Statistical Analysis Plan

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

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Protocol details:

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Review and Approval

The undersigned have approved this Statistical Analysis Plan for use in this study.

Trial Runners, LLC Approval:

Andrew Mao **Biostatistician**	**Date**
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Amydis, Inc Approval:

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1 INTRODUCTION

This statistical analysis plan (SAP) provides a comprehensive and detailed description of analyses and reporting of data for study protocol AMDX-2011P-101P Version 1.0-Master containing sub-study protocols: AMDX-2011P-001 Version 1.0 and AMDX-2011P-101 Version 5.0, Amendment 5.0. The purpose of the SAP is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of study data prior to data base lock.

1.1 Changes from Protocol

The visit window for analysis was changed from +2 days to +6 days.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary study objective is:

- to assess safety and tolerability of single IV dose of AMDX-2011P in subjects with neurodegenerative diseases (Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), multiple system atrophy (MSA), and Cerebral Amyloid Angiography (CAA)).

2.2 Secondary Objective

The secondary study objectives are:

- to assess the ability of AMDX-2011P to identify α-syn in retina of subjects with Parkinson's disease and MSA.
- to assess the ability of AMDX-2011P to identify TDP-43 in retina of subjects with ALS.
- to assess the ability of AMDX-2011P to identify Amyloid Beta in the retina of CAA subjects.
- 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, ALS, MSA, and CAA).

3 STUDY DESIGN

This is a Phase I PROBE basket design study to evaluate safety, tolerability, and the ability of AMDX-2011P to detect amyloid deposits, α-syn, TDP-43, and Amyloid Beta through retinal examination, as well as PK of single bolus IV of AMDX-2011P in subjects with Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Cerebral Amyloid Angiopathy (CAA), and multiple system atrophy (MSA). The study contains two sub-studies under separate protocols.

List of sub-studies included in Master Protocol Basket Design:

Study Protocol	Study Population	Protocol Appendix for further details	Dose Levels	Number of Cohorts	Subjects Per Cohort

Study AMDX-2011P-001 Version 1.0	Cerebral Amyloid Angiopathy (CAA)	Section 7.1	50 mg (2mL), 100 mg, 150 or 200 mg (tentative), Cohort 4 (TBD)	4	3-5
Study AMDX-2011P-101 Version 5.0, Amendment 5.0	Amyotrophic Lateral (ALS) / Parkinson's Disease, Multiple System Atrophy (MSA)	Section 7.2	25 mg (1mL), 50 mg (2mL), 100 mg (4 mL), 150 mg or 200 mg (6-8 mL)	4	3-9 for cohorts 1-2, 12 for cohorts 3-4

Approximately 9 subjects will be enrolled in each of the ALS and MSA studies. Additional subjects may be enrolled into any cohort per DSMB recommendation for CAA sub-study and all sub-study 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 into that cohort. Tentative doses will be determined and confirmed by DSMB based on emerging data from prior cohorts. Additional cohorts may be explored per DSMB recommendations.

3.1 Sample Size Considerations

A total sample size of approximately 36 subjects and assuming approximately 10% subjects are false negative, the sub-study will provide test sensitivity of at least 0.8 for each individual subject population and the pooled populations in protocol AMDX-2011P-101 Version 5.0, Amendment 5.0. The study will enroll approximately 36 subjects with confirmed diagnosis of Parkinson's disease, ALS, or MSA; the prevalence of disease is assumed at 0.75 for pooled population and 0.5 for each individual disease population. For the CAA group, a total sample size of up to 20 subjects with confirmed diagnosis of CAA, and assuming approximately 10% subjects are false negative, the sub-study will provide test sensitivity of approximately 0.8 for the target population in protocol AMDX-2011P-001.

3.2 Randomization

The study is open-label and cohort assignment will be based on current dose level and disease status.

3.3 Schedule of Evaluations and Analysis Visit Windows

The schedule of visits and procedures are provided in the study protocol AMDX-2011P-101, Version 5.0, Amendment 5, Section 1.3 and AMDX-2011P-001, Version 1.0, Section 1.3. The study consists of a screening visit, treatment day, and 1 follow-up visit. For purposes of analysis, nominal time points will be used in data summarizations. The following target windows are expected for each visit:

Visit	Target Day of Visit	Visit Window (Days) for Analysis
Screening	-42 to -1	-42 to -1
Treatment (Day 1)	1	1
Follow-up (Day 8)	8	8-14

4 ANALYSIS POPULATIONS

4.1 Safety Population

The Safety (SAF) Population includes all enrolled subjects who receive any amount of study drug. The Safety Population will be used for the safety and exposure analyses.

