PROTOCOL TITLE PAGE

STUDY NUMBER CNMAu8.202

REPAIR-PD

A PHASE 2, OPEN LABEL, SEQUENTIAL GROUP, INVESTIGATOR BLINDED STUDY OF MAGNETIC RESONANCE SPECTROSCOPY (31P-MRS) TO ASSESS THE EFFECTS OF CNM-Au8 FOR THE BIOENERGETIC IMPROVEMENT OF IMPAIRED NEURONAL REDOX STATE IN PARKINSON'S DISEASE (PD)

NCT:03815916

RELEASE DATE: 09SEP2020

CLINICAL STUDY PROTOCOL

REPAIR-PD:
A Phase 2, Pilot Open Label, Sequential Group, Investigator Blinded Study of Magnetic **Re**sonance Spectroscopy (^{31}**P**-MRS) to **A**ssess the Effects of CNM-Au8 for the Bioenergetic **I**mprovement of Impaired Neuronal **R**edox State in **P**arkinson's **D**isease (PD)

U.S. IND Number:	142,773
Protocol Number:	CNMAu8.202
Clinical Phase:	Phase 2
Investigational Drug:	CNM-Au8
Protocol Version No.:	6.0 (Amendment 5.0)
Current Protocol Version Date:	09-September-2020

PROTOCOL APPROVAL PAGE

Study Title:	**REPAIR-PD**: A Phase 2, Pilot Open Label, Sequential Group, Investigator Blinded Study of Magnetic **Re**sonance Spectroscopy (^{31}**P**-MRS) to **A**ssess the Effects of CNM-Au8 for the Bioenergetic **I**mprovement of Impaired Neuronal **R**edox State in **P**arkinson's **D**isease (PD)
Protocol Number:	CNMAu8.202
Current Protocol Version:	Version 6.0
Current Protocol Date:	09-September-2020
Sponsor Name and Address:	Clene Nanomedicine, Inc. 3165 East Millrock Drive, Suite 325 Salt Lake City, UT 84121 United States

I, the undersigned, have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practices, the Declaration of Helsinki in the latest relevant version, and the applicable legal and regulatory requirements.

Sponsor Signature:

Sponsor's Medical Representative

10 Sept 2020
(Date)

INVESTIGATOR PROTOCOL AGREEMENT

Protocol Title: **REPAIR-PD**: A Phase 2, Pilot Open Label, Sequential Group, Investigator Blinded Study of Magnetic **Re**sonance Spectroscopy (^{31}**P**-MRS) to **A**ssess the Effects of CNM-Au8 for the Bioenergetic **I**mprovement of Impaired Neuronal **R**edox State in **P**arkinson's **D**isease (PD)

Protocol Number: CNMAu8.202
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By my signature, I:

- Confirm that my staff and I have carefully read and understand this protocol and the Investigator's Brochure (IB) and are thoroughly familiar with the appropriate use of the investigational drug described herein.
- Agree to comply with the conduct and terms of the study specified herein and with any other study procedures provided by the Sponsor, Clene Nanomedicine, Inc. (herein referred to as "Clene").
- Agree to comply with US Food and Drug Administration (FDA), as applicable, the International Conference on Harmonization (ICH) GCP guidelines, the Declaration of Helsinki, and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human subjects.
- Agree not to implement changes to the protocol without agreement from the Sponsor and prior written approval (where required) from the Institutional Review Board (IRB) except when necessary to eliminate an immediate hazard to the subjects.
- Agree to onsite monitoring of the case report forms (CRFs) and source documents by Clene or designee and to audit by Clene or designee and appropriate regulatory authorities, including, but not limited to, the FDA and IRB/HREC inspectors.
- Agree to supervise the conduct of the study and maintain responsibility for training and supervising all personnel who have delegated responsibilities under my leadership. The protocol and other important study materials will be available to study staff throughout the conduct of the study.

Investigator's Signature **Date**

Print Name

PROTOCOL SYNOPSIS

Category	Description
Sponsor	Clene Nanomedicine, Inc.
Investigational Drug Product	CNM-Au8
Name of Active Ingredient	Faceted clean-surfaced Au nanocrystals
Phase of Development	Phase 2
Study Title	A Phase 2, Pilot Open Label, Sequential Group, Investigator Blinded Study of Magnetic **Re**sonance Spectroscopy (^{31}**P**-MRS) to **A**ssess the Effects of CNM-Au8 for the Bioenergetic **I**mprovement of Impaired Neuronal **R**edox State In **P**arkinson's **D**isease (PD) (**REPAIR-PD**)
Protocol Version	6.0
Study Center(s)	UT Southwestern Medical Center Dallas, Texas
Principal Investigator(s) & Sub-investigators	***Principal Investigator*** Richard B. Dewey, Jr., MD Professor Director of the Clinical Center for Movement Disorders. Department of Neurology and Neurotherapeutics UT Southwestern Medical Center ***Sub-investigators*** Jimin Ren, PhD *(Imaging)* Associate Professor Advanced Imaging Research Center Department of Radiology UT Southwestern Medical Center Ben Greenberg, MD *(Biomarkers)* Professor, Cain Denius Scholar in Mobility Disorders Department of Neurology and Neurotherapeutics UT Southwestern Medical Center

Study Objectives	To assess the CNS metabolic biomarker profile and safety of CNM-Au8 for the treatment of Parkinson's Disease (PD): • Metabolic effects will be assessed as an improvement of ^{31}P Magnetic Resonance Spectroscopic (^{31}P-MRS) assessment of brain tissue cellular Redox potential defined by the measured tissue ratio of NAD+:NADH concentrations and various bioenergetic markers. Safety will be assessed via adverse events, serious adverse events, discontinuations due to adverse events, and the Columbia Suicide Severity Rating Scale (C-SSRS).
Overall Study Design and Plan	This is a single-center open label pilot, sequential group, investigator blinded study of the CNS metabolic effects, safety, pharmacokinetics, and pharmacodynamics of CNM-Au8 in patients who have been diagnosed with Parkinson's Disease (PD) within three (3) years of Screening. Patients will be screened over a 6-week period. Patients who meet the inclusion criteria and none of the exclusion criteria will be enrolled into the clinical study. The Sponsor will select a starting treatment dose of 15mg or 30mg CNM-Au8 for the initial treatment. Investigators and patients will be blinded to each cohort's study dose. Upon completion of the first treatment cohort, the Sponsor will select a single dose or two different doses for the subsequent second cohort from a prespecified dosing selection plan based on the evaluation of the ^{31}P-MRS changes versus baseline in the first cohort. Up to a total of two treatment cohorts may be studied. All patients will receive daily oral treatment over twelve (12) consecutive weeks during each cohort's Treatment Period. There will be three study periods per treatment cohort: 1. A (6) six week screening period (Screening Period); 2. A (12) twelve week treatment period (Treatment Period); 3. A (4) four-week follow-up period (End-of-Study Assessment). The primary study outcome, CNS metabolic changes, will be assessed based upon each patient's Week 12 study visit versus the pre-treatment baseline. The study investigators and patients will remain blinded until the study database is formally locked. Patients will be contacted by phone to assess safety and tolerability at Week 2. At Weeks 4, 8, and 12 patients will return to the clinic to complete ^{31}P-MRS imaging (Week 12 only), PK, PD, and safety assessments. At the end of the Treatment Period, patients will complete an end-of-study (EOS) visit. All patients who are discontinued from treatment will complete the end-of-study visit.

Imaging Plan	MRI/MRS scans will be conducted on a Philips Achieva 7 Tesla human MRI scanner located in the Advanced Imaging Research Center at UTSW. Patients will undergo ^{31}P MRS scans to measure brain phosphorous metabolites and redox state. **Whole Brain Metabolite Measurements:** *Preparation:* A cylinder bird-cage shaped ^{31}P T/R volume coil insert (into a 1H T/R volume coil) will be used for imaging the brain stem. The head of the patient will be positioned in the center of the coil with soft cushion pads placed under and on the sides of the head to secure the positioning of the head and reduce potential movement during the scans. After a scout image scan, axial, sagittal and coronal T2-weighted spin-echo multi-slice brain images will be collected using the typical parameters: TR 3.5 sec, TE 80 ms, slice thickness 8 mm, gap 2 mm. A 2nd-order volume-based shimming will be conducted prior to ^{31}P MRS data acquisition. *^{31}P MRS scans:* Whole brain ^{31}P spectra will be acquired using three-dimensional chemical shift imaging (3D CSI) approach. Typical MRS parameters will be TR = 0.5 sec, NP 2 k, BW 8 kHz, NA = 4 (short scan) and 16 (long scan), with spatial resolution of 2x2x2 cm^3. If subjects are able to complete both short and long scans without movement artifact, the data will be combined. Otherwise, only the completed dataset without movement artefacts will be used for data analysis. Voxel-based ^{31}P spectra will be pooled together based on the functional regions (frontal, temporal, parietal and occipital) to evaluate the spatial distributions of metabolites. Data analysis will be performed by lineshape fitting of the ^{31}P resonances of all phosphorous metabolites using a previously developed Matlab program. **Partial Volume Coil Measurement:** *Preparation:* A half-cylinder-shaped ^{1}H/^{31}P dual-tuned T/R partial volume coil will be used to image the posterior brain (occipital and parietal lobes) for evaluation of the brain redox state based on the measurement of NAD+/NADH ratio. Axial, sagittal and coronal T2-weighted spin-echo multi-slice images will be collected from the posterior head using the typical parameters TR 3.5 sec and TE 80 ms, slice thickness 8 mm, gap 2 mm. A 2nd-order volume-based shimming will be conducted over the posterior brain region prior to ^{31}P MRS data acquisition. *^{31}P MRS scans:* To obtain ^{31}P spectra with resolved NAD from overlapping α-ATP signals, an inversion-recovery-based NAD spectral editing technique will be used. Briefly, acquisition of a reference ^{31}P spectrum using pulse-acquire sequence is conducted plus the acquisition of an additional spectrum using a sequence containing an adiabatic inversion pre-pulse followed by a short delay is

	done to selectively nullify the NAD signal. A spectral subtraction will be performed to obtain an NAD-edited spectrum with resolved NAD signal from the reference spectrum. The contribution of NAD+ and NADH to the resolved NAD signal will be quantified by lineshape fitting based on prior knowledge of the NADH signal being a singlet and the NAD+ signal being a quartet (as defined by ^{31}P coupled AB-spin system resonance pattern). The brain redox state will be calculated by the NAD+/NADH ratio. Typical ^{31}P MRS parameters for data acquisition will be TR 1.0 sec, TD 0.17 ms, NP 4k and zero filled to 8k, BW 8 kHz, data accumulated in blocks of NA 256 or 512. For quantitative comparison of different metabolites in the posterior brain region, an additional scan will be performed, using the partial volume coil, under the fully relaxed T1 condition with long TR of 15 sec and 16 acquisition averages.
Estimated Participant Study Duration	The planned study duration for each participant will be approximately twenty-two (22) weeks from the start of Screening. This assumes up to a 6-week Screening period; a 12-week Treatment Period; and a 4-week Follow-Up Safety Period.
Number of Patients	Up to 30 patients planned between both cohorts; Up to 15 patients per cohort with 13 patients evaluable (assuming a ~20% drop-out rate): • Cohort Dose 1 (n = 15) • Cohort Dose 2 (n = 15) The Cohort 2 sample size may be re-calculated based on the mean change from baseline in NAD+/NADH Redox Ratio to Week 12 and standard deviation of the change from Cohort 1.
Inclusion Criteria	The patients to be enrolled in this study must meet the following inclusion criteria: 1. Able to understand and give written informed consent and follow study procedures. 2. Male or female, aged 30 – 80 years or age (inclusive) at the time of PD diagnosis. 3. PD subjects will be recruited in accordance with the MDS Clinical Diagnostic Criteria for PD: a. Parkinsonism present (bradykinesia + either rest tremor or rigidity) b. 2 of the following 4 supportive criteria: i. Clear and dramatic beneficial response to dopaminergic medication ii. Presence of levodopa induced dyskinesias iii. Rest tremor of a limb iv. Olfactory loss or cardiac sympathetic denervation seen on prior MIBG SPECT 4. Duration of PD since diagnosis is $\leq$ 3 years (inclusive)

	5. Modified Hoehn and Yahr stage ≤ 3 6. Treatment with dopaminergic therapy for at least 12-weeks and with no change in current medications within the prior 6-weeks
Exclusion Criteria	Patients will be excluded from the study if they meet any of the following criteria: 1. Atypical parkinsonism, including that due to drugs, metabolic disorders, encephalitis, cerebrovascular disease, normal pressure hydrocephalus, or other neurodegenerative disease. 2. The presence of any of the following: a. Unequivocal cerebellar abnormalities b. Downward vertical gaze limitation or slowing of downward saccades c. Diagnosis of behavioral variant frontotemporal dementia or primary progressive aphasia d. Parkinsonian features restricted to the lower limbs for > 3 years e. Treatment with dopamine blockers or depleters in a time course consistent with drug induced parkinsonism f. Absence of an observable response to high dose levodopa despite moderate disease severity g. Expert considers a diagnosis of alternative syndrome more likely than PD h. Rapid progression of gait impairment requiring wheelchair within 5 years of onset i. Complete absence of progression of motor symptoms over 3 years unless due to treatment j. Early bulbar dysfunction within the first 5 years since diagnosis k. Inspiratory respiratory dysfunction (stridor or frequent sighs) l. Severe autonomic failure in the first 5 years m. Recurrent falls (>1 per year) because of impaired balance in the first 3 years n. Disproportionate dystonic anterocollis or hand contractures of hands or feet within 10 years o. Absence of any of the common non-motor features of PD despite 5 years of disease p. Otherwise unexplained pyramidal tract signs (weakness, hyperreflexia, or extensor toe signs) q. Bilateral symmetric parkinsonism 3. Mini-Mental State Examination (MMSE) score of less than 19. 4. Patient with a history of any clinically significant or unstable medical condition based on the Investigator's judgment. 5. History of human immunodeficiency virus (HIV), hepatitis C (HepC) virus antibody, or hepatitis B (HepB) virus antibody.

	6. Based on the investigator's judgment, patients who may have difficulty complying with the protocol and/or study procedures. 7. Patient with clinically significant abnormalities in hematology, blood chemistry, ECG, or physical examination not resolved by the Baseline visit which according to Investigator may interfere with study participation. 8. Patients with clinically significant hepatic or renal dysfunction or clinical laboratory findings that would limit the interpretability of change in liver or kidney function, or those with low platelet counts (<150 x 10^9 per liter) or eosinophilia (absolute eosinophil count of ≥500 eosinophils per microliter) at Screening. 9. Patient participating in any other investigational drug trial or using investigational drug (within 12 weeks prior to screening and thereafter) 10. Positive screen for drugs of abuse or known history of alcohol abuse. 11. Women of child-bearing potential, or men, who are unwilling or unable to use accepted methods of birth control during the study and for 6 months following completion of study participation. 12. Women with a positive pregnancy test, are lactating, or are planning to become pregnant during the study or within 6 months of the end of this trial. 13. Patients with implanted metal objects in their body that may be affected by an MRI procedure. 14. Patients who are claustrophobic or otherwise unlikely to be able to complete the MRI scanning procedures. 15. History of allergy to gold in any form. 16. Patient is considered a suicide risk in the opinion of the Investigator, has previously made a suicide attempt, or is currently demonstrating active suicidal ideation. Subjects with intermittent passive suicidal ideation are not necessarily excluded based on the assessment of the Investigator.
Concomitant Medications	Disease-specific medications allowed per the inclusion/exclusion criteria. To ameliorate procedure-related anxiety, all patients will receive 5 mg of diazepam, PO, 45-60 minutes prior to planned ^{31}P-MRS scan at the Baseline and Week 12 Visits. Otherwise, except for acetaminophen, ibuprofen, naproxen, and 2nd generation antihistamines (including fexofenadine, loratadine, and cetirizine); patients may not take any new prescription medications, OTC, or dietary supplements from 14-days prior to Baseline through the end of study follow-up unless used to manage a treatment emergent adverse event, in which case the patient should report to his/her Study Physician as soon as possible, the occurrence of the adverse event and the medication(s) they are taking to treat the adverse event. The Investigator will make every effort to contact the Sponsor's Medical Monitor prior to administration of a new concomitant therapy (prescription or OTC) after treatment assignment, unless the concomitant therapy is needed immediately for patient safety.

Duration of Treatment	Each treatment cohort will receive twelve (12) weeks of consecutive once daily dosing. In the event that collection of the ^{31}P-MRS at the Week 12 visit is delayed due to imaging issues or COVID-19 pandemic related concerns, participants may remain on study drug beyond the Week 12 visit, so long as the extension is agreeable to both the study PI and Sponsor's Medical Monitor, until it is deemed safe to return for collection of the Week 12 efficacy assessments. Safety assessments that are able to be captured should be completed every 4 weeks beyond the planned Week 12 visits prior to dispensation of unscheduled study drug.
Test Product, Dose, and Mode of Administration	CNM-Au8 is an aqueous suspension of clean surfaced faceted nanocrystals consisting of gold atoms self-organized into crystals of various geometrical shapes (hexagonal bi-pyramid, pentagonal bi-pyramid, tetrahedron, decahedron, planar spheroids). Highly pure elemental Au nanocrystals are suspended in USP purified water buffered with 0.546 mg/mL (6.5 mM) sodium bicarbonate ($NaHCO_3$) nominally concentrated to 0.0625 mg/L (62.5 ppm) for the 7.5 mg CNM-Au8 dose, 0.125 mg/L (125 ppm) for the 15 mg CNM-Au8 dose, 0.25 mg/L (250 ppm) for 30 mg CNM-Au8, or 0.5 mg/L (500 ppm) for 60 mg CNM-Au8. CNM-Au8 will be administered orally in a total volume of 120 mL from two single-unit sterile 60 mL HDPE containers.
Primary Outcome *Brain Redox Potential*	**Measure of Brain Bioenergetic Changes** *Change in ^{31}P-MRS Redox Ratio (NAD+/NADH):* • Mean change in average NAD+/NADH measured brain Redox Ratio per treatment cohort from Baseline to Week 12.
Exploratory Outcomes *^{31}P-MRS CNS Bioenergetic Metabolites and Membrane Markers*	**Other Measures of Brain Bioenergetic Changes** *Change in ^{31}P-MRS Bioenergetic Metabolites* • Regression of baseline values versus mean percentage change of the average tissue concentration by subject per dosing group from Baseline to Week 12 for: ○ ATP (α, β, γ) ○ NAD+/NADH pool ○ Phosphocreatine (PCr) ○ Intracellular inorganic phosphate (Pi(in)) ○ Extracellular inorganic phosphate (Pi(ex))

	Change in ^{31}P-MRS Membrane Components • Regression of baseline values versus mean percentage change of the average tissue concentration by subject per dosing group from Baseline to Week 12 for: ○ Phosphoethanolamine (PE) ○ Phosphocholine (PC) ○ Glycerolphosphoethanolamine (GPE) ○ Glycerophosphocholine (GPC)
Functional Exploratory Outcomes	**Exploratory Efficacy Measures of Gait, Balance, and Mobility** • Mean change in average difference between Baseline to Week 12 for: ○ APDM Instrumented Timed Up and Go (TUG) Test ○ APDM Instrumented Postural Sway Test ○ APDM Instrumented Walk Test **Exploratory Efficacy Measurements of Global Impression of Disease Severity and Improvement** • Mean change in average difference between Baseline to Week 12 for: ○ Clinical Global Impression Scale (CGI) ○ Patient Global Impression Scale (PGI) **Exploratory Efficacy Measurements of Disease Progression** • Mean change in total score and sub-scales between Baseline to Week 12 for: ○ Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
Safety Endpoints	• Columbia Suicide Severity Rating Scale (C-SSRS)
Pharmacodynamic (PD) Endpoints	**Measures of Blood and Cerebrospinal Fluid Biomarkers (CSF optional)** Whole blood and plasma will be collected at baseline and at Weeks 4, 8, and 12 and stored for subsequent pharmacodynamic analyses. CSF will be collected on a voluntary basis only at baseline and at Week 12 and stored for subsequent pharmacodynamic analyses. PD analyses will be specified in a separate PD Analysis Plan.
Pharmacokinetic (PK) Endpoints	A sample for the measurement of whole blood concentrations of Au will be collected before (pre-dose) administration of CNM-Au8 at Weeks 4, 8, and the end of study follow-up Week 16 visit. At the Week 12 visit, whole blood samples for the measurement of whole blood concentrations of Au will be collected before

	(pre-dose) and at 1, 2, 4, and 6 hours, after administration of the last dose of the study drug. The study site shall contact the patient and document the time at which the patient administered his/her prior day's dose of study drug in order to impute a 24-hour trough value. The data will be used to estimate an apparent C_{max} and T_{max} and area under the curve (AUC) over the 24-hour dosing interval [$AUC_{(0-24)}$].
Statistical Methods	The primary efficacy endpoint is mean change in NAD Redox Ratio from Baseline to Week 12. Redox Ratio is defined as: NAD Redox Ratio = mean NAD+/NADH The statistical significance, comparing the result in each CNM-Au8 treatment to Baseline, will be reported. Statistical significance will be determined by two-sided paired t-tests assessed at the $p \leq 0.05$ significance level. The Cohort 2 sample size may be re-calculated based on the mean change from baseline in NAD+/NADH Redox Ratio to Week 12 and standard deviation of the change from Cohort 1. Safety endpoints include incidence of treatment-emergent AEs, deaths, SAEs, AEs leading to discontinuation from the study and changes in the C-SSRS. Changes from baseline in clinical laboratory results and vital signs will be summarized by treatment group and time point. All safety summaries will be descriptive; no statistical significance tests will be performed on safety data and will be based on the safety population. Gold (Au) concentration data in whole blood will be summarized with descriptive statistics by treatment group at each time point of collection.

Table 1: Time and Events Schedule

Time and Events Schedule	Visit	-1	0	1	2	3	4	5
	Phase	Screening	Baseline	Treatment Period				EOS
	Week	-6	0	2	4	8	12	16
	Day	-42 to -1	1[a]	14 [a]	28 [a]	56 [a]	84 [a]	112 [b]
ICF Signed		X						
Eligibility Review		X	X					
Medical History		X						
ConMed/Prior Med Assessment		X	X	X	X	X	X	X
Physical Examination		X	X		X	X	X	X
Height Assessment		X						
Weight Assessment		X	X				X	
Urine Drug Test		X						
HIV/Viral Hepatitis Screen		X						
Serum Pregnancy Test [c]		X						
Urine Pregnancy Test [c]			X		X	X	X	
Vital Signs		X	X		X	X	X	X
12-lead ECG [d]		X	X		X	X	X	X
Clinical Laboratory (Blood)		X	X		X	X	X	X
Urinalysis		X	X		X	X	X	X
MMSE		X						
Treatment Assignment			X					
Dispense/Return Drug			X		X	X	X	
PK Sampling (Whole Blood)					X[e]	X[e]	X[f]	X[e]
PD Sampling (Whole Blood, Plasma)			X[e]		X[e]	X[e]	X[e]	
CSF Sampling (optional)			X				X	
Adverse Events		X	X	X	X	X	X	X
Anxiolytic Administration			X				X	
^{31}P-MRS			X				X	
MDS-UPDRS			X		X	X	X	
PGI			X		X	X	X	
CGI			X		X	X	X	
C-SSRS		X	X	X	X	X	X	X
APDM Instrumented Tests			X				X	
Phone call				X				

Time and Events Schedule Footnotes:

a. Scheduled Visit ± 4 days for Visit 1-4. For Visit 0 (Baseline), the ^{31}P-MRS and PD CSF assessments may be completed over a -7 day window prior to Day 1. All assessments must be completed prior to administration of first study drug dose.
b. Timing for the EOS assessment should occur at four weeks (±3 days) from last dose regardless of early termination or completion of the trial.
c. For females of child bearing potential only.
d. Electrocardiogram (ECG) intervals will be summarized and presented descriptively. ECG rhythm will be interpreted by the Investigator as normal (N), abnormal not-clinically significant (aNCS), or abnormal clinically significant (aCS). Triplicate values will be collected at Baseline and averaged for comparison to single assessments at subsequent visits.
e. Whole blood for PK and PD will be taken pre-dose only (~1 hour prior to the dose of study drug).
f. Whole blood for PK will be taken at pre-dose (T_0) and at 1, 2, 4, and 6 hours after dosing for the visit. The exact time at which the patient took his/her previous day's study drug dose must be recorded in order to impute a 24-hour trough value ($T_{24\text{-imputed}}$).

