CLINICAL STUDY PROTOCOL

Title:	Prospective Randomized Open, Blinded Endpoint (PROBE) Study of AMDX-2011P as a Retinal Tracer in Subjects with Neurodegenerative Diseases Associated with Amyloidogenic Proteinopathy
Protocol Number:	AMDX-2011P-101
Study Drug:	AMDX-2011P
Study Phase:	Phase 1/2a
Short Title:	PROBE Study of AMDX-2011P in Neurodegenerative Diseases
IND Sponsor:	Amydis, Inc. 1800 Century Park East Suite 600, Los Angeles, CA 90067
Approval Date:	24 January 2023
Version:	5.0, Amendment 5

SIGNATURE PAGE

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Protocol Number	AMDX-2011P-101
Version	5.0, Amendment 5, 24 January 2023

I have reviewed and approve the use of this protocol.

Sponsor Representative:

M. Mokhtarani.	24JAN2023
Masoud Mokhtarani, MD Chief Medical Officer	Date

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Version 5.0, Amendment 5	24 January 2023
Version 4.0, Amendment 4	16 September 2022
Version 3.0, Amendment 3	26 July 2022
Version 2.0, Amendment 2	01 July 2022
Version 1.1, Amendment 1	15 June 2022
Original	10 May 2022

Amendment 5.0, 24 January 2023

Overall Rationale for the Amendment:

Changes are shown in order of appearance.

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis 2.1 Study Rationale 2.2 Background 3 Objectives and Endpoints 4 Study Design 5 Study Population 9,1 Sample Size Determination 10.4 Neurodegenerative Diseases Diagnosis Criteria	• Add MSA indication	• To examine additional patient population with alpha-synuclein deposits
10.1.6 Clinical Monitoring	• Update process for monitoring	• For clarity
Global	• Update protocol to ensure consistency across protocol • Correct formatting and style • Move prior amendment to Section 10.6 • Update footer, title page and signature page with date/amendment number	• For clarity and consistency

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with International Council for Harmonization Good Clinical Practice (ICH-GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study, unless the protocol change is intended to eliminate an apparent immediate hazard to subjects. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Prospective Randomized Open, Blinded Endpoint (PROBE) Study of AMDX-2011P as a Retinal Tracer in Subjects with Neurodegenerative Diseases Associated with Amyloidogenic Proteinopathy

Study Phase: Phase 1/2a

Study Description: AMDX-2011P is a novel retinal tracer in clinical development for the detection of neurodegenerative diseases associated with amyloidogenic proteinopathy by detecting alpha-synucleinopathies (α-syn) in Parkinson's disease, and transactive response DNA-binding protein 43 (TDP-43) in amyotrophic lateral sclerosis (ALS). Nonclinical studies have demonstrated that AMDX-2011 binds selectively to protein with high beta sheet content, such as retinal α-syn and TDP-43 deposits, enabling visualization through conventional retinal imaging. There is currently a significant need for an accessible diagnostic measure to facilitate early diagnosis and patient management in these conditions.

The current prospective randomized open, blinded endpoint (PROBE) study will evaluate the activity, safety, tolerability, and pharmacokinetics (PK) as well as detect amyloidogenic deposits through retinal imaging following escalating doses of bolus intravenous (IV) AMDX-2011P administration in subjects with Parkinson's disease, multiple system atrophy (MSA) and ALS. Assessments of retinal images will be conducted by central masked assessors in a randomized fashion.

Objectives and Endpoints:

OBJECTIVES	ENDPOINTS
Primary	
• To assess safety and tolerability of single IV dose of AMDX-2011P in subjects with neurodegenerative diseases (Parkinson's disease, MSA and ALS).	• Incidence, nature, and severity of adverse events (AEs) and serious adverse events (SAEs).
Secondary	
• To assess the ability of AMDX-2011P to identify α-syn in retina of subjects with Parkinson's disease or MSA.	• Detection of α-syn in the retina after AMDX-2011P administration.

• To assess the ability of AMDX-2011P to identify TDP-43 in retina of subjects with ALS.	• Detection of TDP-43 in the retina after AMDX-2011P administration.
• To characterize the PK profile of AMDX-2011P and AMDX-2011 following administration of a single IV dose of AMDX-2011P in subjects with neurodegenerative diseases (Parkinson's disease, MSA and ALS).	• PK parameters of AMDX-2011P and AMDX-2011 calculated from concentrations in plasma.

Overall Study Design

This is a PROBE study designed to evaluate the safety, tolerability, and the ability of AMDX-2011P to detect amyloid deposits, α-syn and TDP-43 (collectively called amyloidogenic deposits) through retinal imaging, as well as PK of single bolus IV of AMDX-2011P in subjects with Parkinson's disease, MSA and ALS. Study schema is shown in Section 1.2.

The study will enroll up to 4 dose cohorts as follows:

Cohort	Number of Subjects with neurodegenerative diseases (Parkinson's Disease and/or MSA and/or ALS) [a]	Proposed dose level
1	3-9 [b]	25 mg (1 mL)
2	3-9 [b]	50 mg (2 mL)
3	12 [b]	100 mg[c] (4 mL)
4	12 [b]	150 or 200 mg[c] (6-8 mL)

Abbreviation: ALS, amyotrophic lateral sclerosis; SMC, Safety Monitoring Committee; TBD, to be determined.
a. Additional subjects (from any subject population) may be enrolled into any cohort per SMC discretion. Approximately 12 ALS subjects will be enrolled in the study.
b. All cohorts will include 1 sentinel subject who will be observed for 8 hours and evaluated at 24 hours for safety before enrolling the remaining subjects in that cohort.
c. Tentative dose and will be confirmed by SMC based on emerging data from the prior cohorts; intermediate or higher dose level may also be explored in these cohorts

A Safety Monitoring Committee (SMC), comprised of the Study Medical Monitor (or designee), neurologists with experience in these patient populations, an ophthalmologist with retinal imaging expertise, and the Sponsor's clinical and technical representatives, will review safety data throughout the study. The SMC may recommend modifying the duration

of observation or timing of study procedures based on safety and other available data from the prior cohorts.

All cohorts will include subjects with neurodegenerative diseases (Parkinson's disease, MSA and/or ALS). The decision to escalate to the next dose level will be made based on review of all available safety and tolerability data collected including AEs, electrocardiograms (ECGs), vital signs, and laboratory safety results of the previous dose levels after single dose administration in at least 3 subjects from each cohort. Based on emerging data, additional cohort(s) and/or an intermediary dose may be investigated with prior approval by the SMC. The study may progress to the next cohort if approved upon satisfactory review of available safety data of the previous cohort(s) by the SMC. During each safety review, the SMC will make a decision to either continue enrollment to the next planned cohort, add additional subjects to the current cohort, or change the dose of the next cohort.

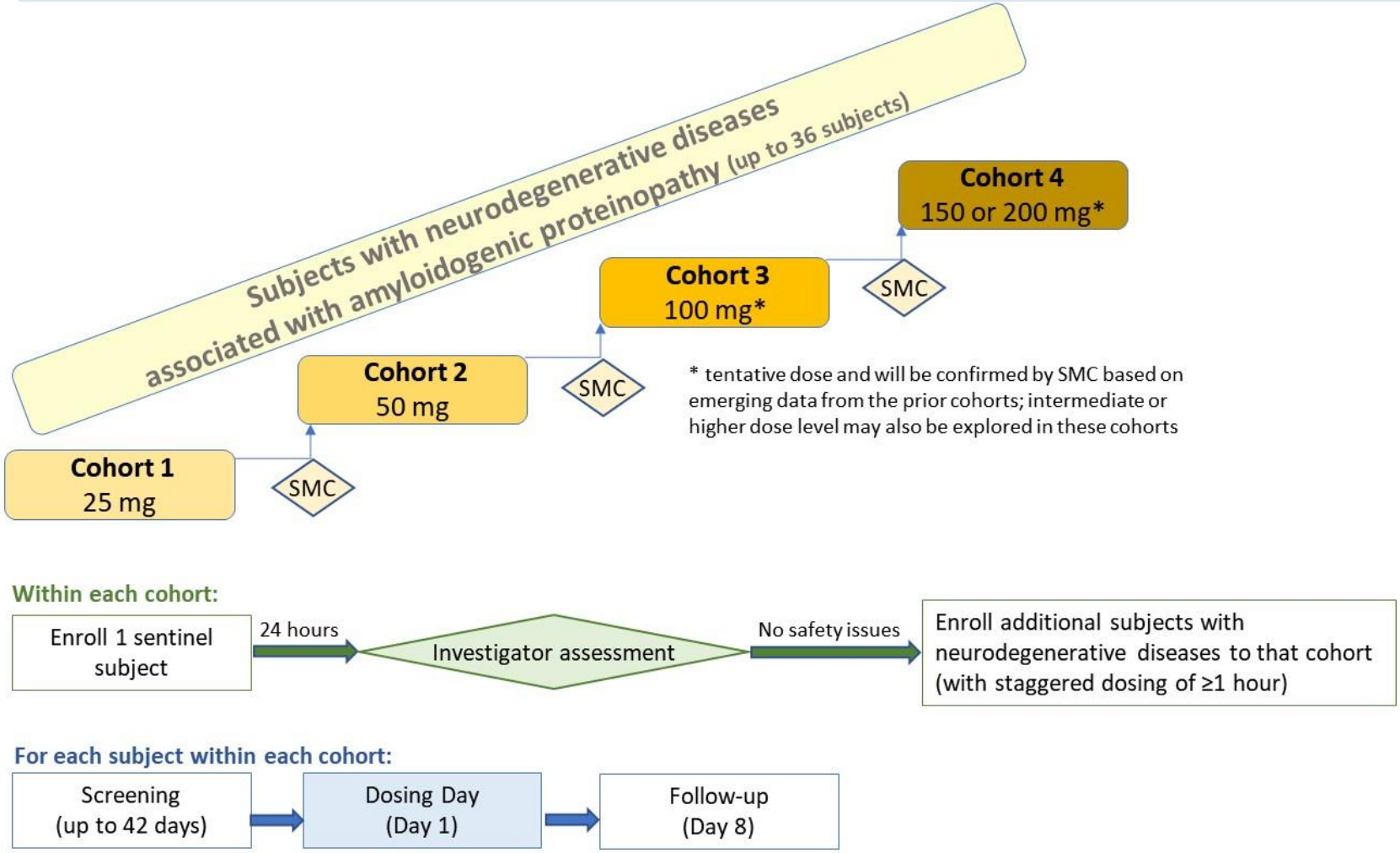
Enrollment into each cohort will begin with 1 sentinel subject who will be observed for at least 8 hours at the clinic and evaluated by the investigator at 24 hours following dosing by the investigator. If no safety issues are observed, the remaining subjects will be enrolled into that cohort. Dosing of remaining subjects at the same center will be staggered by at least 1 hour. Dosing within a cohort will continue unless stopping rules are met.

For all dose cohorts, after signing the informed consent, subjects will be screened. If needed, the screening procedures may occur over a period of up to 42 days prior to dosing. On Day 1, subjects will be admitted to the clinical research unit for 8 hours confinement. Eye examination and retinal imaging will be conducted before administration of the study drug. AMDX-2011P will be administered through a single IV bolus injection followed by repeated retinal imaging and PK and safety assessments as outlined in the Schedule of Activities (SoA; Section 1.3). On Day 8 (end of study), there will be a follow-up visit for retinal imaging and safety assessments.