4.2 Imaging Population

The Imaging (IMG) Population includes all enrolled subjects who receive any amount of study drug and who have at least one set of acceptable color fundus photographs and OCT scans pre-dose and at least 3 acceptable quality fundus autofluorescence images during each of the following imaging sessions: pre-dose, post-dose: 5 minutes to 1 hour. Acceptability of the images will be determined as defined in the Imaging Manual.

4.3 Pharmacokinetic (PK) Population

The Pharmacokinetic (PK) Population includes all enrolled subjects who receive any amount of study drug and who have at least one post-baseline PK measurable samples.

5 STUDY VARIABLES/PARAMETERS

5.1 Primary (Safety) Endpoints

The primary endpoint is defined to be:

- Incidence, nature, and severity of treatment-emergent adverse events (TEAEs)
- Incidence, nature, and severity of serious adverse events (SAEs)

5.2 Secondary Endpoints

The secondary endpoints are defined to be:

- Detection of α-syn (numbers per field) in the retina after AMDX-2011P administration by OCT/OCT A and Fundus Fluorescence
- Detection of TDP-43 (numbers per field) in the retina after AMDX-2011P administration by OCT/OCT A and Fundus Fluorescence
- Detection of Amyloid Beta (numbers per field) in the retina after AMDX-2011P administration by OCT/OCT A and Fundus Fluorescence
- The PK parameters of AMDX-2011P and AMDX-2011 calculated from concentrations in plasma through blood sampling

5.3 Pharmacokinetic Endpoints

The following PK parameters will, where possible, be determined (to a precision minimum of 3 significant figures):

Parameter	Definition
C_0 (ng/mL)	Extrapolated concentration at time equal to zero
C_{max} (ng/mL)	Maximum observed drug concentration
T_{max} (hr)	Time of the maximum drug concentration

Parameter	Definition
$AUC_{0\text{-last}}$ (hr*ng/mL)	Area under the drug concentration-time curve (AUC) from time zero to the last measurable concentration.
AUC_{INF} (hr*ng/mL)	AUC from zero extrapolated to infinity
$AUC_{(0\text{-t})}$	AUC from time zero to time t
CL/F (L)	Apparent total body clearance following IV administration
$t_{½}$ (hr)	Apparent terminal half-life
λz (1/hr)	Apparent terminal elimination rate constant
Vss (L)	Steady-state volume of distribution

6 STATISTICAL ANALYSIS METHODS

No formal statistical tests or inference will be performed. All summaries will be presented using descriptive statistics for each cohort, and overall. Data processing, summarization, and analyses will be performed using SAS Version 9.4 or higher. Unless otherwise discussed, continuous variables will be summarized by the number of available data points (N), mean, standard deviation, median, minimum, and maximum. 90% confidence intervals, least-square means, and ratio for geometrics LS means will be displayed when appropriate. The mean, median, minimum, and maximum will be displayed with the same number of decimals as raw data, and the standard deviation will be displayed with two more decimals than raw data. Categorical variables will be summarized by the number and percentage of subjects in each level, where the denominator for each percentage is the number of subjects in the relevant population and specified cohort. Percentages will be displayed to one decimal place and proportions will be displayed to three decimal places. Data listings of all data collected on the eCRF will be presented, sorted by subject number and visit, where applicable.

6.1 Subject Disposition

The number and percentage of subjects screened, enrolled, cohort assignment, completed treatment, completed study, and analysis populations will be summarized for each cohort, by neurodegenerative disease population, and overall. The time in study (in days) and follow-up time will also be summarized for each cohort and overall.

6.2 Protocol Deviations

The number and percentage of protocol deviations will be presented by cohort and overall.

6.3 Missing Data and Imputation

There will be no imputation of missing data.

6.4 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized for each cohort and overall, including age (years), sex (male, female), child-bearing potential? (Yes, No: bilateral oophorectomy, bilateral tubal ligation, hysterectomy, post-menopausal, and other), contraception (Yes, No), ethnicity (Hispanic or

Latino, Not Hispanic or Latino), Race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, Other), height (inches), weight (lbs), and iris color (Blue, Green, Brown, Black, Grey, Hazel, Other).

6.5 Prior and Concomitant Medications

Prior and concomitant medications will be listed by subject and coded using the Word Health Organization (WHO) drug dictionary, Version September 2021, for the Safety (SAF) Population. Any medication or vaccine taken 7 days prior to study drug administration will be recorded. The number and percentage of subjects who reported taking prior and/or concomitant medications will be summarized overall. Individual subject prior and concomitant medications data will be listed.