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ABBREVIATIONS

Abbreviation	Definition
^{31}P-MRS	Phosphrus-31 magnetic resonance spectroscopy
6-OHDA	Oxidopamine
α -SYN	Alpha-synuclein
aCS	Abnormal clinically significant
AD	Alzheimer's Disease
AE	Adverse event
AESI	Adverse events of special interest
aNCS	Abnormal not clinically significant
ASTM	American Society for Testing and Materials
ATP	Adenosine Trinucleotide Phosphate
Au	Gold
AUC	Area under the curve
$AUC_{(0-24)}$	Area under the curve for 24 hours
βhCG	Beta-human chorionic gonadotropin
BID	Twice a day (*bis in die*)
C_0	Initial or back-extrapolated plasma drug concentration at time zero following bolus intravenous injection
CA	Competent authority
CFR	Code of Federal Regulations
CGI	Clinical Global Impression
CL	Apparent total body clearance of the drug
CL/F	Apparent total clearance of the drug from whole blood after oral administration
CLr	Renal clearance
Cmax	Peak plasma concentration, observed
CNM-Au8	Aqueous suspension of clean surfaced nanocrystals consisting of gold atoms self-organized into crystals of various faceted, geometrical shapes
CNS	Central nervous system
CRF	Case report form
CRO	Contract research organization
CRU	Clinical research unit
CSF	Cerebrospinal fluid or cerebral spinal fluid
CTCAE	Common terminology criteria for adverse events
CV%	Coefficient of variation

Abbreviation	Definition
DNA	Deoxyribonucleic acid
DO	Doctor of osteopathic medicine
EC	Ethics committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EOS	End of study
F	Bioavailability (systemic availability of the administered dose)
FDA	Food and Drug Administration
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practices
HepB	Hepatitis B
HepC	Hepatitis C
HDPE	High density polyethylene
HIV	Human immunodeficiency virus
hr	Hour
HREC	Human Research Ethics Committee
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICH-GCP	International Conference on Harmonization – Good Clinical Practice
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ISO	International Organization for Standardization
IV	Intravenous
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
kg	Kilogram
LOQ/LLOQ	Limit of quantitation or Lower limit of quantitation
m	meter
M:E	Ratio of myeloid to erythroid precursors in bone marrow
MAD	Multiple ascending dose
max	Maximum
MD	Doctor of medicine

Abbreviation	Definition
MDS-UPDRS	Movement Disorders Society – Unified Parkinson's Disease Rating Scale
MFD	Maximum feasible dose
mg	Milligram
MIBG-SPECT	Metaiodobenzylguanidine scintigraphy
min	Minute(s)
mL	Milliliter
mmHg	Millimeters of mercury
MMSE	Mini-Mental State Examination
MPTP	Methyl-4-phenyl-1,2,3,6 tetrahydropyridine
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
ms	Milliseconds
MTD	Maximum tolerated dose
N	Normal
NAD+	Oxidized form of nicotinamide adenine dinucleotide
NADH	Reduced form of nicotinamide adenine dinucleotide
NADP+	Oxidized form of nicotinamide adenine dinucleotide phosphate
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Baking soda (Sodium Bicarbonate)
NIST	National Institutes of Standards and Technology
ng	nanogram
nm	nanometer
NOAEL	No observed adverse effect level
O_2	Oxygen
OG	Oral gavage
OL	Oligodendrocyte
OPC	Oligodendrocyte progenitor cells
OTC	Over-the-counter
PA	Physician assistant
PD	Pharmacodynamic
PGI	Patient Global Impression
pH	Potential hydrogen (relative acidity or alkalinity)
PK	Pharmacokinetic
PO	Oral administration (*per os)*
QD	Once daily (*quaque die*)

Abbreviation	Definition
ROS	Reactive oxygen species
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of the mean
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamate-pyruvic transaminase
SOD	Superoxide dismutase
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
$T_{½}$	Elimination half-life
T1	Time constant for the longitudinal relaxation time following an MRI radio-frequency pulse
T2	Time constant for the transverse relaxation time (perpendicular to the main field) following an MRI radio-frequency pulse
TEAE	Treatment emergent adverse event
TEM	Transmission electron microscopy
TID	Three times daily (*ter in die*)
TK	Toxicokinetic
T_{max}	Time to peak plasma concentration, observed
Ue	Urinary excretion
USP	United States Pharmacopeia
V_{ss}	Volume of distribution at steady state
V_z	Apparent volume of distribution (Vz)
V_z/F	Apparent volume of distribution during terminal phase after non-intravenous administration

1 BACKGROUND INFORMATION

1.1 Disease Background and Pathophysiology

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by tremors, bradykinesia, limb rigidity and gait and balance problems. Approximately one in one hundred individuals over the age of 60 is affected by PD. Both genetic and environmental factors are thought to contribute to the development of PD, and a combination of factors results in the progressive loss of dopaminergic neurons in the substantia nigra area of the midbrain.
PD is currently incurable; existing treatments for PD, such as dopamine agonists, COMT and MAO-B inhibitors, and deep brain stimulation, produce only a modest and temporary amelioration of symptoms. Specifically, long-term use of levodopa, a commonly-prescribed dopamine precursor used to treat Parkinsonian symptoms, results in dyskinesia that in itself becomes disabling. The global burden of PD is expected to further increase owing to population ageing (GBD 2016 Parkinson's Disease Collaborators 2018). PD results in a large economic burden on society. Current major challenges in PD drug development include the lack of reliable biomarkers for its early diagnosis and for disease progression, the lack of a clear understanding of pathophysiologic mechanism(s) of PD, and lack of well-validated clinical trial tools to demonstrate neuroprotection (Arnerić, Kern, and Stephenson 2018; Olanow, Kieburtz, and Katz 2017).

Despite an enormous effort over the past several decades, no disease-modifying or neuroprotective therapeutic for PD has been developed. A therapeutic that alters or slows the clinical progression, and thus improves PD patient quality of life and lifespan, represents a huge unmet need (Olanow, Kieburtz, and Katz 2017).

The pathophysiology of genetic and sporadic variants of PD, though multifactorial, appear to share common pathogenetic mechanisms, including impaired mitochondrial and lysosomal functions, neural sensitivity to glutamate toxicity, accumulation of oxidative stress, autophagic failure in clearing misfolded proteins, and loss of synapse integrity (Plotegher and Duchen 2017). These pathogenetic mechanisms all together are representative of processes of neuronal bioenergetic failure (Camandola and Mattson 2017). As such, improvement of cellular

bioenergetic efficiency represents an important and previously unaddressed therapeutic target for this disease.

Several published studies suggest that elevating brain NAD+ levels and improving cellular redox may be therapeutic for the treatment of PD and other neurodegenerative diseases (De Jesus-Cortes et al., 2012; Gong et al., 2013; Kim et al., 2017; Tesla et al., 2012; Zhou et al., 2015). As cells age, energy production capabilities decrease, mitochondria become damaged, and the redox potential of energy-producing molecules such as nicotine adenine dinucleotide (NAD/NADH) declines. NAD/NADH serve as a key redox couple in energy-producing pathways of the cell, including glycolysis and oxidative phosphorylation.

An age-dependent decline of NAD/NADH redox has been previously demonstrated by ^{31}P magnetic resonance spectroscopy of the visual cortex in young, middle-aged, and aged healthy humans (Zhu et al. 2015). In other studies, increasing intracellular levels of NADH and NAD+ have been demonstrated following administration of the precursor, nicotinamide, in cell and animal models of PD resulting in improvements in mitochondrial function and resistance to oxidative stress (Jia et al. 2008; Delgado-Camprubi et al. 2017; S. Lehmann et al. 2016; Susann Lehmann, Loh, and Martins 2017; Thirtamara-Rajamani et al. 2017; Schwab et al. 2017; Sison and Ebert 2018; Ocampo, Liu, and Barrientos 2013). Similarly, nicotinamide supplementation of culture media improved SK-N-MC neuroblastoma cell viability in response to the dopaminergic neural toxin MPTP, and feeding nicotinamide to transgenic alpha-synuclein (α-syn) overexpressing Drosophila improved climbing behavior (Jia et al. 2008). More recently, Schöndorf et al. demonstrated that increasing intracellular NAD+ using nicotinamide riboside supplementation rescued mitochondrial ultrastructure, reduced ROS levels, restored mitochondrial membrane potential, and increased the potential for mitophagy in PD iPSC-derived neurons (Schondorf et al. 2018). Taken together, these data all suggest that bioenergetic failure plays an important role in the pathophysiology of PD and that agents that improve NAD/NADH redox states have the potential to treat this neurodegenerative disease.

1.2 Investigational Product Rationale and Characteristics

1.2.1 Investigational Product Background

CNM-Au8 is an aqueous suspension of clean-surfaced nanocrystals consisting solely of Au atoms organized into crystals of highly faceted substantially uniform geometrical shapes. CNM-Au8 is supplied for dosing orally in sodium bicarbonate buffered USP purified water. Historically, monomolecular gold complexes (e.g., injectable sodium aurothiomalate, aurothioglucose; oral auranofin) have been widely used historically as immunomodulating therapies in the treatment of autoimmune disorders, predominantly rheumatoid arthritis (Dabrowiak, 2009). However, multiple forms of toxicity, including pruritus, dermatitis, stomatitis, diarrhea, proteinuria, and less frequently hematological effects compromised the clinical use of gold complexes (Dabrowiak, 2009; Menninger et al., 1998). While the role of gold in the pattern of toxicity remains unclear, it is hypothesized that adverse events (AEs) related to gold complexes may be specifically related to the monomolecular covalent formulations of the gold complexes rather than from the activity of gold *per se* (Dabrowiak, 2009; Yei Ho and Tiekink, 2005).

When functionalized as nanocrystals, gold adopts novel electrochemical characteristics unlike those of gold complexes. In addition, as clean-faceted nanocrystals CNM-Au8 has unique properties including a clean safety and tolerability profile as well as high level of biocatalytic activity in cells that is unlike that of gold nanoparticles manufactured using traditional methods that require stabilizing or residual chemical modifications to the particles' surfaces.

The faceted gold nanocrystals of CNM-Au8 generally consist of shapes including hexagonal bi-pyramids, pentagonal bi-pyramids, tetrahedrons, and octahedrons. These crystalline shapes, absent organic residues on their surfaces, are central to the biologic activity of CNM-Au8. In contrast to molecular covalently bound gold compounds (e.g., historical gold salts), or colloidal gold nanoparticles containing surface residue or surfactants as a surface layer (e.g., polyethylene glycol (PEG), cetyl trimethylammonium bromide (CTAB), citrate, thiols, other surfactants), CNM-Au8 consists solely of nanocrystals grown through an electro-crystal chemistry process, which produces consistently faceted catalytically active geometrical shapes (without utilizing surfactants such as those derived from the reduction of chloroauric acid ($HAuCl_4$)).

The characteristics of a nanoparticle's size, shape, and surface chemistry impact, and can define, the biological activity of the nanoparticles. Residual surface chemistry also affects the biological activity of nanoparticles and directly impacts cellular uptake. Nanoparticle surfactants and residual chemical monolayers are also well-known to cause nanoparticle toxicity (Balasubramanian et al., 2010; Freese et al., 2012; Qiu et al., 2010).

In summary, CNM-Au8 was rationally designed to eliminate the need for residual surface chemistry, and to promote biocatalytic activity on it's clean-surfaced, faceted nanocrystalline surfaces. CNM-Au8 is a novel therapeutic paradigm as a suspension of non-covalently bound faceted clean-surfaced Au nanocrystals.

1.2.2 Investigational Product Characteristics

The median diameter of CNM-Au8 nanocrystals is approximately 13 nm, as determined by transmission electron microscopy (TEM). Based upon the distributed range (D_n5 – D_n95: 6.5 – 15.8 nm) of measured nanocrystal diameters and approximate geometrical shapes (e.g., low volume estimate: disc-like approximation, aspect ratio of 0.2; maximum volume estimate: spheroid, aspect ratio of 1.0), each Au nanocrystal has an approximate composition ranging from 13,000 – 66,000 Au atoms per nanocrystal at the 13 nm median diameter with a corresponding molar mass ranging between 2.7 x 10^3 kDA to 1.3 x 10^4 kDA. Summary estimates for CNM-Au8 mass, volume, and particle characteristics are described below in Table 2. Each mL of CNM-Au8 suspension at 500 µg/mL is estimated to contain between 100 - 500 trillion highly faceted Au nanocrystals.

Table 2. Estimated Volume, Mass, and Particle Characteristics of CNM-Au8

Metric (CNM-Au8 500 µg/mL, 60 mL Dose)	**Disc-Like Approximation Minimum** *(Aspect 0.2)*	**Spherical Approximation Maximum** *(Aspect 1.0)*
Median Au Nanocrystal Diameter (nm)	13	
Au Nanocrystal Volume (nm^3)	2.3×10^2	1.2×10^3
Au Nanocrystal Surface Area (nm^2)	3.2×10^2	5.3×10^2
Au Atoms per Nanocrystal (count)	1.4×10^4	6.8×10^4
Au Nanocrystal Molecular Weight (kDa)	2.7×10^3	1.3×10^4
Total Au Nanocrystal Surface Area per mL (cm^2)	3.6×10^2	1.2×10^2
Au Nanocrystals per mL CNM-Au8 (count)	1.1×10^{14}	2.3×10^{13}
Au Nanocrystals per 60 mL Dose of CNM-Au8 (count)	3.4×10^{15}	6.8×10^{14}

1.3 CNM-Au8 Bioenergetic Mechanism of Action

CNM-Au8 passes into the systemic circulation via intestinal absorption. Cellular uptake of CNM-Au8 has been demonstrated in a variety of cells and tissue matrices including macrophages, oligodendrocytes, neurons, and brain tissue using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyses and high resolution TEM visualization of nanocrystals.

Preclinical characterization of the *in vitro* and *in vivo* properties of CNM-Au8 have demonstrated that it acts to protect neuronal populations from chemical, inflammatory, and hypoxic insults through a series of unique catalytic mechanisms involving 1) the nicotine adenine dinucleotide redox couple (NAD+/NADH) to boost glycolytic energy production, 2) the nicotine adenine dinucleotide phosphate redox couple (NADP+/NADPH) to influence anabolic processes associated with differentiation, and 3) the SOD-like inactivation of reactive oxygen species and nitric oxide to protect cells from oxidative damage and mitochondrial dysfunction.

The nicotinamide adenine dinucleotide redox couple (NAD+, NADH) plays a central role in the energy metabolism of all living cells. NAD+, NADH and their relative intracellular ratio (NAD+/NADH) are linked to regulation of energy homeostasis, neuroprotection, immune

function, chromosome stability, DNA repair mechanisms, sleep and circadian rhythms, and longevity (Canto et al., 2015; Imai, 2010a, b; Nikiforov et al., 2015; Ying, 2008). Fundamentally, NAD+ and NADH are essential coenzymes in the adenosine triphosphate (ATP)-generating reactions driving both glycolysis and oxidative phosphorylation. More specifically, in aerobic glycolysis, NAD+ availability is integral to the enzymatic cascade from glyceraldehyde 3-phosphate via glyceraldehyde phosphate dehydrogenase to 1,3-bisphosphoglyceric acid. Similarly, two electrons are removed via NADH oxidation in Complex I of the electron transport chain during oxidative phosphorylation, resulting in NAD+, and ultimately these electrons are transferred to a lipid-soluble carrier, ubiquinone.

In addition, NAD+ and its metabolites act as binding substrates for a wide range of proteins including the metabolic and transcription-regulating sirtuins, the poly-ADP-ribose polymerases (PARPs) involved in DNA repair, and the cyclic ADP-ribose synthases (cADPRs) such as CD38 that serve to regulate Ca^{2+} signaling involved in cell cycle control and insulin signaling (Canto et al., 2015).

NAD+ and NADH are essential for life; dietary deficiency of vitamin B3, a NAD+ precursor, gives rise to a life-threatening condition known as pellagra, which is characterized by a dark, pigmented skin rash, diarrhea, and dementia (Sydenstricker, 1958). Transgenic mice lacking both copies of the NAD+ salvage enzyme *Nmnat1* resulting in NAD+ deficiency, do not live past embryonic stages (Conforti et al., 2011). Conversely, the heightened expression of NMNAT1, resulting in increased NAD+ in the Wallerian Degeneration Slow Dominant Mutant Mouse, has been attributed to neuronal protection against axon degeneration observed in these mice (Sasaki et al., 2009).

Quantitative measurement of regional brain area NAD+ concentrations and redox states in healthy and diseased humans has recently been demonstrated using a novel ^{31}P-Magnetic Resonance Spectroscopy (MRS) imaging technique (Chouinard et al., 2017; Lu et al., 2016). In humans, brain NAD+/NADH redox potential is inversely correlated with age. This age-related decrease in cerebral energy metabolism is believed to be intimately associated with the bioenergetic failure that may underpin the pathophysiology of all neurodegenerative diseases, because NAD+/NADH redox potential is essential for fundamental ATP-generating processes

such as glycolysis and mitochondrial oxidative phosphorylation. These bioenergetic processes power all cellular processes including 'housekeeping' functions that are responsible for maintaining overall cellular health including processes of autophagy, apoptosis, and the unfolded protein response (UPR) (Villanueva-Paz et al. 2016; Chua and Tang 2013; Cantó, Menzies, and Auwerx 2015). Boosting brain NAD+ levels in a mouse model of AD reduced DNA damage, neuroinflammation, and apoptosis while improving cognitive function in multiple behavioral tests and restored hippocampal synaptic plasticity (Hou et al., 2018). In humans, ^{31}P-MRS of the visual cortex of young (21-26 year old), middle-aged (33-36 year old), and aged (59-68 year old) healthy individuals has been show to demonstrate a measurable linear decline of NAD+ redox potential that occurs with increasing age (Zhu et al., 2015).

The first demonstration of oxidation of NADH to NAD+ by gold nanoparticles was demonstrated by Huang et al. (2005) using a simple cell-free assay. In order to compare the catalytic oxidation rate of clean-surfaced CNM-Au8 against similarly-sized gold nanoparticles made using citrate reduction, the same cell-free assay utilized by Huang et al. was repeated comparing gold nanoparticles sourced from the U.S. National Institutes of Standards and Technology (NIST). The resulting change in the 339 nm NADH peak was assessed while investigating each of the gold nanoparticles at the same NADH and Au starting concentrations. In all cases, CNM-Au8 nanocrystals consistently showed significantly superior catalytic activities regardless of size and/or method of preparation of the comparator gold nanoparticles, (Figure 1).

Figure 1. CNM-Au8 NADH Catalysis Rates

Figure 1A. CNM-Au8 Catalytic Effects vs. NIST Standard Citrated AuNP

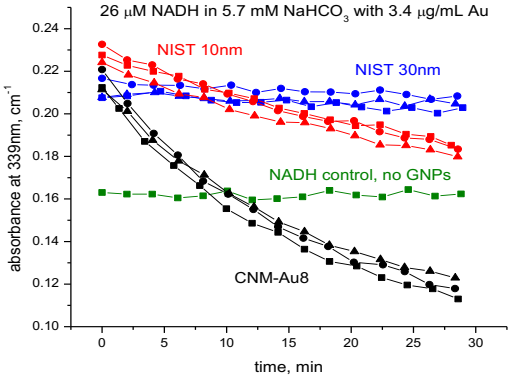
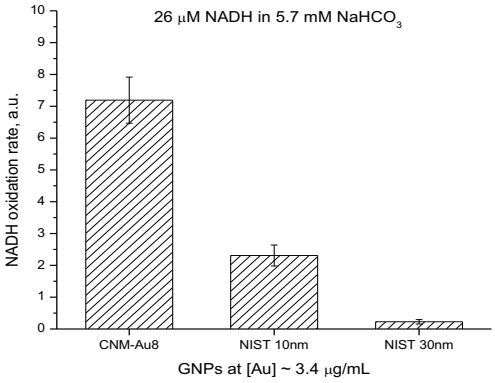


Figure 1B. Relative NADH Oxidation Rates vs. NIST Citrated AuNP



CNM-Au8 treatment of rodent central nervous system cells *in vitro* also significantly increases levels of both NAD+ and NADH (Figure 2).

Figure 2. Effects of CNM-Au8 on NAD Levels in Primary Rodent Mesencephalic Cultures

Figure 2A. Effect of CNM-Au8 on NAD+ Levels

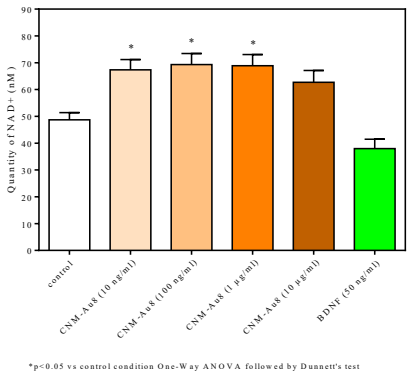
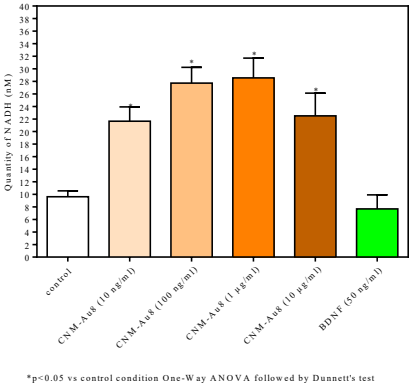


Figure 2B. Effect of CNM-Au8 on NADH Levels

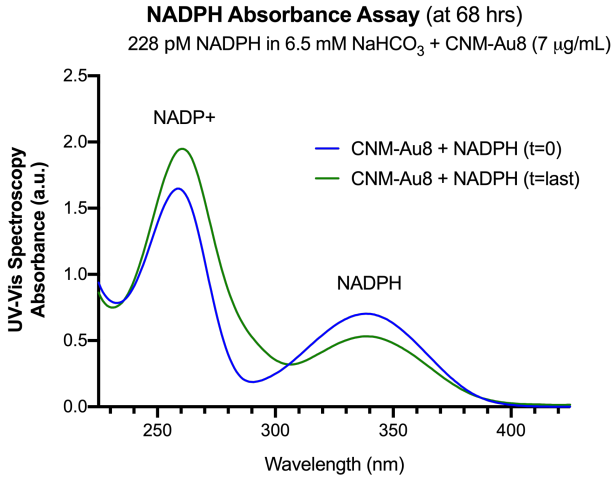


The redox couple NADP+/NADPH, similar to NAD+/NADH, plays a fundamental role in energy homeostasis, anabolic processes, and protection against oxidative stress. NADP+ and NADPH function in the pentose phosphate pathway (PPP), serves to provide precursors for nucleotide and amino acid biosynthesis (Stincone et al., 2015). In addition, NADP+ and NADPH serve as the redox equivalents for both the thioredoxin and glutathione systems (Grant, 2008; Pollak et al., 2007), which in turn serve as the cell's primary defenses against oxidative stress.

CNM-Au8 catalyzes the oxidation of NADPH to NADP+ (Figure 3). While the time course of catalysis of NADPH to NADP+ is longer than that observed for conversion of NADH to NAD+, the magnitude of the effect is similar. Thus, CNM-Au8 plays an important role in providing NADP+ to the enzyme glucose 6-phosphate dehydrogenase for the conversion of 6-phosphogluconate to ribulose 5-phosphate and NADPH + CO_2, which represents the rate-limiting step of the oxidative branch of the PPP.

Figure 3. Effects of CNM-Au8 on NADPH Oxidation

Figure 3. NADPH Oxidation Rates



Excessive reactive oxygen exposure disrupts cellular redox homeostasis, damages intracellular organelles as well as DNA and proteins, and may also underpin pathophysiologic mechanisms of PD (Puspita, Chung, and Shim 2017). Super oxide dismutases (SODs) evolved as a component of the cell's antioxidant defense system to regulate the levels of reactive oxygen species (ROS) and prevent damage from oxidative stress. SOD enzymes are found in the cytoplasm and mitochondria where they convert oxygen radicals to O_2 or H_2O_2, which can in turn be converted to water and O_2 by catalases. A dose-dependent reduction of ROS levels was observed in differentiating oligodendrocyte precursor cells in primary culture with CNM-Au8 treatment (Figure 4).