All retinal images will be deidentified and sent in batches to a reading center for masked assessment. Assessors will be masked to type of subjects, their disease, dose, clinical status of subjects, and sequence of post-injection images. This provides an objective assessment of the ability of AMDX-2011P to identify amyloidogenic deposits. During the interim analysis, the SMC may also review representative images to confirm the optimum detection and the residence time and make potential recommendations to the retinal imaging time point(s) and/or potential safety assessment modifications.

Study Population:	Adult subjects with neurodegenerative diseases: Up to 36 adult male and female subjects diagnosed with Parkinson's disease, MSA or ALS.
Description of Sites/Facilities Enrolling Subjects:	The study will be conducted at 4-5 Phase 1 ophthalmology experienced sites located in the United States.
Description of Study drug:	AMDX-2011P is a novel retinal tracer indicated for the diagnosis of amyloid angiopathy. It is a phosphate prodrug hydrolyzed in vivo to the pharmacologically active molecule AMDX-2011.
Study Duration:	Estimated time to complete study from enrollment to completion of data analyses will be 7 months.
Subject Duration:	For each subject, study participation is expected to last up to 8 days: one day of dosing, 7 days of follow-up, and a window of up to 42 days to complete the screening procedures.

1.2 SCHEMA



Abbreviations: SMC, Safety Monitoring Committee.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Table 1 Schedule of Activities (Subjects with Neurodegenerative Diseases, All Dose Cohorts)

Study Procedures	**Screening[1]**	**Treatment**	**Follow-up**	**Notes**
Study Visit	**-42 to -1**	**Day 1**	**Day 8/EOS/ET**	**1.** Note: Procedures such as diagnostic status determination and ophthalmological examinations performed by subject's referring physicians can be used as screening procedures at the discretion of the investigator.
Visit window (days)	**42**		**+2**	
Informed consent	X			
Domiciled		X[a]		**a.** Subjects will be admitted to the clinical research unit in the morning for 8 hours confinement
Medical/ocular history/Physical exam	X			On Day 1, PE may be repeated if abnormalities are detected at screening. Relevant ocular and disease-specific history will be captured up to date of diagnosis.
Eligibility (inclusion/exclusion criteria)	X			
Body weight and Height	X			
Eye examination/	X	X	X	Refer to Section 8.1.1.
Color fundus photography	X			
Vital signs	X	X[b]	X	**b**. To be collected predose, and at 0.25, 0.5, 1, 2, 8 hours postdose. A window of ±5 minutes will be allowed for measurements up to 2 hours, and ±10 minutes beyond 2 hours.
12-lead safety (ECG)	X	X[c]	X	**c.** Triplicate at the following timepoints: predose (within 1 hour), and at 90 minutes (±15 minutes) and 8 hours (± 60 minutes) postdose or before discharge
Safety laboratories	X	X[d]	X	Refer to Section 10.2. **d.** To be collected predose on Day 1 and before discharge
Urinalysis	X	X	X	
Pregnancy test	S	U (predose)	U	S = Serum; U = Urine
Adverse event reporting	X	X	X	

Study Procedures	Screening[1]	Treatment	Follow-up	Notes
Study Visit	**-42 to -1**	**Day 1**	**Day 8/EOS/ET**	**1.** Note: Procedures such as diagnostic status determination and ophthalmological examinations performed by subject's referring physicians can be used as screening procedures at the discretion of the investigator.
Visit window (days)	**42**		**+2**	
Concomitant medications	X	X	X	
Retinal fundus fluorescence (FF) imaging		X[e]	X	**e.** Refer to Section 8.1.2
Retinal optical coherence tomography (OCT) and OCT angiography (OCTA)	X		X	
Blood PK sampling		X[f]		**f.** On Day 1, PK blood samples will be collected at the following time points: 0 (predose), and at 3, 6, 15, and 30 minutes postdose and 1, 2, 4, 6, and 8 hours postdose. A window of ±5 minutes will be allowed for samples collected from 30 minutes to 2 hours, and ±10 minutes beyond 2 hours.
Study drug administration		X		

Abbreviations: AE, adverse events; ECG, electrocardiogram; EOS, end of study; ET, early termination; FU, follow-up; PE, physical examination; PK, pharmacokinetics.

2 INTRODUCTION

2.1 STUDY RATIONALE

There is currently a significant need for an accessible diagnostic measure to facilitate early diagnosis and treatment management of subjects with neurodegenerative diseases associated with amyloidogenic proteinopathy, such as Parkinson's disease, multiple system atrophy (MSA), and amyotrophic lateral sclerosis (ALS).

AMDX-2011P is a novel retinal tracer indicated for the detection of alpha-synucleinopathies (α-syn) in Parkinson's disease and MSA, and transactive response DNA-binding protein 43 (TDP-43) in ALS. AMDX-2011P is a phosphate prodrug hydrolyzed in vivo to the pharmacologically active molecule, AMDX-2011.

Nonclinical studies have demonstrated that AMDX-2011 binds selectively to protein with high beta sheet content, such as retinal amyloid beta, α-syn, and TDP-43 deposits associated with human amyloid-related diseases. When AMDX-2011 binds to amyloid or amyloid-like peptides, it fluoresces with excitation and emission wavelengths, enabling visualization of amyloid deposits through conventional retinal imaging instruments widely available in ophthalmology clinics.

Pharmacokinetic (PK) studies in rats and dogs demonstrated rapid and incomplete hydrolysis of AMDX-2011P to AMDX-2011 in plasma; and only AMDX-2011 (and not AMDX-2011P) was quantifiable in retinal tissues in rats after intravenous (IV) administration. After a bolus IV injection of 15 mg/kg, a pharmacologically active dose of AMDX-2011P in rats, peak AMDX-2011 concentrations were achieved in the retina by 2 minutes postdose and were below the level of detection within 30 minutes postdose. Similarly, in rabbits, after an IV injection of 15 mg/kg AMDX-2011P, only AMDX-2011 was detected in the retina, and the majority of AMDX-2011 was cleared from the retina within 60 minutes.

The safety of AMDX-2011P has been evaluated in a program of Good Laboratory Practice (GLP) toxicology and safety pharmacology studies, conducted using a single bolus IV injection of AMDX-2011P to mimic the clinical dosing regimen. In the definitive GLP single-dose rat study, no adverse target organ toxicity and no evidence of ocular or cutaneous phototoxicity were identified at up to 50 mg/kg. Injection site reactions occurred in rats after administration into the tail vein, which were considered adverse at 50 mg/kg and not adverse at ≤ 15 mg/kg. Therefore, in rats, the dose showing no adverse systemic toxicity was 50 mg/kg, and the overall study no-observed-adverse-effect level (NOAEL) was 15 mg/kg. In safety pharmacology studies conducted in rats, there were no acute neurobehavioral changes or effects on respiratory function at up to 50 mg/kg. In the definitive GLP single-dose dog study, reversible clinical ophthalmic signs, including mydriasis, minimal hyperemic conjunctiva and minimal to mild chemosis, occurred in male dogs within 24 hours postdose at 60 mg/kg. Other non-adverse clinical signs at 60 mg/kg included intermittent emesis and soft feces, which resolved by Day 2 and were not considered adverse. There was histologic evidence of injection site reactions in dogs at all doses, which were not considered adverse. There was no adverse target organ toxicity in dogs at 60 mg/kg, which was considered the NOAEL in females; the NOAEL in males was 20 mg/kg

based on ophthalmic changes at 60 mg/kg. In a dog cardiovascular safety study, transient reductions in blood pressure (systolic, diastolic, and mean arterial pressure) and heart rate occurred at 15-30 minutes after a single bolus injection of 30 mg/kg, but not at ≤ 10 mg/kg, and there were no effects on electrocardiogram (ECG) profile at any dose. In vitro, AMDX-2011 caused a dose-dependent inhibition of the human Ether-à-go-go-related gene (hERG) cardiac potassium channel of up to 44% at a concentration of 10 µM. This concentration is expected to be at least 10-fold above human peak plasma concentration (C_{max}) values of the pharmacologically relevant doses; therefore, a risk for QTc prolongation in humans is considered low. Overall, the nonclinical safety studies support the safe administration of AMDX-2011P in this study.

The current prospective randomized open, blinded endpoint (PROBE) study is designed to evaluate the ability of AMDX-2011P to assess safety, tolerability, and PK, as well as detect amyloid deposits, α-syn and TDP-43 through retinal imaging following escalating doses of bolus IV AMDX-2011P administration in subjects with Parkinson's disease, MSA and ALS.

2.2 BACKGROUND

2.2.1 Parkinson's Disease

Parkinson's disease is a neurodegenerative disorder that mostly presents in later life with generalized slowing of movements (bradykinesia) and at least one other symptom of resting tremor or rigidity (Zafar, 2021). The prevalence of Parkinson's disease increases with age and is more common in males than females (de Lau, 2006; Dorsey, 2018; GBD, 2018). The neuropathological hallmarks of Parkinson's disease are neuronal loss in the basal ganglia, specifically the substantia nigra and globus pallidus (Prakash, 2016), which leads to striatal dopamine deficiency, and intracellular inclusions containing aggregates of α-syn. Therefore, Parkinson's disease belongs to the group of α-synucleinopathies (including Parkinson's disease, dementia with Lewy bodies and multiple system atrophy) that share the abnormal aggregation of α-syn as their key-molecular hallmark (Malfertheiner, 2021).

Currently, there are no specific tests to detect Parkinson's disease. Diagnosis is determined with a clinical evaluation by identifying and monitoring symptoms associated with the disease. Recent advances in brain imaging (magnetic resonance imaging [MRI], computed tomography [CT], dopamine transport scan [DaTscan]) can only be used to rule out structural and other causes of parkinsonian symptoms (Nemade, 2021).

α-syn is an abundant neuronal protein that is primarily found in the presynaptic nerve terminals (Lashuel, 2013). Under pathological conditions α-syn is phosphorylated and accumulates and forms insoluble aggregates (Heras-Garvin, 2020). Pathological aggregates of α-syn has been reported in retinal layers of Parkinson's disease patients in postmortem examination and levels of a-syn deposition in retina may provide an in vivo indicator of disease severity (Ortuño-Lizarán, 2018). Furthermore, 80% of Parkinson's disease patients report at least one visual symptom that often occurs in early disease stages preceding cardinal Parkinson's disease symptoms and correlates with disease progression (Veys, 2019). However, there are currently no positron emission tomography (PET) ligands that can selectively label α-syn

deposits, and detection is thus far only possible by histological examination of postmortem brain tissue (Korat, 2021).

2.2.2 Multiple System Atrophy

MSA is a fatal progressive neurodegenerative disease characterized by the variable combination of autonomic dysfunction, parkinsonian and cerebellar features (Fanciulli, 2015a; Jellinger, 2018). The dominant display of specific symptomatology is used to stratify cases of disease into two subtypes: a parkinsonian variant (MSA-P) associated with features such as bradykinesia, muscle rigidity, tremors, and postural instability, and a cerebellar variant (MSA-C) characterized by cerebellar ataxia, speech difficulties and problems controlling eye movement (Fanciulli, 2015b). Movement Disorder Society (MDS) criteria for MSA diagnosis were recently updated (Wenning, 2022); however, MSA and Parkinson's disease can be difficult to differentially diagnose due to large overlaps in presenting symptoms and the lack of specific tests for either condition (Stankovic, 2019).

