/symptoms ^a , or ALT or AST ≥ 5 x ULN
ALP <1.5x ULN	ALP ≥ 3 x ULN
TBL <1.5x ULN	TBL ≥ 2 x ULN (except for patients with Gilbert's syndrome)
ALT or AST ≥ 1.5 x ULN	ALT or AST ≥ 2 x baseline with hepatic signs/symptoms ^a , or ALT or AST ≥ 3 x baseline
ALP ≥ 1.5 x ULN	ALP ≥ 2 x baseline
TBL ≥ 1.5 x ULN	TBL ≥ 2 x baseline (except for patients with Gilbert's syndrome)

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; TBL = total bilirubin; ULN = upper limit of normal.

^a Hepatic signs/symptoms are severe fatigue, nausea, vomiting, right upper quadrant abdominal pain, fever, rash, and/or eosinophilia >5%.

At a minimum, this evaluation should include physical examination and a thorough medical history, as outlined above, as well as tests for Prothrombin Time and International Normalized Ratio (PT-INR); tests for viral hepatitis A, B, C, or E; tests for autoimmune hepatitis; and an abdominal imaging study (for example, ultrasound or CT scan).

Based on the patient's history and initial results, further testing should be considered in consultation with the Lilly-designated medical monitor, including tests for hepatitis D virus (HDV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), acetaminophen levels, acetaminophen protein adducts, urine toxicology screen, Wilson's disease, blood alcohol levels, urinary ethyl glucuronide, and blood phosphatidylethanol. Based on the circumstances and the investigator's assessment of the participant's clinical condition, the investigator should consider referring the participant for a hepatologist or gastroenterologist consultation, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), cardiac echocardiogram, or a liver biopsy.

Additional hepatic data collection (hepatic safety CRF) in study participants who have abnormal liver tests during the study

Additional hepatic safety data collection in hepatic safety CRF should be performed in study participants who meet 1 or more of the following 5 conditions:

1. Elevation of serum ALT to ≥ 5 x ULN on 2 or more consecutive blood tests (if baseline ALT < 1.5 x ULN)
 - a. In participants with baseline ALT ≥ 1.5 x ULN, the threshold is ALT ≥ 3 x baseline on 2 or more consecutive tests
2. Elevated TBL to ≥ 2 x ULN (if baseline TBL < 1.5 x ULN) (except for cases of known Gilbert's syndrome)
 - a. In participants with baseline TBL ≥ 1.5 x ULN, the threshold should be TBL ≥ 2 x baseline
3. Elevation of serum ALP to ≥ 2 x ULN on 2 or more consecutive blood tests (if baseline ALP < 1.5 x ULN)
 - a. In participants with baseline ALP ≥ 1.5 x ULN, the threshold is ALP ≥ 2 x baseline on 2 or more consecutive blood tests
4. Hepatic event considered to be a SAE
5. Discontinuation of study drug due to a hepatic event

Note: the interval between the two consecutive blood tests should be at least 2 days.

8.2.6. Quality of Life

Measurement of Health-Related Quality of Life is a valuable measure of therapeutic efficacy and will utilize the ALSSQOL-SF questionnaire. This assessment will be administered at timepoints displayed in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4).

8.2.6.1. ALSSQOL-SF questionnaire

The ALSSQOL-SF is a 20-item instrument that measures overall quality of life (QOL) and 6 specific domains for individuals with ALS (Felgoise et al. 2011)

8.2.7. Suicidal Ideation and Behavior Risk Monitoring

Suicidal ideation and behavior risk monitoring will be assessed using the C-SSRS, which is a semi-structured clinical interview that assess suicidal ideation severity, suicidal ideation intensity, and suicidal behavior.

The C-SSRS will be assessed at the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4).

8.3. Adverse Events, Serious Adverse Events, and Investigational Product Complaints

The definitions of the following events can be found in Appendix 5:

- AEs
- SAEs
- Investigational Product complaints (IPCs)

These events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet these definitions and remain responsible for following up events that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study (see Section 7).

Care will be taken not to introduce bias when detecting events. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about event occurrences.

After the initial report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AEs of special interest (as defined in Section 8.3.2) will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). For investigational product complaints, the investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality. Further information on follow-up procedures is provided in Appendix 5, Section 10.5.

Adverse events will be assessed by the investigator using a 3-grade scale outlined below as suggested in ICH E3.

- Mild
- Moderate
- Severe

Further information is provided in Appendix 5, Section 10.5.4 (Recording and Follow-Up of AE and/or SAE and Investigational Product Complaints).

8.3.1. Timing and Mechanism for Collecting Events

This table describes the timing, deadlines, and mechanism for collecting events.

Event	Collection Start	Collection Stop	Timing for Reporting to Sponsor or Designee	Primary Mechanism for Reporting	Back-Up Method of Reporting
Adverse Event					
AE	Signing of the informed consent form (ICF)	The final follow-up visit	As soon as possible upon site awareness	AE eCRF	N/A
Serious Adverse Event and Adverse Events of Special Interest					
SAE and SAE updates/AESI – prior to start of study intervention and deemed reasonably possibly related with study procedures	Signing of the ICF	The final follow-up visit	Within 24 hours of awareness	SAE paper form or AESI form	SAE paper form
SAE ^a and SAE updates/AESI – after start of study intervention	Start of intervention	Participation in study has ended	Within 24 hours of awareness	SAE paper form or AESI form	SAE paper form
SAE ^a /AESI – after participant's study participation has ended and the investigator becomes aware	After participant's study participation has ended	N/A	Promptly	SAE paper form or AESI form	N/A
Pregnancy					
Pregnancy in female participants and female partners of male participants	After the start of study intervention	6 months after last dose	Within 24 hours of learning of the pregnancy	Pregnancy paper form	SAE paper form

Event	Collection Start	Collection Stop	Timing for Reporting to Sponsor or Designee	Primary Mechanism for Reporting	Back-Up Method of Reporting
Investigational Product Complaints					
PC associated with an SAE or might have led to an SAE	Start of study intervention	End of study intervention	Within 24 hours of awareness	Investigational Product Complaint form	N/A
PC not associated with an SAE	Start of study intervention	End of study intervention	Within 1 business day of awareness	Investigational Product Complaint form	N/A
Updated PC information	—	—	As soon as possible upon site awareness	Originally completed Investigational Product Complaint form with all changes signed and dated by the investigator	N/A
PC (if investigator becomes aware)	Participation in study has ended	N/A	Promptly	Investigational Product Complaint form	

Abbreviations: AE = adverse event; AESI = adverse event of special interest; eCRF = electronic case report form; ICF = informed consent document; PC = product complaint; SAE = serious adverse event.