6.6 Medical History

Medical history will be listed by subject and coded using the MedDRA, Version 25.0, using System Organ Class and Preferred Term for the Safety Population. Individual subject medical history data will be listed.

6.7 Primary (Safety) Analysis

The primary analysis for this study will assess the safety of study drug, AMDX-2011P. Adverse Events will be coded by System Organ Class and Preferred Term using MedDRA, Version 25.0. The number and percentage of subjects experiencing treatment-emergent adverse events, serious adverse events, maximum severity to study drug, and highest relationship to study drug will be summarized by cohort and listed by subject ID for both eyes. Treatment emergent adverse events and serious adverse events leading to discontinuation will also be presented by count. Treatment emergent adverse events are defined as any AEs with an onset date on or after the date of the first dose of the investigational product or is pre-existing and worsens after administration. TEAEs severity will be measured by the severity scale: mild, moderate, severe, life-threatening, and fatal. The degree of certainty of causality will be graded using the following categories: definite, probable, possible, unlikely, and not related. A line listing of all treatment emergent adverse events will be provided including as reported verbatim term, SOC, PT, onset date, end date, severity, seriousness, causality, dose level, outcome and action taken with investigational product. Subjects with 1 or more than one TEAE per system organ class and preferred term will be counted once and be reported with the highest severity. In cases where severity or relationship are missing, the most conservative approach will be taken (i.e., highest severity and assumed to be related). Laboratory evaluations, vital sign assessments, and ECG parameters will be summarized using appropriate statistics at protocol-specified time points, as well as change-from-baseline at all protocol-specified post-baseline timepoints. Non-ocular adverse events will be summarized separately and listed by subject ID for both eyes.

6.7.1 Electrocardiogram (ECG)

Baseline, post-dose, and change from baseline for ECG parameters (heart rate (HR), RR interval, QRS interval, PR interval, QT interval, and QTcF) along with clinically significant abnormalities on T and U waves will be presented by cohort and overall. Triplicate ECGs will be conducted on Day 1 (at pre-dose, 90 minutes post-dose, and 8 hours post dose) and single ECGs at other timepoints. Results will also be listed by subject ID.

Clinically significant findings will be presented based on the following categories:
• The proportion of patients obtaining a heart rate change from baseline > 25% decrease resulting in a heart rate < 50 beats per minute (bpm) and heart rate change from baseline > 25% increase resulting in a heart rate > 100 bpm.
• The proportion of patients obtaining a QRS change from baseline > 25% increase resulting in QRS > 120 ms.
• The proportion of patients obtaining a PR interval change from baseline > 25% increase reaching a value of > 220 ms.
• The proportion of patients obtaining a QTcF increase from baseline values > 30 ms and ≤ 60 ms; and > 60 ms.
• The proportion of patients obtaining treatment emergent absolute QTcF values > 450 ms and ≤ 480 ms; > 480 ms and ≤ 500 ms; and > 500 ms.

6.7.2 Physical Exam

Complete physical exam at screening includes assessment of height (in/cm), weight (lb/kg) at screening. General appearance, cardiovascular, respiratory, head, eyes, ears, nose, throat, neurologic, abdomen will be assessed on a scale of normal, abnormal (NCS), abnormal (CS), and not done.

6.7.3 Vital Signs

Vital signs assessment include systolic blood pressure (mmHG), diastolic blood pressure (mmHG), temperature (F), heart rate (beats/min), and respiration (per/minute) for each protocol specified timepoint.

6.7.4 Clinical Laboratory Assessments

Clinical laboratory assessments including hematology, chemistry, coagulation, and urinalysis will be summarized by cohort and overall for each protocol specified timepoint.

6.7.5 Intraocular Pressure

Intraocular pressure (mmHG) using contact or non-contact tonometry will be assessed and summarized for both eyes for each protocol specified timepoint.

6.8 Secondary Analysis

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 neurodegenerative disease population (e.g PD, ALS, MSA, CAA) and dose levels.

- Detection of α-syn in the retina after AMDX-2011P administration, defined as the number of distinct new spots that are visible to the naked eye post-injection only.
- Detection of TDP-43 in the retina after AMDX-2011P administration, defined as the number of distinct new spots that are visible to the naked eye post-injection only.

- Detection of Amyloid Beta in the retina after AMDX-2011P administration, defined as the number of distinct new spots that are visible to the naked eye post-injection only.
- PK parameters of AMDX-2011P and AMDX-2011 calculated from concentrations in plasma.

Diagnostic test summary statistics will be presented overall. Calculated values will include:

- A true 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.