MSA is characterized by inclusions of abnormal α-syn protein that, for unknown reasons, build up in cells in many parts of the brain and spinal cord (Koga, 2021). There are key differences in the nature of a-syn deposition in MSA and Parkinson's disease, and how these manifests in retina. At the cellular level, MSA is distinguished from Parkinson's disease in that α-syn deposits occur almost exclusively as cytoplasmic inclusions in oligodendrocytes – so called "glial cytoplasmic inclusions" (Koga, 2018; Schweighauser, 2020). Oligodendrocytes are excluded from the retina (presumably to avoid light scattering effects of myelination) but line the retrolaminar aspect of the optic nerve. In addition, recent data indicate that amyloid configurations of a-syn may differ among synucleinopathies (Meade, 2019), which may yield different fluorescence emission profiles for each disease. Detecting a-syn early (before symptoms arise) and being able to determine whether it represents Parkinson's or MSA would be a tremendous advance in the field. It would enable earlier and more accurate therapeutic intervention, and greatly facilitate the clinical testing of novel, disease-modifying therapeutics (Osaki, 2009; Stankovic, 2019; Wenning, 2022).

2.2.3 Amyotrophic Lateral Sclerosis

ALS is the most common adult-onset motor neuron disease, clinically and pathologically characterized by degeneration of upper and lower motor neurons leading to progressive paralysis and death within 3–5 years of diagnosis (Dhasmana, 2021). The estimated prevalence of ALS in the United States (US) and Europe is 5 cases per 100,000 population (Chiò, 2013; Mehta, 2018).

TDP-43 inclusions occur in neurons and glia in approximately 97% of ALS cases (de Boer, 2020). TDP-43 is a DNA/RNA-binding protein that has various roles in RNA metabolism, including trafficking, splicing, and degradation. Misfolding of TDP-43 into an "amyloid-like" or true amyloid configuration promotes aggregate assembly and prion-like spread (McAlary, 2019; Jo, 2020).

Currently, there are no specific tests to detect ALS. The diagnosis is determined by excluding other conditions and utilizing clinical examinations, laboratory and genetic tests and nerve conduction/needle electromyography studies. Needle electromyography records abnormal

activities at rest and looks for neurogenic patterns during muscle contraction. Motor evoked potentials after transcranial magnetic stimulation remain the test of choice to identify impairment of upper motor neurons (Štětkářová, 2021). Recent advances in brain imaging (MRI and PET) can only be used to rule out other neurodegenerative diseases. There are no PET ligands that can selectively label TDP-43 deposits (Young, 2020; Rao, 2021).

2.2.4 AMDX-2011P Product Development

AMDX-2011P is a small molecule intended for IV administration. AMDX-2011P is a prodrug and rapidly converts to AMDX-2011, its active moiety, after injection. In vitro and in vivo pharmacology studies have demonstrated that AMDX-2011 binds to human brain and α-syn associated with Parkinson's disease and MSA, and TDP-43 in ALS.

In vivo studies in mice indicated that amyloidogenic deposits in the retina can be visualized as hyper-fluorescent signals after systemic doses of AMDX-2011P. The principle for the sensitive and selective detection of amyloid-like peptides by AMDX-2011 and other molecules sharing the same molecular core is the enhanced fluorescence occurring after binding (Cao, 2018). Compound binding to the beta-pleated sheet structure of the peptide increases fluorescence by restricting the torsional rotation of the sigma bond between the piperidine and naphthalene rings and enhancing the electronic transition upon photoexcitation. The primary de-excitation mechanism of enhanced fluorescence can then be detected visually.

A detailed description of the chemistry, pharmacology, efficacy (activity), and safety of AMDX-2011P is provided in the Investigator's Brochure (IB).

2.3 Risk/Benefit Assessment

Detailed information about the known and expected benefits and risks and reasonably expected adverse events of AMDX-2011P may be found in the IB.

2.3.1 Known Potential Risks

This is the first-in-human (FIH) study with AMDX-2011P. Though similar and higher doses of AMDX-2011P have been tested in multiple animal studies and results support the dose level to be tested in humans, this will be the first clinical exposure of the study drug; thus, there may be risks that are currently unknown.

There are no identified or potential risks of particular severity or seriousness anticipated on the basis of the mechanism of action of AMDX-2011P. The potential risks are based on theoretical considerations and findings (Table 2) from completed GLP nonclinical safety studies.

Table 2 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Potential Risks which may be associated with Study Drug		
Phototoxicity	Both AMDX-2011P and AMDX-2011 were suspected of being phototoxic in the in vitro	Subjects will be instructed to avoid direct sunlight and wear

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	3T3 neutral red uptake phototoxicity test; however, there was no evidence of ocular or cutaneous phototoxicity after IV administration of AMDX-2011P to pigmented rats at doses of up to 50 mg/kg followed by exposure to ultraviolet B, ultraviolet A, and visible light from a xenon lamp.	protective gear (e.g., sunglasses) for 24 hours after dosing. Ophthalmologic examinations will be conducted throughout the study.
Effect on eye	In male dogs, single IV bolus injections of 60 mg/kg AMDX-2011P resulted in mydriasis, minimal hyperemic conjunctiva and minimal to mild chemosis within 24 hours postdose, which was reversible within 6 days.	Ophthalmologic examinations will be conducted throughout the study.
Effect on cardiovascular function	In dogs, single IV bolus injections of 30 mg/kg AMDX-2011P resulted in transient reductions in systolic, diastolic, and mean arterial pressure and heart rate 15-30 min after injection. In vitro, inhibition of hERG channel was seen at high concentrations of AMDX-2011P.	Vital sign assessments, triplicate ECG recording
Mutagenicity	AMDX-2011P and AMDX-2011 were mutagenic in bacteria only in the presence of an exogenous metabolic system, suggesting a metabolite was the mutagenic and not the parent drugs. AMDX-2011P and AMDX-2011 did not cause chromosomal aberrations in mammalian cells with or without metabolic activation.	Healthy volunteers are excluded from clinical studies until additional information on human risk is obtained.
Potential Risks which may be associated with Study Procedures		
Injection site reactions (ISRs) such as redness, swelling, itching and tenderness	As with any IV administration procedure, ISR is possible. ISRs were seen in nonclinical studies (rats)	IV administration will be done by trained personnel. In the case of ISRs, observe and treat as needed.
Retinal imaging.	Potential mild discomfort, fatigue and temporary dry eyes	For temporary dry eyes, artificial tears may be used to alleviate the symptoms. Retinal imaging will be performed by trained personnel to minimize the time of data acquisition.

Abbreviations: ECG, electrocardiogram; hERG, human Ether-à-go-go-related gene; ISR, injection site reaction; IV, intravenous

2.3.2 Known Potential Benefits

The results from completed nonclinical studies with AMDX-2011P suggest a potential benefit of AMDX-2011P as a novel compound to facilitate diagnosis and patient management in these conditions.

2.3.3 Assessment of Potential Risks and Benefits

Considering the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with AMDX-2011P are justified, given the significant need for an accessible diagnostic measure for patients with neurodegenerative diseases.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
• To assess safety and tolerability of single intravenous (IV) dose of AMDX-2011P in subjects with neurodegenerative diseases (Parkinson's disease, MSA and ALS).	• Incidence, nature, and severity of adverse events (AEs) and serious adverse events (SAEs).
Secondary	
• To assess the ability of AMDX-2011P to identify α-syn in retina of subjects with Parkinson's disease or MSA. • To assess the ability of AMDX-2011P to identify TDP-43 in retina of subjects with ALS.	• Detection of α-syn in the retina after AMDX-2011P administration. • Detection of TDP-43 in the retina after AMDX-2011P administration.
• To characterize the PK profile of AMDX-2011P and AMDX-2011 following administration of a single IV dose of AMDX-2011P in subjects with neurodegenerative diseases (Parkinson's disease, MSA and ALS).	• The PK parameters of AMDX-2011P and AMDX-2011 calculated from concentrations in plasma.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a PROBE study designed to evaluate safety, tolerability, and the ability of AMDX-2011P to detect amyloid deposits, α-syn and TDP-43 (collectively called amyloidogenic deposits) through retinal imaging, as well as PK of single bolus IV of AMDX-2011P in subjects with Parkinson's disease, MSA and ALS. Study schema is shown in Section 1.2.

The study will enroll up to 4 dose cohorts, as shown in Table 3:

Table 3 Cohorts and Planned Doses

Cohort	Number of Subjects with neurodegenerative diseases (Parkinson's Disease and/or MSA and/or ALS)[a]	Proposed dose level
1	3-9[b]	25 mg (1 mL)
2	3-9 [b]	50 mg (2 mL)
3	12 [b]	100 mg[c] (4 mL)
4	12 [b]	150 or 200 mg[c] (6-8 mL)

Abbreviation: ALS, amyotrophic lateral sclerosis; SMC, Safety Monitoring Committee; TBD, to be determined.
a. Additional subjects (from any subject population) may be enrolled into any cohort per SMC discretion. Approximately 9 ALS subjects will be enrolled in the study. Approximately 9 MSA subjects will be enrolled.
b. All cohorts will include 1 sentinel subject who will be observed for 8 hours and evaluated at 24 hours for safety before enrolling the remaining subjects in that cohort.
c. Tentative dose and will be confirmed by SMC based on emerging data from the prior cohorts; intermediate or higher dose level may also be explored in these cohorts.

A Safety Monitoring Committee (SMC), comprised of the Study Medical Monitor (or designee), neurologists with experience in these diseased populations, an ophthalmologist with retinal imaging expertise, and the Sponsor's clinical and technical representatives, will review safety data throughout the study. The SMC may recommend modifying the duration of observation or timing of study procedures based on safety and other available data from the first cohort. For additional details, refer to Section 10.1.5.

All cohorts will include subjects with neurodegenerative diseases (Parkinson's disease, MSA and/or ALS). The decision to escalate to the next dose level will be made based on review of all available safety and tolerability data collected, including AEs, ECGs, vital signs, and laboratory safety results of the previous dose levels after single dose administration in at least 3 subjects from each cohort. Based on emerging data, additional cohort(s) and/or an intermediary dose may be investigated with prior approval by the SMC. The study may progress to the next cohort if approved upon satisfactory review of available safety data of the previous cohort(s) by the SMC. During each safety review, the SMC will make a decision to either continue enrollment to the next planned cohort, add additional subjects to the current cohort, or change the dose of the next cohort.

Enrollment into each cohort will begin with 1 sentinel subject who will be observed for at least 8 hours at the clinic and evaluated by the investigator at 24 hours following dosing by the investigator. If no safety issues are observed, the remaining subjects will be enrolled into that cohort. Dosing of remaining subjects at the same center will be staggered by at least 1 hour. Dosing within a cohort will continue unless stopping rules are met.