^a Serious adverse events, including death, caused by disease progression should not be reported unless the investigator deems them to be possibly related to study treatment.

8.3.2. Adverse Events of Special Interest

Adverse Events of Special Interest (AESI) are to be reported by the site within 24 hours of awareness. The primary mechanism for reporting an AESI will be the paper AESI form.

8.3.2.1. Hypersensitivity Reactions

Hypersensitivity reaction may occur after administration of any monoclonal antibody.

Immediate/acute hypersensitivity reactions, including infusion reactions or anaphylaxis, may occur during or within hours of the infusion. Sites should have appropriately trained medical staff and appropriate medical equipment available when study participants are receiving study drug. It is recommended that participants who experience a systemic hypersensitivity reaction be treated per national and international guidelines.

In the case of generalized urticaria or anaphylaxis, additional blood samples should be collected as described in Appendix 3, Section 10.3, “Recommended Laboratory Testing for Hypersensitivity Events”. Laboratory results are provided to the sponsor via the central laboratory.

Subjects should be monitored for hypotension, fever, chills, bronchospasm, angioedema, and other symptoms or signs of anaphylaxis. Infusion should be slowed or stopped, and reactions managed as indicated in study protocols. If such a reaction occurs, additional data describing each symptom should be provided to the sponsor in the eCRF.

Delayed hypersensitivity reaction can develop days or weeks after infusion. Subjects should be monitored for fever, rash, arthralgia, myalgia, hematuria, changes in hematology, liver or renal tests. Delayed hypersensitivity reactions can be managed symptomatically and treated with corticosteroids.

Subjects should not receive additional doses of AP-101 for any confirmed/suspected case of delayed hypersensitivity reaction to AP-101.

Hypersensitivity reactions, as well as other topics which may be subsequently determined by the sponsor, will be closely monitored as an AESI. Additionally, AESIs will be analyzed for presentation in the clinical study report (CSR) in accordance with the statistical analysis plan (SAP).

8.3.3. Lumbar Puncture

Lumbar puncture (LP) to obtain CSF will be performed at several timepoints during this study. Headache can occur in up to 60% of patients who undergo LP, and backache can occur in up to 40% of patients.

Serious adverse events are rare and include cerebral herniation, intracranial subdural hemorrhage, spinal epidural hemorrhage, and infection.

Lumbar puncture adverse events will be closely monitored and documented during the study..

8.4. Treatment of Overdose

For this study, any dose of AP-101 greater than 2500 mg of AP-101 within a 24-hour time period ($\pm$ 4 hours) will be considered an overdose. The planned dosing on days 1 and 2 will not be considered an overdose.

An overdose is not anticipated, as the study drug will be administered by a trained staff member.

In the event of an overdose, the investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for any AE/SAE and laboratory abnormalities until AP-101 can no longer be detected systemically (at least 100 days). Refer to Section 8.3 for reporting details.

3. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

8.5. Pharmacokinetics

At the visits and times specified in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4), venous samples will be collected to determine the serum concentrations of AP-101. CSF sampling will also be collected at baseline and at defined timepoints during the study, according to the SoA. The sampling times may be modified at the discretion of the sponsor, based on review of interim PK data as they become available. Actual PK sample collection dates and times will be recorded.

8.5.1. Bioanalysis

Samples will be analyzed at a laboratory approved by the sponsor and stored at a facility designated by the sponsor.

Concentrations of AP-101 will be assayed from serum and CSF using a validated immunoassay.

Bioanalytical samples collected to measure study drug concentrations will be retained for a maximum of 1 year following last participant visit for the study. [Table 8.1](#) presents a sampling summary for this study.

8.6. Pharmacodynamics

Pharmacodynamic assessments in this study include changes in total SOD1, mSOD1, pNfH, and NfL as well as additional exploratory assessments.

Levels of mSOD1 will be measured retrospectively from the CSF of participants at baseline and after at least 1 timepoint after treatment with AP-101. This is an exploratory endpoint that will be used to investigate the relationship between levels of mSOD1 and response as well as whether treatment with AP-101 can reduce the levels of detectable mSOD1 in participants.

8.6.1. SOD1 and mSOD1 Assays

Levels of total SOD1 and misfolded SOD1 will be measured retrospectively from the CSF of participants at baseline and after at least 1 timepoint after treatment with AP-101. This is an exploratory endpoint that will be used to investigate whether there are correlations with levels of SOD1 with response as well as whether treatment with AP-101 can reduce the levels of detectable SOD1 in participants. Correlations with other exploratory biomarkers and endpoints will also be performed.

8.6.2. Neurofilament Assays

Levels of pNfH and NfL will be measured from both CSF and serum at various timepoints during the 6-month study. This endpoint will be used to determine the effects of AP-101 on pNfH and NfL levels. pNfH and NfL will also be examined for correlations with other exploratory biomarkers and endpoints.

8.7. Genetics

Mandatory genetic testing for all participants to identify common variants associated with ALS will be conducted by a certified genetics laboratory.

A blood sample for DNA isolation will be collected from participants.

See Appendix 7, Section 10.7 for Information regarding genetic research and details about sample retention and custody

8.8. Biomarkers

Additional biomarkers will include transcriptional and microRNA profiles from biological fluids, CSF, and serum. These are exploratory biological markers of disease activity and will be examined at various timepoints during the study as displayed in the double-blind phase SoA (Section 1.3) or the OLE SoA (Section 1.4).

- This study will analyze biomarkers relevant to the mechanism of action for AP-101. Samples collected for these analyses may also be used to develop related research methods or to validate diagnostic tools or assays.
- Additional samples may be collected during the study if warranted and agreed upon by the investigator and sponsor.

8.9. Immunogenicity Assessments

Antibodies to AP-101 will be evaluated in serum samples collected from all participants according to the SoA. Additionally, serum samples should also be collected at the final visit from participants who discontinued study intervention or were withdrawn from the study. In the event of a systemic hypersensitivity reaction, unscheduled blood samples will be collected for additional immunogenicity analysis. These additional samples will be collected as per Appendix 3, Section 10.3, “Recommended Laboratory Testing for Hypersensitivity Events”. These samples will be tested by the sponsor or sponsor's designee.

Serum samples will be screened for antibodies binding to AP-101 and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to AP-101 and/or further characterize the immunogenicity of AP-101.

