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Trial title	N-DOSE: A randomized, double blind, dose optimization trial of nicotinamide riboside in Parkinson's disease.
Trial ID	The N-DOSE PD study.
Trial registration number	NCT05589766

## SAP and protocol version:

SAP version and date:	This SAP is version 1.0, dated 16 June 2025
Protocol version	This document has been written based on information contained in the study protocol version 3.1, dated 5th June 2025.

## SAP revision history:

Protocol version	SAP version	Section number changed	Description and reason for change	Date changed
3.1	1.0	NA	NA	16.06.2025

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## ABBREVIATIONS

PD	Parkinson's disease
AE	Adverse Event
ATC	Anatomical/Therapeutic/Chemical
BMI	Body Mass Index
BPM	Beats Per Minute
CI	Confidence Interval
DMC	Data Monitoring Committee
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
MedDRA	Medical Dictionary for Regulatory Activities
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
PL-group	Placebo group
DS-group	Dose-stable group
DE-group	Dose-escalation group
SOC	System Organ Class
Persons with Parkinson's disease	PwPD
NAD	Nicotinamide adenine dinucleotide
NR	Nicotinamide riboside
PD	Parkinson's disease
<sup>31</sup> P-MRS	<sup>31</sup> phosphorus magnetic resonance spectroscopy
CSF	Cerebrospinal fluid
NRRP	NR related pattern
PDRP	PD related pattern
LC-MS	Liquid-chromatography mass spectrometry
MDS-UPDRS	Movement disorders society unified Parkinson's disease rating scale
B-SIT	Brief Smell Identification Test
SAM	S-adenosyl methionine
MOCA	Montreal Cognitive Assessment
MDS-NMS	International Parkinson and Movement Disorder Society – Non-Motor Rating Scale
mGIDS-PD	Modified Gastrointestinal Dysfunction Scale for Parkinson's Disease
LME	Linear mixed model
ANCOVA	Analysis of covariance
RBD	Rapid Eye Movement Sleep Disorder

**TABLE OF CONTENTS**

1	INTRODUCTION.....	5
1.1	Background and Rationale .....	5
1.2	Intervention(s).....	5
1.3	Trial Objectives .....	5
2	TRIAL METHODS .....	9
2.1	Trial Design.....	9
2.2	Randomisation.....	9
2.3	Statistical Framework .....	9
2.4	Timing of Outcome Assessments.....	10
2.5	Statistical Interim Analyses and Stopping Guidance.....	10
2.6	Timing of Main Analysis.....	10
3	TRIAL POPULATION.....	11
3.1	Screening Data, Eligibility and Recruitment .....	11
3.2	Baseline Patient Characteristics.....	12
3.3	Withdrawal/Follow-up .....	12
3.4	Adherence and Protocol Deviations .....	12
3.5	Analysis Populations .....	13
4	ENDPOINT DEFINITIONS.....	14
4.1	General Definitions and Derived Variables .....	14
4.2	Primary Endpoint Definition .....	14
4.3	Secondary Endpoint Definition .....	14
4.4	Exploratory Endpoint Definitions .....	15
4.5	Overview of Endpoints.....	16
5	ANALYSIS METHODS.....	18
5.1	Methods for Primary Endpoint.....	18
5.2	Methods for Continuous Secondary Endpoint.....	19
5.3	Methods for Continuous Exploratory Endpoints.....	21
5.4	Additional Explorative Analyses .....	21
5.5	Sample size .....	22
6	SAFETY ANALYSES .....	23
6.1	Adverse Events .....	23
6.2	Clinical Laboratory Parameters .....	23
6.3	Vital Signs.....	23
7	STATISTICAL SOFTWARE.....	23
8	REFERENCES.....	24
8.1	Literature References .....	24
8.2	Reference to Data Handling Plan .....	25
8.3	Reference to the Trial Master File and Statistical Documentation .....	25
8.4	Reference to other Standard Operating Procedures or Documents .....	25

## 1 Introduction

### 1.1 Background and Rationale

Increasing evidence supports that boosting cellular levels of nicotinamide adenine dinucleotide (NAD) confers neuroprotective effects in both healthy aging and neurodegeneration<sup>1</sup>. Increasing NAD via supplementation of precursors have shown beneficial effects on life- and health span in multiple model systems, and evidence of neuroprotection in models of neurodegeneration and other age-related diseases<sup>2-4</sup>. NAD can be replenished via supplementation of nicotinamide riboside (NR), a vitamin B3 analog and biosynthetic precursor of NAD<sup>2,5</sup>. NR has undergone extensive preclinical testing<sup>6</sup> and is well tolerated by adult humans, showing no evidence of toxicity with doses up to 2000 and 3000 mg daily<sup>7,8</sup>. In two recent trials of NR in PD, increase in cerebral and whole blood NAD and an association with clinical improvement was seen<sup>8,9</sup>. This response was heterogeneous, and it is unknown if this was due to inadequate dosing<sup>9</sup>. We are therefore conducting the N-DOSE trial, investigating the dose-response and effect of an increasing dose of NR dose from 1000mg to 3000mg.