For all dose cohorts, after signing the informed consent, subjects will be screened. If needed, the screening procedures may occur over a period of up to 42 days prior to dosing. On Day 1, subjects will be admitted to the clinical research unit for 8 hours confinement. Eye examination and retinal imaging will be conducted before administration of the study drug. AMDX-2011P will be administered through a single IV bolus injection followed by repeated retinal imaging and PK and safety assessments as outlined in the Schedule of Activities (SoA; Section 1.3). On Day 8 (end of study), there will be a follow-up visit for retinal imaging and safety assessments.

All retinal images will be deidentified and sent in batched to a reading center for masked assessment. Assessors will be masked to type of subjects, their disease, dose, clinical status of subjects, and sequence of post-injection images. This provides an objective assessment of the ability of AMDX-2011P to identify amyloidogenic deposits. During the interim analysis (see Section 9.3.8), the SMC may also review representative images to confirm the optimum detection and the residence time and make potential recommendations to the retinal imaging timepoint(s) and/or potential safety assessment modifications.

4.2 Scientific Rationale for Study Design

This is a FIH study of AMDX-2011P and the study aims to recruit subjects with a diagnosis of neurodegenerative diseases (Parkinson's disease, MSA or ALS) to assess the activity, safety, tolerability, and PK of AMDX-2011P and AMDX-2011 in the target population. AMDX-2011, formed from AMDX-2011P, is intended to visualize the amyloidogenic deposits not abundantly present in healthy individuals.

The PROBE design (Hansson, 1992) provides an objective assessment of the ability of AMDX-2011P to identify α-syn (Parkinson's disease and MSA) and TDP-43 (ALS).

Conducting a FIH study in a target patient population provides essential information to establish the amyloidogenic deposits visualization and fluorescence residence time in the retina after administration of study drug. As an older population (40 to 90 years old) may have undiagnosed amyloidogenic proteinopathy associated diseases of up to ~28%.

4.3 Justification for Dose

The proposed starting dose is based on recommendations from an FDA Guidance for Industry: *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, July 2005* (FDA, July 2005). Table 4 shows NOAEL doses and systemic AUC and C_{max} exposures from the pivotal rat and dog toxicity studies, as well as projected human exposures at proposed clinical dose levels. Clinical PK parameters (clearance and volume of distribution) for AMDX-2011P were projected by model fitting of dog PK data, then scaling to human values. AMDX-2011 parameters were projected based on rates of formation and

observed metabolite/parent ratios in animals. Human PK profiles were then simulated at various doses based on projected parameter values.

The lowest NOAEL for dogs (20 mg/kg, males) equates to a human equivalent dose (HED) for a 60-kg adult male of 667 mg, and the NOAEL for systemic toxicity in rats (50 mg/kg) equates to a HED of 484 mg. Using an ~20-fold safety margin, the starting dose in the proposed clinical study will be 25 mg. The escalating doses will be 25 mg, 50 mg, 100 mg, and either 150 mg or 200 mg.

The doses proposed for this study will cover the anticipated pharmacologically active dose range and are predicted to be significantly below AUC and C_{max} exposures for AMDX-2011 and AMDX-2011P achieved at NOAEL doses in rats and dogs.

Table 4 Projected Human Dose Levels and Corresponding AUC and C_{max} Exposures Based on Nonclinical Data

	AUC (hr ••ng /mL)		C_{max} (ng/mL)	
Dose	**AMDX-2011P[a]**	**AMDX-2011[a]**	**AMDX-2011P[a]**	**AMDX-2011[a]**
Human (mg)				
25	49.0	19.1	135	43.8
50	97.8	38.2	269	87.5
75	144	57.4	403	131
100	192	76.6	539	175
150	288	115	806	263
200	383	153	1070	350
Dog NOAEL[b]				
20 mg/kg (Male) *(HED 667 mg)*	400	182	2270	937
60 mg/kg (Female) *(HED 2000 mg)*	13,700	2200	45,500	5930
Rat NOAEL[c]				
15 mg/kg (injection site reaction) *(HED 145 mg)*	923	138	7,175	1,019
50 mg/kg (Systemic) *(HED 484 mg)*	17,900	1,345	141,500	8,930

[a] human AUC and C_{max} values are estimated from projected PK at each dose level
[b] NOAEL in dogs based on ophthalmic clinical observations at 60 mg/kg in males and no adverse toxicity in females. AUC values are AUC_{last}

[c] NOAEL in rats based on adverse injection site reactions at 50 mg/kg, but no adverse target organ (systemic) toxicity at up to 50 mg/kg . AUC_{last} and C_{max} are means of male and female values.
Abbreviation: AUC, area under the curve; HED, human equivalent dose; NOAEL, no observed adverse effect level.

4.4 STUDY DURATION

For each subject, the study is expected to last up to 8 days: one day of dosing, 7 days of follow-up, and a window of up to 42 days to complete the screening procedures.

4.5 END OF STUDY DEFINITION

A subject is considered to have completed the study if he or she has completed the study, including the last visit or the last scheduled procedure shown in the SoA, Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the study globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Subjects are eligible to be included in the study only if they meet the criteria relevant to their disease status:

Type of Subject and Disease Characteristics

Subjects with Parkinson's Disease

1. Clinically established Parkinson's disease based on Movement Disorder Society (MDS) Clinical Diagnostic Criteria for Parkinson's disease (Table 9) and a modified Hoehn & Yahr scale of 1-3 (Table 10).
2. No suspected atypical parkinsonian syndromes due to drugs, metabolic disorders, encephalitis, or degenerative diseases.

Subjects with ALS

3. Confirmed diagnosis of ALS with both upper and lower motor neuron involvement (Table 11).

Subjects with MSA

4. Diagnosis of clinically established MSA according to MDS 2022 Criteria (Wenning, 2022); refer to Appendix 4, Section 10.4.2.

Age, Gender, Informed Consent, Pregnancy and Contraception

5. Male and female subjects 18 years and older at the time of signing the informed consent.
6. Ability to undergo retinal imaging.
7. Subject or legally authorized representative must provide signed informed consent (or signed assent form) prior to study entry and have the ability and willingness to attend and comply with the necessary study procedures and visits at the study site. For subjects unable to physically sign the informed consent, a guardian or trusted care giver can sign on their behalf in presence of an independent witness.

Contraception

8. Contraception use by study subjects of childbearing potential (male and female) and female partners of childrearing potential male subjects should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. For details, refer to Section 10.2.
9. Female subjects of childbearing potential and female partners of childbearing potential male subjects must refrain from oocyte donation for up to 30 days after study drug administration.

10. Male subjects must refrain from sperm donation for 90 days after study drug administration.
11. Females of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day 1 (predose). Females not of childbearing potential must be surgically infertile or postmenopausal (defined as cessation of regular menstrual periods for at least 12 months) at Screening.

5.2 EXCLUSION CRITERIA

Subject meeting ANY of the following exclusion criteria are NOT eligible to be enrolled into the study:

Medical Conditions

1. Presence of any underlying physical or psychological medical condition that, in the opinion of the investigator, would make it unlikely that the subject will complete the study per protocol.
2. Clinically significant laboratory abnormalities assessed by the investigator.
3. Active malignancy and/or history of malignancy in the past 5 years, with the exception of completely excised non-melanoma skin cancer or low-grade cervical intraepithelial neoplasia.
4. Prolonged QTcF (>450 ms for males and >470 ms for females), cardiac arrhythmia, or any clinically significant abnormality in the resting ECG, as judged by the investigator.
5. Presence of any ocular condition that, in the opinion of the investigator, would significantly hinder the ability to detect and quantify hyper-fluorescent puncta (e.g., eyes with significant hyper-autofluorescence that would mask the ability to detect, quantify, and discern post-injection hyper-fluorescent signal from pre-injection hyper-autofluorescence signal). These conditions may include, but are not limited to; age-related macular degeneration, central serous chorioretinopathy, diabetic retinopathy, macular dystrophies such as Stargardt disease, retinitis pigmentosa, choroideremia, white dot syndromes, and drug toxicities such as hydroxychloroquine toxicity.

Prior/Concomitant Prohibited Therapy

6. Use of any new prescription therapies or vaccines within 7 days prior to the study drug administration.
7. Drugs with potential phototoxicity per Package Insert are prohibited within 48 hours or 5 half-lives, whichever is longer, prior to first study drug until End-of-study (EOS) visit, except for those required for treatment of underlying disease. Examples of such drugs include the following: Chloroquine (Aralen), hydroxychloroquine (Plaquenil), Thioridazine (Mellaril), Topiramate (Topamax), vemurafenib, voriconazole,

doxycycline, hydrochlorothiazide, amiodarone, furosemide, allopurinol, phenothiazine, and chlorpromazine.

Prior/Concurrent Clinical Study Experience

8. Administration of investigational product in another study within 30 days prior to the first study drug administration, or five half-lives, whichever is longer.

Other Exclusions

9. Females who are pregnant or breastfeeding.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable.

5.4 SCREEN FAILURES

Subjects will be assigned a subject number upon entry of data into the Electronic Data Capture (EDC) system.

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently assigned to a cohort. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects 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 not met, and any SAE.

Potential candidates who do not meet the criteria for participation in this study (screen failure) may be rescreened.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

The Sponsor (or designee) will identify clinical sites with:

- Access to subjects with neurodegenerative diseases (Parkinson's disease, MSA and ALS).
- Ability to perform ophthalmological procedures.

The clinical study is designed to recruit subjects through a network of physicians who routinely manage patients with neurodegenerative diseases and make referrals to study sites. The investigator will perform qualification assessments including confirmed diagnosis of neurodegenerative diseases.

6 STUDY DRUG

6.1 STUDY DRUG(S) ADMINISTRATION

6.1.1 Study Drug Description

AMDX-2011P, a retinal tracer (small molecule), is a phosphate prodrug hydrolyzed in vivo to the pharmacologically active molecule AMDX-2011.

	Active
Study Drug Name (INN)	AMDX-2011P
Type	Retinal tracer (small molecule)
Dose Formulation	Solution for injection
Unit Dose Strength(s)	mg
Dosage Level(s)	• Cohort 1 – 25 mg (1 mL) • Cohort 2 – 50 mg (2 mL) • Cohort 3 – tentative 100 mg (4 mL) • Cohort 4 – tentative 150 or 200 mg (6-8 mL) For Cohorts 3-4, doses to be confirmed by SMC based on emerging data from prior cohorts. Intermediate and/or higher dose levels may be explored.
Route of Administration	IV bolus injection over 2 minutes
Use	Experimental
Sourcing	The drug product is manufactured by Pace (Salem, NH), and provided centrally by the Sponsor or subsidiary, or designee.
Preparation	Individual doses of AMDX-2011P will be prepared by pharmacy staff and will be provided to study personnel for administration. Procedures relating to study drug preparation will be outlined in the Pharmacy Manual. AMDX-2011P will be provided frozen (at -20°C) in a 10 mL amber vial containing 5.5 mL of solution at a concentration of 25 mg/mL. It is a single dose vial. Vials must not be shared between subjects. The solution is light sensitive and must be protected from light at all times. AMDX-2011P must be thawed to room temperature prior to administration; once thawed, it must be used within 3 hours.
Post administration instructions	Subjects to be instructed to avoid direct sun and wearing light protective eye gear such as sunglasses for 24 hours after the dose. Subjects will be evaluated for signs and symptoms of phototoxicity.
Packaging and Labeling	The study drug will be dispensed in single administration vials. Study drug will be labeled as required per local regulations. All packaging and labeling operations for the study drug will be performed according to

	Active
	current Good Manufacturing Practices and the relevant regulatory requirements.
Storage Condition	Study drug is to be stored in a secure freezer (-20°C) and monitored (manual or automated) in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug. Only subjects enrolled in the study may receive study drug, and only authorized site staff may administer study drug.