Figure 4 . Effects of CNM-Au8 on SOD and ROS Generation

Figure 4A. Effects on SOD Activity Assay

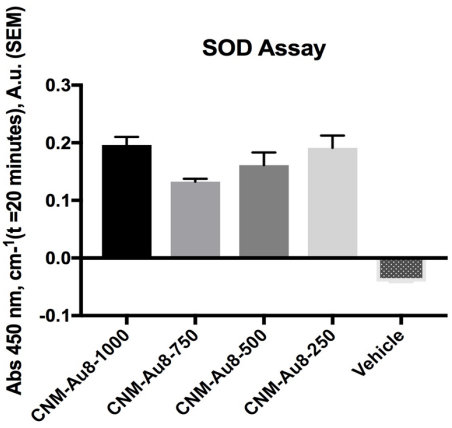


Figure 4B. Effects on ROS Generation in Purified Murine OPC cultures

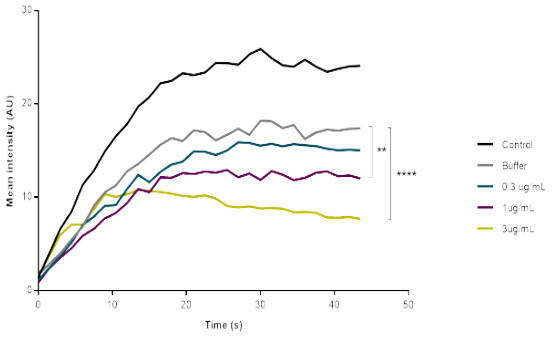
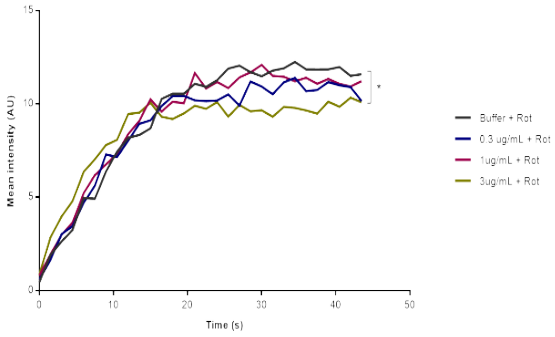


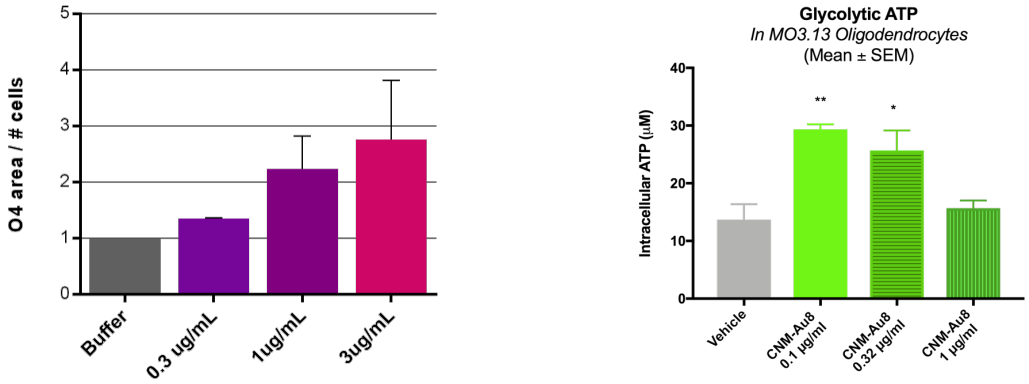
Figure 4C. Effects on ROS Generation in Murine OPC Cultures Plus Rotenone



Demonstration of SOD-like catalytic activity with CNM-Au8 treatment has several important implications. First, neurons and glial cells are energetically demanding and generate large amounts of ROS as byproducts of the energetic processes necessary for their function. Theories of aging and neurodegeneration suggest that as CNS cells age, they are less able to efficiently remove ROS and thereby succumb to the consequences of long-term ROS exposure. As CNS cells are exquisitely sensitive to ROS, they are among the first cell types to degenerate, leading to the cognitive, psychomotor, and movement impairments observed in diseases such as Alzheimer's disease and Parkinson's disease (Angelova and Abramov, 2018). In addition, SOD1 may act not only as a regulator of ROS but also as a part of a glucose-oxygen sensing mechanism of a cell to determine whether the cell utilizes oxidative phosphorylation or aerobic glycolysis for ATP production (Reddi and Culotta, 2013). A study of oligodendrocyte (OL) energetics showed that human OLs preferentially use aerobic glycolysis during differentiation and myelination

(Rone et al., 2016). Data with CNM-Au8 demonstrate that treatment switches OPCs from proliferation to differentiation, while simultaneously stimulating aerobic glycolysis production of ATP likely through NADH oxidation (Figure 5). Stimulation of SOD-like activity therefore is consistent with a role for CNM-Au8 in regulating cellular bioenergetics.

Figure 5. Effect of CNM-Au8 on OPC Differentiation and ATP Production



Effect of CNM-Au8 on a) OPC differentiation (O4+ cells) in isoldated murine OPCs and b) ATP production in MO3.13 oligodendrocytes (72hr). Statistical analysis performed using one-way ANOVA ($p < 0.05$).

1.4 CNM-Au8 Parkinson's Disease Neuroprotection Models

1.4.1 *In vitro* Dopaminergic Neuroprotection Models

I*n vitro* models of neuroprotection were conducted to assess the neuroprotective role of CNM-Au8's bioenergetic mechanism of action. Treatment with the neurotoxins: methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) results in oxidative stress and impairment of neuronal mitochondrial function (Dauer and Przedborski 2003).

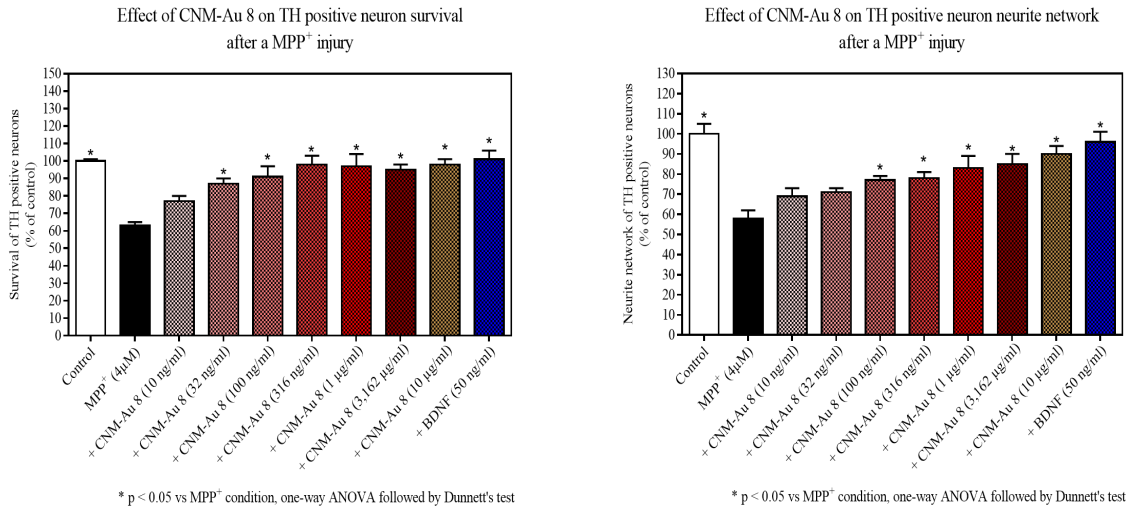
CNM-Au8 was studied in rat primary mesencephalic neurons injured with MPP+ or 6-OHDA. The primary assessment was protection of tyrosine hydroxylase (TH)-expressing neurons and neurite network preservation. In addition, impairment of mitochondria function induced by these neurotoxins was studied including measures of energy production (ATP pool), oxidative stress (ROS), and mitochondria function.

MPP+ (4 µM, 48 h) induced significant TH+ neuronal death (~35 %) and loss of the neurite network (~40 %) in primary rat dopaminergic neurons. In the presence of CNM-Au8 (pre-incubated 48 hours before MPP+), a significant dose-dependent neuroprotective effect was

observed (Figure 6) . A dose-dependent neuroprotective effect on neurite network area was also observed. Furthermore, addition of the neurotoxin MPP+ induced a significant decrease of number of mitochondria in the cytoplasm of TH+ neurons. Pretreatment with CNM-Au8 for 48 hours significantly protected neurons from depletion of mitochondria (Figure 7).

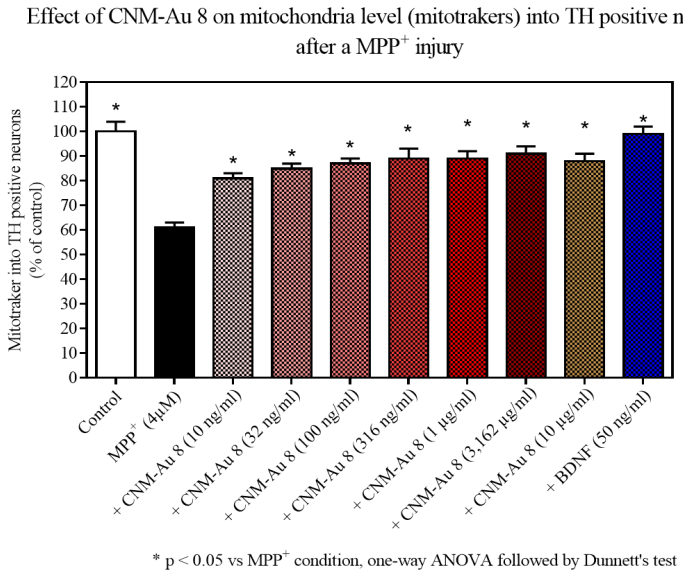
Consistent with the MPP+ results, 6-OHDA (20 µM, 48h) also induced a significant TH+ neuronal death (~30 %) and a large loss of the neurite network (~40%). The presence of CNM-Au8 (pre-incubated 48 hours before 6-OHDA), also showed a significant dose-dependent protective effect on neuronal survival (Figure 8).

Figure 6. Effect of CNM-Au8 on TH+ Neuron Survival and Neuron Network Area Following MPP+ Injury



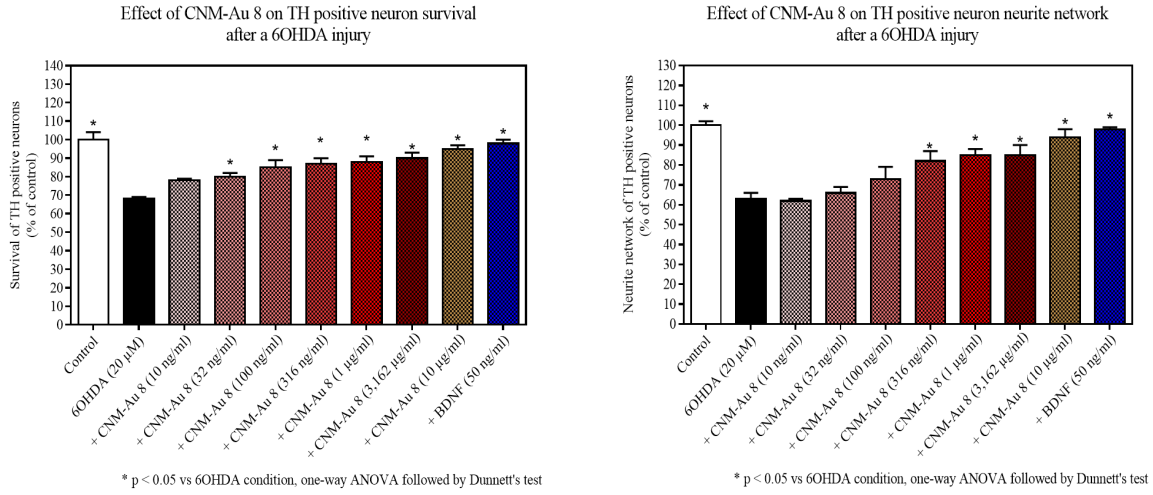
*Effect of CNM-Au8 on a) TH positive neuron survival and b) neurite network of primary rat dopaminergic neurons injured with MPP+ (48h, 4 µM). Data expressed as percentage of control show the mean ± SEM (100% = no MPP+, no compound). One-way ANOVA followed by Dunnett's test, n=6. * p< 0.05 was considered significant (vs MPP+ condition).*

Figure 7. Effect Of CNM-Au8 On Mitochondria Level (Mitotracker) In TH Positive Neuron After MPP+ Injury



*Effect of CNM-Au8 on mitochondria level in primary rat dopaminergic neurons injured with MPP+ (48h, 4 µM). Data expressed as percentage of control show the mean ± SEM (100% = no MPP+, no compound). One-way ANOVA followed by Dunnett's test, n=6. * p< 0.05 was considered significant (vs MPP+ condition).*

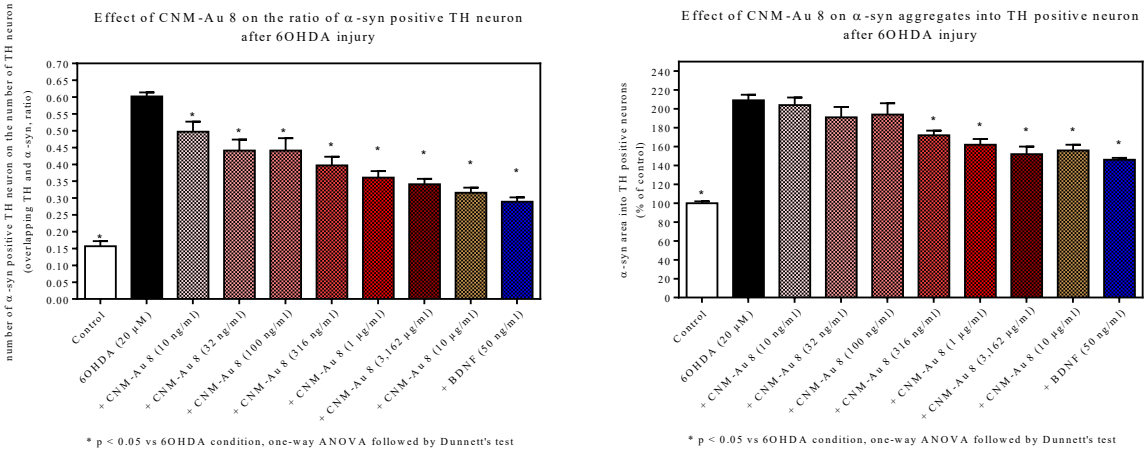
Figure 8. Effect Of CNM-Au8 On TH+ Neuron Survival And Neurite Network Following 6-OHDA Injury



*Effect of CNM-Au8 on a) TH positive neuron survival, and b) neurite network, in primary rat dopaminergic neurons injured with 6OHDA (48h, 20 µM). Data expressed as percentage of control show the mean ± SEM (100% = no 6OHDA, no compound). One-way ANOVA followed by Dunnett's test, n=6. * p< 0.05 was considered significant (vs 6-OHDA condition).*

TH positive neurons demonstrated α-syn aggregates in their cytoplasm after 6-OHDA treatment and treatment with CNM-Au8 significantly and dose-dependently prevented intracellular α-synuclein aggregation. CNM-Au8 also significantly reduced the size of the α-synuclein aggregates (Figure 9).

Figure 9. Effect Of CNM-Au8 On α-Synuclein Presence In TH+ Neurons and α-Syn Aggregates Following 6-OHDA Injury



*Effect of CNM-AU 8 on a) ratio of TH positive neuron showing α-syn aggregates and b) size of the α-syn aggregates in primary rat dopaminergic neurons injured with 6OHDA (48h, 20 µM). Data expressed as percentage of control show the mean ± SEM (100% = no 6-OHDA, no compound). One-way ANOVA followed by Dunnett's test, n=6. * p< 0.05 was considered significant (vs 6-OHDA condition).*

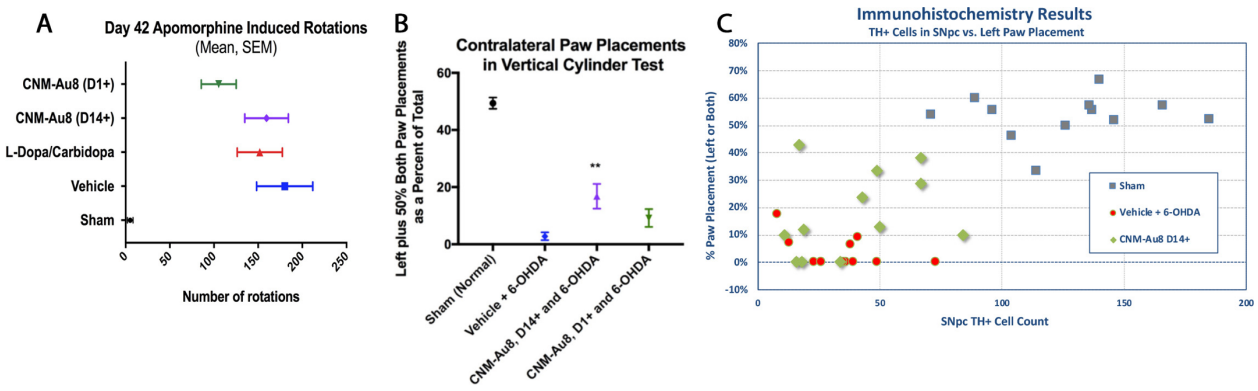
Taken together, these *in vitro* data demonstrate a significant neuroprotective effect of CNM-Au8 on dopaminergic neurons in neurotoxin models of Parkinson's Disease.

1.4.2 *In vivo* Dopaminergic Neuroprotection Models

CNM-Au8's was studied in a behavioral model of Parkinson's Disease Rats were unilaterally lesioned by stereotactic injection of 6-OHDA into the right striatum on Day 1. Apomorphine-induced rotations were quantified during a 30-minute window on a weekly basis starting on Day 15. Cylinder paw placement was quantified on Day -1 (baseline), and then weekly. In the apomorphine-induced rotation study (Figure 10A), early treatment (D1+) with CNM-Au8 resulted in a trend of reduced number of apomorphine-induced rotations by 42% compared to vehicle, numerically outperforming the reduction seen with the positive control L-Dopa/Carbidopa (CNM-Au8 early treatment vs. vehicle; p=0.06, unpaired t-test). In the

functional paw placement test (Figure 10B), both early treatment demonstrated a trend and late treatment with CNM-Au8 showed a statistically significant contralateral paw placement improvement over vehicle (Early: p=0.08; late: p=0.009, unpaired t-tests).

Figure 10. CNM-Au8 Efficacy in Reducing Apomorphine-Induced Rotations and Contralateral Paw Placements in 6-OHDA Unilaterally Lesioned Rats



Taken together, these *in vivo* data demonstrate a significant neuroprotective effect of CNM-Au8 in behavioral models of Parkinson's Disease.

In summary, CNM-Au8 has demonstrated neuroprotective effects in both *in vitro* and *in vivo* dopaminergic toxin-induced models of PD. Treatment with CNM-Au8 significantly increases cell viability while significantly reducing detectable intracellular alpha-synuclein (a-syn) aggregates within dopaminergic neurons exposed to the toxins MPTP and 6-OHDA. Functional *in vivo* studies using a standard 6-OHDA unilateral lesion rat model have further demonstrated CNM-Au8's efficacy. The unique intracellular catalytic mechanism of action, positions CNM-Au8 as a promising therapeutic for the treatment of PD.

2 SUMMARY OF NONCLINICAL SAFETY & TOXICOLOGY STUDIES

The Sponsor has conducted multiple safety and toxicology studies with CNM-Au8 in accordance with ICH M3 (R2) guidelines across three species including rats, minipigs, and canines, in addition to standard *in vitro* mutagenicity studies. Initial *in vivo* dosing levels were modeled on delivering comparable amounts of gold as the historical gold complex, auranofin, to a 50-kg patient on a milligram per kilogram basis without adjustments for body surface area variance between species. This resulted in target nonclinical daily doses of Au at an estimated 0.0348 mg/kg/day, which were increased 10-fold (0.348 mg/kg) and 100-fold (3.48 mg/kg) to provide an anticipated safety margin for planned human dosing.

2.1 Summary of Safety Mutagenicity Studies

Three safety mutagenicity studies were conducted to address the mutagenic potential of CNM-Au8 including *in vivo* Micronucleus testing, and the in vitro Mammalian Cell Gene Mutation Test and Bacterial Reverse Mutation Test. These studies each demonstrated no effect of CNM-Au8 on cytotoxicity or mutation frequency as summarized below in Table 3.

Table 3. Summary of Safety Mutagenicity Studies

Study ID (Internal ID/ CRO ID)	GLP Status	Mutagenicity Model	Brief Design [Route; Frequency]	Effective Dose	CNM-Au8 Conc. (µg/mL)	Summary/Notes
AB06525	GLP	L5178Y Mouse Lymphoma Cells (Thymidine Kinase+/-)	Mammalian Cell Gene Mutation Test (In vitro)	1.7, 3.5, 5.2, 8.7 and 17 µg/mL	348	No signs of cytotoxicity; no biologically significant increases in the mutant frequency
AB05703	GLP	Salmonella typhimurium	Bacterial Reverse Mutation Test (In vitro)	17, 35, 70, 104, 174 µg/plate	348	No precipitate and no sign of cytotoxicity were noted in any strain and at any dose level, either with or without metabolic activation
AB17832 (sub-study)	GLP	Rat Micronucleus Test	Bone Marrow Micronucleus Test after 4-weeks of treatment (In vivo)	N/A	0.0348, 0.348, 3.48	No significant increase in the frequency of micronucleated polychromatic erythrocytes; no decrease in the PCE/NCE ratio
CNM-Au8-MPI-17-01	GLP	Rat Micronucleus Test	Bone Marrow Micronucleus Test after 2-days of treatment (In vivo)	N/A	10, 20, 40 mg/kg/d	No significant increase in the incidence of micronuclei in the test article dosed animals compared to the vehicle control. The test article CNM-Au8 was evaluated as negative (non-clastogenic)

2.2 Summary of Safety Pharmacology Studies

Safety pharmacology assessments included testing of three doses of CNM-Au8 (0.0348, 0.348 and 3.48 mg/kg) in rodents in the standard CNS Irwin test and the same dosing regimen for assessment of renal function following saline overload. Cardiovascular safety was assessed by constant telemetry in minipigs. The CNS Irwin testing protocol provided no evidence CNM-Au8 affected central and peripheral neurologic function. Similarly, renal function was not affected in Han Wistar rats following saline overload. Telemetry recording of cardiovascular responses in minipigs including heart rate, blood pressure, ECG, and body temperature demonstrated no negative effects of CNM-Au8 on cardiovascular function. These safety pharmacology results are summarized in Table 4.

Table 4. Summary of Safety Pharmacology Studies

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Summary/Notes**
AB17835	GLP	Han Wistar Rat (N=24)	CNS Irwin Test Following Single-Dose [OG, QD]	0.0348	3.3	No relevant effect on a battery of behavioral and physiological parameters, covering the main central and peripheral nervous system functions, evaluated for 24 hours after dosing
				0.348	36.6	
				3.48	346.5	
AB17836	GLP	Han Wistar Rat (N=40)	Renal Function Following Saline Overload [OG, QD]	0.0348	3.3	No mortality or clinical signs; no change in the serum concentrations of sodium, potassium, chloride or creatinine or in the serum osmolality; no changes in urine volume, pH, osmolality, electrolytes, or creatinine; no change in GF, free water clearance, or excretion fractions of electrolytes
				0.348	36.6	
				3.48	346.5	
AB17837	GLP	Göttingen Minipig (N=6)	Cardiovascular Telemetry (11-Day + Washout + PK) [OG, QD]	0.0348	3.3	No effect on general health status, body weight gain, or body temperature; no relevant effects on the arterial blood pressure, heart rate, or ECG parameters
				0.348	36.6	
				3.48	346.5	

2.3 Summary of Toxicology and Toxicokinetic Studies in Rodents and Minipigs

2.3.1 Initial Toxicokinetic Studies in Rodents and Minipigs

Two initial toxicokinetic studies were completed in the Göttingen Minipig and two initial studies were completed in rats as summarized in Table 5.

These initial studies were conducted up to a maximum of 3.48 mg/kg or approximately 100-fold higher delivery of gold when compared to comparable doses of auranofin in humans. These studies did not identify any treatment related toxicities, so a maximum tolerated dose was not defined. The no-adverse-effect-level (NOAEL) was identified at 3.48 mg/kg in rats and minipigs.