The detection and characterization of antibodies to AP-101 will be performed using a validated assay method by or under the supervision of the sponsor. All samples collected for detection of antibodies to study intervention will also be evaluated for AP-101 serum concentration to enable interpretation of the antibody data. Antibodies may be further characterized and/or evaluated for their ability to neutralize the activity of the study intervention(s). Samples may be stored for a maximum of 2 years (or according to local regulations) following the last participant's last visit for the study at a facility selected by the sponsor to enable further analysis of immune responses to AP-101.

8.10. Health Economics

Not applicable.

9. Statistical Considerations

9.1. Statistical Hypotheses

Let θ represent the average change in serum NfL from baseline to the 21-day follow-up visit in the Double-Blind portion of the study as [21-day follow-up] - [baseline]. Further, denote θ_{placebo} and $\theta_{\text{AP-101}}$ to denote θ when subset to the placebo and AP-101 treatment arms, respectively. Then, the following hypothesis will be tested: _

$$H_0: \theta_{\text{AP-101}} - \theta_{\text{placebo}} \geq 0$$

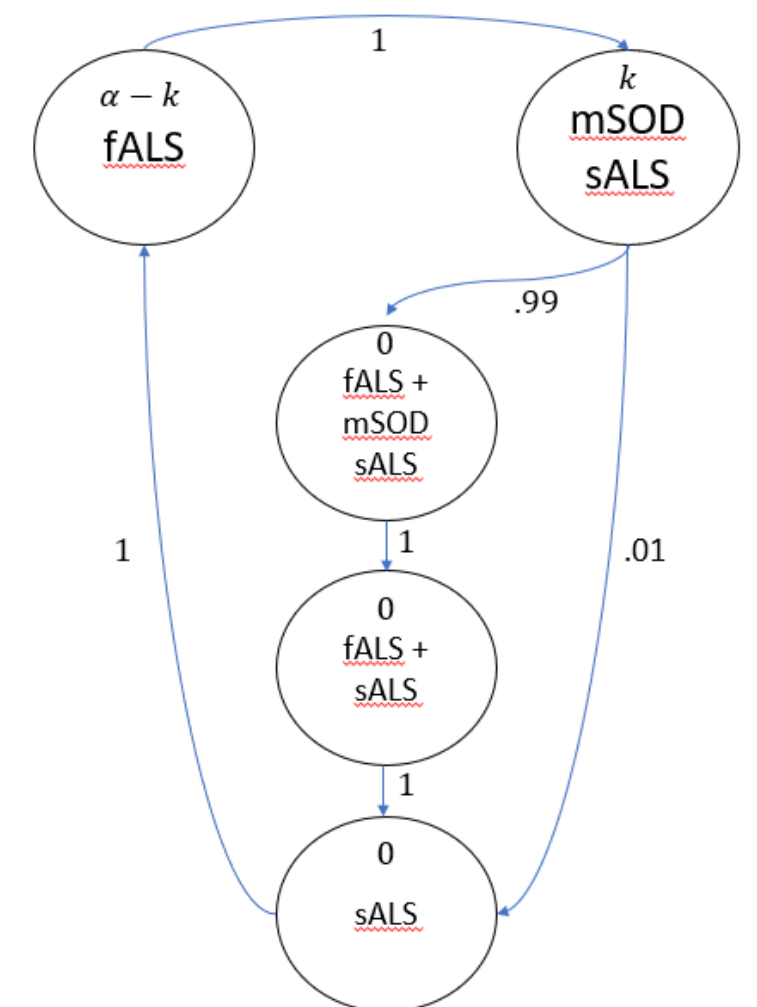
$$H_1: \theta_{\text{AP-101}} - \theta_{\text{placebo}} < 0$$

In other words, the null hypothesis is that the reduction in NfL is the same or greater in the placebo group than the AP-101 group. This hypothesis will be tested in the following subgroups using a graphical α -spending approach:

- fALS cohort.
- sALS cohort in participants who exhibit positive (e.g., detectable) mSOD1 (referred to as the mSOD1 cohort)
- fALS and mSOD1 cohort.
- fALS and sALS cohorts (e.g., all participants).
- sALS cohort.

Otherwise, descriptive statistics will be used for statistical evaluation of efficacy, safety, and tolerability.

The graphical approach to control the family-wise type I error at $\alpha = 0.025$ (one sided) will be described fully in the SAP (e.g., initial distribution of α along with any adjustments for correlated hypotheses) and follow the below figure:



Overall $\alpha=.025$ one sided $k \in [0, .025]$

9.2. Sample Size Determination

Approximately 63 participants (21 with fALS and $42 \pm 20\%$ with sALS) will be randomly assigned in a 2:1 randomization ratio to study intervention or placebo such that approximately 18 evaluable participants with fALS and 36 evaluable participants with sALS will complete the study. This sample size is based on clinical considerations for assessing the primary endpoint of safety. With this sample size, power analyses for the hypothesis testing of NfL changes show >90% power for the fALS group if the NfL effect of AP-101 is similar to that of tofersen (Miller et al. 2022).

9.3. Populations for Analyses

The following populations are defined for the double-blind phase of the study:

Population	Description
Intent to Treat (ITT)	All participants assigned to treatment, regardless of whether they take any doses of study treatment, or if they took the

	correct treatment. Participants will be analyzed according to the treatment group to which they were assigned.
Per-Protocol (PP)	All participants in the safety population who have completed the study, defined as completing Week 24 dosing and the 21 -day follow-up visit. Participants and visits may be excluded due to major protocol violations. Participants will be analyzed according to the intervention which they actually received.
Safety	All participants randomly assigned to study intervention and who take at least 1 dose of study intervention. Participants will be analyzed according to the intervention which they actually received.

All PK and PD (including neurofilament) analyses will be on performed on the safety population.

The following populations are defined for the OLE:

Population	Description
OLE Population	All participants who complete the double-blind phase of the study and receive at least one dose in the OLE period of the study. For safety analyses, participants will be analyzed as a single comprehensive group. For all other analyses, participants will be analyzed according to the intervention they were assigned to within the double-blind phase.

9.4. Statistical Analyses (Double-blind Phase)

Statistical analysis of this study will be the responsibility of sponsor or its designee.

All CIs will be given at a 2-sided 90% level.

Any change to the data analysis methods described in the protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol, and the justification for making the change, will be described in the statistical analysis plan and the clinical study report. Additional exploratory analyses of the data will be conducted as deemed appropriate.

The statistical analysis plan will be finalized prior to unblinding and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.4.1. General Considerations

Statistical analysis of this study will be the responsibility of the sponsor or its designee. Statistical analyses will be detailed in the statistical analysis plan.