The investigator, or designee is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). A record will be maintained by the investigational site staff member that will account for all dispensing and return of any used and unused study drug. Study drug administration will be at the clinical site. At the end of the study, the study drug will be reconciled, and a copy of the record given to the study monitor.

Clinical supplies, including study drug and inventory records, must be made available upon inspection by Sponsor or regulatory agency upon request. The study monitors will review the records along with other study records during monitoring visits.

Upon completion of the study, and after completion of study drug accountability by the study monitor, any surplus supplies will be destroyed upon receipt of written approval from the Sponsor (following the site's destruction Standard Operating Procedures) and evidence of destruction supplied to the study monitor.

Further guidance and information for the final disposition of unused study drugs are provided in the relevant manuals.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This study is open-label, assessor masked. All retinal images will be sent in batches to a central reading center for masked assessment. Assessors will be masked to type of subjects, their disease status, dose, and sequence of post-injection images. Investigators will not be masked to study drug. Please see Section 8.1.4 for further details on the central reading center procedures.

6.4 STUDY DRUG COMPLIANCE

Study drug will be administered at the study site by the investigator or designee, as per the SoA (Section 1.3).

A record of the dose of AMDX-2011P administered to each subject must be maintained and reconciled with study drug and compliance records. Study drug start time will also be recorded in the electronic case report form (eCRF).

6.5 TREATMENT OF OVERDOSE

For this study, any dose of AMDX-2011P greater than the assigned dose will be considered an overdose.

There is no specific treatment recommended to treat an overdose of study drug, and the subject should receive treatment directed towards any symptoms manifested.

In the event of an overdose, the investigator/treating physician should:

1. Contact the Study Medical Monitor immediately.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities until AMDX-2011P can no longer be detected systemically (at least 48 hours).
3. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

6.6 CONCOMITANT THERAPY

Any medication or vaccine, including over-the-counter or prescription medicines, vitamins and/or herbal supplements, taken during the 7 days prior to the study drug administration will be recorded and reviewed by the investigator (or designee) to determine whether the subject is suitable for inclusion.

Treatment with another study drug, investigational device, or approved therapy for investigational use within 30 days (or five half-lives, whichever is longer) before anticipated dosing is prohibited.

The Study Medical Monitor (or designee) should be contacted if there are any questions regarding concomitant medications.

6.6.1 Prohibited Medications

Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing study this include:

- Use of any new prescription therapies and vaccines within 7 days prior to study drug administration.
- Use of drugs with potential phototoxicity per Package Insert are prohibited within 48-hrs or 5-half-lives, whichever is longer, prior to first study drug until EOS visit, except for those required for treatment of underlying disease and/or AEs. Examples of such drugs include the following: Chloroquine (Aralen), hydroxychloroquine (Plaquenil), Thioridazine (Mellaril), Topiramate (Topamax), vemurafenib, voriconazole,

doxycycline, hydrochlorothiazide, amiodarone, furosemide, allopurinol, phenothiazine, and chlorpromazine.

The investigator is expected to discuss any questions regarding prohibited medications with the Study Medical Monitor (or designee) and/or referring physician if applicable. The final decision on any supportive therapy rests with the investigator.

6.6.2 Rescue Medicine

Not applicable.

7 STUDY DRUG DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 STOPPING RULES

The SMC will monitor safety throughout the study. The SMC will meet periodically to review available safety data to make decisions regarding continuation, modification, suspension, or termination of the study.

Study Stopping Rules

Dose escalation or administration of study drug in a dose cohort may be stopped and additional subjects will not receive study drug until a consultation has taken place among the investigator, the Study Medical Monitor (or designee), and SMC if one of the following circumstances occurs in subjects treated with AMDX-2011P unless it is determined by the SMC in consultation with investigator and the Study Medical Monitor that the occurrence is not related to the administration of the study drug:

- Two or more subjects in a cohort experience a Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or higher clinical or laboratory abnormality that is considered to be related to study drug by the investigator.
- An AE or group of AEs that singularly or in aggregate suggests to the investigator or Sponsor that the study drug is poorly tolerated and further treatment per protocol may not be safe.
- If one or more subject(s) in a cohort experience any treatment-related, treatment-emergent SAEs, dose escalation will be stopped to re-evaluate the dose in question.

If any of these criteria occur, the SMC will review all available data; if an event is determined to be related to a specific dose level, doses in subsequent cohorts may be modified or dose escalation may be terminated. Prior to any required dose level adjustment, the clinical and laboratory safety parameters from the previous dosing cohort(s) will be reviewed and discussed by the SMC to allow a recommendation on advancing to the next dose level. The suggested next doses may be the protocol-defined escalation dose, an equivalent dose, an intermediate dose, or an adjusted-downward dose based on evaluation of safety and tolerability data from previous cohorts.

The Sponsor, investigator, and the IRBs reserve the right to terminate or suspend the study at any time; the reason for such decision must be recorded. Should this occur, all data available will also be recorded in eCRFs. The investigator should notify the IRB in writing of the study's completion or early termination.

7.1.1 Pregnancy

A female subject or a female partner of a male subject who becomes pregnant will be followed as described in Section 10.3.

7.2 Discontinuation of Study Drug

Not applicable; this is a single dose study.

7.3 Subject Withdrawal from the Study

- A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or compliance reasons.
- At the time of withdrawing from the study, if possible, an early termination visit should be conducted, as shown in the SoA (Section 1.3). 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 subject will be permanently discontinued from the study drug and the study at that time.
- If the subject 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 subject 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.

7.4 Lost to Follow-Up

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

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

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

8 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (Section 1.3). Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness.
- 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 subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (e.g., diagnostic status determination, ophthalmological examinations, blood count) and obtained before signing of the informed consent form may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.
- Where study procedures coincide at the same time point, order of collection should be retinal imaging, ECGs, vital signs, blood draws where possible, provided that PK samples are collected within permitted windows. When ECG and vital signs occur at the same time, the rest time may be shared by the assessments.

8.1 OPHTHALMOLOGICAL AND RETINAL IMAGING ASSESSMENTS

8.1.1 Ophthalmological Exam

Eye exam (both eyes) will include:

- Slit lamp examination of the anterior segment of the eye
- Refraction (manual, autorefractor, lensometer, or historical)
- Visual acuity with Snellen eye chart
- Ophthalmoscopy examination, which may include OCT imaging as part of routine clinical care
- Intraocular pressure (contact or non-contact tonometry)
- Color fundus photograph

8.1.2 Triplicate Fundus Fluorescence (FF) Imaging

FF imaging enables visualization of fluorescent signals in the retina. Conventional and routinely used FF retinal cameras have the appropriate technical specifications to detect the hyper-fluorescent signal generated by AMDX-2011P–bound amyloidogenic proteins.

Predose images will be captured using fundus autofluorescence (FAF) imaging. Immediately after IV injection with study drug, FAF images and/or videos will be continuously captured until 2 minutes post-injection to assess the influx of the dye into the retina. After that, the remainder of the static images will be captured using FAF imaging. FF images (excluding conventional fundus photos) at all time points will be captured with the Heidelberg Spectralis 55-degree lens and the FAF settings of the device, using the central fixation target. Additional FF images may also be captured using the Optos UWF imaging modality or offset (no central fixation target) 55-degree Heidelberg FAF modality at select time points. Complete instructions information will be provided in the imaging section of the Study Reference Manual. Images will be quality checked and exported per the respective retinal imaging device User Manual. All FF images will be prepared and transferred to a central reading center for review. Each FF image will be reviewed by the masked reading center assessors using the respective retinal imaging device's native review software. The images will be assessed for image quality based on the respective devices image quality criteria.

On Day 1, retinal imaging will be performed with both Spectralis and Optos devices in triplicate as shown in Table 5. Due to intensity of imaging, images taken outside the windows will not be considered protocol deviation.

Table 5 Collection Timepoints for Fundus Fluorescence (FF) Imaging

Day 1 Time point	Window	Spectralis	Optos
Predose		X	X
0-1 min		Video	
2 min		X	
5 min		X	
10 min	±2	X	X
30 min	±5	X	X
1hr	±10	X	X
2hr	±10	X	X
4hr	±10	X	X
8hr	±10	X	X

8.1.3 Optical Coherence Tomography (OCT) and OCT Angiography (OCT-A) Imaging

OCT is a non-contact imaging technique which generates high resolution cross-sectional images of the retina and its corresponding layers. OCT images will be acquired as specified in the SoA (Section 1.3). The Heidelberg Spectralis device will be used to capture volumetric structural

OCT and OCT-A images. The combination of FF and OCT/OCT-A images will enable for detection and mapping of amyloidogenic protein distribution across the fundus and across different retinal layers and retinal plexuses. For each imaging modality, OCT and OCT-A, the images will be assessed for image quality based on the respective device's image quality criteria.

8.1.4 Central Retinal Image Center (CRIC)

All acquired images will be checked for quality, and subjects' identifiers will be removed by study personnel at investigational site. Deidentified images will be sent to a central imaging center specialized in assessing retinal images for detection and quantification of hyper-fluorescent puncta. Each assessor at the CRIC will be masked to the subject's disease status and sequence of post-injection images. Images will be batched and read at time of the interim analysis and final analysis, or more frequently at the request of SMC. Upon receiving deidentified images from the clinical site, quality assurance (QA) officer at the CRIC will review all images to confirm that the images sent meet the quality, quantity, and image acquisition criteria defined in the protocol. The QA officer will confirm that the images are labeled appropriately and that they were obtained by a certified imager. Once the QA officer reviews and confirms the integrity of each image batch, the images will be randomized and submitted to the masked assessors for grading. At the time of the interim and final analyses, the randomized masked images submitted to the assessors will be evaluated for image quality and for the number of distinct hyper-fluorescent puncta. Additional information will be provided in the imaging section of the Study Reference Manual.

8.2 Safety and Other Assessments

Safety assessments will include physical examination, ophthalmologic examination, vital signs, electrocardiogram (ECG), and laboratory assessments. Adverse events and concomitant medications will be monitored throughout the study.

8.2.1 Physical Examinations

- A complete physical examination will include, at a minimum, assessment of the Cardiovascular, Respiratory, Gastrointestinal, and Neurological systems.
- Height and body weight will be measured and recorded at screening.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2 Vital Signs

- Systolic and diastolic blood pressure, heart rate, oral temperature, respiratory rate, and oximetry will be assessed.
- Blood pressure and heart rate measurements will be assessed using a completely automated device, with cuff wrapped around the non-dominant arm. Manual techniques will be used only if an automated device is not available.