Table 5. Initial Acute and Subchronic Dosing Studies in Rats and Minipigs

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Safety Summary/ Toxicity Notes**
AB17839	Non-GLP	Göttingen Minipig (N=2)	1-Day & 5-Day Acute Toxicity; 2-phases [OG; QD]	0.0348	3.7	No mortality; no treatment related clinical signs; no related macroscopic abnormalities
				0.348	35.3	
				3.48	343.3	
AB17834	GLP	Göttingen Minipig (N=24; 6/group)	28-Day Repeat Dose Toxicity [OG; QD]	0.0348	3.3	No mortality; no treatment-related clinical signs or ophthalmological findings; no treatment-related effects on hematology, serum clinical chemistry, and urine parameters; no treatment-related macroscopic or histological findings
				0.348	36.6	
				3.48	346.5	
AB17831	Non-GLP	Han-Wistar Rat (N=40; 10/group)	7-Day Acute Toxicity [OG; QD]	0.0348	3.7	No mortality; no clinical signs; no treatment-related changes in body weight, body weight gain and food consumption; no effects on hematology and serum clinical chemistry; no treatment-related changes in organ weights; no macroscopic and histopathological changes
				0.348	35.3	
				3.48	343.3	
AB17832	GLP	Han-Wistar Rat (N=100; 25/group)	28-Day Repeat Dose Toxicity [OG; QD]	0.0348	3.3	No mortality; no clinical signs; no treatment-related changes in body weight, body weight gain and food consumption; no effects on hematology, coagulation, serum clinical chemistry and urine parameters; no treatment-related ophthalmological findings at any dose level; no organ weight, macroscopic changes or histological findings
				0.348	36.6	
				3.48	346.5	

2.3.1.1 Study AB17834 (28-Day Repeat Dose Toxicity in Göttingen Minipig)

At the end of the treatment period in Study AB17834, a lower platelet count from the pretest baseline was noted in 3 animals as described in Table 6, below. One female in Group 2 (low dose) and one in Group 3 (intermediate dose) had very low levels of platelets counts, and one male in Group 4 (high dose) group had a significant reduction.

Table 6: Study AB17834 Platelet Change from Day -12 to Day 27 in Select Animals

Group	Animal ID	Platelets (Giga/L)	
		Day -12	Day 27
Low Dose (0.0348 mg/kg)	Female 310	470	16
Intermediate Dose (0.348 mg/kg)	Female 317	401	6
High Dose (3.48 mg/kg)	Male 321	561	109

While the reduction in platelet counts were not attributed as related to the test article, given the history of hematological adverse events associated with gold complexes the Sponsor organized a series of measures to explore the potential relationship to test article, including:

- Review of historical data from the minipig supplier and contact research organization (CRO) laboratory values for pre-dose and control minipigs to evaluate whether platelet reductions or low platelet counts had been previously observed.
- Specialty hematological assessment of bone marrow sections from these animals and repeat examination of bone marrow smears taken at necropsy.

Review of historical data sets revealed that the platelet count in the peripheral blood of the minipig is highly variable and low platelet counts have sporadically been observed in untreated minipigs at the CRO laboratory (Eurofins). Further examination of bone marrow smears revealed no treatment-related differences in group means or individual Myeloid:Erythroid (M:E) ratios. Female no. 317 had a low M:E ratio due to higher proportion of erythroid precursors, which correlated with the histopathological observation of a high cellularity of the bone marrow. These changes were consistent with a hyperplastic change of the erythroid compartment, not accompanied by any increase in reticulocyte count in the peripheral blood as might be expected. There were no cytological abnormalities correlating with the severe decrease in platelet counts observed at necropsy in treated female nos. 310 and 317 and at lower severity in male no. 321. It

could not be excluded that the low platelet counts may be part of the background variation in this species or may have resulted from hemorrhage occurring accidentally at blood sampling. There was no evidence of qualitative or quantitative treatment-related effects on lymphoid cells, monocytes, reticulum, mast cells, or megakaryocytes. There were also no treatment-related differences in M:E ratio (group mean and individual values) or in absolute and relative counts of myeloid, erythroid, and lymphoid cell series.

To date, no significant platelet reductions have been observed in any of the toxicology studies conducted in rodents or canines.

2.3.2 Maximum Feasible Dose Study and High Dose Subchronic Toxicity Studies in Rodents and Minipigs

Upon submission of the Investigational New Drug (IND) application to the U.S. FDA, the Agency requested additional higher dose studies to identify a maximum tolerated dose, or alternatively a maximum feasible dose. Subsequently, a 7-day maximum feasible dose study was conducted in rats with subcutaneous (SC) dosing compared to 90 mg/kg oral gavage (OG) dosing. The maximal feasible dose (MFD) represented the highest concentration of CNM-Au8 (3000 parts per million, 3 mg/mL) that could be manufactured with currently available techniques dosed at the maximum permissible dosing volume delivered via gavage or SC administration. Of note, the 3 mg/mL concentration used in the two MFD studies had deviations out of product specification (hydrodynamic radius of 54.38 nm, which exceeded the production specification range of 10 – 30 nm) likely indicating nanoparticle agglomeration.

In rodents, doses of 5, 15, 30 and 90 mg/kg/day over seven consecutive days, were administered in a divided dose, i.e., twice daily subcutaneously (2.5, 7.5 and 15 mg/kg, BID) or, orally, three times daily (30 mg/kg TID; 90 mg/kg/day). The three times daily oral gavage administration was well tolerated by all rodents and not associated with any adverse findings. Test-article related findings that were not adverse, included discolored feces (black), decreased body weight gain and food consumption when compared to controls, and anatomic pathology findings of extramedullary hematopoiesis noted in the spleen and/or liver. This study defined 90 mg/kg/day as the NOAEL with oral gavage administration.

The daily subcutaneous administration was associated with adverse events related to subcutaneous administration of the test article. The skin was thickened/leathery in all treatment groups with associated changes in clinical pathology, defined by increased neutrophils and fibrinogen levels and decreases in albumin; and anatomic pathology, defined by subacute/chronic inflammation, edema, and cavitation in the injection sites, which were considered adverse in their totality. Systemic test article-related changes not considered adverse were noted in body weights (decreased gain), food consumption (decreased), and anatomic pathology (increased spleen weights, and extramedullary hematopoiesis in the spleen and liver). Accordingly, a NOAEL could not be established in the rodents receiving daily SC administration up to 30 mg/kg.

With twice-daily SC administration of CNM-Au8, blood Au exposures increased proportionally or slightly greater than proportionally from 5 to 30 mg/kg/day on Day 1 and Day 7. Blood Au exposures after SC administration were considerably higher than after oral gavage administration with the dose normalized $AUC_{(0\text{-}Last)}$ ratio (SC:OG) ranging from approximately 9x – 50x higher dose-normalized AUC depending upon animal gender and the SC dose. Despite substantially greater Au accumulation, no systemic toxicities were observed with SC administration.

Table 7 below summarizes the design and results of the maximum feasible dose and subsequent high dose subchronic toxicity studies in rodents.

Table 7. Maximum Feasible Dose Study and High Dose Subchronic Toxicity Studies

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Safety Summary/ Toxicity Notes**
CNAU-MPI-14-04 (2257-004)	Non-GLP	Sprague-Dawley Rat (N=60; 12/group)	7-Day Max Feasible Dose Study [SC; BID] [OG; TID]	5 [SC; 2.5 mg/kg BID]	500	Discolored feces (black), decreased body weight gain and food consumption; non-adverse anatomic pathology findings consisting of extramedullary hematopoiesis noted in the spleen and/or liver. Skin thickened/leathery in SC injection groups associated with changes in clinical pathology (increased neutrophils and fibrinogen levels; decreases in albumin) and anatomic pathology (subacute/ chronic inflammation, edema, and cavitation in the injection sites).
				15 [SC; 7.5 mg/kg BID]	1,500	
				30 [SC; 15 mg/kg BID]	3,000	
				90 [OG; 30mg/kg, TID]	3,000	
CNM-Au8-MPI-16-01 (2257-008)	GLP	CD Rat (N=146)	21-Day Repeat Dose Toxicity [OG; QD/BID]	10	1,000	No treatment-related signs of toxicity; no effects on body weights, food consumption or clinical pathology parameters; no treated related ophthalmologic effects; examinations; no organ weights changes; no treatment-related observations during necropsy and no histopathologic changes.
				20	1,000	
				40 [20mg/kg, BID]	1,000	

2.3.3 Chronic Rodent Toxicology (6-Month Dosing)

The objective of this study was to evaluate the toxicity of the test article in rats after administration for 13- and 26-weeks and to evaluate reversibility, progression, or delayed appearance of any observed changes following a 4-week postdose observation period after each dosing termination.

Assessment of toxicity was based on mortality, clinical observations, body weight, and food consumption; ophthalmoscopic examinations; and clinical and anatomic pathology.

Toxicokinetic (TK) assessment [whole blood, urine, cerebral spinal fluid (CSF), and designated frozen tissues] was conducted for gold content.

There were no definitive test article-related mortalities and no test article-related effects on body weight, food consumption, ophthalmoscopic examinations; clinical pathology, macroscopic examinations and organ weights. Based on the data from the detailed clinical observations, body weight, food consumption, clinical and anatomical pathology, oral doses of CNM-Au8 at 10, 20, and 40 mg/kg/day resulted in no adverse findings of these rats. Therefore, the NOAEL for this study was considered to be 40 mg/kg/day, the highest dose tested.

Table 8. Chronic Rodent Toxicity Study

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Safety Summary/ Toxicity Notes**
CNM-Au8-MPI-16-04 (2257-006)	GLP	CD Rat (N=306)	26-Week Repeat Dose Toxicity with 4-Week Recovery [OG; QD] [OG; BID]	10 [OG QD]	1,000	There were no definitive test article-related mortalities. No test article-related effects on body weight, food consumption, ophthalmoscopic examinations; clinical pathology, organ weights macroscopic and microscopic examinations.
				20 [OG QD]	1,000	
				40 [OG; 20 mg/kg BID]	1,000	

2.4 Summary of Toxicology and Toxicokinetic Studies in Canines

Five toxicokinetic studies have been conducted in Beagle dogs including:

- Single Dose Toxicokinetic Study
- Maximum Tolerated Dose study (7-Day)
- 28-Day Subchronic Repeat Dose Toxicokinetic Study
- 21-Day Maximum Feasible Dose Study
- 9-Month Chronic Toxicity Study

Doses of CNM-Au8 up to 90 mg/kg by oral gavage have not demonstrated any acute or subchronic toxicity, and accordingly, a MTD has not been identified to date. All dosing levels and all concentrations (ranging from 350 – 3,000 µg/mL) explored in canines have produced NOAELs.

Table 9 below summarizes the design and results of the toxicokinetic studies conducted to date in Beagle dogs.

Table 9. Acute and Subchronic Dosing Studies in Canines

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Safety Summary/ Toxicity Notes**
CNAU-MPI-14-01 (2257-001)	GLP	Beagle Dog (N=18; 6/group)	Single Dose Toxicity and Toxicokinetics (With 9 Day Follow-Up)	0.5 mg/kg IV	100	No mortality and no clinical observations noted during study.
				3.5 mg/kg [OG, QD]	350	
				10 mg/kg [OG, QD]	1,000	
CNAU-MPI-14-02 (2257-003)	GLP	Beagle Dog (N=48; 12/group)	28-Day Repeat Dose Toxicity [OG, QD]	0.35	35	No mortality; no treatment-related toxicity; no effects on body weights, food consumption or clinical pathology parameters; no treatment-related effects on ophthalmologic or physical examinations; no effects on organ weights or histopathologic changes
				3.5	350	
				10	1,000	
CNAU-MPI-14-03 (2257-002)	Non-GLP	Beagle Dog (N=8; 2/group)	Ascending Dose and 7-Day Repeat Dose Toxicity [OG, QD]	10	1,000	No mortality and no clinical observations noted during study; discolored feces were observed in all treated animals; no related treatment effects on food consumption, body weight, or physical exam.
				20	2,000	
				30	3,000	
CNAU-MPI-14-05 (2257-005)	GLP	Beagle Dog (N=24; 6/group)	21-Day Repeat Dose Toxicity (Maximum Feasible Dose) [OG; QD, BID, TID]	30 [30mg/kg QD]	3,000	No mortality; treatment-related salivation and discolored feces at 60 and 90 mg/kg/day. No treatment-related effects on body weights, food consumption, ophthalmology, electrocardiograms, clinical pathology parameters, organ weights or macroscopic examinations. Non-adverse changes in the kidneys in three animals at 90 mg/kg/day with minimal bilateral tubular cell hypertrophy, characterized by a diffuse increase in the size of tubular epithelial cells.
				60 [30mg/kg BID]	3,000	
				90 [30mg/kg TID]	3,000	

2.4.1 Single Dose Canine Toxicokinetic Summary

After intravenous (IV) administration, the mean CNM-Au8 whole blood estimated concentration at time zero (C_0) was calculated as 9.39 ng/mL (range 4.97 to 18.4 ng/mL) extrapolated using a log-linear regression from the first two measured concentrations. The first blood sample after IV dosing was collected at 30 minutes postdose, so it is possible that the concentration at 30 minutes underestimates C_0. After oral administration, maximum CNM-Au8 plasma concentrations were observed at a T_{max} range of 4 – 48 hours and 12 – 48 hours postdose for the 3.5 and 10 mg/kg cohorts, respectively.

Due to limited interpretable data points above the lower limit of quantitation (LLOQ) in the IV dosing group, AUC_∞ was not calculated, and absolute bioavailability (F) was therefore calculated using the mean values for $AUC_{0\text{-}336}$ from two of six animals with quantifiable Au whole blood concentrations to 336 hours. As shown in Table 10 was 30.5% for the 3.5 mg/kg group and 20.5% for the 10 mg/kg group. The difference may be due to the use of different animals in the 3 cohorts rather than a true difference in bioavailability. The mean C_{max} value at 3.5 mg/kg includes two animals with CNM-Au8 concentrations all below the lower limit of quantitation with subsequent C_{max} values set to zero, so the reported C_{max} value may be an underestimate in this dosing group.

Table 10. Summary of C_{max}, AUC, $T_{1/2}$, and Bioavailability Results Following Single Dose Administration of CNM-Au8 in Study CNAU-MPI-14-01

CNM-Au8 Dose (mg/kg)	Statistic	C_{max} or C_0 (ng/mL)	$AUC_{0\text{-}336hr}$ (hr*ng/mL)	$AUC_{0\text{-}336/Dose}$ (kg*hr*ng/mL/mg)	$T_{1/2}$ (hr)	Bioavailability (F%) (Dose Normalized AUC Ratio (PO/IV)
0.5 (IV)	N	6	2	2	2	N/A
	Mean	9.39	146	291	31.0	
	SD	5.25	N/A	N/A	N/A	
	CV%	55.9	N/A	N/A	N/A	
3.5 (PO)	N	6	4	4	N/A Insufficient Data)	30.5%
	Mean	1.80	311	88.8		
	SD	1.65	234	66.8		
	CV%	92%	75%	75%		
10.0 (PO)	N	6	5	5	4	20.5%
	Mean	4.04	596	59.6	150	
	SD	2.99	457	45.7	51.5	
	CV%	74%	77%	77%	34%	

The urine volume and feces mass in each collection interval was not determined, therefore, comparisons among dosing groups were limited to urine and fecal concentration data. In the IV dosing group, feces concentrations were all below the LLOQ (<150 ng/mL); however, identification of CNM-Au8 above the lower limit of detection (LLOD) in the feces over the 2-week period established the feces as a route of elimination. Quantifiable urine concentrations were observed between 36 to 48 hours postdose in the 0.5 mg/kg IV dosing group, confirming urine as an elimination pathway. Urinary excretion data were not collected at consistent intervals with whole blood concentration, so renal clearance could not be calculated. Peak urine concentrations in the 3.5 and 10 mg/kg groups occurred between 12 – 24 hours and 36 – 48 hours, respectively, and exceeded whole blood concentrations.

Mean values for half-life ($T_{1/2}$), clearance (CL), volume of distribution (V_z), and volume of distribution at steady state (V_{ss}), respectively, for the 0.5 mg/kg IV dosing group are shown in Table 11.

Table 11. Summary of CL, $T_{1/2}$, V_z, and V_{ss} after a Single 0.5 mg/kg IV Dose of CNM-Au8 (Study CNAU-MPI-14-01)

CNM-Au8 Dose	Statistic	$T_{1/2}$ (hr)	CL (L/hr/kg)	V_z (L/hr/kg)	V_{ss} (L/hr/kg)
0.5 mg/kg (IV)	N	2	2	2	2
	Mean	31.0	5.64	249	242
	$Animal_{102}$	33.6	4.72	229	224
	$Animal_{105}$	28.5	6.57	270	261

In the oral gavage dosing groups, dose normalized systemic exposure ($AUC_{0\text{-}336hr/Dose}$) and C_{max} values for CNM-Au8 showed less than proportional increases. An approximate 1:3-fold increase in dose (3.5 mg/kg to 10 mg/kg) resulted in an approximate 1:1.5-fold decrease in mean dose normalized $AUC_{0\text{-}336hr/Dose.}$ Mean values for terminal half-life ($T_{1/2}$), oral clearance (CL/F), and oral volume of distribution (V_z/F), for the 10 mg/kg dosing group are reported in Table 12.

Table 12. Summary of CL/F, $T_{1/2}$, and Vz/F after a Single 10 mg/kg PO Dose of CNM-Au8 (Study CNAU-MPI-14-01)

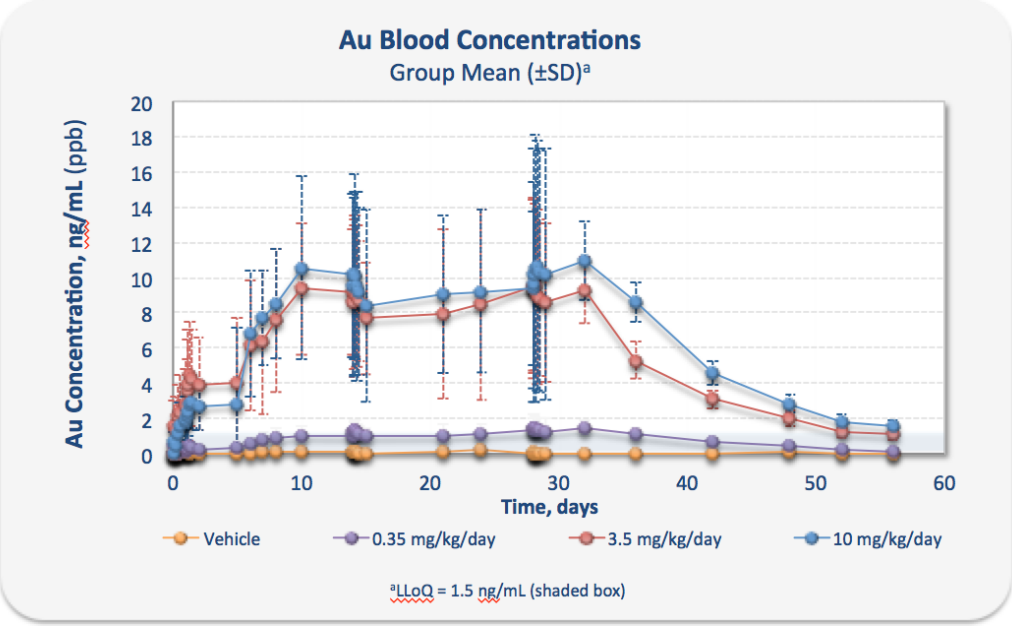
CNM-Au8 Dose	Statistic	$T_{1/2}$ (hr)	CL/F (L/hr/kg)	V_z/F (L/hr/kg)
10 mg/kg (PO)	N	4	4	4
	Mean	150	13.1	2,970
	SD	51.5	7.3	2,080
	CV%	34%	56%	70%

2.4.2 Repeat Dose Canine Toxicokinetic Summary

In brief, in the subchronic toxicity study with a 4-week recovery period, 48 beagles were dosed for 28-days to assess toxicity and toxicokinetics. CNM-Au8 was well tolerated when administered at gold concentrations of 0.35, 3.5, and 10 mg/kg/day as a single daily dose of 1mg/mL CNM-Au8 via oral gavage, and the10 mg/kg/day dose was considered the NOAEL. There were no significant changes in serum chemistry or hematology measures or in the macroscopic or histologic assessments post-necropsy. In terms of toxicokinetics, systemic exposure appeared independent of gender. There was an increase in C_{max} from 0.35 to 3.5 mg/kg, but no apparent change from 3.5 to 10 mg/kg, suggesting a plateau in exposure to 3.5 and 10 mg/kg. Mean systemic exposure $AUC_{(0\text{-}24\ hr)}$ accumulated in whole blood following repeated administration over the first two weeks, with no significant change in accumulation ratio (R_{ac}) after that time, indicating that steady-state was reached within 2 weeks of dosing. Elimination

half-life ($T_{1/2}$) was long; 333 hours (13.9 days) in the 10 mg/kg dose group. During the recovery elimination phase, gold levels were still quantifiable at 4-weeks post-dosing in both blood and tissue. Whole blood Au concentrations are shown in Figure 11.

Figure 11. Whole Blood Concentrations by Day in 28-Day Repeat Dosing Canine Study



A noteworthy finding in the three-animal species studied was a disproportionate tissue concentration of gold measured in the kidney. This renal accumulation was not accompanied by changes in renal function, as measured by serum biochemistry, urinalysis, or structural changes identified in the histologic examination of the kidney. In the 4-week recovery period in the 28-day dog study, gold concentrations in the kidney decreased significantly over that recovery period, and continuing urinary excretion of gold was measured throughout the recovery period.

2.4.3 Maximum Feasible Dose Canine Toxicokinetic Study

In the canine maximum feasible dose study, the doses administered were 30 mg/kg/day (QD), 60mg/kg/day (30 mg/kg BID), or 90 mg/kg/day (30 mg/kg TID) over three consecutive weeks (21-days). Dosing was well tolerated in the dogs over the 21 consecutive days of dosing. At the highest dose, 90mg/kg/day, minimal bilateral tubular cell hypertrophy was observed in the kidneys of males and females but was considered non-adverse by the MPI study pathologist-toxicologist.

No deaths occurred. Treatment-related salivation and black discolored feces were observed at 60 and 90 mg/kg/day. No treatment-related effects were observed on body weights, food consumption, ophthalmology, ECGs, clinical pathology parameters, organ weights or macroscopic examinations. There was slightly less than a proportional increase in gold exposure when the dose was increased from 30 to 60 mg/kg/day. Increasing the dose from 60 to 90 mg/kg/day was not generally accompanied by an increase in gold exposure. Thus, maximal systemic exposure in the dogs may occur with a dose of 60mg/kg/day divided into two daily oral doses of 30 mg/kg. Accumulation appeared to plateau by Day 7, with no substantive increase in exposure on Days 14 and 21. Blood gold exposures at steady-state were approximately 1.1 to 3.3 times higher than on Day 1, and the extent of the accumulation tended to be lower with higher doses. Systemic gold concentration appears to be approximately at steady state after 1 to 2 weeks of dosing. Systemic exposure appears to be higher in female dogs than in male dogs.

2.4.4 Chronic Canine Toxicology (9-Month Dosing)

The objective of this study was to evaluate the potential toxicity of the test article in beagle dogs when administered orally via gavage once daily for 13 and 39 weeks, and to evaluate reversibility, progression, or delayed appearance of any observed changes following a 4-week post dose observation period after each termination.
Assessment of toxicity was based on mortality, clinical observations, body weight, and food consumption; ophthalmoscopic, and electrocardiographic examinations; and clinical and anatomic pathology. Blood, urine, CSF, and designated frozen tissues were analyzed for gold content. Toxicokinetic assessment for gold content in blood and urine was conducted. Results are summarized in Table 13.

No early deaths occurred. There were no treatment-related clinical signs of toxicity observed, no effects on body weight or food consumption, or during ophthalmoscopic observations or electrocardiographic examinations. There were no test article-related effects among clinical pathology parameters noted during the interim dosing and recovery periods. No organ weight changes were observed and no test article related macroscopic or microscopic findings were observed at the interim or recovery necropsies.

CNM-Au8 was well tolerated in dogs when administered at doses of 0.35, 3.5 and 10 mg/kg/day for 39-weeks. No adverse signs of toxicity were observed at any dose level. Therefore, the dose of 10 mg/kg/day was considered the NOAEL in dogs following oral administration.