We refer the reader to the N-DOSE protocol for a more detailed description.

### 1.2 Intervention(s)

#### 1.2.1 Brief description of the study intervention(s)

**Dose-escalation group (DE-group):** This group consists of 40 persons with Parkinson's disease who will receive an escalating dose of NR over three months: month one NR 1000 mg daily, month two NR 2000 mg daily, month three NR 3000 mg daily.

**Dose-stable group (DS-group):** This group consists of 20 persons with Parkinson's disease who will receive a dose of NR 1000 mg daily for three months.

#### 1.2.2 Control settings (if applicable)

**Placebo (PL-group):** This group consists of 20 persons with Parkinson's disease who will receive placebo for three months.

### 1.3 Trial Objectives

#### 1.3.1 Primary Objective

The primary objective of this study is to compare the effect of orally administered nicotinamide riboside (NR), escalated to 1500 mg twice per day (3000 mg/day) in the dose-escalation group (DE-group) versus stable dosing of 500 mg twice per day (1000 mg/day) in the dose-stable group (DS-group) on cerebral NAD-levels at week 12.

#### 1.3.2 Secondary Objectives

The secondary objectives of this study are to:

- Assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in cerebral NAD levels from baseline to weeks 4, 8 and 12.

- Compare the effectiveness of orally administered nicotinamide riboside (NR) 1500 mg twice per day versus 500 mg twice per day in augmenting the NAD-metabolome in the central nervous system (CNS) at week 12.

### 1.3.3 Exploratory Objectives

The exploratory objectives of this study are:

- To compare the effect of orally administered NR in the DE-group versus DS-group on the NR related metabolic pattern (NRRP) expression at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in NRRP expression from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR DE-group versus DS-group on the PD-related pattern (PDRP) expression at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in PDRP expression from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on the NAD metabolome\* in the blood, urine and central nervous system (CNS) at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in the NAD metabolome\* in blood and urine from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on clinical severity of PD symptoms at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and change in clinical severity of PD symptoms from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on severity of non-motor symptoms of daily living in PD at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and change in severity of non-motor symptoms of daily living in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on severity of motor aspects of experiences of daily living in PD at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in severity of motor aspects of experiences of daily living in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on severity of PD motor symptoms at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in severity of PD motor symptoms from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on severity PD motor complications at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in severity of PD motor complications from baseline to weeks

- 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on clinical severity of PD non-motor symptoms at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in clinical severity of PD non-motor symptoms from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on clinical severity of gastrointestinal non-motor dysfunction in PD at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in clinical severity of gastrointestinal non-motor dysfunction in PD from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on cognition at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in cognition from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on quality of life in PD at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in quality of life in PD from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on gene expression at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in gene expression from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on protein expression at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in protein expression from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on serum and CSF inflammatory markers at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in serum inflammatory markers from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on histone acetylation in PD at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in histone acetylation in PD from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on H3K27 and H4K16 histone acetylation in PD at week 12.
  - To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in on H3K27 and H4K16 histone acetylation in PD from baseline to weeks 4, 8 and 12.
  - To compare the effect of orally administered NR in the DE-group versus DS-group on the genomic distribution of H3K27 and H4K16 histone acetylation in PD at week 12.

- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in the genomic distribution of H3K27 and H4K16 histone acetylation in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on folate and one-carbon metabolism in PD at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in folate and one-carbon metabolism in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on methyl donors in PD at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in methyl-donors in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on DNA methylation at week 12.
- To assess the dose-response relationship between NR dose (1000 mg, 2000 mg, 3000 mg per day) and changes in methyl-donors in PD from baseline to weeks 4, 8 and 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on synthesis of neurotransmitters in PD at week 12.
- Determine whether NR-therapy affects the gut microbiome in a dose-responsive manner at week 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on the gut metabolome at week 12.
- To compare the effect of orally administered NR in the DE-group versus DS-group on the sense of smell at week 12.
- To determine the safety and tolerability of NR at a dose of 1000 mg, 2000 mg, and 3000 mg per day in PD.

**\*The NAD metabolome is comprised of:** Nicotinamide adenine dinucleotide oxidized (NAD<sup>+</sup>), Nicotinamide adenine dinucleotide reduced (NADH), NAD<sup>+</sup>/NADH ratio, total NAD (sum of NAD<sup>+</sup> and NADH), Nicotinamide adenine dinucleotide phosphate oxidized (NADP<sup>+</sup>), Nicotinamide adenine dinucleotide phosphate reduced (NADPH), NADP<sup>+</sup>/NADPH ratio, total NADP (sum of NADP<sup>+</sup> and NADPH), 1-methyl nicotinamide (Me-Nam), nicotinic acid-adenine dinucleotide (NAAD), N1-methyl-2-pyridone-5-carboxamide (Me-2-PY), Nicotinamide (Nam), Nicotinamide N-oxide (Nam N-oxide), ADP-ribose (ADPR), Nicotinic acid riboside (NAR), Nicotinamide riboside (NR), Nicotinamide mononucleotide (NMN), Nicotinic acid (NA).