Data for sALS participants and fALS participants will be analyzed separately. Interim analyses will be performed once all sALS patients and all fALS patients have completed the 6-month double-blind phase of the study. These interim analyses are expected to occur approximately 6 months apart. A final analysis will occur when all enrolled patients have completed the OLE. The interim analyses are being performed to allow the Sponsor to plan for the next phase of development and to allow for engagement with regulators. The interim analyses will be performed under a strict Unblinding Plan to minimize impact on the integrity of the ongoing study.

Safety analyses will be conducted for all dosed participants, whether or not they completed all protocol requirements as per the Safety Population.

For continuous variables, summary statistics will include number of participants, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized using number of participants, frequency, and percentages.

A detailed description of participant disposition will be provided at the end of the study. All participants who discontinued from the study will be identified, and the extent of their participation in the study will be summarized. If known, a reason for their discontinuation will be provided.

Participant demographics and baseline characteristics (age, gender, race, ethnicity, height, weight, and body mass index [BMI]) will be summarized by treatment. Summaries for safety and will also be provided.

9.4.2. Primary Endpoint(s)

The primary endpoints in this study are the incidence of AEs and SAEs, including immunogenicity, and the incidence of abnormalities in vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms, and changes in weight.

All treatment and protocol procedure AEs will be listed. Summary statistics for AEs and SAEs will be provided by treatment arm and sorted by system organ class and preferred term. Summaries will also be provided by relationship to study drug and maximum severity. AEs leading to discontinuation of study drug or of the study will be summarized. AEs will be summarized for the Safety Population. Abnormal vital signs, clinical laboratory assessments, physical and neurological examinations, electrocardiograms, and changes in weight will be provided as listings. The number of participants experiencing abnormalities and the number of abnormalities will be summarized using the Safety Population.

9.4.3. Secondary Endpoints

9.4.3.1. AP-101 Pharmacokinetics in Serum

The PK analyses will only be performed for participants who received at least 1 full dose of study treatment and evaluable concentrations, and will be performed on the Safety Population. All serum PK samples will be utilized in a compartmental model to describe the PK for these patients.

PK parameters will be reported with descriptive statistics which include: arithmetic mean, geometric mean, standard deviation, coefficient of variation (CV)%, geometric CV%, median, minimum, and maximum (Table 9.1).

Table 9.1. Pharmacokinetic Parameters

Multiple-dose PK parameters	Definition
$C_{at\ EOI}$	concentration at the end of infusion
$T_{at\ EOI}$	time to concentration at the end of infusion
$AUC_{0-tlast}$	area under the plasma concentration-time curve from time 0 to the last quantifiable concentration
V	central volume of distribution
CL	total body clearance
Q	intercompartmental clearance
V_2	peripheral compartment volume
$t_{1/2}$	elimination half-life

9.4.3.2. Pharmacokinetics in CSF

During the 6-month treatment period, the level of AP-101 in the CSF will be summarized by time point using the safety population.

9.4.3.3. Pharmacodynamics: pNfH and NfL Levels in CSF and Plasma

Both pNfH and NfL will be summarized by type (either CSF or plasma) for each available treatment visit as well as the change from baseline at each visit for both the ITT and per-protocol (PP) populations.

The average reduction at the 21-day follow-up visit in serum NfL, along with the estimated difference between treatment arms, will be calculated for various subgroups as described in Section 9.1.

Plots showing the baseline levels of pNfH and NfL and their average change per month will be provided.

9.4.4. Exploratory Endpoints

All exploratory endpoints will be summarized by treatment using the ITT population. A more in-depth description of all endpoint summaries will be provided in the SAP.

9.4.4.1. Clinical Effect of AP-101 on the Rate of Functional Decline as Measured by the ALSFRS-R

Total ALSFRS-R scores and change from baseline will be summarized at visits according to the SoA.

9.4.4.2. Clinical Effect of AP-101 on Lung Function (SVC) or Equivalent Alternative, Muscle Strength (HHD), and Disease Staging (King's Staging Tool)

These will be summarized by visit and by change from baseline. Change from baseline in the King's staging tool will be summarized as the number of stages decreased since baseline.

9.4.4.3. The Combined Assessment of Function and Survival (CAFS)

The mean rank score for each treatment group will be summarized, using the last known survival time for the survival portion, and the 21-day follow-up visit (after the Week 24 visit) for the change in ALSFRS-R portion. Confidence intervals for each treatment's mean rank will be provided.

9.4.4.4. Composite Time to Event Measurement Comprising Time to Death or Permanent Ventilator Use

A Kaplan-Meier analysis will be performed comparing the treatment group to the placebo group. The 25th, 50th (median), and 75th quartiles will be presented along with their 90% confidence intervals, as well as a plot of survival curves. The individual components of the composite endpoint will be similarly individually analyzed.

9.4.4.5. Quality of Life by ALSSQOL-SF

The ALSSQOL-SF will be summarized by treatment visit.

9.4.4.6. Total SOD1 and Misfolded SOD1 in the CSF after Multiple IV Doses of AP-101

Total and misfolded SOD1 from the CSF will be summarized at each available visit as well as the change from baseline. Two-sided 90% confidence intervals will be included in the summaries.

Plots showing the baseline levels of SOD1 and mSOD1 and their change over time will be provided.

9.4.4.7. Other Biological Markers of Disease Activity Including Creatinine and Transcriptional Markers

All other biological markers of disease activity will be summarized at each available visit as well as the change from baseline. Two-sided 90% confidence intervals will be provided.

9.4.5. Other Safety Analyses

All safety analyses will be made on the Safety Population and provided as listings.

9.4.6. Other Analyses

Subgroup analyses pooling fALS and sALS participants may be performed for various endpoints, including neurofilaments (NfL and pNfH) and measures of mSOD1 and SOD1. This will be fully detailed in the SAP.

Additional exploratory subgroup analyses may be performed.

9.5. Interim Analyses

An interim safety review will occur after 10 participants have been randomized to AP-101, received the loading dose and at least one dose of 2500 mg, and been followed for at least 30 days following this initial treatment. It is anticipated that most participants will have at least received their 2nd treatment infusion 3 weeks after their initial dose.

Once all sALS participants have completed the 6-month double-blind period, an interim analysis will be performed on sALS participant data, including hypothesis testing as appropriate. The fALS participants will continue in the study and an additional database lock and analysis will be performed once all fALS patients complete the 6-month double-blind treatment period.

If any unplanned interim analysis is deemed necessary for reasons other than a safety concern, the protocol must be amended.

9.6. Safety Review Committee

For details on the SRC, refer to the Safety Review Committee charter. The Safety Review Committee will remain blinded throughout the study. For the planned interim safety review, SRC may be unblinded to study treatment.