Table 13. Chronic Canine Toxicity Study

Study ID (Internal ID/ CRO ID)	**GLP Status**	**Species** (N)	**Brief Design** [Route; Frequency]	**Nominal Dose** (mg/kg/day)	**CNM-Au8 Conc.** (µg/mL)	**Safety Summary/ Toxicity Notes**
CNM-AU8-MPI-16-03 (2257-007)	GLP	Beagle Dog (N=80; 8 or 12 (TK)/ group)	39-Week Repeat Dose Toxicity with 4-Week Recovery [OG; QD]	0.35 [OG, QD]	1,000	No treatment-related clinical signs of toxicity observed, no effects on body weight or food consumption, or during ophthalmoscopic observations or electrocardiographic examinations. There were no test article-related effects among clinical pathology parameters noted during the interim dosing and recovery periods. No organ weight changes were observed and no test article related macroscopic or microscopic findings were observed at the interim or recovery necropsies.
				3.5 [OG, QD]	1,000	
				10 [OG, QD]	1,000	

3 PRIOR HUMAN DOSING EXPERIENCE

A Phase 1, First-Time-In-Human study was previously conducted under a clinical trial application (CTA) in the Netherlands (AU8.1000-14-01). The study was a 2-phase, randomized, double-blind, single- and multiple-dose escalating study to evaluate the safety, tolerability, and PK of CNM-Au8 in healthy male and female volunteers. There were 2 phases to this study: a single ascending dose (SAD) Phase and a multiple ascending dose (MAD) Phase. The SAD Phase was conducted first, followed by the MAD Phase of the study.

3.1 AU8.1000-14-01 Design

A total of 8 subjects per cohort were randomly assigned (3:1 ratio) to receive a single dose of either CNM-Au8 (n = 6) or placebo (n = 2) at an initial dose level of 15 mg CNM-Au8. Additional cohorts of 8 subjects were enrolled to investigate single-escalating doses of CNM-Au8 at 30 mg, 60 mg, and 90 mg. Cohorts were to be balanced by gender with no more than two-thirds of subjects being of one gender. Safety parameters included findings from electrocardiograms (ECGs), vital signs, clinical laboratory panels, physical examinations, urinalysis, AEs, and concomitant medications. A follow-up visit was conducted for final safety assessments on Day 17 ± 1.

After the SAD Phase was completed, a total of 12 subjects per cohort were randomly assigned (3:1 ratio) to receive a multiple dose of either CNM-Au8 (n = 9) or placebo (n = 3) once daily for 21 days at an initial dose level of 15 mg CNM-Au8. Additional cohorts of 12 subjects were enrolled to investigate multiple escalating doses of CNM-Au8 at 30 mg, 60 mg, and 90 mg. Cohorts were balanced by gender, with no more than two-thirds of subjects being of one gender. Following the final dose of study drug on Day 21, subjects entered a 28-Day follow-up phase and returned to the clinical research unit (CRU) for PK sampling on Days 23, 24, 25, 26, 27, 28, 32, 36, 40, and 49, which completed study participation. Safety parameters included findings from ECGs, vital signs, clinical laboratory panels, physical examinations, urinalysis, AEs, and concomitant medications.

3.2 AU8.1000-14-01 Pharmacokinetic Results

The PK Population included all randomized subjects who received CNM-Au8 study drug and had sufficient samples collected for estimation of PK parameters.

- The PK Population for the SAD Phase included 24 subjects who received a single dose of CNM-Au8 study drug at 15 mg, 30 mg, 60 mg, and 90 mg.
- The PK Population for the MAD Phase included a total of 35 subjects: 8 subjects in the 15 mg dosing group and 9 subjects each in the other 3 cohorts who received multiple doses of CNM-Au8 study drug at 30 mg, 60 mg, and 90 mg (Cohorts 5–8).

In the SAD Phase, PK analyses were not completed since the majority of whole blood Au concentrations were below the LLOQ. Since the lack of quantitation of Au concentrations may have been related to ICP-MS assay sensitivity, the Sponsor upgraded to an advanced, more sensitive, ICP-MS instrument for the PK analyses in the MAD Phase.

In the MAD Phase:

- The geometric mean whole blood concentrations from 1 week onward increased in a dose related, but not a dose-proportional manner. Based on Days 14 and 21, the increases in both C_{max} and $AUC_{(0-24)}$ were less than dose proportional.
- Based on pre-specified fit criteria, the elimination $t_{½}$ ranged from 277 to 628 hr (11.5 to 26.2 days).
- Steady-state for all cohorts, based on the geometric mean whole blood concentrations, was reached by the end of the second week of dosing (Day 14), which was substantially less than predicted by the $t_{½}$ [range of 46 to 105 days (6.6 to 15 weeks)].

PK parameters related to urinary excretion (Ue, CL_r) could not be calculated. Only 1 urine Au concentration ≥ LLOQ (3 ng/mL) was within limits of the assay.

3.3 AU8.1000-14-01 Safety Results

The Safety Population included randomized subjects who received at least 1 dose of study drug and had at least 1 post-baseline safety assessment, and was used to perform all safety analyses.

The Safety Population included 40 subjects in the SAD Phase, 46 subjects in the MAD Phase, and 86 subjects in the Pooled (SAD + MAD) group.

All planned dose cohorts were completed, and the Safety Review Committee (SRC) agreed to escalate to each subsequent dose cohort per the protocol. An MTD was not identified. All subjects in the SAD Phase completed a single oral dose per protocol. In the MAD Phase, the mean duration of treatment was 21.0 days of consecutive oral dosing for the 15 mg, 30 mg, and 60 mg Cohorts and placebo. Subjects in the 90 mg Cohort received on average 20.1 days of consecutive dosing. One subject (subject 0085-MAD 90 mg) in the MAD Phase did not complete the 21 days of consecutive dosing per protocol due to a reported pregnancy after 13 days of consecutive dosing, and had a medically induced abortion 7 days later.

For the SAD and MAD Phases of the study, the overall incidence of TEAEs was comparable between treatments, including placebo; overall incidence of TEAEs considered related to study drug by the Investigator was also comparable between treatments, including placebo. The most frequently reported TEAEs were in the classes of Nervous system disorders and Gastrointestinal disorders. The majority of TEAEs were Grade 1 severity (mild). There were no serious TEAEs, TEAEs leading to discontinuation of treatment, or TEAEs considered severe, life threatening, or resulting in death. Overall, no dose response relationship to TEAEs was observed in the SAD or MAD Phase of the study; however, the frequency of headache and gastrointestinal TEAEs was higher in the 90 mg MAD treated subjects.

In the SAD Phase, TEAEs considered related to study drug by the Investigator were reported by 2 (16.7%) out of 12 subjects in the 15 mg group, 2 (33.3%) out of 6 subjects in the 30 mg group, 4 (66.7%) out of 6 subjects in the 60 mg group, and 5 (83.3%) out of 6 subjects in the 90 mg group compared with 7 (70.0%) out of 10 subjects in the placebo group. All related TEAEs were Grade 1 severity (mild) in the SAD Phase, except for 2 related TEAEs (diarrhea and abdominal pain: both in Subject 0024-SAD 60 mg) that were considered Grade 2 severity (moderate).

In the MAD Phase, TEAEs considered related to study drug by the Investigator were reported by 5 (62.5%) out of 8 subjects in the 15 mg, 4 (44.4%) out of 9 subjects in the 30 mg, 3 (33.3%) out

of 9 subjects in the 60 mg, 8 (88.9%) out 9 subjects in the 90 mg, and 6 (54.5%) 11 subjects in the placebo group. All related TEAEs in the MAD Phase were Grade 1 severity (mild).

Modified treatment emergent events were defined as a treatment emergent adverse event that started within 24 hours from first dose of study medication. Modified TEAEs for all CNM-Au8 doses were reported by 11 (36.7%) out of 30 subjects in the SAD Phase, 7 (20.0%) out of 35 subjects in the MAD Phase, and 18 (27.7%) out of 65 subjects in the Pooled (SAD + MAD) group. A total of 9 (42.9%) out of 21 placebo treated subjects reported modified TEAEs. The most frequently reported modified TEAEs included headache, blood creatinine increased, dizziness, diarrhea, and nausea.

The Sponsor performed a pre-specified search of TEAE preferred terms potentially associated with gold complex compounds to determine adverse events of special interest (AESIs). All AESIs were considered Grade 1 severity (mild), except for 2 TEAEs of Grade 2 severity (moderate): diarrhea and abdominal pain in the same subject (Subject 0024-SAD 60 mg). Incidence rates were comparable between all dosing groups including placebo.

Dosing with either a single dose or multiple doses of CNM-Au8 was generally well tolerated over the course of this study. The most frequently reported TEAEs in the pooled population were headache, somnolence, fatigue, abdominal pain upper, diarrhea, nausea, abdominal pain, and dizziness.

Oral administration of either a single (SAD) or repeated dose (MAD) of CNM-Au8 over 21 consecutive days in healthy volunteers was safe and well tolerated. The TEAEs observed across the 4 doses (15 mg, 30 mg, 60 mg, and 90 mg) of CNM-Au8 were mostly mild in severity. While no dose response relationship to TEAEs was observed overall in the SAD or MAD Phase of the study, increases in headache and gastrointestinal related TEAEs were observed in the 90 mg cohort of the MAD Phase.

Overall, for both the SAD and MAD Phases, routine clinical laboratory assessments (hematology, serum chemistry, and urinalysis), vital signs, ECGs, and physical examinations did not reveal clinically notable findings or trends; none resulted in serious TEAEs or TEAEs leading to discontinuation of treatment.

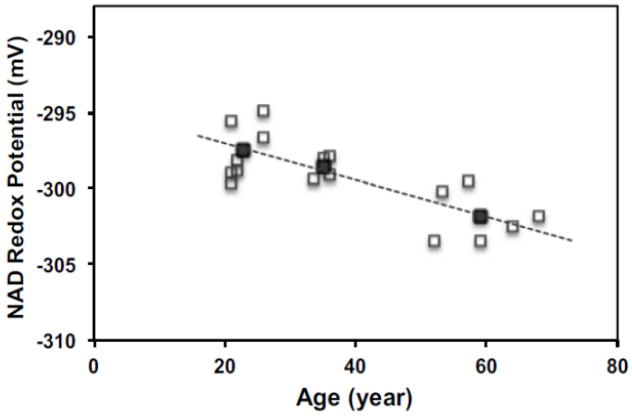
4 INVESTIGATIONAL PLAN

4.1 Study Rational

CNM-Au8 has been studied in several preclinical Parkinson's Disease Models and has demonstrated significant neuroprotection in these models. Through a mechanism of action targeting restoration of cellular redox homeostasis, CNM-Au8 addresses pathophysiologic bioenergetic failure in neurons that may underlie the pathogenesis of Parkinson's Disease and other neurodegenerative diseases. CNM-Au8 acts on bioenergetics to protect sensitive neuronal populations from chemical, inflammatory, and hypoxic insults through a unique mechanism promoting cellular redox homeostasis that involves the catalytic oxidation of NADH resulting in enhanced aerobic glycolytic energy production.

The primary objective of this investigator-blinded, open labeled, randomized, Clinical Phase 2 trial is to study the effects on brain NAD+/NADH redox potential of 12-weeks of treatment with CNM-Au8 using ^{31}P-Magnetic Resonance Spectroscopy in patients with Parkinson's Disease. Measurement of regional brain NAD+/NADH concentrations in healthy human subjects and in patients with schizophrenia has been demonstrated using ^{31}P-MRS (Chouinard et al., 2017; Lu et al., 2016). In humans, brain NAD/NADH redox potential is inversely correlated with age and has been shown to decrease linearly with advancing age in healthy human subjects (Zhu et al. 2015) shown in Figure 12 below.

Figure 12. NAD Redox Potential In Adult Humans



Age dependences of NAD+/NADH redox potential observed in healthy human brains. The open symbols represent individual subject data, and the filled symbols display the average data from three age groups of younger (21–26 y old; n = 7), middle (33–36 y old; n = 4), and older (59–68 y old; n = 6) subjects.

This age-related decrease in brain cellular redox potential is believed to be associated with the bioenergetic failure that underpins neurodegenerative diseases such as PD in susceptible people. NAD+/NADH redox potential is essential for fundamental ATP-energy-generating bioenergetic processes such as glycolysis and mitochondrial oxidative phosphorylation. Proper bioenergetic functioning is required for normal cellular activities including many 'cellular housekeeping' processes such as autophagy, apoptosis and the unfolded protein response (UPR) that have been shown to be abnormal in neurodegenerative diseases (Villanueva-Paz et al. 2016; Chua and Tang 2013; Cantó, Menzies, and Auwerx 2015). Improvement of brain NAD+/NADH redox potential demonstrated via ^{31}P-MRS will provide Proof of Concept for the ability of CNM-Au8 treatment to potentially treat bioenergetic failure associated with the neurodegeneration observed in patients with Parkinson's Disease.

4.2 Study Objectives

To assess the CNS metabolic biomarker profile and safety of CNM-Au8 for the treatment of Parkinson's Disease (PD):

- Metabolic effects will be assessed as an improvement of ^{31}P Magnetic Resonance Spectroscopic (^{31}P-MRS) assessment of brain tissue cellular Redox potential defined by

the measured tissue ratio of NAD+:NADH concentrations and various bioenergetic markers.

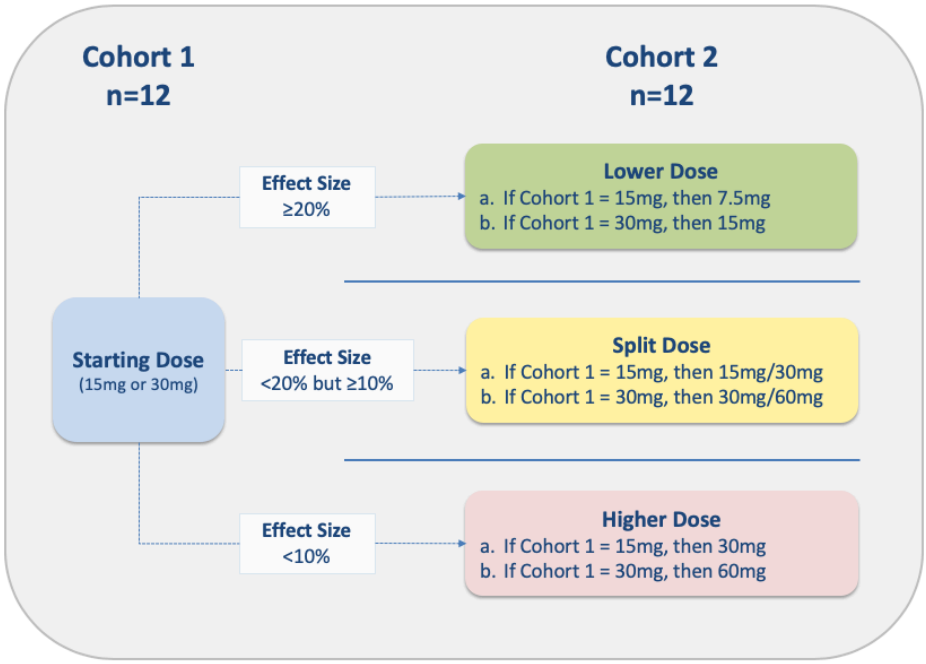
- Safety will be assessed via adverse events, serious adverse events, discontinuations due to adverse events, and the Columbia Suicide Severity Rating Scale (C-SSRS).

4.3 Overall Study Design and Plan

This is a single-center open label pilot, sequential group, investigator-blinded study of the CNS metabolic effects, safety, pharmacokinetics, and pharmacodynamics of CNM-Au8 in patients who have been diagnosed with Parkinson's Disease (PD) within three (3) years of Screening. Patients will be screened over a 6-week period. Patients who meet the inclusion criteria and none of the exclusion criteria will be enrolled into the clinical study.

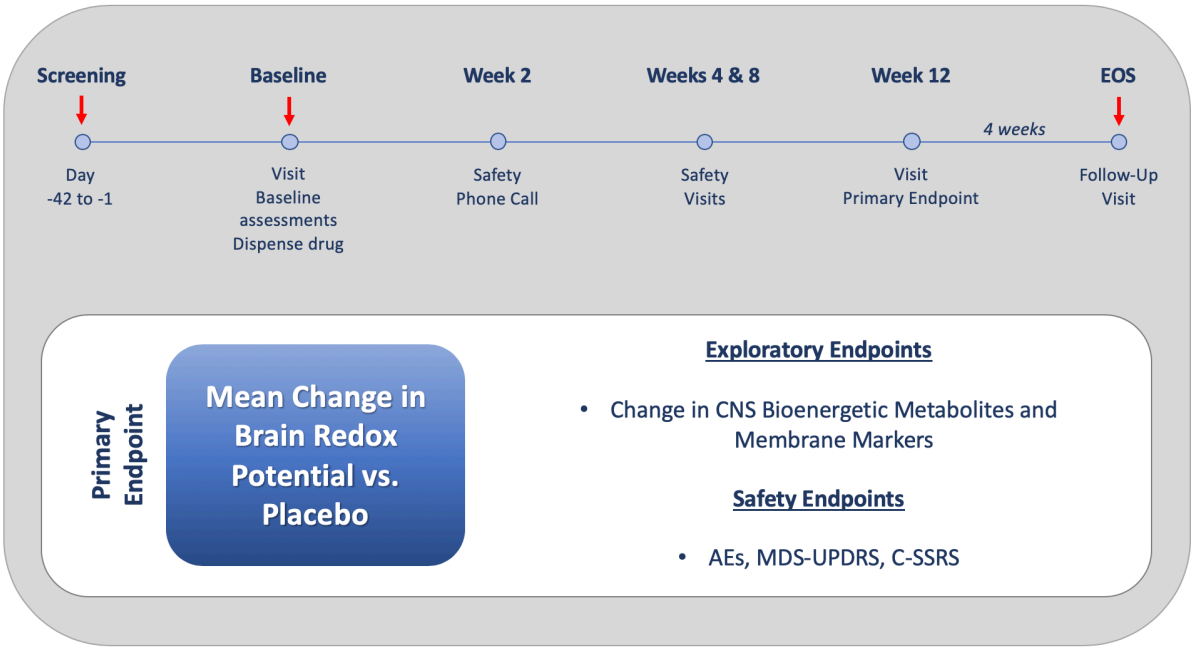
The Sponsor will select a starting treatment dose for the initial treatment cohort of 15 mg or 30 mg CNM-Au8. Investigators and patients will remain blinded to each cohort's study dose. Upon completion of a treatment cohort, the Sponsor will select a single dose or two doses from the prespecified dosing selection plan for the next treatment cohort based on the evaluation of the ^{31}P-MRS changes versus baseline in NAD+/NADH (Figure 13). Up to a total of two treatment cohorts may be initiated.

Figure 13. Dose Selection Plan



All patients will receive daily oral treatment over twelve (12) consecutive weeks during each cohort Treatment Period according the study schematic in Figure 14.

Figure 14. Study Scheme



The primary efficacy outcome measure will be assessed based upon each patient's Week 12 study visit. All patients who are discontinued from treatment will complete the EOS assessment. At the end of the study, patients will complete an EOS Visit 4-weeks following their last study visit before exiting the study.

All patients will receive once daily oral treatment over twelve (12) consecutive weeks during each cohort Treatment Period. In the event that collection of the ^{31}P-MRS at the Week 12 visit is delayed due to imaging issues or COVID-19 pandemic related concerns, participants may remain on study drug beyond the Week 12 visit, so long as the extension is agreeable to both the study PI and Sponsor's Medical Monitor, until it is deemed safe to return for collection of the Week 12 efficacy assessments. Safety assessments that are able to be captured should be completed every 4 weeks beyond the planned Week 12 visits prior to dispensation of unscheduled study drug.

There will be three study periods per treatment cohort:

1. A (6) six-week screening period (Screening Period);
2. A (12) twelve-week treatment period (Treatment Period);
3. A (4) four-week follow-up period (End-of-Study Assessment).

The study investigators and patients will remain blinded for the duration of the study.

5 INVESTIGATORS AND KEY STUDY PERSONNEL

Key study personnel and investigators include the following:

- Sponsor's Medical Monitor:

  Name: Robert Glanzman, MD, FAAN
  Title: Chief Medical Officer
  Address: 3165 E. Millrock Drive
  Suite 325
  Salt Lake City, Utah 84121
  United States
  Email: Robert@clene.com or mm@clene.com
  Telephone: +1 (801) 676 –9695

- Principal Investigator

  Name: Richard Dewey, Jr., MD
  Title: Professor, Neurology and Neurotherapeutics
  Director, Clinical Center for Movement Disorders
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  Email: Richard.Dewey@UTSouthwestern.edu
  Telephone: +1 (817) 424-5567

- Coordinating Core Biomarkers Investigator; Sub-investigator

  Name: Ben Greenberg, MD
  Title: Professor, Cain Denius Scholar in Mobility Disorders
  Department of Neurology and Neurotherapeutics
  Address: UT Southwestern Medical Center
  Dallas, Texas
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- Coordinating Core Imaging (MRS) Investigator; Sub-investigator

  Name: Jimin Ren, PhD
  Title: Associate Professor
  Advanced Imaging Research Center, Department of Radiology
  Address: UT Southwestern Medical Center
  Dallas, Texas
  Email: Jimin.Ren@UTSouthwestern.edu

All other study personnel not included in this section are identified in a separate personnel list (not part of this clinical study protocol) as appropriate. This list will be updated as needed; an abbreviated version with personnel relevant for the centers will be available in each center's Investigator site file.

Whenever the term 'Investigator' is noted in the protocol text, it may refer to either the Principal Investigator at the site or an appropriately qualified, trained and delegated individual of the investigational site.

The Principal Investigator of each site/study center must sign the protocol signature sheet before patient recruitment may occur at the respective center. Similarly, all protocol amendments and/or revised integrated protocols must be signed and dated by the site's Principal Investigator before coming into effect at the respective center. A complete list of all participating centers and their investigators, as well as all required signature documents, will be maintained in the Sponsor study file.

6 STUDY ENDPOINTS

6.1 Efficacy Endpoints

6.1.1 Primary Efficacy Endpoint

Measure of Brain Bioenergetic Improvement

Change in ^{31}P-MRS Redox Ratio (NAD+/NADH):

- Mean change in average NAD+/NADH measured brain Redox Ratio by treatment group from Baseline to Week 12

6.1.2 Exploratory Efficacy Endpoints

Measures of Brain Bioenergetic Improvement

Change in ^{31}P-MRS Bioenergetic Metabolites:

- Regression of baseline values versus mean percentage change in of the average tissue concentration by subject per dosing group from Baseline to Week 12 for:
    - ATP (α, β, γ)
    - NAD+/NADH pool
    - Phosphocreatine (PCr)
    - Intracellular inorganic phosphate (Pi(in))
    - Extracellular inorganic phosphate (Pi(ex))

Change in ^{31}P-MRS Membrane Components:

- Phosphoethanolamine (PE)
- Phosphocholine (PC)
- Glycerolphosphoethanolamine (GPE)
- Glycerophosphocholine (GPC)

Exploratory Functional Measures

Change in APDM Instrumental Tests of Gait, Balance, and Mobility

- Mean change in average difference between Baseline to Week 12 for:
    - APDM Instrumented Timed Up and Go (TUG) Test
    - APDM Instrumented Postural Sway Test
    - APDM Instrumented Walk Test

Change in Global Impression of Disease Severity and Improvement

- Mean change between Baseline to Week 12 for:
    - Clinician Global Impression Scale (CGI)
    - Patient Global Impression Scale (PGI)

Change in Disease Severity and Progression

- Mean change in total score and sub-scales between Baseline to Week 12 for:
    - Unified Parkinson's Disease Rating Scale (MDS-UPDRS)

6.1.3 Pharmacokinetic Endpoints

Samples for the measurement of whole blood concentrations of Au will be collected before (pre-dose) administration of the investigational drug product during the Week 4, 8, and Week 12 Visit.

At the Week 12 Visit, whole blood for PK will be taken at pre-dose (T0) and at 1, 2, 4, and 6 hours after dosing for the visit. The exact time at which the patient took his/her previous day's study drug dose must be recorded in order to impute a 24-hour trough value (T_{24}-imputed).

The data will be used to construct a composite whole blood concentration-time profile for the Week 12 visit over the assigned treatment period. The Week 12 data will be used to estimate an apparent C_{max} and T_{max} and area under the curve (AUC) over the 24-hour dosing interval [$AUC_{(0-24)}$].