## 2 Trial Methods

### 2.1 Trial Design

The N-DOSE study is designed as a randomized, double blind, placebo-controlled, parallel-group, single-centre, phase II dose-optimization study. Treatment allocation is a 1:1:2 ratio. Patients are randomized to either placebo, dose escalation up to 3000 mg NR or stable dose of 1000 mg NR for three months.

### 2.2 Randomisation

Eligible patients are allocated in a 1:1:2 ratio between placebo, DS and DE groups, using the electronic case report form (eCRF) vieDoc.com (www.viedoc.com, version 4.77.8648.13864).

The randomization process is described in full within the clinical trial protocol. Details of the randomization including the final random allocation list are held securely and unavailable to unauthorized trial personnel.

### 2.3 Statistical Framework

#### 2.3.1 Hypothesis Test

This trial is designed to investigate the dose-responsiveness of NR treatment in a dose escalation group compared to a dose stable group and compared to placebo over three months.

The primary null hypothesis (H<sub>0</sub>) is that the NR-induced increase in cerebral NAD levels (measured by <sup>31</sup>P-MRS) is not dose-responsive.

The alternative hypothesis (H<sub>A</sub>) is that NR-induced increase in cerebral NAD levels is dose responsive.

There is only one identified primary analysis in this trial. All other efficacy analyses will be regarded as secondary or exploratory.

#### 2.3.2 Confidence Intervals and p-values

All calculated p-values will be two-sided and compared to a 5% significance level. If a p-value is less than 0.05, the corresponding treatment group difference will be denoted as statistically significant. All efficacy estimates will be presented with two-sided 95% confidence intervals. As there is only one primary null hypothesis to be tested in this trial, there will be no adjustments for multiplicity.

#### 2.3.3 Decision Rule

This trial is designed to address a single primary outcome. Non-inferiority is claimed if the primary null hypothesis is rejected on the significance level (alpha) of 0.025 (one-sided). That is, if the upper limit of the 95% two-sided confidence interval for the treatment difference is less than 15%.

## 2.4 Timing of Outcome Assessments

For all clinically planned measures, visits should occur within a window of the scheduled visit. Visits outside visit window is regarded as a protocol deviation. Visits more than 7 days outside the visit window are regarded as a major protocol deviation. The target day and visit window are defined in the protocol as:

Visit Label	Target Day	Definition (Hour window)
Screening	-90 Days	Prior to V1 (Baseline)
V1 (Baseline)	Day 0	Day 0
V2 (Week 4)	Day 28	Target day $\pm$ 72 hours
V3 (Week 8)	Day 56	Target day $\pm$ 72 hours
Last study visit* (Week 12)	Day 84	Target day $\pm$ 72 hours

\*The last study visit is defined as the visit following the last visit with randomised treatment, and where there is a study end statement.

For analysis and tabulation purposes, we define study time points as

Time Point Label	Target Day	Definition (Day window)
V1 (Baseline)	Day 0 (Randomisation)	Information up to randomisation
V2 (Week 4)	28	Days 25 to 31
V3 (Week 8)	56	Days 53 to 59
V4 (Week 12)	84	Days 81 to 87

If more than one visit fall into the same time point interval, information on all visits will be used in the analyses.

## 2.5 Statistical Interim Analyses and Stopping Guidance

There will be no interim analyses in this trial due to the short duration for each participant and relatively small sample size.

## 2.6 Timing of Main Analysis

The main analysis is planned when all participants have concluded  $84 \pm 7$  days of treatment, all data up to 84 days have been entered, verified and validated and the primary database has been locked.

### 3 Trial Population

#### 3.1 Screening Data, Eligibility and Recruitment

The total number of screened patients and reasons for not entering the trial will be summarised and tabulated.

A CONSORT flow diagram will be used to summarise the number of patients who were:

- assessed for eligibility at screening
- eligible at screening
- ineligible at screening\*
- eligible and randomised
- eligible but not randomised\*
- received the randomised allocation
- did not receive the randomised allocation\*
- lost to follow-up\*
- discontinued the intervention\*
- randomised and included in the primary analysis
- randomised and excluded from the primary analysis\*

\*Reasons will be provided.

##### **Inclusion criteria:**

The following condition must apply to the prospective patient at screening prior to receiving study agent:

- Clinically established diagnosis of idiopathic PD according to the MDS criteria<sup>10</sup>.
- 123I-Ioflupane dopamine transporter imaging (DAT-scan) or 18-F-FDOPA positron emission tomography (18F-FDOPA PET) confirming nigrostriatal degeneration.
- Hoehn and Yahr score < 4 at enrollment.
- Age ≥ 40 years at time of enrollment.
- Able to undergo lumbar puncture.
- Able to undergo MRI.