9.7. Statistical Analysis (Open Label Extension)

Similar analyses and reporting conventions as described in the sections above for the double-blind phase will be performed for the OLE using the OLE population (including summaries of safety, efficacy, and PK/PD parameters as per assessments included the SoA (Section 1.4). For some summaries, confidence intervals may be produced, but no formal comparisons or p-values will be performed for the OLE portion of the study. Unless otherwise specified in the statistical analysis plan, changes from baseline will not be reported.

Safety measures, including adverse events and laboratory parameters, will be summarized for the OLE only and will not repeat information from the double-blind phase of the study.

For statistical purposes, the OLE Treatment Baseline will be defined as the visit where the first dose of AP-101 is given during the OLE portion of the study (Day 1 or Day 22, depending on randomization assignment in the double-blind phase). This baseline will be used for determining which adverse events are treatment-emergent in the OLE portion of the study and any changes from baseline.

Efficacy, PK, and PD measures may also include data from the double-blind phase of the study.

The sponsor may decide to analyze sALS and fALS patients who complete the OLE separately. This will be decided prior to database lock for the OLE and the SAP will be updated accordingly.

A full description of all summaries, listings, and analyses for the OLE will be provided in the OLE SAP.

10. Supporting Documentation and Operational Considerations

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. 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 (CIOMS) International Ethical Guidelines
 - Applicable International Council for Harmonization (ICH) GCP Guidelines
 - Applicable laws and regulations
- The protocol, protocol amendments and addenda, ICF, Investigator's Brochure, and other relevant documents (for example, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments or addenda to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
 - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
 - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
 - Providing oversight of study conduct for participants under their responsibility and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations
- Investigator sites are compensated for participation in the study as detailed in the clinical trial agreement.

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study, including the risks and benefits, to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative and is kept on file.

A participant who is rescreened is not required to sign another ICF if the rescreening occurs within 7 days from the previous ICF signature date.

10.1.4. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records, datasets or tissue samples that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant 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 participant who will be required to give consent for his/her data to be used as described in the informed consent.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The sponsor has processes in place to ensure data protection, information security and data integrity. These processes include appropriate contingency plan(s) for appropriate and timely response in the event of a data security breach.

10.1.5. Dissemination of Clinical Study Data Reports

The sponsor will disclose a summary of study information, including tabular study results, on publicly available websites where required by local law or regulation.

10.1.6. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or eCRF unless transmitted to the sponsor or designee electronically (for example, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- 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/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (for example, risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organizations).
- 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 participants 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 for the time period outlined in the Clinical Trial Agreement (CTA) 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.
- In addition, sponsor or its representatives will periodically check a sample of the participant data recorded against source documents at the study site. The study may be audited by sponsor or its representatives, and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

Data Capture System

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor.

An EDC will be used in this study for the collection of eCRF data. The investigator maintains a separate source for the data entered by the investigator or designee into the sponsor-provided

EDC system. The investigator is responsible for the identification of any data to be considered source and for the confirmation that data reported are accurate and complete by signing the eCRF.

Data collected via the sponsor-provided data capture system(s) will be stored at a third-party vendor. The investigator will have continuous access to the data during the study and until decommissioning of the data capture system(s). Prior to decommissioning, the investigator will receive an archival copy of pertinent data for retention.

Data managed by a central vendor, such as laboratory test data, will be stored electronically in the central vendor's database system and electronic transfers will be provided to the investigator for review and retention. Data will subsequently be transferred from the central vendor to the sponsor data warehouse.

10.1.7. Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- The investigator will keep records of all original source data. This might include laboratory tests, medical records, and clinical notes. If requested, the investigator will provide the sponsor, applicable regulatory agencies, and applicable ERBs with direct access to the original source documents.

10.1.8. Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants.

The sponsor designee 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. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

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 IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and assures appropriate participant therapy and/or follow-up.

10.1.9. Publication Policy

In accordance with the sponsor's publication policy, the results of this study will be submitted for publication by a peer-reviewed journal.

10.1.10. Investigator Information

Physicians with a specialty in neurodegenerative disease, and specifically ALS, will participate as investigators in this clinical trial.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed below will be performed by the central laboratory
- Local laboratory results are only required in the event that the central laboratory results are not available in time for either study intervention administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either a study intervention decision or response evaluation, the results must be entered into the eCRF.
- In circumstances where the sponsor approves local laboratory testing in lieu of central laboratory testing (in the table below), the local laboratory must be qualified in accordance with applicable local regulations.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.
- Pregnancy Testing as described in the inclusion criteria (Section 5.1)

Investigators must document their review of the laboratory safety results.

Laboratory results that could unblind the study will not be reported to investigative sites or other blinded personnel.

Safety Clinical Laboratory Tests	
Hematology	Clinical Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count (RBC)	Bicarbonate
Mean cell volume	Chloride
Mean cell hemoglobin	Calcium
Mean cell hemoglobin concentration	Phosphate, inorganic
Leukocytes (WBC)	Glucose [random]
Platelets	Blood urea nitrogen (BUN)
Differential WBC [Absolute counts and/or %] of:	
Neutrophils	Total cholesterol
Lymphocytes	Total protein
Monocytes	Albumin
Eosinophils	Total bilirubin
Basophils	Alkaline phosphatase (ALP)
	Aspartate aminotransferase (AST)
	Alanine aminotransferase (ALT)
	Creatinine
Urinalysis	
Specific gravity	
pH	
Protein	
Glucose	
Ketones	Ethanol testing ^{a,b}
Bilirubin	Urine drug screen ^{a,b}
Urobilinogen	Hepatitis B surface antigen ^a
Blood	Hepatitis C antibody ^a
Nitrite	HIV ^a
	Prothrombin time/ activated partial thromboplastin time (aPTT)
	Pregnancy test [if applicable]
	FSH [if applicable] ^a
	Thyroid stimulating hormone [optional]

Abbreviations: FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; RBC = red blood cells; WBC = white blood cells.

^a Performed at screening only

^b Urine drug screen and ethanol level may be repeated prior to admission to the clinical research unit and at other times indicated in the SoA.

10.3. Appendix 3: Recommended Laboratory Testing for Hypersensitivity Events

Lab testing should be performed at the time of a Systemic Hypersensitivity Event. The management of the adverse event may warrant lab testing beyond that described below and should be performed as clinically indicated.