PK collection procedures are described in Section 9.2.8.

6.1.4 Pharmacodynamic Endpoints

Blood samples for the measurement of pharmacodynamic (PD) assays will be collected at Baseline (pre-dose) and following the PK collection during the Weeks 4, 8, and 12 Visits. An optional CSF collection for PD analysis will occur at the Baseline Visit (pre-dose) and Week 12 for subjects who consent to the procedure. PD samples will be stored for future analyses to be defined in a separate PD analysis plan.

PD blood and CSF collection procedures are detailed in Section 9.2.8

Blood and CSF collection for PD assays will be collected according to the schedule listed in Table 1.

7 STUDY POPULATION

The study selection criteria were chosen to exclude patients who may potentially be exposed to specific risks after administering the study drug as well as patients with conditions that may have an impact on assessing the objectives of the study.

7.1 Study Inclusion Criteria

The patients to be enrolled in this study must meet the following inclusion criteria at the time of screening:

1. Able to understand and give written informed consent and follow study procedures.
2. Male or female, aged 30 – 80 years or age (inclusive) at the time of PD diagnosis.
3. PD subjects will be recruited in accordance with the MDS Clinical Diagnostic Criteria for PD:
    a. Parkinsonism present (bradykinesia + either rest tremor or rigidity)
    b. 2 of the following 4 supportive criteria:
        i. Clear and dramatic beneficial response to dopaminergic medication
        ii. Presence of levodopa induced dyskinesias
        iii. Rest tremor of a limb
        iv. Olfactory loss or cardiac sympathetic denervation seen on prior MIBG SPECT
4. Duration of PD since diagnosis is $\leq$ 3 years (inclusive)
5. Modified Hoehn and Yahr stage $\leq$ 3
6. Treatment with dopaminergic therapy for at least 12-weeks and with no change in current medications within the prior 6-weeks

7.2 Study Exclusion Criteria

Patients will be excluded from the study if they meet any of the following criteria:

1. Atypical parkinsonism, including that due to drugs, metabolic disorders, encephalitis, cerebrovascular disease, normal pressure hydrocephalus, or other neurodegenerative disease.
2. The presence of any of the following:
    a. Unequivocal cerebellar abnormalities

b. Downward vertical gaze limitation or slowing of downward saccades
c. Diagnosis of behavioral variant frontotemporal dementia or primary progressive aphasia
d. Parkinsonian features restricted to the lower limbs for > 3 years
e. Treatment with dopamine blockers or depleters in a time course consistent with drug induced parkinsonism
f. Absence of an observable response to high dose levodopa despite moderate disease severity
g. Expert considers a diagnosis of alternative syndrome more likely than PD
h. Rapid progression of gait impairment requiring wheelchair within 5 years of onset
i. Complete absence of progression of motor symptoms over 5 years unless due to treatment
j. Early bulbar dysfunction within the first 5 years since diagnosis
k. Inspiratory respiratory dysfunction (stridor or frequent sighs)
l. Severe autonomic failure in the first 5 years
m. Recurrent falls (>1 per year) because of impaired balance in the first 3 years
n. Disproportionate dystonic anterocollis or hand contractures of hands or feet within 10 years
o. Absence of any of the common non-motor features of PD despite 5 years of disease
p. Otherwise unexplained pyramidal tract signs (weakness, hyperreflexia, or extensor toe signs)
q. Bilateral symmetric parkinsonism

3. Mini-Mental State Examination (MMSE) score of less than 19.
4. Patient with a history of any clinically significant or unstable medical condition based on the Investigator's judgment.
5. History of human immunodeficiency virus (HIV), hepatitis C (HepC) virus antibody, or hepatitis B (HepB) virus antibody.
6. Based on the investigator's judgment, patients who may have difficulty complying with the protocol and/or study procedures.

7. Patient with clinically significant abnormalities in hematology, blood chemistry, ECG, or physical examination not resolved by the Baseline visit which according to Investigator may interfere with study participation.
8. Patients with clinically significant hepatic or renal dysfunction or clinical laboratory findings that would limit the interpretability of change in liver or kidney function, or those with low platelet counts (<150 x 10^9 per liter) or eosinophilia (absolute eosinophil count of ≥500 eosinophils per microliter) at Screening.
9. Patient participating in any other investigational drug trial or using investigational drug (within 12 weeks prior to screening and thereafter)
10. Positive screen for drugs of abuse or known history of alcohol abuse.
11. Women of child-bearing potential, or men, who are unwilling or unable to use accepted methods of birth control during the study and for 6 months following completion of study participation.
12. Women with a positive pregnancy test, are lactating, or are planning to become pregnant during the study or within 6 months of the end of this trial.
13. Patients with implanted metal objects in their body that may be affected by an MRI procedure.
14. Patients who are claustrophobic or otherwise unlikely to be able to complete the MRI scanning procedures.
15. History of allergy to gold in any form.
16. Patient is considered a suicide risk in the opinion of the Investigator, has previously made a suicide attempt, or is currently demonstrating active suicidal ideation. Subjects with intermittent passive suicidal ideation are not necessarily excluded based on the assessment of the Investigator.

A patient who, for any reason (e.g., failure to satisfy the selection criteria), terminates the study before treatment assignment at the Baseline Visit (Visit 0) will be regarded as a Screen Failure. Screen Failures may be rescreened on a case by case basis after discussion and Sponsor approval.

7.3 Reproductive Potential

Female patients of child bearing potential must have a negative serum β-hCG pregnancy test at the Screening Visit (Visit -1) and a negative urine pregnancy test at the Baseline Visit (Visit 0)

prior to treatment assignment. Male and female patients must abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception during the study and or 6-months following their last dose. Condoms should be used with the following acceptable contraceptives:

- Intrauterine devices
- Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring).

Other acceptable contraception methods are:

- Double barrier methods (e.g., condoms and diaphragms with spermicidal gel or foam).

All patients must be advised to use acceptable contraceptives throughout the study period and for 180 days following the last dose of investigational product. If hormonal contraceptives are used, they should be administered according to the package insert. Male and female patients who are not currently sexually active must agree to use acceptable contraception, as defined above, if they become sexually active during the period of the study and 180-days following the last dose of investigational product.

7.4 Removal of Patients from Therapy/Premature Discontinuation

A patient may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or the institution. The Investigator or Sponsor may withdraw the patient at any time (e.g., in the interest of patient safety). The withdrawal of a patient from investigational product by the Investigator should be discussed where possible with the Sponsor's Medical Monitor before the patient stops the investigational product. Any patient removed from the study will complete an end of treatment visit and remain under medical supervision until study discharge is medically acceptable.

If the investigational product is prematurely discontinued, regardless of the reason, the final study evaluations are to be performed as completely as possible. The information should be collected in the eCRF until the safety follow-up visit 28 ± 3 days after the last intake of study drug. Adverse events that occur within 28 ± 3 days after the last dose of study drug will be followed up until resolution, if possible.

All discontinued patients should also undergo the protocol-specified follow-up and should complete the end of study assessments. Comments (spontaneous or elicited) or complaints made by the patient must be recorded in the appropriate source documents (e.g., an adverse event must be recorded and entered into the eCRF). The reason for termination, date of stopping investigational product, and the total amount of investigational product taken must be recorded on the electronic case report form (eCRF) and source documents for all discontinued patients.

Patients will be encouraged to complete the study and all assessments.

Patients *will be* discontinued from the study for the following medical and/or administrative reasons:

- Patient request or at the request of the patient's legally acceptable representative
- Pregnancy, breast feeding, or repeat non-compliance with the scheduled pregnancy testing
- The Investigator judges that continuation of the study would be harmful to the patient's well-being
- TEAE or SAE that limits the patient's ability to continue the study
- Treatment interruption for 7 days or more

Patients *may be* discontinued from the study for the following medical and/or administrative reasons:

- At the specific request of the Sponsor and in liaison with the Investigator (e.g., obvious non-compliance, safety concerns)
- If any exclusion criterion applies during the treatment periods
- Substantial non-compliance with planned study procedures
- Use of illicit drugs or other substances that may, in the opinion of the Investigator have a reasonable chance of contributing to toxicity or otherwise confound the study results

7.4.1 Patient Replacement

Patients who withdraw or are withdrawn following administration of the investigational product will be replaced under this protocol at the discretion of the Sponsor and Investigator.

7.4.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the Investigator and recorded in the patient's medical record and on the eCRF. If a patient is withdrawn for more than one (1) reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

If a TEAE is the reason for discontinuation, then TEAE must be recorded on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event
- Protocol violation
- Withdrawal by patient
- Lost to follow-up
- Pregnancy
- Other (must be specified)

7.4.2.1 Lost to Follow-Up

At least 3 documented attempts must be made to contact any patient lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). One of these documented attempts must include a written communication sent to the patient's last known address via courier or mail (with an acknowledgement of receipt request) asking that the patient return any unused investigational product and return to the site for final safety evaluations, as applicable.

7.5 Patient Identification

After a patient has signed an informed consent form (ICF), the patient identification number for each patient will be assigned by site staff. Patients will be identified by a unique number (e.g., 840-001-001), which consists of:

- First three (3) digits: International Organization for Standardization (ISO) UN M49 Country code (e.g., United States [840])
- Second three (3) digits: Site code (e.g., [001] UTSW)

- Last three (3) digits: Current sequential patient number within the center

Patient identification numbers must be used in sequence and no number should be skipped, substituted, or re-used.

8 INVESTIGATIONAL DRUG PRODUCT

8.1 Treatments Administered

Patients will either receive CNM-Au8 at doses of 7.5 mg, 15 mg, 30 mg, or 60 mg during the study. Study drug will be self-administered by patients who will be directed to take the study drug each day in the morning (7:00 – 10:00 am) at approximately the same time (e.g., 8:00 a.m. ± 1 hour) at least 30 minutes before planned food intake.

The drug formulations will be identical in appearance (size, shape, volume, color) and smell. The packaging and labeling will be designed to maintain blinding to the Investigator's team and to patients. There are no visible differences between the 7.5 mg, 15 mg, 30 mg, and 60 mg CNM-Au8 dosing units.

8.2 Identity of Investigational Products

8.2.1 CNM-Au8

CNM-Au8 is a dark red/purple-colored liquid formulation consisting of a stable suspension of faceted clean surfaced elemental gold nanocrystals in buffered deionized water with a concentration of up to 1 mg/mL of gold. The formulation is buffered by sodium bicarbonate ($NaHCO_3$), present at a concentration of 0.546 mg/mL. The suspension is initially created as an in-process bulk suspension with a gold nanocrystal concentration of 5.5 – 8.5 mg/L, which is then further processed to increase the gold concentration up to 1000 mg/L. The $NaHCO_3$ is present to assist in the manufacturing process and the concentration of $NaHCO_3$ remains nominally unchanged throughout processing. The pH of the suspension is between 7.5 and 10.0. There are no other excipients. The drug product is formulated to be taken orally and will be provided in single dose HDPE containers. The study doses vary by the concentration of gold nanocrystals per milliliter in a volume of 60 mL as described in Table 14.

The CNM-Au8 suspension is filtered using sterile, nonfiber-releasing 0.20 µm filters. The filtration occurs in a clean room with either ISO 7 or ISO 8 standards (depending upon the specific room utilized). The filtered solution is dispensed from the bulk container into clean, food-grade HDPE bottles.

The bottles will be labeled as described in Section 8.2.2.

Manufacturing, testing, product characterization, release of CNM-Au8 will be carried out at the Sponsor's facility at 500 Principio Parkway West, Suite 400, North East, MD 21901, USA.

Table 14. CNM-Au8 Investigational Product Dosing Administration

Description	CNM-Au8			
Daily Dosage	7.5 mg	15 mg	30 mg	60 mg
Concentration	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL
Volume per Bottle	60 mL	60 mL	60 mL	60 mL
Bottles per Day	2	2	2	2
Daily Volume	120 mL	120 mL	120 mL	120 mL
Route of Administration	Oral	Oral	Oral	Oral
Time and Frequency	Once Daily; Same Time Each Day (± 1 hour)	Once Daily; Same Time Each Day (± 1 hour)	Once Daily; Same Time Each Day (± 1 hour)	Once Daily; Same Time Each Day (± 1 hour)

The investigational product components and quality standards are described in Table 15 below.

Table 15. CNM-Au8 Components and Quality Standards

Description	Quality Standard	CNM-Au8 7.5 mg	CNM-Au8 15 mg	CNM-Au8 30 mg	CNM-Au8 60 mg
$NaHCO_3$ (mg) per bottle	ACS, USP identity	32.8 mg	32.8 mg	32.8 mg	32.8 mg
Au (mg) per Bottle	Conforms with ASTM B562-95 and USP <233>	3.75 mg	7.5 mg	15 mg	30 mg
USP Purified Water	USP for total organic carbon, and conductivity	60 mL	60 mL	60 mL	60 mL

8.2.2 Labeling

All investigational drug product will be labeled according to applicable local and legislative requirements. Label text will be approved according to the Sponsor's agreed procedures, and a copy of the labels will be made available to the study site upon request. For all study drugs, a system of numbering in accordance with all requirements of Good Manufacturing Practice (GMP) will be used, ensuring that each dose of study drug can be traced back to the respective bulk ware of the ingredients. The Sponsor's Quality Assurance group will maintain lists linking

all numbering levels. A complete record of batch numbers and expiry dates of all study treatment as well as the labels will be maintained in the Sponsor study file.

Study drug label may include, but is not limited to, the following information:

- Batch number
- Storage information
- Site identification
- Expiry date
- Unique number/code

8.2.3 Storage and Handling

Investigational product will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and GMP requirements and will be inaccessible to unauthorized personnel. A complete record of batch numbers and expiry dates can be found in the Sponsor study file; the site-relevant elements of this information will be available in the Investigator site file. The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the study drug must be properly documented according to the Sponsor's agreed and specified procedures.

All study drug will be kept in a locked area with limited access and stored at 15°- 25°C (59°-77°F). Mean kinetic temperature should not to exceed 25°C. Excursions between 15°C and 30°C (59° and 86° F) that may be experienced in pharmacies, hospitals, or warehouses, and during shipping are allowed.

8.3 Method of Assigning Patients to Treatment Groups and Dose Selection

8.3.1 Treatment Assignment

Patients who complete all screening procedures and meet the eligibility criteria to enter the study will be allocated by an unblinded study pharmacist to treatment per the pre-specified dosing schedule (Figure 13).

8.3.2 Selection of Doses in the Study

The original planned CNM-Au8 dose levels in the first-time-in-human study were 15, 30, 60, and 90 mg CNM-Au8. The initial dose level selected was calculated based on the FDA guidance document "Estimating the Maximum Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers". The lowest NOAEL was in the rat and was 40 mg/kg. Based on this NOAEL, an equivalent human dose was determined to be approximately 6.4 mg/kg. In a 60-kg human patient, the selected starting dose of 15 mg CNM-Au8 represented a safety margin of approximately 26-fold.

The protocol for Study AU8.1000-14-01 was submitted to an Independent Ethics Committee and Competent Authority in the Netherlands and was subsequently approved for initiation under a Clinical Trial Application (CTA). The study was conducted in the Netherlands, at a Phase 1 facility, The Center for Human Drug Research (CHDR) and has been completed.

A total of 40 patients were randomized in the SAD phase and 46 patients in the MAD phase. Oral administration of either a single (SAD) or repeated dose (MAD) of CNM-Au8 over 21 consecutive days in healthy volunteers was safe and well tolerated. The TEAEs observed across the 4 doses (15 mg, 30 mg, 60 mg, and 90 mg) of CNM-Au8 were mostly mild in severity. While no dose response relationship to TEAEs was observed overall in the SAD or MAD Phase of the study, increases in headache and gastrointestinal related TEAEs were observed in the 90 mg cohort of the MAD Phase. No safety trends or safety signals were observed in the study.

In the SAD phase, PK analyses were not completed because the majority of whole blood Au concentrations were below the LOQ. However, a more sensitive ICP-MS instrument was employed for the MAD phase.

In the MAD Phase:

- The geometric mean whole blood concentrations from 1 week onward increased in a dose related, but not a dose-proportional manner. Based on PK results from Days 14 and 21, the increases in both C_{max} and $AUC_{(0-24)}$ were less than dose proportional.
- Based on pre-specified fit criteria, the elimination $t_{½}$ ranged from 277 to 628 hr (11.5 to 26.2 days).

- Steady-state for all cohorts, based on the geometric mean whole blood concentrations, was reached by the end of the second week of dosing (Day 14), which was substantially less than predicted by the $t_{½}$ [range of 46 to 105 days (6.6 to 15 weeks)].
- PK parameters related to urinary excretion (Ue, CLr) could not be calculated. Only one urine Au concentration ≥ LOQ (3 ng/mL) was within limits of the assay.

Based upon the maximum doses (mg/kg/day) evaluated in the nonclinical GLP repeat-dose 21-day toxicokinetic studies, the completed first-time-in-human Phase 1 study (AU8.1000-14-01) doses of 15, 30, 60, 90 mg provided a safety margin to the NOAEL based on dose ratios (mg/m^2) ranging from 26x – 4x in rodents, and 195x – 32x in canines, as described in the tables below. Therefore, at the top dose of 90 mg tested in humans, there was a minimum 4x safety margin to the NOAEL in rats.

Table 16. Summary of CNM-Au8 Conversion of Animal 21-Day Repeat Doses To Human Equivalent Dose (HED) Based On Body Surface Area (mg/m^2) for 60 kg Human

Species	**21-Day NOAEL Dose** (mg/kg/day)	**21-Day NOAEL Dose** (mg/m^2)	**Safety Margin Based on Dosing Ratios (mg/m^2)** *(For 21-Day dosing Studies)*			
			15 mg (9.3 mg/m^2)	**30 mg** (18.5 mg/m^2)	**60 mg** (37.0 mg/m^2)	**90 mg** (55.5 mg/m^2)
Rat	40	**240**	25.9	13.0	6.5	4.3
Canine	90	**1800**	194.6	97.3	48.6	32.4

Further, when evaluating Au exposure based on the end of study Day 21 $AUC_{(0\text{-}24)}$ (ng*hr/mL) in the canine, and rodent studies in comparison with the human 21-day MAD study, the human doses provided an exposure safety margin ranging from 3.3x – 1.6x compared with rodents, and 18.5x – 9.0x compared with canines, as described in Table 17 below.

Table 17. Summary of CNM-Au8 Exposure Safety Margin Based on End of Study $AUC_{(0-24)}$ ng*hr/mL. Ratio of Animal Toxicokinetic to Human Pharmacokinetic AUC Results From 21-Day Repeat Dose Studies.

Species	21-Day NOAEL Dose (mg/kg/day)	Animal End of Study $AUC_{(0-24)}$ (ng*hr/mL)[a]	Safety Margin Based on Animal/Human $AUC_{(0-24)}$ Ratio *(For 21-Day Dosing Studies)*			
			Human 15 mg AUC (32.3 ng*hr/mL)	Human 30 mg AUC (41.4 ng*hr/mL)	Human 60 mg AUC (50.3 ng*hr/mL)	Human 90 mg AUC (66.0 ng*hr/mL)
Rat	40	**106**	3.3	2.6	2.1	1.6
Canine	90	**596**	18.5	14.4	11.8	9.0

Notes: [a] Average of Male and Female $AUC_{(0-24)}$ ng*hr/mL values at End of Study

CNM-Au8 exposure at the NOAEL at the end of the chronic toxicokinetic studies in the 9-Month canine and 6-Month rodent chronic dosing studies, provided an exposure safety margin ranging from 6.5x – 3.2x, and 13.6x – 6.7x in rodents and canines, respectively in comparison with the human exposures in the 21-day MAD study, as described below in Table 18.

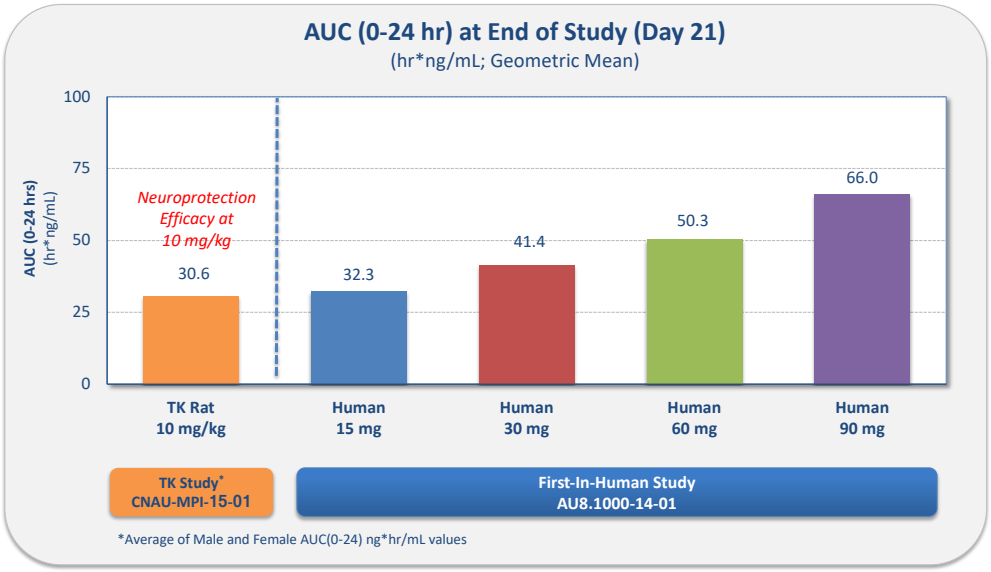
Table 18. Summary of CNM-Au8 Exposure Safety Margin Based on End of Study $AUC_{(0-24)}$ ng*hr/mL Ratio of Chronic Animal Toxicokinetic 6 and 9-Month Rodent and Canine Repeat Dose Studies to Human 21-Day Pharmacokinetic Results

Species (Study)	NOAEL Chronic Dosing (mg/kg/day)	Animal End of Study $AUC_{(0-24)}$ (ng*hr/mL)[a]	Safety Margin Based on Chronic Animal End-of-Study/ Human 21-Day $AUC_{(0-24)}$ Ratio			
			Human 15 mg AUC (32.3 ng*hr/mL)	Human 30 mg AUC (41.4 ng*hr/mL)	Human 60 mg AUC (50.3 ng*hr/mL)	Human 90 mg AUC (66.0 ng*hr/mL)
Rat (6-Month)	40	**209**	6.5	5.0	4.2	3.2
Canine (9-Month)	10	**440**	13.6	10.6	8.7	6.7

[a] Average of Male and Female $AUC_{(0-24)}$ ng*hr/mL values at End of Study

Based on CNM-Au8 exposures at the NOAELs in the chronic rodent and canine studies along with observed CNM-Au8 exposures in 21-day multiple dosing in humans while also considering safety and tolerability, the doses chosen for this Phase 2 study in patients with Parkinson's Disease will be up to 60 mg. Furthermore, the blood gold exposure observed at this dose in humans, is similar to the exposure observed in rodent toxicokinetic (TK) studies where significant remyelination and neuroprotection benefits were observed in preclinical neuroprotection models at 10 mg/kg, thus suggesting a positive bioenergetic response to CNM-Au8 at this relative dose (e.g. 15 – 60 mg).

Figure 15. 21-Day AUC Exposure Ranges Between Rodent Toxicokinetic Studies, Preclinical Efficacy Models, and First-In-Human Dosing



8.4 Procedures for Blinding

Patients and investigators will remain blinded to the study dose through the duration of the study. An unblinded study pharmacist at the clinical site will allocate patients to a dose of study medication per the pre-specified dosing schedule in Figure 13. The initial starting dose will be communicated to the sites unblinded representative prior to treatment assignment, and subsequent doses as outlined in Figure 13 will be only communicated to unblinded staff in order to maintain the single blind.

The pharmacist at each site will maintain a secured copy of the pre-specified dosing scheme according to the site's SOP and dispense medication based on the scheme for each patient. In the event the PI requires knowledge of the assigned dose for immediate care, the site's unblinded pharmacist or Sponsor's Medical Monitor can be contacted.