##### **Exclusion criteria:**

The participant cannot have any of the following prior to receiving study agent:

- Dementia or other neurodegenerative disorder at baseline visit.
- Diagnosed with atypical parkinsonism (PSP, MSA, CBD) or vascular parkinsonism.
- Any psychiatric disorder that would interfere with compliance in the study.
- Metabolic, neoplastic, or other physically or mentally debilitating disorder at baseline visit.
- Use of high dose vitamin B3 supplementation within 30 days of enrollment.

## 3.2 Baseline Patient Characteristics

The patient demographics and baseline characteristics to be summarized include:

- Age (in years).
- Sex (assigned according to electronic patient records).
- Time since PD diagnosis at baseline (in years).
- Weight (in kilograms).
- Height (in centimeters).
- BMI (in kg/m<sup>2</sup>).
- Smoking status (Current/Previously/Never, number of pack years (0 - 10 years, > 10 years)).
- Subjective symptom duration of PD (in months).
- Anamnestic RBD (Yes/No).
- Duration of RBD at baseline (in years).
- Anamnestic loss of smell (Yes/No).
- Duration of loss of smell at baseline (in years).
- MDS-UPDRS total score and subsections I-IV<sup>11</sup>.
- MDS-NMS score<sup>12</sup>.
- MoCA score<sup>13</sup>.
- Modified GIDS-PD score<sup>14</sup>.
- Total EQ-ED-5L score<sup>15</sup>.
- EQ-ED-5L subsection EQ VAS score<sup>15</sup>.
- Hoehn and Yahr score<sup>16</sup>.
- B-SIT (Sensonics International, Haddon Heights, NJ, USA) score for subset where available.

Patient demographics and baseline characteristics will be summarized by randomized treatment arm and overall using descriptive statistics (N, mean, standard deviation) for continuous variables, and number and percentages of patients for categorical variables. No formal statistical analysis will be performed between groups as participants were randomized during the trial.

## 3.3 Withdrawal/Follow-up

The status of eligible and randomised patients at trial end will be tabulated by treatment group according to

- completed intervention and assessments
- completed assessments but not intervention
- withdrew consent
- lost to follow-up

## 3.4 Adherence and Protocol Deviations

### 3.4.1 Adherence to Allocated Treatment

Compliance is assessed based on the percentage of subjects who have taken the scheduled number of pills. It is defined as:

% compliance = (number of capsules taken / number of capsules supposed to have been taken) x 100%.

The number of capsules supposed to have been taken will be calculated as the duration of treatment (end of study medication – start of study medication) multiplied by 12 per dispensation of study medication. In this study 6 capsules are taken in the morning and 6 capsules in the evening.

The mean  $\pm$  SD of study drug compliance over the whole study period and between individual visits will be presented in a table by treatment group.

### **3.4.2 Protocol Deviations**

The following are pre-defined major protocol deviations regarded to affect the efficacy of the intervention:

- Entering the trial when the eligibility criteria should have prevented trial entry.
- Discontinuation of intervention prior to  $84 \pm 7$  days.
- Major change in concomitant anti-Parkinson medication.
- Received or used other intervention than allocated.
- Adherence to allocated treatment below 85%.
- Visit date interval larger than  $28 \pm 7$  days between individual visits (i.e.: V1 and V2, V2 and V3, and V3 and V4).
- Visit date interval larger than  $84 \pm 7$  days from V1 to V4.
- Not fasting before neuroimaging.

Protocol deviations are classified prior to unblinding of treatment. The number (and percentage) of patients with major and minor protocol deviations will be summarised by treatment group with details of type of deviation provided. The patients that are included in the FAS analysis data set (see point 3.5 below) will be used as the denominator to calculate the percentages. No formal statistical testing will be undertaken.

## **3.5 Analysis Populations**

The Full Analysis Set (FAS) will be performed using the intention to treat population, defined as all patients randomly assigned to a treatment group and attended the baseline visit.

The Safety Analysis Set will include all patients having received at least one study treatment infusion after randomisation.

The Per Protocol Analysis Set (PPS) will include all randomised patients meeting the study eligibility criteria and with no major protocol deviations affecting the treatment efficacy.

## 4 Endpoint Definitions

### 4.1 General Definitions and Derived Variables

#### 4.1.1 Body Mass Index

Body Mass Index (BMI) = Body weight in kilograms divided by the square of the height in meters.

### 4.2 Primary Endpoint Definition

Change in cerebral NAD/ATP- $\alpha$  ratio measured by 31 Phosphorus magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) in the posterior brain (encompassing the occipital, parietooccipital and posterior parts of the temporal cortex). The variables are continuous.