Laboratory testing during a Systemic Hypersensitivity Event is not performed for diagnostic purposes. Its intent is several fold:

- To help characterize and classify systemic hypersensitivity reactions
- To meet regulatory expectations
- To improve subsequent clinical management by helping to distinguish between the various mechanistic bases of anaphylaxis

Labs should be obtained in the presence of generalized urticaria or if anaphylaxis is suspected. After the subject has been stabilized, obtain a sample within 1-2 hours of the event; however, samples may be obtained as late as 12 hours after the event as analytes can remain altered for an extended period of time. Record the time at which the sample was collected. At the next regularly scheduled visit or after 4 weeks obtain a follow-up sample.

Clinical tests should include the following*

- Tryptase**
- ADA and AP-101 concentration (PK)
- Complement
 - C3, C3a and C5a
- Cytokines
 - IL-6, IL-1 β , IL-10 (or any cytokine panel that includes these 3 cytokines)

* these labs are bundled in the Clinical Laboratory Operations Hypersensitivity Lab Testing Kit

** If a tryptase sample is obtained more than 2 hours after the event (i.e., within 2-12 hours), or is not obtained because more than 12 hours have elapsed since the event, obtain urine for *N*-methylhistamine (NMH) testing. Note that for tryptase serum samples obtained within 2-12 hours of the event, urine NMH testing is performed in addition to tryptase testing. Collect the first void urine following the event. Obtain a follow-up urine for NMH testing at the next regularly scheduled visit or after 4 weeks, whichever is later.

10.4. Appendix 4: Hepatic Monitoring Tests for Treatment-Emergent Abnormality

Selected tests may be obtained in the event of a treatment-emergent hepatic abnormality and may be required in follow-up with subjects in consultation with Lilly or its designee clinical research physician.

Hepatic Monitoring Tests

Hepatic Hematology^a	Haptoglobin^a
Hemoglobin	
Hematocrit	Hepatic Coagulation^a
RBC	Prothrombin time
WBC	Prothrombin time, INR
Neutrophils	
Lymphocytes	Hepatic Serologies^{a,b}
Monocytes	Hepatitis A antibody, total
Eosinophils	Hepatitis A antibody, IgM
Basophils	Hepatitis B surface antigen
Platelets	Hepatitis B surface antibody
	Hepatitis B core antibody
Hepatic Chemistry^a	Hepatitis C antibody
Total bilirubin	Hepatitis E antibody, IgG
Conjugated bilirubin	Hepatitis E antibody, IgM
Alkaline phosphatase	
ALT	Anti-nuclear Antibody^a
AST	Alkaline Phosphatase Isoenzymes^a
GGT	Anti-smooth Muscle Antibody (or Anti-actin
CPK	Antibody)^a

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatinine phosphokinase; GGT = gamma-glutamyl transferase; Ig = immunoglobulin; INR = international normalized ratio; RBC = red blood cell; WBC = white blood cell.

^a Assayed by Lilly-designated or local laboratory.

^b Reflex/confirmation dependent on regulatory requirements and/or testing availability.

10.5. Appendix 5: Adverse Events:

Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.5.1. Definition of AE

AE Definition
An AE is any untoward medical occurrence in a participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Events Meeting the AE Definition
- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (for example, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (that is, not related to progression of underlying disease). - Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. - New conditions detected or diagnosed after study intervention administration even though they may have been present before the start of the study. - Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. - Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdose should be reported regardless of sequelae. “Lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events <u>NOT</u> Meeting the AE Definition
Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant’s condition.

- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (for example, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.5.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (for example, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

SAE is defined as any untoward medical occurrence that, at any dose:	
a. Results in death	
b. Is life-threatening	The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization	• In general, hospitalization signifies that the participant has been admitted to hospital for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity	• The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (for example, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.5.3. Definition of Investigational Product Complaints**Investigational Product Complaint**

- An investigational product complaint is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness or performance of a study intervention. When the ability to use the study intervention safely is impacted, the following are also investigational product complaints:
 - Deficiencies in labeling information, and
 - Use errors for device or drug-device combination investigational products due to ergonomic design elements of the investigational product.
- Investigational product complaints related to study interventions used in clinical trials are collected in order to ensure the safety of participants, monitor quality, and to facilitate process and investigational product improvements.
- Investigators will instruct participants to contact the site as soon as possible if he or she has an investigational product complaint or problem with the study intervention so that the situation can be assessed.
- An event may meet the definition of both an investigational product complaint and an AE/SAE. In such cases, it should be reported as both an investigational product complaint and as an AE/SAE.

10.5.4. Recording and Follow-Up of AE and/or SAE and Investigational Product Complaints

AE, SAE, and Product Complaint Recording
- When an AE/SAE/product complaint occurs, it is the responsibility of the investigator to review all documentation (for example, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. - The investigator will then record all relevant AE/SAE/product complaint information in the participant's medical records, in accordance with the investigator's normal clinical practice. AE/SAE information is reported on the appropriate eCRF page and product complaint information is reported on the Product Complaint Form. Note: An event may meet the definition of both a product complaint and an AE/SAE. In such cases, it should be reported as both a product complaint and as an AE/SAE. - It is not acceptable for the investigator to send photocopies of the participant's medical records to sponsor or designee in lieu of completion of the eCRF page for AE/SAE and the Product Complaint Form for product complaints. - There may be instances when copies of medical records for certain cases are requested by sponsor or designee. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to sponsor or designee. - The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories: - Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living. - Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant. - Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A “reasonable possibility” of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the IB in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to sponsor or designee. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to sponsor or designee.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-Up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by sponsor or designee to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

10.5.5. Reporting of SAEs

SAE Reporting Mechanism
• The primary mechanism for reporting an SAE will be paper SAE form data collection tool. • The paper SAE data collection tool should be sent to the sponsor within 24 hours. • Contacts for SAE reporting can be found at the top of the SAE form as follows: pharmacovigilance@simbecorioncro.com or fax +441753 695 101 (Back-up fax: +44 1753 695 124) • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.

10.5.6. Regulatory Reporting Requirements

SAE Regulatory Reporting
• Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities toward the safety of participants and the safety of a study intervention under clinical investigation are met. • The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators. • An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

10.6. Appendix 6: Contraceptive Guidance and Collection of Pregnancy Information**Definitions:****Woman of Childbearing Potential (WOCBP)**

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (for example, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

1. Premenarchal
2. Premenopausal female with 1 of the following:
 - Documented hysterectomy
 - Documented bilateral salpingectomy
 - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (for example, mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

Note: Determination can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

1. Postmenopausal female is defined as women with:
 - 12 months of amenorrhea for women >55, with no need for FSH
 - 12 months of amenorrhea for women >40 years old with FSH ≥ 40 mIU/mL and no other medical condition such as anorexia nervosa and not taking medications during the amenorrhea (e.g., oral contraceptives, hormones, gonadotropin releasing hormone, anti-estrogens, SERMs, or chemotherapy that induced amenorrhea).