8.5 Unblinding

The treatment allocation of a patient will be made available to the Investigator in case of emergency and when knowledge of the treatment allocation is important in the medical

management of AEs. All unblinding events must be reported to the IRB/HREC in accordance with IRB/HREC timelines. Unblinding may occur in the following circumstances:

8.5.1 Unblinding by Sponsor's Pharmacovigilance Representative

In compliance with applicable regulations, in the event of a suspected unexpected serious adverse reaction (SUSAR), which is deemed related to blinded treatment, the patient's treatment code may be unblinded by the Sponsor before reporting to the health authorities, HRECs, IRBs, and investigators if the SUSAR is related to the blinded treatment.

8.5.2 Emergency Unblinding By The Investigator

In case of emergency and where knowledge of assigned treatment allocation is required for the acute management of a TEAE, the Investigator may unblind the case by contacting the site's unblinded pharmacist or Sponsor's Medical Monitor. Investigators should note that the occurrence of an AE or SAE should not routinely precipitate the immediate unblinding. If possible, the Investigator should always consult with the Sponsor's Medical Monitor prior to breaking the blind. The Investigator must contact the Sponsor's Medical Monitor in the event that the blind is broken and document the reason for unblinding.

8.6 Prior and Concomitant Therapy

Disease-specific medications allowed per the inclusion/exclusion criteria.

To ameliorate procedure-related anxiety, all patients will receive 5 mg of diazepam, PO, 45-60 minutes prior to planned 31P-MRS scan at the Baseline and Week 12 Visits.

Otherwise, except for acetaminophen, ibuprofen, naproxen, and 2nd generation antihistamines including fexofenadine, loratadine, and cetirizine; patients may not take any new prescription or OTC medications or dietary supplements from 14 days prior to Baseline through the end of study follow-up unless used to manage a TEAE.

Patients must be on a stable dose of any chronic medications for at least six weeks prior to Screening. Any changes in dose or newly prescribed medications started during the study must be brought to the attention of the Investigator and reported immediately to the Sponsor's Medical Monitor.

The Investigator will make every effort to contact the Sponsor's Medical Monitor prior to administration of a new concomitant therapy (prescription or OTC) after treatment assignment, unless the concomitant therapy is needed immediately for patient safety.

Any use of new prescription medicines, OTC medications, or dietary supplements during the study period must be reported on the eCRF.

8.7 Treatment Compliance

Patients will receive study drug dispensed per visit schedule in Table 1.

Patients will be requested to return any unused study drug including empty packaging and used bottles at the Week 4, 8 and 12 Visits. Treatment compliance will be assessed at study visits through bottle counts and will be documented and summarized by a drug-dispensing log for each patient. Overall treatment compliance with study drug intake for the *per protocol* analysis set should be between 80% and 120% of the planned dose and will be assessed at each study visit. In the event patients are not compliant within this range, discontinuation may be considered by the Investigator in consultation with the Sponsor. In the event of site logistical issues or safety concerns due to global pandemics, study participants may remain on study drug beyond the Week 12 visit if agreeable to both the PI and Sponsor's Medical Monitor. Treatment compliance will be assessed for each 4-week extension beyond the Week 12 visit. The date of dispensing the study drug to the patient will be documented in the CRF.

If a dose of study drug is missed, the patient should take the dose that day and continue with the planned dosing interval for the following day. The dose should not be doubled to make up for a missed dose within the same day.

8.7.1 Study Drug Accountability

Study drug will be administered in accordance with the procedures of this protocol. Only authorized site personnel may supply study drug, and only patients enrolled in the study may receive study drug, in accordance with applicable regulatory requirements.

A sponsor representative will perform ongoing drug accountability assessments during interim study monitoring visits. Following completion of the trial, unused study drug and empty

containers may be returned to the sponsor or upon request from the sponsor, destroyed per site process.

9 STUDY PROCEDURES

9.1 Study Schedule

A Time and Events Schedule is provided in Table 1. Assessments should be conducted at each visit indicated in Table 1, unless the collection is unable to occur due to logistical or safety concerns associated with the COVID-19 pandemic. In these unique cases, missed assessments should be attributed to COVID-19 on the respective CRFs.

Screening will occur on Days -42 through -1. The Baseline visit will occur on Day 1. The treatment period will include Day 1 through the last dose of study medication.

9.1.1 Screening Phase (Day -42 through Day -1) – VISIT (-1)

The initial Screening Visit may be conducted up to 42 days prior to Baseline on Day 1. The following procedures/assessments will be performed at Screening:

- Informed consent (must be done prior to any of the following procedures)
- Medical history and patient demographics (confirm PD diagnosis)
- Prior medication assessment
- Physical examination
- Confirm patient meets all eligibility criteria
- Height and weight
- Vital Signs
- Urinalysis
- Urine drug screen (cocaine, marijuana, opiates, benzodiazepines, methamphetamines)
- Venous blood for clinical laboratory tests
- Venous blood for infectious disease screen (HIV, hepatitis B, and hepatitis C)
- Serum pregnancy test (for females of childbearing potential)
- Mini Mental State Exam (MMSE)

- 12-Lead ECG
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Adverse Events
- Schedule next study visit (Baseline Visit)

9.1.2 Baseline (Day 1) – VISIT 0

Patients will present to the study site for the Baseline assessments prior to dosing initiation. All Baseline assessments should be completed prior to the subject receiving their first dose of study medication. Magnetic resonance spectroscopy and/or PD CSF collection may be performed within a -7 day window from Day 1. The following assessments are to be completed for the Baseline visit:

- Physical exam
- Concomitant medication assessment
- Vital signs
- Weight
- 12-Lead ECG in triplicate
- Urine pregnancy test (for females of childbearing potential)
- Re-confirm patient meets all eligibility criteria
- Venous blood for clinical laboratory tests
- PD blood collection
- PD CSF collection (optional)
- Urinalysis
- Adverse events
- Anxiolytic Administration

- Magnetic Resonance Spectroscopy (MRS)
- Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
- Clinical Global Impression Scale (CGI)
- Patient Global Impression Scale (PGI)
- Columbia Suicide Severity Rating Scale (C-SSRS)
- APDM Instrumented Timed Up and GO (TUG) Test
- APDM Instrumented Postural Sway Test
- APDM Instrumented Walk Test
- Treatment Assignment
- Dispense Study Drug Package
- Take First Study Dose at the Site from the dispensed supply (following all Baseline assessments)
- Schedule next study visit

9.1.3 Treatment Phase (Day 1 through EOS)

During this period, patients will return to the study site for evaluations of safety and efficacy as outlined in Table 1.

9.1.3.1 VISIT 1 (Week 2)

At Week 2, clinical site staff will contact patients by telephone and assess for the following items:

- Adverse Events
- Concomitant medication assessment
- Treatment compliance assessment
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Schedule next visit

9.1.3.2 VISIT 2, 3 (Week 4 and 8)

Study activities include:

- Physical exam
- Concomitant medication assessment
- Vital signs
- 12-Lead ECG
- Urine pregnancy test (for females of childbearing potential)
- Venous blood for clinical laboratory tests
- PK blood collection
- PD blood collection
- Urinalysis
- Adverse events
- Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
- Clinical Global Impression Scale (CGI)
- Patient Global Impression Scale (PGI)
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Return/Dispense Study Drug Package
- Take Study Dose at the Site from the dispensed supply
- Schedule next study visit

9.1.3.3 VISIT 4 (Week 12)

Study activities include:

- Physical exam
- Concomitant medication assessment

- Vital signs
- 12-Lead ECG
- Urine pregnancy test (for females of childbearing potential)
- Venous blood for clinical laboratory tests
- PK blood collection
- PD blood collection
- PD CSF collection (optional)
- Urinalysis
- Adverse events
- Anxiolytic Administration
- Magnetic Resonance Spectroscopy (MRS)
- Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
- Clinical Global Impression Scale (CGI)
- Patient Global Impression Scale (PGI)
- Columbia Suicide Severity Rating Scale (C-SSRS)
- APDM Instrumented Timed Up and GO (TUG) Test
- APDM Instrumented Postural Sway Test
- APDM Instrumented Walk Test
- Return Study Drug Package
- Take Last Study Dose at the Site from the dispensed supply (at least 1 hour prior to MRS)
- Schedule next study visit

9.1.3.4 VISIT 5 (Week 16/EOS)

- Physical exam
- Concomitant medication assessment
- Vital signs
- 12-Lead ECG
- Venous blood for clinical laboratory tests
- PK blood collection
- Urinalysis
- Adverse events
- Columbia Suicide Severity Rating Scale (C-SSRS)

9.1.4 End of Study Visit – Follow Up Period (Period 3)

Timing for the end-of-study assessment (Visit 5) should occur at 28-days (±3 days) following study discontinuation for: 1) patients who discontinue therapy prior to the end of study, or 2) who complete the study.

Any AEs/SAEs occurring up to the time of the follow-up visit will be recorded. Appropriate follow-up should continue until all safety concerns, in the Investigator's opinion, are resolved, or return to baseline.

9.1.5 Unscheduled Safety Assessments

In the event that collection of the 31P-MRS at the Week 12 visit is delayed due to imaging issues or COVID-19 pandemic related concerns, participants may remain on study drug beyond the Week 12 visit, so long as the extension is agreeable to both the study PI and Sponsor's Medical Monitor, until it is deemed safe to return for collection of the Week 12 efficacy assessments. Safety assessments that are able to be captured should be completed every 4 weeks beyond the planned Week 12 visits prior to dispensation of unscheduled study drug. These safety assessments include:

- Adverse Events
- Concomitant Medications
- ECG (if able to collect)
- Vitals (if able to collect)
- Physical Exam (if able to collect via remote visit)
- Clinical Laboratory Tests (if able to collect via remote visit)
- Patient Global Impression Scale (PGI)
- Columbia Suicide Severity Rating Scale (C-SSRS)

9.2 Study Measurements and Assessments

Measurements and assessments are to be performed according to the schedule shown in Table 1.

Measurements and assessments should only be performed by trained and qualified personnel and whenever possible, the same person at each site should perform each of these assessments for all Patient visits.

If a patient terminates treatment prematurely, all assessments listed in Table 1 for the End-of-Study Assessment should be completed and dispensed study drug should be returned to the clinical site.

9.2.1 Demographic and Other Baseline Characteristics

Demographic characteristics such as age, sex, weight, height, ethnicity, and body mass index (BMI) will be collected during the study according to the schedule in Table 1.

9.2.2 Assessment of Imaging Biomarkers

Imaging of metabolic biomarkers will be assessed at each visit noted in the Study Schedule (Table 1) using the measures described below. All efficacy measurements are to be performed by a trained and qualified person.

In vivo ^{31}P Magnetic Resonance Spectroscopy (MRS) measures phosphorus-containing metabolites such as NADH and ATP that are key molecules in cellular bioenergetic processes. By measuring the ratios of NAD+/NADH and ATP, neuronal redox potential can be assessed and the effects of CNM-Au8 on redox homeostasis can be studied. Patients will have two ^{31}P-MRS studies: at Baseline (Visit 0) and at Week 12 (Visit 4).

9.2.3 Imaging Procedures

MRI/MRS scans will be conducted on a Philips Achieva 7 Tesla human MRI scanner located in the Advanced Imaging Research Center, at UTSW. Patients will undergo ^{31}P MRS scans to measure brain phosphorous metabolites and redox state. 45-60 minutes prior to ^{31}P-MRS scans, a standard anxiolytic dose will be administered to ameliorate procedure related anxiety. The patient will remain under medical supervision until deemed safe for discharge by the Investigator.

9.2.3.1 Whole Brain Metabolite Measurements:

Preparation: A cylinder bird-cage shaped ^{31}P T/R volume coil insert (into a 1H T/R volume coil) will be used for imaging the brain stem. The head of the patient will be positioned in the center of the coil with soft cushion pads placed under and on the sides of the head to secure the positioning of the head and reduce potential movement during the scans.

After a scout image scan, axial, sagittal and coronal T2-weighted spin-echo multi-slice brain images will be collected using the typical parameters: TR 3.5 sec, TE 80 ms, slice thickness 8 mm, gap 2 mm. A 2nd-order volume-based shimming will be conducted prior to ^{31}P MRS data acquisition.

^{31}P MRS scans: Whole brain ^{31}P spectra will be acquired using three-dimensional chemical shift imaging (3D CSI) approach. Typical MRS parameters will be TR = 0.5 sec, NP 2 k, BW 8 kHz, NA = 4 (short scan) and 16 (long scan), with spatial resolution of 2x2x2 cm^3. If subjects are able to complete both short and long scans without movement artifact, the data will be combined. Otherwise, only the completed dataset without movement artefacts will be used for data analysis. Voxel-based ^{31}P spectra will be pooled together based on the functional regions (frontal, temporal, parietal and occipital) to evaluate the spatial distributions of metabolites. Data

analysis will be performed by lineshape fitting of the ^{31}P resonances of all phosphorous metabolites using a previously developed Matlab program.

9.2.3.2 Partial Volume Coil Measurement:

Preparation: A half-cylinder-shaped ^{1}H/^{31}P dual-tuned T/R partial volume coil will be used to image the posterior brain (occipital and parietal lobes) for evaluation of the brain redox state based on the measurement of NAD+/NADH ratio.

Axial, sagittal and coronal T2-weighted spin-echo multi-slice images will be collected from the posterior head using the typical parameters TR 3.5 sec and TE 80 ms, slice thickness 8 mm, gap 2 mm. A 2nd-order volume-based shimming will be conducted over the posterior brain region prior to ^{31}P MRS data acquisition.

^{31}P MRS scans: To obtain ^{31}P spectra with resolved NAD from overlapping α-ATP signals, an inversion-recovery-based NAD spectral editing technique will be used. Briefly, acquisition of a reference ^{31}P spectrum using pulse-acquire sequence is conducted plus the acquisition of an additional spectrum using a sequence containing an adiabatic inversion pre-pulse followed by a short delay is done to selectively nullify the NAD signal. A spectral subtraction will be performed to obtain an NAD-edited spectrum with resolved NAD signal from the reference spectrum. The contribution of NAD+ and NADH to the resolved NAD signal will be quantified by lineshape fitting based on prior knowledge of the NADH signal being a singlet and the NAD+ signal being a quartet (as defined by ^{31}P coupled AB-spin system resonance pattern). The brain redox state will be calculated by the NAD+/NADH ratio. Typical ^{31}P MRS parameters for data acquisition will be TR 1.0 sec, TD 0.17 ms, NP 4k and zero filled to 8k, BW 8 kHz, NA 512x2. For quantitative comparison of different metabolites in the posterior brain region, an additional scan will be performed, using the partial volume coil, under the fully relaxed T1 condition with long TR of 15 sec and 16 acquisition averages.

9.2.4 APDM Gait and Postural Analysis

Gait abnormalities and postural instability are features of PD that worsen with disease severity and can both be used as indicators of fall risk. Gait abnormalities and postural instability will be assessed using the APDM Mobility Lab and wearable Opal sensors.

Measurements of gait abnormality will be collected by conducting two instrumented tests. A Timed Up and Go (TUG) test will be performed, which involves standing from a seated position, walking six meters, turning 180°, walking back to the chair and sitting. The time it takes to complete this action will be recorded, and the wearable Opal sensors will provide multiple measures pertaining to gait analysis (e.g., lean angle, duration, velocity, steps, speed, etc.). Gait abnormality will also be measured by the Instrumented 2-Minute Walk test, which involves walking back and forth on a 7-meter course for a period of two minutes. Multiple variables will be measured by the Opal sensors, and the distance walked recorded.

Measurements of postural instability will be collected by performing an Instrumented Postural Sway test in which the subject will be instructed to stand still for a period of time while the wearable sensors measure parameters such as mean sway area, path length, jerk, and sway distance in the mediolateral and anteroposterior directions. This assessment will be conducted while the subject remains standing with their eyes open, and repeated standing with their eyes closed, both when standing on a firm surface (e.g., a laminate floor).

Both the TUG, Postural Sway and 2-Minute Walk assessments will be conducted at the Baseline and Week 12 Visits.

9.2.5 Assessment of Safety

Safety assessments will include the following:

- Treatment emergent AEs and SAEs, both reported and observed
- Physical examination
- Vital sign measurements: Ear temperature, respiration, blood pressure, and heart rate (blood pressure and heart rate will be measured after the patient has been supine for 5 minutes)
- 12-Lead ECG will be conducted in triplicate at the Baseline Visit and averaged. A single ECG collection will occur at each additional scheduled time point
- Venous blood for clinical laboratory tests will include hematology, coagulation, and blood chemistry. Blood for analysis will be drawn after an overnight fast when possible
- C-SSRS

Outlier criteria for safety results including chemistry, urine, hematology, ECG, vital signs are based upon the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0, November 27, 2017. This outlier criteria document has been authored and approved by the Sponsor safety physician to enhance review of safety data. The outlier criteria will be used as an additional review tool for TEAE reporting.

9.2.6 Assessment of Disease Improvement

9.2.6.1 Clinical Global Impression Scale (CGI)

The CGI scale provides a brief assessment of the clinician's view of the patient's global functioning and severity of current disease state and can be utilized to assess for changes in disease progression over the course of a clinical trial. The CGI scale assessing disease severity (CGI-S) will be performed at the Baseline Visit and at Weeks 4, 8, and 12. A second CGI scale assessing improvement (CGI-I) will be performed at the Weeks 4, 8, and 12 visits.

9.2.6.2 Patients Global Impression Scale (PGI)

The PGI scale provides a brief assessment of the patient's view global functioning and severity of current disease state and can be utilized to assess for changes in disease progression over the course of a clinical trial. The PGI scale assessing disease severity (PGI-S) will be performed at the Baseline Visit and at Weeks 4, 8, and 12. A second PGI scale assessing improvement (PGI-I) will be performed at the Weeks 4, 8, and 12 visits.

9.2.6.3 Unified Parkinson's Disease Rating Scale (MDS-UPDRS)

The Unified Parkinson's Disease Rating Scale (UPDRS) is a rating tool used to gauge the course of Parkinson's disease in patients. The UPDRS is composed of four parts: part I assesses behavioral problems such as intellectual decline, hallucinations, and depression; part II assesses patients' perceptions of their ability to carry out activities of daily living, including dressing, walking, and eating; part III covers the motor evaluation of disability and includes ratings for tremor, slowness (bradykinesia), stiffness (rigidity), and balance; part IV covers a number of treatment complications including ratings of involuntary movements (dyskinesias), painful cramps (dystonia), and irregular medication responses (motor fluctuations).

The UDPRS will be performed at the Baseline Visit and Weeks 4, 8 and 12.

9.2.7 Assessment of Disease Related Safety

9.2.7.1 Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a semi-structured interview that captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period (Posner et al. 2011). The interview includes definitions and suggested questions to solicit the type of information needed to determine if a suicide-related thought or behavior occurred.

The C-SSRS contains 2 required items pertaining to suicidal ideation, 4 required items pertaining to suicidal behavior, and 1 required item pertaining to non-suicidal self-injurious behavior. There are 8 additional suicidal ideation items and 2 additional suicidal behavior items which are completed in cases of positive responses for other items, as well as 2 items for completed suicide and suicide behavior present during the interview. Thus, there is a maximum of 19 items to be completed.

The C-SSRS must be performed by an individual who is medically responsible for the subject.

Two versions of the C-SSRS are used in this study:

- The “Baseline” version will be administered at the Screening Visit (Visit 0) and will be completed for all subjects.
- The “Since Last Visit” version will be completed for all subjects at all study visits after the Screening Visit (Visit 0).

9.2.8 PK and PD Assessments

Blood samples for pharmacokinetic (PK) and pharmacodynamics (PD) analyses and the optional CSF samples for PD analyses will be collected during the Treatment Phases as outlined in the Time and Events Schedule in Table 1.

During PK and PD study visits patients should present themselves to the clinical site or hospital without taking the morning dose of study medication. During these visits the PK and PD blood samples should always be collected before administration of the morning dose of the study

medication. Accurate time of blood sampling and administration times for PK and PD collection will be documented in the eCRF.

For patients participating in the optional CSF collection, CSF will be sampled by via lumbar puncture by a board certified clinician. Following sterile collection, the CSF sample should immediately be transported to the laboratory and processed per the study's Lab Manual. CSF sampling should occur after the subject has performed all other assessments associated with the visit.

Pharmacokinetic analyses, and pharmacokinetic/pharmacodynamics (PK/PD) modeling as applicable, using population approaches to describe Au whole blood pharmacokinetics including potential influence of relevant patient co-variables (e.g. age, gender, body weight, etc.) or potentially to relate parameters of clinical safety and efficacy response with Au whole blood concentrations will be investigated under separate detailed PK and/or PD evaluation plan(s).

9.2.8.1 Collecting, Processing, and Shipping of PK and PD Samples

For PK analyses, 4 mL of whole blood will be collected for each blood draw for bioanalytical analyses of Au content. Blood samples for PK analyses will be collected in tubes with dipotassium ethylenediaminetetraacetic acid (K_2EDTA), which should not exceed 1.6 mg K_2EDTA per mL of whole blood). PK samples must be placed on wet crushed ice immediately and stored at -70 °C or lower within 15 minutes.

For PD analyses, up to an additional 20 mL of whole blood will be collected following the collection of the PK in two divided samples. PD samples will be collected in citric acid tubes, placed on wet crushed ice immediately, and stored at -70 °C or lower, as quickly as feasible.

Additional processes surrounding collection, processing and shipment of collected samples will be defined in the study's Lab Manual.

9.2.8.2 Bioanalytical Methods

Blood concentrations of gold (Au) will be determined by using validated Inductively Coupled Plasma Mass Spectrometer (ICP-MS) analytical methods. The lower limit of quantification, deviation of calibration standards from the theoretical value, and precision have been established

using standard methods. Performance of the assay will be assessed by monitoring the analysis of spiked samples with known concentrations of CNM-Au8.

Blood samples will be analyzed under the responsibility of the Sponsor's bioanalytical laboratory at:

Clene Nanomedicine, Inc.
Bioanalytics Laboratory
500 Principio Parkway West, Suite 400
North East, MD 21901-2912

9.2.9 Clinical Laboratory Tests

All routine samples will be analyzed by a central licensed clinical laboratory unless otherwise specified below. At the end of the study when all planned analyses are completed, all clinical laboratory blood samples will be destroyed per the central clinical laboratory SOPs.

The clinical laboratory tests should include, but are not limited to:

- Hematology: hemoglobin, hematocrit, white blood cell count with differential, red blood cell count, platelet count.
- Serology (Screening Visit only): human immunodeficiency virus (HIV), hepatitis C (HepC), and hepatitis B (HepB).
- Blood Chemistry: Alanine aminotransferase (ALT; SGPT) and Aspartate aminotransferase (AST; SGOT), total bilirubin, direct bilirubin, Gamma-Glutamyl transferase (GGT), blood urea nitrogen (BUN), creatinine, alkaline phosphatase, lactate dehydrogenase (LDH), sodium, potassium, calcium, chloride, glucose, albumin, bicarbonate, uric acid, total protein, creatine kinase (CK).
- Serum and urine pregnancy test: Beta-human chorionic gonadotropin (βhCG) test for females of childbearing potential.
- Urinalysis: Dipstick analyses will include: Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, and glucose. Urinalysis will include macroscopic analysis and microscopic analysis only when indicated by dipstick.
- Urine Drug Screen: Cocaine, cannabinoids, methamphetamines, benzodiazepines, and opiates. Patients with positive urine drug screen test results, without an active

prescription, will be excluded from the study. Urine Drug Screen kits will be supplied for sites to complete testing onsite. Repeat urine drug screens for validation of initial findings may be conducted at the discretion of the site investigator.

The core clinical laboratory will supply appropriate blood collection tubes for each study visit as specified in the study's Lab Manual.

9.2.10 Physical Examinations

A full physical examination will consist of assessments of the following: skin, ears, nose, throat, head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, neurologic.

9.2.11 Vital Signs

Vital signs will include respiration rate (breaths per minute), temperature, blood pressure (mmHg) and heart rate (beats per minute [bpm]). Blood pressure and heart rate will be obtained after the patient has been resting in supine position for 5 minutes. The same arm must always be used for blood pressure and heart rate measurements. Noninvasive measurement should be conducted preferably with a mercury sphygmomanometer or a validated electronic device in accordance with published guidelines (e.g. American Heart Association: Recommendations for Blood Pressure Measurement in Humans and Experimental Animals) (Pickering et al., 2005).

9.2.12 12-Lead ECG

12-lead ECGs will be performed successively in triplicate after the patient has rested quietly for at least 5 minutes in a supine position at the Baseline Visit. The average corrected QT interval (QTc) of the triplicate ECG measurements collected prior to Day 1 dosing will serve as that patient's baseline for use in ongoing safety assessment. At all other scheduled timepoints, a single ECG will be collected after the patient has rested quietly for at least 5 minutes in a supine position. When the timing of the measurement coincides with a blood collection, the ECG should be obtained prior to blood collection. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads are placed in the same positions each time for consistency.