- Subjects should be resting in a supine or semi-reclined position for at least 5 minutes prior to and during vital signs measurement obtained. When ECG and vital signs occur at the same time, the rest time may be shared by the assessments.
- Vital signs should be checked prior to blood draws when blood draws and vital signs occur at the same time.
- Additional vital signs may be performed at other times if deemed necessary.
- If deemed appropriate by the investigator, clinically significant findings in the vital signs will exclude a subject from study participation. Any abnormal finding related to vital signs that the investigator considers to be clinically significant must be recorded as an AE.

8.2.3 Electrocardiograms

- 12-lead ECGs will be obtained at the time points indicated in the SoA (Section 1.3) with the following parameters: HR, PQ (PR), QRS, QT, RR and QTcF along with information on T- and U-waves. Triplicate ECGs will be conducted on Day 1; single ECGs at all other time points.
- Subject to be resting in a supine position for at least 5 minutes prior to ECG. When ECG and vital signs occur at the same time, the rest time may be shared between the assessments.
- Investigator to record in eCRF if ECG is normal, abnormal (not clinically significant) or abnormal (clinically significant, with description of findings).
- In all cases in which an ECG has a potentially clinically significant finding, it will be repeated in triplicate within about 30 minutes and reviewed by the investigator or designee prior to subsequent dosing or study disposition decisions that do not constitute a subject emergency.
- Clinically significant findings in the ECG obtained at screening should exclude a subject from study participation (as deemed appropriate by the investigator). Any clinically significant change will be reported as an AE.
- Original paper tracings and tracing copies of the ECGs, including the interpretation, should be stored in the source documents. If ECG is thermal paper, it should be photocopied and then signed and dated since it fades over time.

8.2.4 Clinical Safety Laboratory Assessments

- See Section 10.2 for the list of clinical laboratory tests to be performed and the SoA (Section 1.3) for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically

significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

- All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or Study 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 Medical Monitor notified.
  - All protocol-required laboratory assessments, as defined in Section 10.2, must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).
  - If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE), then the results must be recorded in the eCRF.

8.3 PHARMACOKINETIC ASSESSMENT

Blood samples for determination of plasma concentrations of AMDX-2011P and AMDX-2011 will be collected as detailed in the SoA (Section 1.3). The timings of sample collection may be amended during the study conduct on the basis of emerging data to allow optimal characterization of the PK profile of AMDX-2011P and AMDX-2011.

Plasma concentrations of AMDX-2011P and AMDX-2011 will be measured by a specific and validated liquid chromatography-tandem mass spectroscopy (LC-MS/MS) method.

The following pharmacokinetic parameters (where appropriate) will be calculated by noncompartmental analysis from plasma concentrations of AMDX-2011P and for AMDX-2011 as defined in Table 6.

Table 6 Pharmacokinetic Parameters

Parameter	Definition
C0	Back-extrapolated concentration at time = 0 hr (AMDX-2011P only)
C_{max}	Maximum observed drug concentration
T_{max}	Time of the maximum drug concentration
$AUC_{0\text{-last}}$	Area under the drug concentration-time curve from time zero to the last measurable concentration
$AUC_{(inf)}$	Area under the drug concentration-time curve from time zero to infinity
$AUC_{(0\text{-}t)}$	Area under the drug concentration-time curve from time zero to time t
$t_{½}$	Apparent terminal half-life
λz	Apparent terminal elimination rate constant
CL	Systemic clearance (AMDX-2011P only)

Parameter	Definition
Vss	Steady-state volume of distribution (AMDX-2011P only)

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Events (AE)

An AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

AE severity will be evaluated by the investigator in accordance with the National Cancer Institute (NCI) CTCAE v5.0 [1]. For AEs that are not adequately addressed in the NCI CTCAE, the investigator should classify the intensity of the AE using the following guidelines:

- Grade 1: Mild: Aware of sign or symptom, but easily tolerated; no intervention needed
- Grade 2: Moderate: Discomfort enough to cause interference with usual activity, minimal non-invasive intervention indicated (e.g., short course of antibiotics)
- Grade 3: Severe: Medically significant but not immediately life-threatening; incapacitation with inability to work or do usual activity
- Grade 4: Life-threatening: Refers to an event in which the subject was at risk of death at the time of the event, as judged by the investigator; urgent/emergent intervention indicated. This category should not be used for an event that hypothetically might have caused death if it were more severe.
- Grade 5: Fatal outcome.

[1] Please refer to the CTCAE v5 at: *https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. Accessed March 30, 2022*

It will be left to the investigator's clinical judgment to determine whether an AE is of sufficient severity to require the subject's removal from treatment or from the study. A subject may also voluntarily withdraw consent from treatment due to what she/he perceives as an intolerable AE. If either of these situations arises, the subject should be strongly encouraged to undergo an EOS assessment and be under medical supervision until symptoms cease or the condition becomes stable. 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.

8.4.3.2 Relationship to Study Drug

All AEs must have their relationship to study drug assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical study, the study product must always be suspect.

Relationship Scale	Definition
Definite	A reaction that follows a reasonable temporal sequence from administration of the study drug or in which the study drug level has been established in body fluids or tissue; that follows a known or expected response pattern to the suspected study drug; and that is confirmed by improvement on stopping or reducing the dosage of the study drug, and reappearance of the reaction on repeated exposure.
Probable	A reaction that follows a reasonable temporal sequence from administration of the study drug; that follows a known or expected response pattern to the suspected study drug; that is confirmed by stopping or reducing the dosage of the study drug; and that could not be reasonably explained by the known characteristics of the subject's clinical state.
Possible	A reaction that follows a reasonable temporal sequence from administration of the study drug; that follows a known or expected response pattern to the suspected study drug; but that could readily be produced by a number of other factors.
Unlikely	An event that does not follow a reasonable temporal sequence from administration of the study drug; that does not follow a known or suspected response pattern to the suspected study drug; and that could reasonably be explained by known characteristics of the subject's clinical state.
Not Related	Any event that does not meet the above criteria.

8.4.3.3 Expectedness

The investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information provided in the IB.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate eCRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and will be reported in the Medical History eCRF page, not as an AE. However, if the study subject's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator will record all events with start dates occurring any time after informed consent is obtained until 7 days after the study drug administration for non-serious AEs or 30 days after the study drug administration for SAEs. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

Table 7 below summarizes the different reporting periods for AEs, SAEs, and pregnancy.

Table 7 Adverse Event Reporting Periods

Type of Event	Adverse Event	Serious Adverse Event / SUSAR	Pregnancy
Reporting period	From consent until 7 days after study drug administration or until AE is resolved or no further follow-up is required. (from consent to dosing, only collect AEs related to study procedures)	From consent until: 30 days after study drug administration or until SAE is resolved or no further follow-up is required	From dosing until 30 days after study drug administration
Reporting Timelines to the Sponsor	Entered into the clinical database on an ongoing basis	Within 24 hours of the investigator's knowledge	Within 24 hours of the investigator's knowledge
Reporting Method	AE eCRF	AE eCRF and SAE form	Pregnancy Form

Abbreviation: AE, adverse event (AE); eCRF, electronic case report form; SAE, serious adverse event ; SUSAR, serious unexpected suspected adverse reaction.

Events that begin before the start of study drug but after obtaining informed consent will be recorded on the AE section of the eCRF and will be identified in the analysis as non-TEAEs.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours from site awareness. The investigator will submit any updated SAE data to the Sponsor or designee within 24 hours of it becoming available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of a subject's study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has completed the study, and he/she considers the event to be related to the study drug or study participation, the investigator must promptly notify the Sponsor or designee.

8.4.6 Serious Adverse Event Reporting

The investigator will immediately report to the Sponsor any SAE, whether or not considered study drug-related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study drug caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study drug and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the Sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the subject is stable. Other supporting documentation of the event may be requested by Sponsor and should be provided as soon as possible.

The Sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical studies or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor determines that the information qualifies for reporting.

SAE reporting procedures

- The primary mechanism for reporting an SAE to the Sponsor will be the EDC system.
- Investigators will report all SAEs occurring during the study to the contract research organization (CRO) within 24 hours following the site's knowledge of the SAE. All SAEs will be reported using the Serious Adverse Event Report form (SAE Report) in the electronic data system, Medrio. In case that the outcome is death, the investigator will also contact the Medical Monitor via telephone as soon as possible.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.

- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information via contact to the Medical Monitor.
- If the electronic system is unavailable, then within 24 hours, the site will contact the Medical Monitor to report the event or email or fax the SAE form as detailed below.
- Contacts for SAE reporting: Safety@TrialRunners.com

8.4.7 Reporting Events to Subjects

Subjects will be informed about new risks and safety information through updated informed consent during the study.

8.4.8 Reporting of Pregnancy

- Details of all pregnancies in female subjects and of female partners of male subjects will be collected as outlined in Section 10.2.
- If a pregnancy is reported, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 10.3.3.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

9 STATISTICAL CONSIDERATIONS

9.1 SAMPLE SIZE DETERMINATION AND STATISTICAL HYPOTHESES

This clinical study is designed to explore safety and preliminary activity of AMDX-2011P. A total sample size of ~36 subjects and assuming approximately 10% subjects are false negative, the study will provide test sensitivity and test specificity of approximately 0.8 for each individual subject population and the pooled populations. The study will enroll up to 36 subjects with confirmed diagnosis of Parkinson's disease, MSA or ALS (approximately 12 subjects); the prevalence of disease is assumed at 0.5 for each individual disease population.

9.2 POPULATIONS FOR ANALYSES

Analysis Population	Definition
Safety Population	All enrolled subjects who receive any amount of study drug comprise the Safety Population for safety and exposure analyses.
Imaging Population	All enrolled subjects who received any amount of study drug and who have at least one set of acceptable color fundus photographs and OCT scans predose and at least 3 acceptable quality fundus autofluorescence images during each of the following imaging sessions: predose, postdose: 5 minutes to 1 hour.
Pharmacokinetic (PK) Population	All enrolled subjects who receive any amount of study drug and who have at least one post-baseline PK sample will comprise the PK Population.

Abbreviations: OCT, optical coherence tomography.

9.3 STATISTICAL ANALYSES

9.3.1 General Approach

A formal statistical analysis plan (SAP) and data management plan will be developed and finalized before study database lock. The following is a summary of statistical approach:

- Variables used for sample size calculation will be calculated and presented for pooled populations and each subject population.
- All disposition, demographic, baseline characteristic and concomitant medication summaries will be presented by arm.
- Continuous variables: Descriptive statistics will include the number of non-missing values (n), arithmetic mean, standard deviation (SD), median, minimum and maximum

values. 90% Confidence Intervals (CIs), least-square (LS Means) values and Ratio for Geometrics LS means will also be displayed when appropriate.

- Categorical variables: Descriptive statistics will include counts and percentages per category. The denominator in all percentage calculations will be the number of subjects in the relevant analysis population with non-missing data, unless specifically stated otherwise. Percentages will be displayed to one decimal place. Proportions will be displayed to 3 decimal places.

9.3.2 Safety Analyses

Continuous safety data will be summarized with descriptive statistics (arithmetic mean, standard deviation [SD], median, minimum, and maximum) overall, by treatment, and by nominal visit/time point (where applicable).

Categorical safety data will be summarized with frequency counts and percentages overall and within dose level. Adverse events will be coded by system organ class and preferred term using the most current Medical Dictionary for Regulatory Activities (MedDRA) version available. The number of subjects experiencing treatment-emergent adverse events as well as maximum severity and relationship to study drug will be summarized.