Contraception Guidance:

Women of childbearing potential are permitted to enroll in this study if they are and agree to remain completely abstinent as their preferred and usual lifestyle, or exclusively in same sex relationships.

They must not:

- use periodic abstinence methods
 - calendar
 - ovulation
 - symptothermal, or
 - post-ovulation
- declare abstinence just for the duration of a trial, or
- use the withdrawal method

Women of childbearing potential who are NOT completely abstinent as their preferred and usual lifestyle, or exclusive in same sex relationships must agree to use 2 forms of effective contraception, where at least 1 form must be highly effective (less than 1% failure rate) throughout the trial and for 6 months after their last dose of AP-101.

Female participants should refrain from egg donation for the duration of the study and for 6 months following the final administered dose of AP-101.

Examples of different forms of contraception are as follows:

Methods	Examples
Highly effective contraception	combination oral contraceptive pill and mini-pill implanted contraceptives injectable contraceptives contraceptive patch (only women <198 pounds or 90 kg) total abstinence vasectomy (if only sexual partner) fallopian tube implants (if confirmed by hysterosalpingogram) combined contraceptive vaginal ring, or intrauterine devices
Effective contraception	• male or female condoms with spermicide • diaphragms with spermicide or cervical sponges • barrier method with use of a spermicide ○ condom with spermicide ○ diaphragm with spermicide, or ○ female condom with spermicide Note: The barrier method must include use of a spermicide (i.e., condom with spermicide, diaphragm with spermicide, female condom with spermicide) to be considered effective.

Ineffective forms of contraception	• spermicide alone • immunocontraceptives • periodic abstinence • fertility awareness (calendar method, temperature method, combination of above 2, cervical mucus, symptothermal) • withdrawal, • post coital douche • lactational amenorrhea
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Male participants should refrain from sperm donation for the duration of the study and for 6 months following the last dose received.

Men, regardless of their fertility status, with partners who are non-pregnant WOCBP, must agree to either

- remain abstinent (if this is their preferred and usual lifestyle), or
- use condoms during intercourse for the duration of the study, and
- for 6 months after their last dose of AP-101

Contraception for men in exclusively same sex relationships, as their preferred and usual lifestyle, are not required to use contraception.

Collection of Pregnancy Information

Male Participants with Partners Who Become Pregnant

- The investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive AP-101.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Participants Who Become Pregnant

- The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of the gestational age, fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- A spontaneous abortion (occurring at <20 weeks gestational age) or still birth (occurring at >20 weeks gestational age) is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.1. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will be withdrawn from the study. If the participant is discontinued from the study intervention, follow the standard discontinuation process and continue directly to the follow-up phase.

10.7. Appendix 7: Genetics**Use/Analysis of DNA**

- Genetic variation may impact a participant's response to study intervention, susceptibility to, and severity and progression of disease. Variable response to study intervention may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and IRB/IEC allow, a blood sample will be collected for DNA analysis from consenting participants.
- DNA samples will be used for research related to AP-101 or ALS and related diseases. They may also be used to develop tests/assays including diagnostic tests related to ALS. Genetic research may consist of the analysis of one or more candidate genes (as appropriate).

- DNA samples will be analyzed for known genetic mutations associated with ALS. Additional analyses may be conducted if it is hypothesized that this may help further understand the clinical data.
- The samples may be analyzed as part of a multi-study assessment of genetic factors involved in the response to AP-101.
- The results of genetic analyses may be reported in the clinical study report (CSR) or in a separate study summary.
- The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained while research on AP-101 continues but no longer than 10 years or other period as per local requirements.

10.8. Appendix 8: Provisions for Changes in Study Conduct During Exceptional Circumstances

Implementation of this Appendix

The changes to procedures described in this appendix are temporary measures intended to be used only during specific time periods as directed by the sponsor in partnership with the investigator.

Exceptional Circumstances

Exceptional circumstances are rare events that may cause disruptions to the conduct of the study. Examples include pandemics or natural disasters. These disruptions may limit the ability of the investigators, participants, or both to attend on-site visits or to conduct planned study procedures.

Implementing Changes under Exceptional Circumstances

In an exceptional circumstance, after receiving the sponsor's written approval, sites may implement changes if permitted by local regulations.

Ethics Review Boards (ERBs), regulatory bodies and any other relevant local authorities, as required, will be notified as early as possible to communicate implementation of changes in study conduct due to exceptional circumstances. To protect the safety of study participants, urgent changes may be implemented before such communications are made, but all changes will be reported as soon as possible following implementation. If approval of ERBs, regulatory bodies, or both is required per local regulations, confirmation of this approval will be retained in the study records.

In the event written approval is granted by the sponsor for changes in study conduct, additional written guidance, if needed, will be provided by the sponsor.

Considerations for Making a Change

The prevailing consideration for making a change is ensuring the safety of study participants. Additional important considerations for making a change are compliance with Good Clinical Practice, enabling participants to continue safely in the study and maintaining the integrity of the study.

Informed Consent

Additional consent from the participant will be obtained, if required, for:

- participation in remote visits, as defined in section called "Remote Visits,"
- a change in the method, location, or both, of study intervention administration,
- alternate delivery of study intervention and ancillary supplies, and
- provision of their personal or medical information required prior to implementation of these activities.

Changes in Study Conduct During Exceptional Circumstances

Changes in study conduct not described in this appendix, or not consistent with applicable local regulations, are not allowed.

The following changes in study conduct will not be considered protocol deviations.

1. Remote Visits

In source documents and the eCRF, the study site should capture the visit location and method, with a specific explanation for any data missing because of missed in-person site visits.

Telemedicine: Telephone or technology-assisted virtual visits, or both, are acceptable to complete appropriate assessments. Assessments to be completed in this manner include, but are not limited to, adverse event monitoring, ALSFRS-R, ALSSQOL and concomitant medication.

Regardless of the type of remote visits implemented, the protocol requirements regarding the reporting of AEs, SAEs, and investigational product complaints remain unchanged. Furthermore, every effort should be made to enable participants to return to on-site visits as soon as reasonably possible, while ensuring the safety of both the participants and the site staff.

2. Local Laboratory Testing Option

Local laboratory testing may be conducted in lieu of central laboratory testing. However, central laboratory testing must be retained for biomarker assessments. The local laboratory must be qualified in accordance with applicable local regulations.