The **primary analysis** of the **primary endpoint** will be:

- The comparison between the dose stable (DS-group) and dose escalation group (DS-group), in cerebral NAD levels measured by  $^{31}\text{P}$ -MRS at week 12 depending on randomization group and adjusted for  $^{31}\text{P}$ -MRS at baseline.

The **secondary analyses** of the **primary endpoint** will be:

- The comparison of change over time from baseline to week 4, week 8 and week 12, between the DE-group, PL-group and the DS-group, in cerebral NAD levels measured by  $^{31}\text{P}$ -MRS, depending on randomization group, time and their interaction adjusted for random individual intercept.
- The comparison between the dose stable (DS-group) and placebo group (PL-group), in cerebral NAD levels measured by  $^{31}\text{P}$ -MRS at week 12 depending on randomization group and adjusted for  $^{31}\text{P}$ -MRS at baseline.

### 4.3 Secondary Endpoint Definition

#### 4.3.1 Determine whether NR-therapy augments NAD levels in the central nervous system (CNS) in a dose-responsive manner, as measured by LC-MS

Change in the cerebrospinal fluid (CSF) levels of NAD or other metabolites of the NAD metabolome\*, measured. If NAD is measured directly this will be compared. If NAD is not detected or not measurable, the metabolite reflecting NAD levels, will be used for analysis. The variables are continuous. Metabolites from the NAD metabolome will be measured by liquid chromatography mass spectrometry. The variables are continuous.

The **primary analysis** of the **secondary endpoint** will be:

- The comparison between the dose stable (DS-group) and dose escalation group (DE-group), in NAD or NAD metabolite\* levels measured by HPLC-MS at week 12 depending on randomization group and adjusted for NAD or NAD metabolite\* at baseline.

The **secondary analysis** of the **secondary endpoint** will be:

- The comparison between the dose stable (DS-group) and placebo group (PL-group), in NAD or NAD metabolite\* levels measured by HPLC-MS at week 12 depending on randomization group and adjusted for NAD or NAD metabolite\* at baseline.

**\*The NAD metabolome is comprised of:** Nicotinamide adenine dinucleotide oxidized (NAD<sup>+</sup>), Nicotinamide adenine dinucleotide reduced (NADH), NAD<sup>+</sup>/NADH ratio, total NAD (sum of NAD<sup>+</sup> and NADH), Nicotinamide adenine dinucleotide phosphate oxidized (NADP<sup>+</sup>), Nicotinamide adenine dinucleotide phosphate reduced (NADPH), NADP<sup>+</sup>/NADPH ratio, total NADP (sum of NADP<sup>+</sup> and NADPH, 1-methyl nicotinamide (Me-Nam), nicotinic acid-adenine dinucleotide (NAAD), N1-methyl-2-pyridone-5-carboxamide (Me-2-PY), Nicotinamide (Nam), Nicotinamide N-oxide (Nam N-oxide), ADP-ribose (ADPR), Nicotinic acid riboside (NAR), Nicotinamide riboside (NR), Nicotinamide mononucleotide (NMN), Nicotinic acid (NA).

#### 4.4 Exploratory Endpoint Definitions

Due to the exploratory nature of the trial these outcomes are subject to change of analysis method. The preliminary plan for analysis is the following:

The following exploratory outcomes were recorded at V1, V2, V3 and V4 and will be analysed using the same methodology as the primary endpoint (see section 4.2):

- Change in the NR-related pattern (NRRP). This is calculated from the FDG-PET scans as described in the imaging protocol section of the trial protocol. The values are continuous variables.
- Change in the PD-related pattern (PDRP). This is calculated from the FDG-PET scans as described in the imaging protocol section of the trial protocol. The values are continuous variables.
- Change in the NAD metabolome in blood and urine as measured by HPLC-MS. These values are continuous variables.
- Change in motor symptoms from PD as measured by the MDS-UPDRS scale. The scale will be analysed both as total MDS-UPDRS scale, combined by subsections I-III and by subsections I-IV individually. The values are continuous variables.
- Change in non-motor symptoms from PD as measured by the mGIDS and MDS-NMS scales. Scales will be analysed both as total scores and by subsections. The values are continuous variables.
- Change in cognition as measured by the MoCA scale. Scales will be analysed both as total scores and by subsections. The values are continuous variables.
- Change in quality of life in PD, as measured by the EQ-5D-EL scale. Scales will be analysed both as total scores and by subsections. The values are continuous variables.
- Change in inflammatory markers in blood and/or urine. These values are continuous variables.
- Change in proteostasis, measured by RNA expression analysis of lysosomal and proteasomal pathways. These values are continuous variables.
- Change in gene and protein expression in PD. These values are continuous variables.

- Change in methylation metabolism via decreased availability of methyl-donors such as for example S-adenosyl methionine (SAM). These values are continuous variables.
- Change in methylation metabolism via decreased DNA methylation globally or at specific sites. These values are continuous variables.
- Change in methylation metabolism via decreased DNA methylation globally or at specific sites. These values are continuous variables.
- Change in methylation metabolism via aberrant folate and one-carbon metabolism. These values are continuous variables.