Laboratory evaluations, vital signs assessments, and ECG parameters will be summarized at protocol-specified collection time point. A summary of change-from-baseline at each protocol-specified time point will also be presented.

Concomitant medications will be listed by subject and coded using the most current World Health Organization drug dictionary.

Medical history will be listed by subject and coded using MedDRA.

Further details will be provided in the SAP.

9.3.3 Analysis of the Secondary Endpoint (Activity)

The number, diameter, and area of post-injection hyper-fluorescent puncta detected on fundus fluorescence images upon injection of AMDX-2011P that were not present prior to injection will be summed and descriptive statistics will be presented for each study eye per cohort.

A positive diagnosis is defined as detection of hyper-fluorescent puncta on fundus fluorescence imaging that were not present prior to injection of AMDX-2011P.

A false negative is defined as an eye from a subject with definite diagnosis and no new hyper-fluorescent puncta detected post-injection of AMDX-2011P via fundus fluorescence imaging that were not present prior to injection.

9.3.4 Pharmacokinetic Analysis

The actual blood sampling dates and times will be listed by subject and nominal sampling time, with time deviation calculated, for all subjects with available plasma concentration data, including subjects excluded from the PK population. Individual (for each subject) and arithmetic

mean concentrations over time will be displayed graphically on linear and semi-logarithmic axes, by treatment group. For individual subjects, the latter will include line segments indicating the range of data used to estimate the elimination rate constant (lz). The actual collection time will be used for individual plasma concentration curves, and the nominal time will be used for the plots of mean plasma concentration curves.

For PK concentration data, the arithmetic mean, standard deviation, median, minimum, maximum, and coefficient of variation (CV%) values will be presented. For the calculation of summary statistics, unrounded data will be used and reported to 3 significant figures except for n and CV%, which will be presented to the nearest integer and one decimal place, respectively.

For PK parameter data, the number of non-missing values, arithmetic mean, standard deviation, median, minimum and maximum, coefficient of variation (CV%), geometric mean and geometric coefficient of variation (geo CV%) values will be presented. Individual PK parameters will be presented to a minimum of 3 significant figures.

9.3.5 Sub-Group Analyses

Each disease will be analyzed as a subgroup in an exploratory manner. Further details will be provided in the SAP.

9.3.6 Tabulation of Individual Subject Data

All data collected on the eCRF will be presented in the data listings and will be listed, sorted by subject number and visit, where applicable.

9.3.7 Exploratory Analyses

Exploratory analyses will be defined in the SAP.

9.3.8 Interim Analyses

An exploratory interim analysis will be performed after the end of the 2nd cohort or when 12 subjects have been enrolled to assess safety, PK, and retinal image quality by SMC. Additional information will be provided in the SAP.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 Informed Consent Process

10.1.1.1 Consent/assent and Other Informational Documents Provided to Subjects

Consent forms describing in detail the study drug, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study drug. Master informed consent will be submitted with this protocol.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved, and the subject will be asked to read and review the document. The investigator (or designee) will explain the research study to the subject and answer any questions that may arise. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The subject will sign the informed consent document prior to any procedures being done specifically for the study. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the signed informed consent document will be given to the subjects for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Cognitively impaired subjects will sign an assent form; their legally authorized representative will also sign this form.

In the event a subject is unable to physically sign the informed consent form, an independent witness should be present for the entire consent discussion. The subject should read the consent; if this is not possible due to disability, the consent should be read to the subject. The discussion should allow ample time for the subject to ask questions and for the person obtaining informed consent to assess subject understanding. The subject may verbally provide consent; such verbal consent must be noted in the subject's source documents and by hand on the consent document. The independent witness signs and personally dates the consent form. By doing so, the witness attests that the consent information was accurately explained and that the subject apparently understood and informed consent was given freely.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the Sponsor to investigator, funding agency, and the IND. If the study is prematurely terminated or suspended, the investigator will promptly inform study subjects, the IRB, and Sponsor and will provide the reason(s) for the termination or suspension. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB and/or FDA.

10.1.3 Confidentiality and Privacy

Subject confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the Sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies, or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the CRO. This will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used

by clinical sites and by the CRO research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the CRO.

10.1.4 Key Roles and Study Governance

Refer to the Study Reference Manual.

10.1.5 Safety Oversight – Safety Monitoring Committee

An SMC, appointed by the Sponsor and comprised of the Study Medical Monitor (or designee), neurologists with experience in these diseased populations, an ophthalmologist with retinal imaging expertise, and the Sponsor's clinical and technical representatives, will be established before start of screening in the study. Details for the SMC will be outlined in a separate SMC Charter, the governing document that will supersede this section of the protocol. Composition of the SMC, meeting structure, schedule, and procedures, the content and format of SMC reports, and other relevant details will be determined in consultation with SMC members and detailed in a separate SMC charter.

Safety data to be reviewed by the SMC will be shared in the format of a Safety Data Review Summary. The summary will include details on any clinically significant events experienced on study related to safety parameters, with a table listing of the aggregate of AEs reported on the study. The SMC authorizes escalating the dose and continued enrolment in subsequent cohorts based on all available safety, PK and/or imaging data during the safety review.

At each decision stage, the SMC will review the Safety Data Review Summary and PK data (if available), and together make one of the following recommendations:

- To continue the study as planned, e.g. enroll the next cohort at the planned dose.
- To continue the study with modifications, e.g. modify the next dose, enroll additional subjects into current cohort, or add additional cohorts to the study
- To temporarily suspend or terminate the study. If at any time the study is terminated, a written statement fully documenting the reasons for study termination will be provided to the IRB.

10.1.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of study subjects are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with ICH-GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the CRO.
- The electronic database will be 100% source verified.
- Monitoring visits will be onsite or remote at the discretion of the Sponsor and monitor dependent on enrollment and risk-based metrics.

- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits will be conducted by the CRO to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

10.1.7 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical study is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH-GCP, and applicable regulatory requirements (e.g., GLP, Good Manufacturing Practices [GMP]).

The investigational site will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities.

10.1.8 Data Handling and Record Keeping

10.1.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical study staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each subject enrolled in the study. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into EDC, a 21 CFR Part 11-compliant data capture system provided by the CRO. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

10.1.9 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol or International Council for Harmonisation Good Clinical Practice (ICH GCP) requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH-GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations in a timely manner. All deviations must be addressed in study source documents, reported to Sponsor. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the study manuals.

10.1.10 Publication and Data Sharing Policy

All information supplied by the Sponsor or their representative in association with this study and not previously published, is considered confidential information. Any data collected during the study are also considered confidential. This confidential information shall remain sole property of the Sponsor, shall not be disclosed to others without the written consent of the Sponsor, and shall not be used except in the performance of this study.

The Sponsor follows local regulatory requirements relating to clinical study registration and results disclosure. Thus, this study will be registered on the ClinicalTrials.gov, and results from this study will be submitted to ClinicalTrials.gov. In addition, any other presentation/publication of complete/partial study data by the investigators or any other party is stipulated by written authorization from the Sponsor.

10.1.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who

have a role in the design, conduct, analysis, publication, or any aspect of this study will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this study. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 CLINICAL LABORATORY TESTS

- The tests detailed in Table 8 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.
- Investigators must document their review of each laboratory safety report in subject's source records.

Table 8 Protocol-Required Safety Laboratory Assessments

Laboratory	Parameters
Serum Chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and conjugated bilirubin, alkaline phosphatase (ALP), gamma-glutamyl-transferase (g-GT), creatine phosphokinase (CPK), albumin, creatinine, urea, total protein, sodium, chloride, calcium, phosphate, potassium, triglycerides, total cholesterol, glucose (fasting)
Hematology	Hemoglobin, hematocrit, erythrocytes (RBC), reticulocytes, platelets, leukocytes (WBC), differential (counts): neutrophils, basophils, eosinophils, lymphocytes, and monocytes
Coagulation	International normalized ratio (INR), activated partial thromboplastin time (aPTT), prothrombin time (PT)
Urinalysis	Protein, glucose ketones, bilirubin, urobilinogen, hemoglobin, pH, specific gravity, appearance, color, leukocyte esterase, nitrite Microscopic tests (bacteria, erythrocytes, leucocytes, crystals, casts, epithelial cells, yeast, oval fat bodies, fat, mucous, sperm, trichomonas)
Other laboratory assessments	Human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)

10.3 CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

10.3.1 Definitions

Woman of Childbearing Potential (WOCBP)

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

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study drug, 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, (e.g., mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

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

Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
  - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

10.3.2 Contraception Guidance

Male Subjects

Male subjects are eligible to participate if they agree to the following from informed consent through 90 days after study drug administration:

- Refrain from donating sperm, except for the purpose of fertility analysis as part of this protocol

  PLUS, either**:**

- Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

OR

- Must agree to use double barrier contraception (a male condom with spermicide)

Female Subjects

- A female subject is eligible to participate if she is not pregnant or breastfeeding, and at least one of the following conditions applies:
  - Is not a woman of childbearing potential (WOCBP)

OR

  - Is a WOCBP and using a contraceptive method that is highly effective (with a failure rate of < 1% per year), preferably with low user dependency (see table below), from consent through 30 days after study drug administration, and agrees not to donate eggs (ova, oocytes) for the purpose of reproduction during this period. The investigator should evaluate the effectiveness of the contraceptive method in relationship to the first dose of study drug.
  - A WOCBP must have negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to receiving the first administration of study drug and a negative urine pregnancy test before first administration of study drug. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test is required and results must be negative.

Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies.

Highly Effective Methods[a] That Have Low User Dependency
• Implantable progestogen-only hormone contraception associated with inhibition of ovulation[b]
• Intrauterine device (IUD) (hormonal and non-hormonal)
• Surgical sterilization
• Bilateral tubal occlusion or ligation
• Vasectomized partner *(Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.)*
Highly Effective Methods[a] That Are User Dependent
• Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation – oral – intravaginal – transdermal – injectable
• Progestogen-only hormone contraception associated with inhibition of ovulation[b] – oral – injectable
• Sexual abstinence *(Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.)*

[a] Failure rate of < 1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly.
[b] If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.
Note: Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception for this study. Male condom and female condom should not be used together (due to risk of failure with friction).

10.3.3 Collection of Pregnancy Information

Male subjects with partners who become pregnant

- The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study.
- 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 Subjects who become pregnant

- The investigator will collect pregnancy information on any female subject 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 subject's pregnancy.
- The subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject 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 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 <22 weeks gestational age) or still birth (occurring at >22 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 drug by the investigator will be reported to the Sponsor as described in Section 8.4.8. While the investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue study drug or be withdrawn from the study.

10.4 NEURODEGENERATIVE DISEASES DIAGNOSTIC CRITERIA

Below are diagnostic criteria for neurodegenerative diseases evaluated in this study.