3. Adjustments to Visit Windows

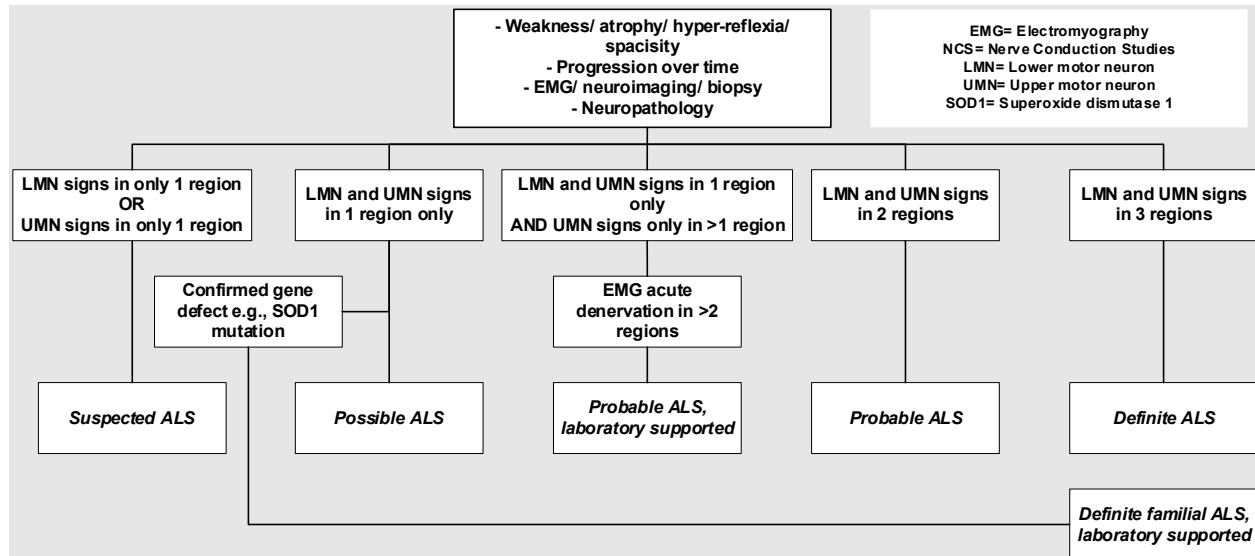
Whenever possible and safe to do so, as determined by the investigator's discretion, participants should complete the usual SoA. To maximize the possibility that these visits can be conducted as on-site visits, the windows for visits may be adjusted, upon further guidance from the sponsor. This minimizes missing data and preserves the intended conduct of the study.

Documentation

Changes to study conduct will be documented:

- Sites will identify and document the details of how participants, visits types, and conducted activities were affected by exceptional circumstances. Dispensing/shipment records of study intervention and relevant communications, including delegation, should be filed with site study records.
- Source documents generated at a location other than the study site should be part of the investigator's source documentation and should be transferred to the site in a secure and timely manner.

10.9. Appendix 9. El-Escorial Criteria for the Diagnosis of ALS



Refer to the following for further information: <http://www.alsa.org/>

10.10. Appendix 10: Abbreviations

Term	Definition
AE	adverse event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.
AESI	Adverse Event of Special Interest
ALS	amyotrophic lateral sclerosis
ALSFRS	amyotrophic lateral sclerosis functional rating scale
ALSFRS-R	amyotrophic lateral sclerosis functional rating scale - revised
ALSSQOL-SF	ALS-Specific Quality of Life- Short Form
ALT	alanine aminotransferase
ALP	alkaline phosphatase
aPTT	activated partial thromboplastin time
AUC	area under the drug concentration-time curve
AUC _{0-tlast}	area under the plasma concentration-time curve from time 0 to the last quantifiable concentration
AST	aspartate aminotransferase
BiPAP	bilevel positive airway pressure
BMI	body mass index
CAFS	combined assessment of function and survival
C _{at EOI}	concentration at end of infusion
CFR	Code of Federal Regulations
CI	confidence interval
CK	creatinine kinase
CL	apparent total body clearance
CONSORT	Consolidated Standards of Reporting Trials
C _{last}	last quantifiable concentration
CRF	Case report form
CSF	cerebrospinal fluid
CSR	clinical study report
CSSR-S	Columbia-Suicide Severity Rating Scale
CTA	Clinical Trial Agreement
D. Bil	direct bilirubin
ECG	electrocardiogram
ED	early discontinuation
EDC	electronic data capture
ERB	ethical review board
fALS	familial amyotrophic lateral sclerosis
FIH	first-in-human
FSH	follicle-stimulating hormone
GCP	good clinical practice
GGT	Gamma-glutamyl transferase
HBsAg	surface antigen of the hepatitis B virus
HCV	hepatitis C virus
HHD	hand-held dynamometry

HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonization
IEC	International Ethics Committee
informed consent	A process by which a patient voluntarily confirms his or her willingness to participate in a particular study, after having been informed of all aspects of the study that are relevant to the patient's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.
INR	international normalized ratio
IPCs	Investigational Product complaints
IRB	Institutional Review Board
IV	Intravenous
ITT	Intent to treat
IWRS	interactive web-response system
LP	lumbar puncture
NIPPV	nasal intermittent positive pressure ventilation
NfL	neurofilament light chain
NOAEL	no-observed-adverse-effect-level
OLE	open-label extension
open-label	A study in which there are no restrictions on knowledge of treatment allocation, therefore the investigator and the study participant are aware of the drug therapy received during the study.
PAV	proportional assist ventilation
PD	pharmacodynamic
PK	pharmacokinetic
pNfH	phospho-neurofilament heavy chain
PP	per-protocol
QOL	quality of life
QTcB	Bazett's formula
QTcF	Fridericia's formula
randomize	the process of assigning subjects/patients to an experimental group on a random basis
SAE	serious adverse event
sALS	sporadic amyotrophic lateral sclerosis
SAP	statistical analysis plan
SERM	selective estrogen receptor modulator
SoA	Schedule of Activities
SOD1	superoxide dismutase 1
SVC	slow vital capacity
SRC	Safety Review Committee
$t_{1/2}$	elimination half-life
$T_{at\ EOI}$	time to concentration at the end of infusion
TBL	total bilirubin
TEAE	treatment-emergent adverse event: Any untoward medical occurrence that emerges during a defined treatment period, having been absent pretreatment, or worsens relative to the pretreatment state, and does not necessarily have to have a causal relationship with this treatment
ULN	upper limit of normal

V	central volume of distribution
WOCBP	woman of childbearing potential

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