The following exploratory outcomes were recorded at V1 and V4 and will be analysed using the same methodology as the secondary endpoint assessing NAD in CSF (see section 4.3):

- Change in olfaction as measured by the B-SIT score. These values are continuous variables.
- Change in the NAD metabolome in the central nervous system as measured by HPLC-MS. These values are continuous variables.
- Change in inflammatory markers in the central nervous system. These values are continuous variables.
- Change in methylation metabolism via decreased synthesis of neurotransmitters like dopamine and serotonin. These values are continuous variables.
- Change in gut microbiome composition as measured by microbial DNA analysis of faeces. These values are continuous variables.

## 4.5 Overview of Endpoints

Endpoint level	Analysis level	Endpoint	Timeframe	Type
<b>Primary Endpoint</b>				
	Primary	Cerebral NAD <sup>31</sup> P-MRS: Difference between DE-group and DS-group.	84 ± 7 Days	Continuous
	Secondary	Cerebral NAD <sup>31</sup> P-MRS: Difference between DE-group-, DS-group, and PL-group.	84 ± 7 Days	Continuous
	Secondary	Cerebral NAD <sup>31</sup> P-MRS: Difference between DS-group and PL-group.	84 ± 7 Days	Continuous
<b>Secondary Endpoint</b>				
	Secondary	NAD levels in CSF: Difference between DE-group and DS-group.	84 ± 7 Days	Continuous



Secondary	NAD levels in CSF: Difference between DS-group and PL-group.	84 ± 7 Days	Continuous
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**Exploratory Endpoints (see section 4.4)**

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**Safety**

N/A	Number and severity of adverse events between treatment groups and study visits.	During trial	Continuous
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## 5 Analysis Methods

### 5.1 Methods for Primary Endpoint

#### 5.1.1 Descriptive Statistics

Cerebral NAD measured by <sup>31</sup>P-MRS will be summarized by group for visits V1, V2, V3 and V4 using descriptive statistics (N, mean, standard deviation). Descriptive statistics will be based on non-imputed data. Data will be presented both in tabular and graphical format.

#### 5.1.2 Primary Inferential Analysis

The *primary analysis* of the *primary endpoint* will be performed using analysis of covariance (ANCOVA) between the dose stable (DS-group) and dose escalation group (DE-group), i.e. the regression of NAD levels measured by <sup>31</sup>P-MRS at week 12 depending on randomization group and adjusted for <sup>31</sup>P-MRS at baseline. No additional adjustments/covariates will be used. As this is the single primary outcome, alpha will be set at 0.05. No correction for multiple testing will be performed.

The formula for this calculation in R is:

```
lm_object = lm(NAD_at_V4 ~ Group + NAD_at_V1)
```

#### 5.1.3 Effect Estimates

The primary effect estimate will be the B-coefficient with confidence intervals and Cohen's d. Cohen's d will be calculated as the difference in the means between two groups and divided by the pooled standard deviation.

The Beta coefficient will be calculated in R in the following manner:

```
Beta_coffecient = summary(lm_object)$coefficients
```

Confidence intervals will be calculated in R in the following manner:

```
Confidence_intervals = confint(lm_object)
```

P-values will be retrieved from the summary of the lm\_object:

```
P_values = summary(lm_object)$coefficients
```

#### 5.1.4 Assumption Checks and Alternative Analyses

Assumptions for ANCOVA will be tested in the following manner:

Normal distribution of residuals: Normality of residuals will be assessed by QQ-plots and the Shapiro-Wilk test.

#### 5.1.5 Missing Data

For the primary outcome, missing data occur if a patient has not completed the study or not completed <sup>31</sup>P-MRS imaging within the time window of the final visit 12 weeks  $\pm$  7 days.

As the primary analysis uses two timepoints (V1 and V4), in the case of a participant missing V4 measurements, this patient will not be included in the analysis.

For the secondary analysis of the primary outcome using a linear mixed model (LME, see point 5.1.7), missing data will not be imputed but treated as missing at random.

### 5.1.6 Sensitivity Analyses

Sensitivity analysis will be performed by comparing the results of analysis with the FAS and PPS populations. If there is a notable difference this will be commented upon.

### 5.1.7 Secondary Analyses of the Primary Endpoint

The *secondary analyses* of the *primary endpoint* will be:

1. Analysis of covariance (ANCOVA) between the dose stable (DS-group) and placebo group (PL-group), i.e. the regression of NAD levels measured by <sup>31</sup>P-MRS at week 12 depending on randomization group and adjusted for <sup>31</sup>P-MRS at baseline. No additional adjustments/covariates will be used. No correction for multiple testing will be performed.

The above-mentioned analysis will be performed in the same manner as the primary analysis for the primary endpoint.