6.9 Pharmacokinetic Analysis

PK Data Reporting and BLQ Imputation:

Individual plasma concentration data will be listed and summarized by cohort and dose levels / nominal time point with descriptive statistics (sample size [N], arithmetic mean, standard deviation [SD], median, minimum, maximum, geometric mean, and percent coefficient of variation [CV%]). Only concentration data collected within window of the scheduled timepoint will be included in summary tables and figures, however all data will be provided in data listings. Individual concentration data and descriptive statistics will be presented to 3 significant figures, except N, which will be reported as whole number CV% will be reported to one decimal place. For the purpose of descriptive statistics on the plasma concentration values and for PK analysis, all non-numerical values reported as being below the lower limit of quantification (BLQ) will be imputed as follows:

- Any BLQ value will be presented as 'BLQ' in the concentration table listings and footnoted accordingly.
- Any BLQ value prior to C_{max} will be set equal to zero. Any BLQ post C_{max} will be kept as "BLQ" and treated as a non-numerical value and excluded from descriptive statistics and PK analysis.
- In the event that two BLQ values are followed by a value within the quantification limit, the quantifiable value may be excluded from the analysis.

PK Parameter Estimation:

The PK parameters will be estimated by non-compartmental analysis (NCA) using Phoenix WinNonlin (v8.3 or later) using the data imputations described above. Actual sampling time points relative to dosing will be used for the NCA. There will be no imputation for missing data. No formal "outlier" analysis is planned. A profile must include at least consecutive quantifiable concentrations to be included in PK analysis. Subject profiles that are excluded from PK analysis (e.g. missing more than 2 samples, etc.) will be included in the concentration tables only and the reason for exclusion will be provided in the PK report.

Peak plasma concentrations (C_{max}) post dose administration and the corresponding T_{max} will be determined by direct assessment (no calculations) of the concentration vs time data.

All AUC calculations will be performed using the linear trapezoidal rule.

If the data permit, the terminal rate constant (λz) will be calculated. The value of λz will be determined by the slope of the regression line of the natural log transformed concentrations vs time with the following constraints:

1. At least 3 data points post the C_{max} should be used in the regression; and
2. The correlation coefficient (R^2) of regression should be > 0.80.

To optimize the reliability of the identified terminal phase (λz), where possible, the data points used to define the λz will be manually selected. The λz profiles that do not meet the guidelines stated above will not report the AUC_{INF}, $t_{1/2}$, CL or Vz parameters for that subject profile.

When possible, AUC_{INF} will be calculated as: AUC_{last} + (C_{last} / λz). CL will be calculated as: Dose / (AUC_{INF}) and Vz will be calculated as: Dose / (AUC_{INF} * λz). Terminal half-life ($t_{1/2}$) will be calculated as: $\log_e(2)$ / λz. If the lambda z range is not at least 2-fold greater than the calculated half-life value, the half-life value will be flagged and excluded from summary descriptive statistics.

If there any deviations from the planned treatment, then the analysis model specified or methods of analysis may be re-evaluated, as appropriate. Details of any deviations from the planned analysis will be documented in the PK report.

Individual subject PK parameters will be listed and summarized by cohort / dose levels with descriptive statistics (e.g. N, arithmetic mean, SD, median, minimum, maximum, geometric mean and CV%). Individual parameters and descriptive statistics will be presented to 3 significant figures, except N, which will be reported as whole number, while individual T_{max} and half-life values will be reported to two significant figures.

PK Figures:
Mean ± SD Concentration vs nominal time profiles will be generated on log/linear scales by cohort. Overlay plots of all individual plasma concentration by actual time (or nominal time) for each cohort will be generated on log/linear axis, with each subject data represented with a different symbol and included in a legend. Individual subject concentration by time plots will also be produced on log/linear axis.

Mean ± SD AUC_{last} versus dose plots will also be generated to assess dose proportionality / linearity.

6.10 Exploratory Analyses

The following endpoints will be considered in the exploratory analysis, to be performed at the time of final analysis.

- time to clearance of all puncta,
- determining the residence time, defined as the time of injection to complete resolution of all identified puncta
- the number of post-injection spots
- distribution and total area of hyperfluorescent between cohorts
- peripheral puncta vs central, estimated number and size within the Macula and outside of the macula as reported by the grading center
- relationship between puncta and blood concentrations and residence time,

- time to maximum number of puncta detected

6.11 Other Assessments

6.11.1 ALS/Parkison's/CAA Disease History

Disease history assessment will capture diagnostic criteria for each disease and also describes disease state for each subject.