10.4.1 Parkinson's Disease Diagnostic Criteria

Table 9 Movement Disorder Society (MDS) Diagnostic Criteria for Clinically Established Parkinson's Disease

Parkinsonism consist of bradykinesia plush either rigidity or rest tumor, as defined below		
Bradykinesia: slowness of movement AND decrement in amplitude or speed (or progressive hesitations/halts) as movements are continued	**Rigidity**: velocity-independent resistance to passive movement not solely reflecting failure to relax (i.e., distinct from spasticity or paratonia)	**Rest tremor:** a 4- to 6-Hz tremor in the fully resting limb, which is suppressed during movement initiation
Clinical established Parkinson's disease is defined as: Absence of absolute exclusion criteria; at least 2 supportive criteria; no 'red flags' (as defined below)		
Absolute Exclusion Criteria	**Red Flags**	**Supportive Criteria**
• Cerebellar signs • Supranuclear gaze palsy • Established diagnosis of BVFTD • Parkinsonism restricted to the lower limbs only for >3 years • Treatment with an antidopaminergic, or with dopamine-depletion agents • Absence of response to levodopa • Sensory–cortical loss • No evidence for dopaminergic deficiency on functional imaging • Other parkinsonism-inducing condition	• Rapid deterioration of gait • Absence of motor symptom progression over 5 years • Early bulbar dysfunction • Respiratory dysfunction • Early severe autonomic failure • Early recurrent falls due to misbalance • Disproportionate anterocollis • Absence of common non-motor features of disease during >5 years • Pyramidal tract signs • Bilateral symmetric presentation	• A clear and dramatic positive response to dopaminergic therapy • Levodopa-induced dyskinesia • Documentation of resting tremor of a limb • A positive diagnostic test of either olfactory loss or cardiac sympathetic denervation on scintigraphy

Abbreviation: BVFTD, behavioral variant frontotemporal dementia.
Source: (Postuma, 2015)

Table 10 Parkinson's Disease Criteria per Hoehn and Yahr (1967)

Stage	Hoehn and Yahr Scale
1	Unilateral involvement only usually with minimal or no functional disability
2	Bilateral or midline involvement without impairment of balance
3	Bilateral disease: mild to moderate disability with impaired postural reflexes; physically independent
4	Severely disabling disease; still able to walk or stand unassisted
5	Confinement to bed or wheelchair unless aided

Source: (Hoehn, 1967)

10.4.2 MSA Diagnostic Criteria

Division into clinically established MSA-P or MSA-C according to predominant motor syndrome

Essential features	A sporadic, progressive adult (>30 years) onset disease		
	Clinically established MSA	**Clinically probable MSA**	
Core clinical features	1. Autonomic dysfunction defined as (at least one is required) ○ Unexplained voiding difficulties with post-void urinary residual volume ≥100 mL ○ Unexplained urinary urge incontinence ○ Neurogenic OH (≥20/10 mmHg blood pressure drop) within 3 minutes of standing or head-up tilt test and at least one of 1. Poorly L-dopa-responsive parkinsonism 2. Cerebellar syndrome (at least two of gait ataxia, limb ataxia, cerebellar dysarthria, or oculomotor features)	At least two of: 1. Autonomic dysfunction defined as (at least one is required): ○ Unexplained voiding difficulties with post-void urinary residual volume ○ Unexplained urinary urge incontinence ○ Neurogenic OH (≥20/10 mmHg blood pressure drop) within 10 minutes of standing or head-up tilt test 2. Parkinsonism 3. Cerebellar syndrome (at least one of gait ataxia, limb ataxia, cerebellar dysarthria, or oculomotor features)	
Supportive clinical (motor or non-motor) features	At least two	At least one[a]	
MRI marker	At least one	Not required	
Exclusion criteria	Absence	Absence	
Supportive clinical features			
Supportive motor features	Rapid progression within 3 years of motor onset Moderate to severe postural instability within 3 years of motor onset Craniocervical dystonia induced or exacerbated by L-dopa in the absence of limb dyskinesia Severe speech impairment within 3 years of motor onset Severe dysphagia within 3 years of motor onset Unexplained Babinski sign Jerky myoclonic postural or kinetic tremor Postural deformities	**Supportive non-motor features**	Stridor Inspiratory sighs Cold discolored hands and feet Erectile dysfunction (below age of 60 years for clinically probable MSA) Pathologic laughter or crying
MRI markers of clinically established MSA			
Each affected brain region as evidenced by either atrophy or increased diffusivity counts as one MRI marker.			
For MSA-P • Atrophy of: ○ Putamen (and signal decrease on iron-sensitive sequences) ○ Middle cerebellar peduncle ○ pons ○ Cerebellum • "Hot cross bun" sign		For MSA-C • Atrophy of: ○ Putamen (and signal decrease on iron-sensitive sequences) ○ Infratentorial structures (pons and middle cerebellar peduncle) • "Hot cross bun" sign	

Continued

• Increased diffusivity of: ○ Putamen ○ Middle cerebellar peduncle	• Increased diffusivity of: ○ Putamen
Exclusion criteria	
Substantial and persistent beneficial response to dopaminergic medications	
Unexplained anosmia on olfactory testing	
Fluctuating cognition with pronounced variation in attention and alertness and early decline in visuoperceptual abilities	
Recurrent visual hallucinations not induced by drugs within 3 years of disease onset	
Dementia according to DSM-V within 3 years of disease onset	
Downgaze supranuclear palsy or slowing of vertical saccades	
Brain MRI findings suggestive of an alternative diagnosis (eg, PSP, multiple sclerosis, vascular parkinsonism, symptomatic cerebellar disease, etc.)	
Documentation of an alternative condition (MSA look-alike, including genetic or symptomatic ataxia and parkinsonism) known to produce autonomic failure, ataxia, or parkinsonism and plausibly connected to the patient's symptoms	

Source: (Wenning, 2022)

10.4.3 ALS Diagnostic Criteria

Table 11 ALS Diagnostic Criteria

Definite ALS	Presence of upper motor neuron and lower motor neuron signs in the bulbar region and at two of the other spinal regions
Probable ALS	Presence of upper motor neuron and lower motor neuron signs in at least two regions with upper motor neuron signs rostral to lower motor neuron signs
Probable ALS, laboratory results supported	Presence of upper motor neuron and lower motor neuron signs in one region with evidence by EMG of lower motor neuron involvement in another region
Possible ALS	Presence of upper motor neuron and lower motor neuron signs in one region or upper motor neuron signs in two or three regions, such as monomelic ALS, progressive bulbar palsy, and primary lateral sclerosis

ALS, amyotrophic lateral sclerosis; EMG, electromyography.

Source: (Brooks, 2000)

10.5 ABBREVIATIONS

Term	Description
α-syn	alpha-synucleinopathies
AE	Adverse event
ALS	Amyotrophic lateral sclerosis
AUC	Area under the curve
CFR	Code of Federal Regulations
CI	Confidence interval
C_{max}	Peak plasma concentration
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CRIC	Central Retinal Image Center
CRO	Clinical Research Organization
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
DaTscan	Dopamine transport scan
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EOS	End-of-study
ET	Early termination
FAF	Fundus autofluorescence
FDA	Food and Drug Administration
FF	Fundus fluorescence
FIH	First-in-human
FSH	Follicle-stimulating hormone
FU	Follow-up
GCP	Good Clinical Practice
geo CV	geometric coefficient of variation
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HED	Human equivalent dose
hERG	Human Ether-à-go-go-related gene
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities

Term	Description
MRI	Magnetic resonance imaging
MRSD	Maximum recommended starting dose
NCI	National Cancer Institute
NOAEL	No observed adverse effect level
OCT	Optical coherence tomography
OCT-A	OCT angiography
PET	Positron emission tomography
PK	Pharmacokinetic
PROBE	Prospective Randomized Open, Blinded Endpoint
QA	Quality assurance
QC	Quality control
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SMC	Safety Monitoring Committee
SoA	Schedule of activities
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TDP-43	Transactive response DNA-binding protein 43
US	United States
WOCBP	Woman of childbearing potential

10.6 PROTOCOL AMENDMENT HISTORY

Prior amendments are listed below in order of occurrence. Current amendment is provided in section above table of contents.

Original	10 May 2022

Amendment 1.0, 15 June 2022

Overall Rationale for the Amendment:

Changes are shown in order of appearance.

Section # and Name	Description of Change	Brief Rationale
1.3 SoA	• Add vital signs measurements on Day 1 at 15 and 30 minutes postdose	• Per request of the IRB
5.1 Inclusion criteria 10.1.1 Informed Consent Process	• Clarify that legally authorized representative will also sign assent form for cognitively impaired subjects	• Per request of the IRB
Global	• Ensure consistency across protocol including typo corrections • Correct formatting and style • Add summary of changes page • Update footer and title page with date/amendment number	• For clarity and consistency

Amendment 2.0, 01 July 2022

Overall Rationale for the Amendment:

Changes are shown in order of appearance.

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 4 Study Design 9.1 Sample Size Determination	• Add 2 sentinel healthy volunteers to cohorts 1-4 • Adjust sample size	• To enhance the safety of participants at each cohort and maintain the blind at the imaging center.
8.1.1	• Indicate that visual acuity will be done by Snellen eye chart	• For clarification
10.2 Clinical Laboratory Tests	• Replace central laboratory with local laboratory	• For logistical and operational reasons

Section # and Name	Description of Change	Brief Rationale
Global	• Ensure consistency across protocol including typo corrections • Correct formatting and style • Move prior amendment to Section 10.6 • Update footer, title page and signature page with date/amendment number	• For clarity and consistency

Amendment 3.0, 26 July 2022

Overall Rationale for the Amendment:

Changes are shown in order of appearance.

Section # and Name	Description of Change	Brief Rationale
Protocol title 1.1 Synopsis 1.2 Schema 1.3 Schedule of Activities 2. Introduction 3 Objectives and Endpoints 4 Study Design 5 Study Population 6 Study Drug 9 Statistical Considerations 10.1.5 Safety Oversight – Safety Monitoring Committee	• Remove healthy volunteers participation in the study	• As a safety precaution per FDA request based on preliminary nonclinical finding requiring additional in vitro testing
2.3.1 Known potential risks 6.1.1 Study Drug Description	• Add that due to potential phototoxicity, subjects will be instructed to avoid direct sun and wear protective gear for 24 hours after dosing. • Add mutagenicity as a potential risk	• As a safety precaution per FDA request
6.1.1 Study Drug Description	• Clarify study drug is a single dose vial not to be shared between subjects.	• To align with the investigator's brochure

Section # and Name	Description of Change	Brief Rationale
Global	• Update protocol to ensure consistency across protocol • Correct formatting and style • Move prior amendment to Section 10.6 • Update footer, title page and signature page with date/amendment number	• For clarity and consistency

Amendment 4.0, 16 September 2022

Overall Rationale for the Amendment:

Changes are shown in order of appearance.

Section # and Name	Description of Change	Brief Rationale
1.3 Schedule of Activities 8.1.2 Retinal fundus fluorescence (FF) imaging	• Clarify timing of when assessments will be performed	• For clarity
1.1 Synopsis 4.1 Study design 9.1 Sample Size Determination	• Indicate that approximately 12 ALS subjects will be enrolled	• For clarity
10.2 Clinical Laboratory Tests	• Delete urine test for drugs of abuse	• This assessment is not conducted in the studied subject population
Global	• Update protocol to ensure consistency across protocol • Correct formatting and style • Move prior amendment to Section 10.6 • Update footer, title page and signature page with date/amendment number	• For clarity and consistency

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