2. Comparison of change over time from baseline to week 4, week 8 and week 12, between the DE-group, DS-group and the PL-group. This will be assessed using a linear mixed model (LME), i.e. NAD (<sup>31</sup>P-MRS) at weeks 4, 8 and 12 depending on randomization group, time and their interaction adjusted for random individual intercept. The above-mentioned analysis will be performed using a linear mixed model (LME) comparing NAD level as a function of group with time and the interaction between group and time as independent variables. Individual subjects will be random effects.

The following formula will be used in R format:

```
NAD_level ~ Group + Visit + Group * Visit + (1 | Subject)
```

Visit will be treated as a numeric variable if there is no plateau effect. If there is a plateau effect in the data, visit will be treated as a factor.

The primary effect estimate will be the B-coefficient with confidence intervals and Cohen's d. Cohen's d will be calculated as the difference in the means between two groups and divided by the pooled standard deviation.

The dose stable group will be set as the reference to which the dose escalation and placebo groups will be compared.

No additional covariates will be included in the LME model.

## 5.2 Methods for Continuous Secondary Endpoint

### 5.2.1 Descriptive Statistics

CSF NAD (or other metabolites of the NAD metabolome) measured by LC-MS will be summarized by group for visits V1 and V4 using descriptive statistics (N, mean, standard deviation). Descriptive

statistics will be based on non-imputed data. Data will be presented both in tabular and graphical format.

### 5.2.2 Primary Inferential Analysis

Analysis of covariance (ANCOVA) between the dose escalation (DE-group) and the dose stable group (DS-group), i.e. the regression of CSF NAD (or other metabolites of the NAD metabolome) at week 12 depending on randomization group and adjusted for CSF NAD (or other metabolites of the NAD metabolome) at baseline. No additional adjustments/covariates will be used. No correction for multiple testing will be performed.

The formula for this calculation in R is:

```
NAD_at_V4 ~ Group + NAD_at_V1
```

### 5.2.3 Effect Estimates

The primary effect estimate will be the B-coefficient with confidence intervals and Cohen's d. Cohen's d will be calculated as the difference in the means between two groups and divided by the pooled standard deviation.

The Beta coefficient will be calculated in R in the following manner:

```
Beta_coffecient = summary(lm_object)$coefficients
```

Confidence intervals will be calculated in R in the following manner:

```
Confidence_intervals = confint(lm_object)
```

P-values will be retrieved from the summary of the lm\_object:

```
P_values = summary(lm_object)$coefficients
```

### 5.2.4 Assumption Checks and Alternative Analyses

Assumptions for ANCOVA will be tested in the following manner:

Normal distribution of residuals: Normality of residuals will be assessed by QQ-plots and the Shapiro-Wilk test.

### 5.2.5 Missing Data

For the primary outcome, missing data occur if a patient has not completed the study or CSF collection has not been successful within the time window of the final visit 12 weeks  $\pm$  7 days.

As the primary analysis uses two timepoints (V1 and V4), in the case of a participant missing V4 measurements, this patient will not be included in the analysis.

### 5.2.6 Sensitivity Analyses

Sensitivity analysis will be performed by comparing the results of analysis with the FAS and PPS populations. If there is a notable difference this will be commented upon.

### 5.2.7 Secondary Analyses of the Secondary Endpoint

The *secondary analysis* of the *secondary endpoint* will be:

- Analysis of covariance (ANCOVA) between the dose stable (DS-group) and the placebo group (PL-group), i.e. the regression of CSF NAD (or other metabolites of the NAD metabolome)

at week 12 depending on randomization group and adjusted for CSF NAD (or other metabolites of the NAD metabolome) at baseline. No additional adjustments/covariates will be used. No correction for multiple testing will be performed.

The above mentioned analysis will be performed in the same manner as the primary analysis for the secondary outcome (see section 5.2.2).

### 5.3 Methods for Continuous Exploratory Endpoints

As described in section 4.4, due to the nature of the exploratory outcomes, the analysis method is subject to change. The preliminary plan is that they will be analysed in the same manner as the primary endpoint if they have observations at V1, V2, V3 and V4 (see sections 5.1.2 and 5.1.7) and the same manner as the secondary endpoint if they only have observations at V1 and V4 (see sections 5.2.2 and 5.2.7).

### 5.4 Additional Explorative Analyses

#### 5.4.1 Correlation analyses

Explorative correlation analyses comparing the following correlations with either Spearman or Pearson correlations are planned:

- Change in cerebral NAD levels measured by <sup>31</sup>P-MRS vs NAD levels in CSF.
- Change in cerebral NAD levels measured by <sup>31</sup>P-MRS vs NRRP expression.
- Change in cerebral NAD levels measured by <sup>31</sup>P-MRS vs PDRP expression.
- Change in NAD levels in CSF vs NRRP expression.
- Change in NAD levels in CSF vs PDRP expression.
- Change in cerebral NAD levels measured by <sup>31</sup>P-MRS vs the following clinical rating scales:
  - o MDS-UPDRS
  - o MDS-NMS
  - o MoCA
  - o mGIDS-PD
  - o EQ-5D-EL
- Change in NAD levels in CSF vs the following clinical rating scales:
  - o MDS-UPDRS
  - o MDS-NMS
  - o MoCA
  - o mGIDS-PD
  - o EQ-5D-EL
- Change in NRRP expression vs the following clinical rating scales:
  - o MDS-UPDRS
  - o MDS-NMS
  - o MoCA
  - o mGIDS-PD
  - o EQ-5D-EL
- Change in PDRP expression vs the following clinical rating scales:
  - o MDS-UPDRS
  - o MDS-NMS

- MoCA
- mGIDS-PD
- EQ-5D-EL
- Change in Blood NAD levels vs NAD levels in CSF
- Change in Blood NAD levels vs cerebral NAD levels measured by <sup>31</sup>P-MRS
- Change in Blood NAD levels vs NRRP expression
- Change in Blood NAD levels vs PDRP expression

## 5.5 Sample size

Our primary null hypothesis ( $H_0$ ) is that the NR-induced increases in cerebral NAD levels (measured by <sup>31</sup>P-MRS), or NRRP expression (measured by FDG-PET) are not dose-responsive. The alternative hypothesis ( $H_A$ ) is that at least one of these measures will be dose responsive. In the NADPARK study, all three measures showed a highly significant increase in the group receiving 1000 mg NR ( $n = 15$ ) compared to the placebo group ( $n = 15$ ). In the NADPARK study<sup>9</sup>, treatment with 1000mg of NR led to an increase in cerebral NAD-levels by a factor of 1.27 from baseline in the treatment group, whereas the change in the placebo group was negligible at -0.43%. Under the  $H_A$ , we assume that cerebral NAD levels will increase in a linear fashion in the 2000 mg NR and 3000 mg NR groups, respectively. Based on these assumptions and given a type I error rate of 5% ( $\alpha = 0.05$ ) and a type II error rate of 10% ( $\beta = 0.1$ , power = 90%), we estimated that a sample size of 30 individuals will be required in the dose escalation group. Accounting for drop-out and statistical safety margin, we estimate that the study requires 40 subjects in this group.

For the secondary outcomes, we assume that the metabolomic analyses will have sufficient power, since they produced very large effect sizes and highly significant results in the NADPARK study with 15 individuals per group.

## 6 Safety Analyses

General safety evaluations will be based on the incidence, intensity, and type of AEs, and clinically significant changes in the patient's physical examination findings, vital signs, and clinical laboratory results. Only AE grade 2 and above will be recorded. Safety variables will be tabulated and presented for all patients in the safety set.

### 6.1 Adverse Events

#### 6.1.1 Descriptive Statistics

The frequency and severity of adverse events (AE) will be presented in tabular format between all three randomization groups and NR dose levels (1000 mg, 2000 mg and 3000 mg). AEs are evaluated as being either unrelated, unlikely, possible, probable or definite in relation to the investigational medicinal product (IMP). AEs will be grouped by status; Moderate or Severe and grouped by relation to the IMP. AEs will be presented by number and by percentage of group. The safety analysis set (SAS) will be used for analysis. The variables are continuous. No further statistical testing will be performed on the AEs. We refer the reader to the study protocol for a more detailed definition of AE severity and registration procedures.

#### 6.1.2 Sensitivity Analyses

Sensitivity analysis of data will be performed by comparing the results of analysis with the SAS and PPS populations. If there is a notable difference this will be commented upon.

### 6.2 Clinical Laboratory Parameters

Safety clinical laboratory parameters were collected and assessed, both to be used to identify adverse events and for exploratory analyses. Clinical laboratory parameters will be summarised by treatment group and visit.

### 6.3 Vital Signs

Changes in vital signs (including systolic and diastolic blood pressure (mmHg), heart rate (beats per minute) and weight (kg)) will be summarised by treatment group and visit.

## 7 Statistical Software

All statistical analyses will be done in R 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

## 8 References

### 8.1 Literature References

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13. Nasreddine, Z. S. *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* **53**, 695–699 (2005).
14. Camacho, M., Greenland, J. C. & Williams-Gray, C. H. The Gastrointestinal Dysfunction Scale for Parkinson's Disease. *Mov Disord* **36**, 2358–2366 (2021).
15. Herdman, M. *et al.* Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* **20**, 1727–1736 (2011).
16. Goetz, C. G. *et al.* Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. *Mov Disord* **19**, 1020–1028 (2004).

## 8.2 Reference to Data Handling Plan

Data handling and management is outlined in the trial protocol: N-DOSE protocol V3.1.

## 8.3 Reference to the Trial Master File and Statistical Documentation

Data handling and management is outlined in the trial protocol: N-DOSE protocol V3.1.

## 8.4 Reference to other Standard Operating Procedures or Documents

The trial protocol: N-DOSE protocol V3.1, was adhered to and followed when writing the SAP.