- ALS Diagnostic Criteria
  - 1 - Definite ALS
  - 2 - Probable ALS
  - 3 - Probable ALS w/ Lab Result Supported
  - 4 - Possible ALS
- ALSRFS-R Score (0-10)
- Parkinson MDS Diagnostic Criteria:
  - 1 – Bradykinesia
  - 2 – Rigidity
  - 3 – Resting Tremor
  - 4 – Positive response to dopaminergic therapy
  - 5 – Levodopa – induced Dyskinesia
  - 6 – Documentation of resting tremor of a limb
  - 7 – Positive diagnostic test for olfactory loss
  - 8 – Positive diagnostic test of cardiac sympathetic denervation on scintigraphy
- Hoehn and Yahr Scale for Parkinson's subjects
  - 1 – Unilateral only
  - 2 – Bilateral or midline without impairment of balance
  - 3 – Bilateral
  - 4 – Severely disabling
  - 5 – Confinement
- MSA Diagnostic Criteria
  - Established MSA
    - MSA-C – Atrophy of: Putamen (and signal decrease on iron-sensitive sequences), infratentorial structures (pons and middle cerebellar pedundle). Hot cross bun sign.
    - MSA-P – Atrophy of Putamen (and signal decrease on iron-sensitive sequences), middle cerebellar pedundle, pons, and cerebellum. Hot cross bun sign.
  - Probable MSA
    - MSA-C – Atrophy of: Putamen (and signal decrease on iron-sensitive sequences), infratentorial structures (pons and middle cerebellar pedundle). Hot cross bun sign.
    - MSA-P – Atrophy of Putamen (and signal decrease on iron-sensitive sequences), middle cerebellar pedundle, pons, and cerebellum. Hot cross bun sign.
- CAA Diagnostic Criteria
  - 1 – Definite CAA
  - 2 – Probable CAA with evidence
  - 3 – Probable CAA
  - 4 – Possible CAA

6.11.2 Ophthalmoscopy

Measurements will be taken of the vitreous, macula, peripheral retina, choroid, optic nerve for both eyes and assessed on a scale of normal, abnormal (NCS), abnormal (CS), and not done.

6.11.3 Slit Lamp Biomicroscopy

Measurements will be taken of the subject's lids/lashes, conjunctiva/sclera, iris/pupil, cornea, anterior chamber, and lens at each protocol specified timepoint for both eyes and assessed on a scale of normal, abnormal (NCS), abnormal (CS), and not done.

6.11.4 Mini-mental state exam (MMSE) for CAA group

Short screening tool to provide overall measure of cognitive impairment in clinical research and will be conducted at screening for the CAA group. The MMSE is a score-based 30-question assessment with a maximum of 30 points possible (PubMed, 2015). MMSE scores will be assessed as a continuous variable.

7 INTERIM ANALYSIS

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.

Individual subject listings will be provided by Trial Runners for the following:

- subject disposition
- number of hyperfluorescent puncta pre and post dose for each subject
- TEAEs
- Clinically significant laboratory results
- Vital signs

No formal statistical analysis will be performed as the nature of the interim analysis is descriptive, to evaluate the quality of images obtained, and to inform the selection of the optimum dose. There will be no interim analysis for the MSA and Glaucoma groups.

8 REFERENCES

Rodriguez, Ingrid-Arevalo, et. al. (2015, Mar). Mini-Mental State Examination (MMSE) for the detection of Alzheimer's disease and other dementias in people with mild cognitive impairment (MCI) PubMed. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6464748/

9 APPENDICES

9.1 Glossary of Abbreviations

Abbreviation	Definition
AE	Adverse event

ALS	Amyotrophic lateral sclerosis
AUC	Area under the curve
CAA	Cerebral Amyloid Angiopathy
CS	Clinical Signs
CV	Coefficient of variation
ECG	Electrocardiogram
EOS	End of Study
IMG	Imaging population
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini-Mental State Exam
MSA	Multiple Systems Atrophy
NCS	No Clinical Signs
OCT	Optical Coherence Tomography
OCT A	Optical Coherence Tomography Angiography
OD	Right Eye
OS	Left Eye
OU	Both Eyes
PK	Pharmacokinetic
PROBE	Prospective Randomized Open Blinded Endpoint
SAE	Serious adverse event
SAF	Safety population
SD	Standard Deviation
SMC	Safety Monitoring Committee
TDP-43	TAR DNA-binding protein 43
TEAE	Treatment-emergent adverse event
WHO	World Health Organization
α-syn	Alpha-synuclein

10 VERSION HISTORY LOG

Version	Date	Implemented Changes	Author
1.0	31-JAN-2023	First approved version	Andrew Mao