ECG intervals will be summarized and presented descriptively. ECG rhythm will be interpreted by the Investigator as normal (N), abnormal not-clinically significant (aNCS), or abnormal clinically significant (aCS).

9.2.13 Assessment Windows

The MRS scan should occur as close to the same time per patient for the Baseline and Week 12 Visit, within a +/- 2 hours window. Collection of blood samples should occur as close to the nominal timepoint as possible. The actual collection time of blood samples will be recorded.

10 ADVERSE EVENTS

Safety will be assessed via adverse events, serious adverse events, discontinuations due to adverse events, and changes in the Columbia Suicide Severity Rating Scale (C-SSRS).

10.1 Safety Reporting Guidelines

AE monitoring and reporting is a routine part of every clinical study. All Investigators, clinical site staff, and the Sponsor's employees share in the responsibility for reporting AEs and SAEs.

10.1.1 Sponsor Guidance

The Sponsor is required to notify all participating Investigators and FDA in a written Investigational New Drug (IND) safety report of any AE associated with the use of the investigational drug that is both serious and unexpected, and any finding from tests in laboratory animals that suggests a significant risk for human patients (21 CFR 312.32(c)(1)(i)(A),(B)). The Sponsor is required to keep Investigators informed of new observations discovered by, or reported to the Sponsor regarding the study drug particularly with respect to AEs and safe use (21 CFR 312.55).

10.1.2 Investigator Guidance

The Investigator is required to promptly report to the Sponsor any AE that may reasonably be regarded as caused by or probably caused by the investigational drug. If the AE is alarming, the Investigator shall report the adverse effect immediately to the Sponsor (21 CFR 312.64). Further, Investigators are required to promptly report to the Human Research Ethics Committee (HREC) and/or Institutional Review Board (IRB) as applicable all unanticipated problems involving risks to human patients or others including AEs that should be considered unanticipated problems (21 CFR 56.108(B)(1), 21 CFR 312.53(c)(1)(vii), and 21 CFR 312.66). All Investigators and clinical site staff who learn about or are notified of a SAE must collect and promptly report to the Sponsor (within 24 hours) data according to the study protocol and relevant regulations (21 CFR 312.64, 21 CFR 312. 32).

10.2 Adverse Events (AE or Adverse Experience)

Adverse events include any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational drug, whether or not considered related to the study drug (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite') (International Conference on Harmonization [ICH] E2A, E6). Adverse events include:

- Exacerbation of a pre-existing disease.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Disease or medical condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at Baseline that worsen following the start of the study.
- Lack of efficacy in the acute treatment of a life-threatening disease.
- Events considered by the Investigator to be related to study-mandated procedures.
- Abnormal assessments (e.g., change on physical examination, ECG findings), if they represent a clinically significant finding that were not present at Baseline or worsened during the course of the study.
- Laboratory test abnormalities if they represent a clinically significant finding, symptomatic or not, which was not present at Baseline or worsened during the course of the study or led to dose reduction, interruption, or permanent discontinuation of the study drug.

Adverse events do not include:

- Medical or surgical procedure not mandated in the protocol (e.g., surgery, endoscopy, tooth extraction). However, the event leading to the procedure is an AE. If this event is serious, the procedure must be described in the SAE narrative on the SAE eCRF.
- Pre-existing disease or medical condition that does not worsen. However, if the condition deteriorates at any time during the study, it should be recorded as an AE.

- Situations in which an adverse change did not occur (e.g., hospitalizations for cosmetic elective surgery or for social and/or convenience reasons).
- Overdose of either study drug without any signs or symptoms. However, overdose of study drug must be reported in the Study Drug Log.

The occurrence of an AE may come to the attention of study personnel during the study visits, during interviews of a study recipient presenting for medical care, or upon review by a study monitor. Information to be collected and recorded include: 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, which would include MD, PA, Nurse Practitioner, DO, or Physician Assistant), and time of resolution/stabilization of the event.

All AEs occurring during the study including local and systemic reactions must be documented appropriately regardless of relationship and recorded on the relevant eCRF. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

10.2.1 Intensity of Adverse Events

The intensity of all AEs will be graded for severity. The investigating clinician will assess all AEs by grading the AE on a three-point scale of 1) mild, 2) moderate, and 3) severe; which are defined as follows:

- **Mild events**: require minimal or no treatment and do not interfere with the patient's daily activities.
- **Moderate events**: result in a low level of inconvenience or concern with the investigational drug. Moderate events may cause some interference with daily functioning.
- **Severe events**: interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

If the intensity of an AE worsens during study drug administration, only the worst intensity should be reported on the relevant AE eCRF. If the AE lessens in intensity, no change in the severity is required. AEs characterized as intermittent require documentation of onset and duration of each episode.

The AE intensity definitions do not apply to clinically significant and asymptomatic laboratory test abnormalities or abnormal assessments (e.g., ECG findings) considered as AEs. The Investigator should tick “non-applicable” on the AE page of the eCRF when identifying the intensity of the AE.

Of note, mild, moderate, or severe AE classifications may or may not be serious (see SAE section). These terms are used to describe the intensity of a specific adverse event (as in mild, moderate, or severe nausea). For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Alternatively, a fever of 39°C that is not considered severe, may however become serious, if it prolongs hospitalization. Seriousness rather than severity serves as a guide for defining regulatory reporting requirements.

10.2.2 Relationship and Attribution of the AE to Study Drug

10.2.2.1 Relationship to Study Drug

Each adverse event must be assessed by the Investigator as to whether or not there is a reasonable possibility of causal relationship to the study drug, and reported as either related or unrelated. The Investigators assessment of an AE's relationship to the study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study.

- Related to Study Drug: This category applies to any AE (whether serious or not) that appears to have a reasonable possibility of causal relationship to the use of the study drug (i.e., a relationship cannot be ruled out). Guidelines to determine whether an event might be considered related include but are not limited to the following:
    - The event occurred in close temporal relationship to study drug administration.
    - The event diminished or disappeared when treatment with the study drug was down titrated, interrupted, or discontinued.
    - The event reoccurred when treatment was reintroduced.
    - Environmental factors such as clinical state and other treatments could not equally have caused the event.
- Unrelated to Study: There is not a reasonable possibility that the administration of the study product caused the event (see above guidelines).

10.2.2.2 Attribution to The Study Drug

After identifying and grading the event, the clinical Investigator must assign an attribution to the AE using the following attribution categories described in the table below. AEs listed as 'possibly, probably' or definitely' related to the investigational drug are considered to have a suspected 'reasonable causal relationship' to the investigational agent (ICH E2A).

Table 19. AE Attribution to the Study Drug

Relationship to Study Drug	Attribution	Description
Unrelated	Unrelated	The AE is clearly NOT related to the study drug
	Unlikely	The AE is doubtfully related to the study drug
Related	Possible	The AE may be related to the study drug
	Probable	The AE is likely related to the study drug
	Definite	The AE is clearly related to the study drug

10.2.3 Serious Adverse Events

The International Conference on Harmonization (ICH) guidelines define a serious adverse event (SAE) as an AE or suspected adverse reaction that in the view of either the Investigator or Sponsor results in any of the following study patient outcomes:

- Death,
- A life-threatening AE,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- A congenital anomaly/birth defect, or
- An important medical event, requiring medical or surgical intervention (treatment) to prevent at least one of the outcomes listed above.

Life threatening refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death had it been more severe.

Important medical events that do not result in death, are not life threatening, or do not require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the SAE definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, a blood dyscrasia or convulsion that does not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Complications that occur during hospitalization may be AEs or SAEs depending upon Investigator judgment (i.e., if a complication prolongs hospitalization it would be considered an SAE). However, the following reasons for hospitalizations are not considered AEs, and are therefore also not SAEs:

- Hospitalizations for cosmetic elective surgery, or social and/or convenience reasons.
- Standard monitoring of a pre-existing disease or medical condition that did not worsen (e.g., hospitalization for coronary angiography in a patient with stable angina pectoris).
- Elective treatment of a pre-existing disease or medical condition that did not worsen (e.g., elective hip replacement for arthritis).

10.2.4 Serious Adverse Events Related To Study-Mandated Procedures

A SAE is defined as related to study-mandated procedures if it appears to have a reasonable possibility of a causal relationship (i.e., a relationship cannot be ruled out) to procedures other than administration of the study drug. Examples of study-mandated procedures include blood sampling, car accident on the way to the study unit for a study visit, etc.

10.3 Reporting Requirements for AEs and SAEs

10.3.1 Reporting of Adverse Events

All AEs collected from the time of treatment initiation up to the last IP dose, or leading to the premature discontinuation of IP must be recorded on the appropriate AE pages of the eCRF. AEs documented between signing of the Informed Consent and treatment initiation, and from last dose to 28 days after study drug discontinuation will be recorded on the appropriate AE source page but will not be entered as an eCRF.

Information to be collected includes event description, date of onset, Investigator assessment of severity, Investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome.

10.3.2 Reporting Abnormal Laboratory Test Values or Abnormal Clinical Findings

Collection of specific safety laboratory data is outlined in the study visit schedule. Toxicity tables are based on the FDA toxicity tables developed for normal healthy adult volunteers. Laboratory values and abnormal clinical findings that are abnormal based on the toxicity tables must be reported on the appropriate AE eCRF.

10.3.3 Reporting of AE Intensity and Causality

Only licensed study clinicians (i.e., Medical Doctor, Doctor of Osteopathy, Nurse Practitioner, Physician's Assistant) should assess the intensity of non-serious AEs. Similarly, only licensed study clinicians as identified on Form FDA 1572 or per ICH E6 standards should assess the causality of AEs.

10.3.4 Follow-up of AEs

All AEs occurring during the AE reporting period of the study will be documented appropriately regardless of relationship. All AEs occurring during the treatment period will be followed to adequate resolution or until the patient is considered stable.

Adverse events still ongoing after study drug discontinuation for a given patient will be followed until 28 days after study drug discontinuation or until AE resolution or patient stabilization.

Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition to the Investigator and/or Sponsor's satisfaction with the expectation that the condition or abnormality may remain chronic. Follow-up procedures, evaluations, and outcomes will be recorded on the patient's CRF.

10.3.5 Reporting Requirements For SAEs

All SAEs occurring during the course of the study (e.g., signing of informed consent by patient to 28 days following the last dose of the study drug) and any SAEs, considered causally related to study drug that occur following completion of the study, must be reported to the Sponsor.

10.3.5.1 Reporting of SAEs

All serious adverse events that occur during the course of study will be recorded on the appropriate eCRF (e.g., SAE eCRF) and will be reported to Sponsor's drug safety physician.

Any AE that meets a protocol-defined criterion as serious must be submitted immediately (within 24 hours of site or Investigator awareness) on an SAE form to the Sponsor's drug safety physician at the following address:

Robert Glanzman, MD, FAAN
Sponsor's Medical Monitor
Clene Nanomedicine, Inc.
3165 East Millrock Drive, Suite 325
Salt Lake City, Utah 84121
United States
Tel: +1 (801) 676-9695
Email: safety@clene.com

Information to be collected includes unique patient ID, event description, date of onset, Investigator assessment of severity, Investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome.

Other supporting documentation of the event may be requested by the Sponsor and should be provided as soon as possible. The Sponsor's drug safety physician will review and assess the SAE for regulatory reporting and potential impact on study patient safety and protocol conduct.

10.3.5.2 SAE Reporting Periods

- Screening period: SAEs occurring between signing the Informed Consent Form and study drug initiation should be reported in the eCRF.

- Treatment Period: All SAEs, regardless of causal relationship, must be reported, including those related to study-mandated procedures. Those SAEs occurring during study drug administration, i.e., between study drug initiation and 28 days after study drug discontinuation, are defined as treatment-emergent SAEs. These SAEs are reported on SAE forms and also on AE pages in the eCRF. They are therefore entered into both the drug safety and clinical databases, and must be reconciled before study closure.

- Follow-Up Period: All SAEs, regardless of causal relationship, occurring after study drug discontinuation until 28 days after study drug discontinuation must be reported.

- Post Follow-Up Period: If the Investigator becomes aware of a new SAE that is suspected of being causally related to the study drug occurring after 28 days of follow-up or post-study completion, the Investigator will report the event within 24 hours to the Sponsor's drug safety physician. These SAEs are only entered in the drug safety database, and therefore will not affect study closure.

10.3.5.3 SAE Reporting Procedures

- All SAEs must be reported by the Investigator to drug safety physician within 24 hours of the Investigator's first knowledge of the event.

- All SAEs must be recorded on the appropriate SAE forms, irrespective of the study drug received by the patient, and whether this event is considered by the Investigator to be related to study drug. These SAE forms must be faxed and emailed to the Clene Nanomedicine, Inc. drug safety physician.

- The Investigator must complete the SAE form in English (unless otherwise specified), and must assess the relationship of the event to study drug. Such reports will be followed

by detailed descriptions that may include copies of hospital case reports, autopsy reports, hospital discharge summaries and other documents when requested and applicable.

- Follow-up information about a previously reported SAE must also be reported within 24 hours of receiving it. The drug safety physician may contact the Investigator to obtain further information.

- The intensity of SAEs may only be assessed by a licensed study physician as described previously. The causality of SAEs may only be assessed by a licensed physician identified on the Form FDA 1572 or per ICH E6 standards.

10.3.5.4 SAE Regulatory Reporting

Following notification from the Investigator, the Sponsor will expedite reporting any suspected adverse reaction that is both serious and unexpected to Health Authorities, HREC, IRBs, and other Investigators, as appropriate.

The Sponsor will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. Sponsor will notify FDA and all participating Investigators (i.e., all Investigators to whom the Sponsor is providing drug under its INDs or under any Investigator's IND) in an IND safety report of potential serious risks from clinical trials 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 as specified in 21 CFR Part 312.32. Further, Sponsor will notify 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. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, Sponsor will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

Un-blinding of serious and unexpected AEs will be performed as necessary and appropriate.

All serious adverse events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

General study reporting periods and safety reporting requirements are summarized in the table below.

Table 20. Summary of Study Safety Reporting Periods

Study Periods:	Screening	Treatment	Follow-Up	Post Follow-up
Timeframe	From signature of informed consent to study drug initiation	During study drug administration	From immediately after study drug discontinuation until 28 days after study drug discontinuation	After 28 days
AE Reporting on AE eCRF	None	All AEs	None[1]	None
SAE Reporting on AE eCRF	Only if related to study- mandated procedures	All SAEs (treatment-emergent)	All SAEs	If considered causally related to the study medication
Reconciliation[2]	Not applicable	Yes	Not applicable	Not applicable
Clinical Study Report/Final Study Report	AEs and SAEs may be described	Analyzed	Reported	AEs and SAEs may be described

[1]Adverse events still ongoing after study drug discontinuation for a given patient must be followed until 28 days after study drug discontinuation or until resolution or stabilization or until the event is otherwise explained.
[2]Reconciliation between clinical and drug safety databases.

10.4 Pregnancy

10.4.1 Prevention of Pregnancy During the Study

Women should not become pregnant during the study and up to 6 months following study drug discontinuation. Accordingly, women of childbearing potential should take appropriate precautions to prevent pregnancy during the study. If a woman becomes pregnant while on study drug, no further treatment will be administered. In the event of a pregnancy, all study-mandated blood samples will be obtained and the patient will continue in follow-up for safety events.

10.4.2 Reporting of Pregnancy

Irrespective of the treatment received by the patient, any pregnancy which occurs in a study patient or the partner of a male study patient, which occurs during study drug administration or 6

months following study drug discontinuation must be reported within 24 hours of the Investigator's knowledge of the event.

Pregnancies must be reported to the Sponsor's drug safety physician and on the AE page of the eCRF, as applicable.

10.4.3 Pregnancy Follow-Up

Pregnancies will be followed to pregnancy outcome pending the patient's permission and reported to the Sponsor's drug safety physician. Follow-up information will only be entered in the drug safety database, and hence will not affect study closure.

10.5 Clinical Monitoring

10.5.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that human patient protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet Sponsor, International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) guidelines and applicable regulatory requirements, and that the study is conducted in accordance with the protocol and Sponsor's SOPs. The Sponsor or its designee will conduct site-monitoring visits as specified in the monitoring plan.

Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, patient source records (i.e., electronic medical records), informed consent forms (ICFs), medical and laboratory reports, and protocol compliance. Study monitors will meet with Investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICS AND DATA MANAGEMENT

Separate PK and statistical analysis plans (SAP) will provide details of planned analyses and summary documents, such as tables, listings and figures. All variables will be analyzed descriptively with appropriate statistical methods: categorical variables by frequency tables and continuous variables by sample statistics (i.e., mean, SD, minimum, median, quartiles and maximum). An overview of the planned analyses is provided here. However, final analyses are not limited to the summaries described herein but will be defined in the SAP prior to final database lock.

11.1 Determination of Sample Size

As an open label pilot study, no formal sample size calculations have been conducted.

11.2 Analysis Populations

The following patient sets are applicable to this study:

- **Intent to Treat Analysis Set** – The Intent To Treat Set (ITT) will consist of Screened patients for whom a treatment assignment number has been assigned at Visit 0.
- **Safety Analysis Set** – The Safety Analysis Set (SAS) will consist of patients in the Treatment Assignment Set who receive at least 1 dose of investigational product.
- **Partial Analysis Set** – The Partial Analysis Set (PAS) will consist of all patients in the Safety Set with at least 1 post-baseline measurement of MDS-UPDRS.
- **Per Protocol Set –** The Per Protocol Set (PPS) will consist of all patients in the Safety Set who have completed at least 12-weeks of treatment, the Week 12 ^{31}P-MRS scan, and who are treatment adherent per Section 8.7.
- **Treatment Cohort –** A Treatment Cohort will be defined as all patients who receive the same dose of CNM-Au8.

11.3 Baseline Characteristics and Patient Disposition

Overall Baseline and demographic data will be summarized using descriptive statistics. Patient disposition (e.g., the number of patients enrolled, completed, and discontinued) will be summarized and medical history and physical examination findings will be listed.

11.4 Prior and Concomitant Medications

Medications taken after the patient receives study drug through Follow-Up will be listed by treatment, dose level and patient.

11.5 Pharmacokinetic Analyses

Pharmacokinetic parameters will be derived using noncompartmental analyses employing appropriate software and graphing tools (e.g. WinNonlin® Professional version 6.3 [Pharsight Corp], SAS® for Windows® Version 9.4, SigmaPlot for Windows Version 12.5; or higher versions).

Au concentrations will be summarized using descriptive statistics (including N, mean, SD, coefficient of variation (CV%), median, minimum, and maximum) for each treatment. Concentrations below the limit of quantification (BLQ) will be treated as zero for the computation of descriptive statistics and for construction of mean concentration-time profiles. Concentrations assigned a value of missing will be omitted from the calculation of descriptive statistics.

The following PK parameters will be estimated by noncompartmental methods from whole blood samples. Actual elapsed time from dosing will be used to estimate all individual PK parameters.

Table 21. Summary of PK Parameters

Parameter	Description
C_{max}	Maximum observed plasma concentration
T_{max}	Time of maximum concentration (h), obtained directly from the observed concentration versus time data.
CL/F	Apparent systemic clearance
$AUC_{(0-24)}$	Area under the plasma concentration-time curve from time 0 to the end of the 24-hour dosing interval

Derived PK descriptive statistics will be tabulated by dosing group and summary statistics. Descriptive statistics for PK parameters will include the arithmetic and geometric mean (for C_{max}, T_{max}, $AUC_{(0-24)}$, and CL/F), CV%, SD of the arithmetic mean, median, minimum, maximum, and N.

Population PK and PK/PD models may be developed to address objectives that require an integrative interpretation of the study results. These include assessment of the dose proportionality, investigation of the nature of the PK/PD relationship, and the use of study results as part of a larger model-based data analysis. If population PK/PD models are developed, a separate Pharmacometric Analysis Plan will be written.

11.6 Safety Analyses

All safety summaries will be descriptive; no statistical significance tests will be performed on safety data and will be based on the safety population.

Safety assessments include extent of exposure, incidence of treatment-emergent AEs, drug-related AEs, deaths, SAEs, AEs leading to discontinuation from the study and changes in the C-SSRS. Changes from baseline in clinical laboratory results, ECGs and vital signs will also be summarized by treatment group and time point.

At each time point, absolute values and change from baseline of each safety endpoint will be summarized with n, mean, SD, standard error of the mean (SEM), median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. Values outside the reference range will be flagged in the listing. 'H' and 'L', denoting values above or below the Investigator reference range (when present), will flag out-of-range results.

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT).

11.7 Efficacy Analyses

11.7.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the mean change in NAD Redox Ratio measured by ^{31}P-MRS from Baseline to Week 12.

Redox Ratio is defined as:

- NAD Redox Ratio = mean NAD+/NADH

11.7.2 Exploratory Efficacy Endpoints

Exploratory ^{31}P-MRS imaging endpoints are as follows:

- Regression of baseline values versus mean percentage change of the average tissue concentration by subject per dosing group from Baseline to Week 12 for:
    - ATP (α, β, γ)
    - NAD+/NADH pool
    - Phosphocreatine (PCr)
    - Intracellular inorganic phosphate (Pi(in))
    - Extracellular inorganic phosphate (Pi(ex))
    - Phosphoethanolamine (PE)
    - Phosphocholine (PC)
    - Glycerolphosphoethanolamine (GPE)
    - Glycerophosphocholine (GPC)

11.7.3 Functional Exploratory Endpoints

Exploratory analyses of Gait, Postural Stability, and Mobility are:

- Mean change by treatment group from Baseline to Week 12 for:
    - APDM Instrumented Timed Up and Go (TUG) Test
    - APDM Instrumented Postural Sway Test
    - APDM Instrumented Walk Test

Exploratory Measurements of Global Impression of Disease Severity and Improvement are:

- Mean change in average difference between Baseline to Week 12 for:
    - Clinical Global Impression Scale (CGI)
    - Patient Global Impression Scale (PGI)

Exploratory Efficacy Measurements of Disease Progression

- Mean change in total score and sub-scales between Baseline to Week 12 for:
    - Unified Parkinson's Disease Rating Scale (MDS-UPDRS)

For primary and exploratory endpoints, the statistical significance comparing the result of each CNM-Au8 treatment versus baseline, will be reported. Statistical significance will be determined by two-sided paired t-tests assessed at the $p \leq 0.05$ significance level. Instrumented APDM tests will be performed at the Baseline and Week 12 study visits to determine the change from Baseline. All analyses will be specified in the Statistical Analyses Plan.

The Cohort 2 sample size may be re-calculated based on the mean change from baseline in NAD+/NADH Redox Ratio to Week 12 and standard deviation of the change from Cohort 1.

The proportion of patients with improvement of additional secondary bioenergetic metabolites will be compared between treatment groups using exact test procedures.

Primary, and exploratory efficacy analyses will be based on the intent to treat (ITT) population. Changes in primary and secondary efficacy parameters (e.g., ^{31}P-MRS Redox Ratio [NAD+/NADH ratio], bioenergetic metabolites) will also be compared to historical age-matched healthy controls.

11.8 Interim analyses

Cohort 1 data will be analyzed prior to initiation of Cohort 2. No other pre-specified interim analyses are planned per protocol.

11.9 Data Handling and Quality Assurance

11.9.1 Data Recording

Limited data may be entered solely into the eCRF (e.g., ethnic group). All other data must have source documentation available. A source document checklist will be used at the site to identify the source data for all data points collected, and the study monitor will work with the site to complete the source documentation verification.

11.9.2 Data Recorded From Screening Failures

For patients who do not meet selection criteria (Screen Failures), source data will be recorded and entered into the eCRF, including the reason for the screen failure, and demographic information (e.g., patient number, date of birth/age, sex, height, weight, race and ethnicity), and the date of the study visit.

For patients classified as Screen Failures with an SAE during the Screening visit (Visit -1), the following additional data should be collected in the eCRF:

- All information about the SAE
- Other related including:
    - Concomitant medication
    - Medical history
    - Any other information needed to complete the SAE eCRF page

11.9.3 Data Monitoring

In accordance with applicable regulations, Good Clinical Practice (GCP), and the Sponsor's and/or the Clinical Research Organization (CRO) procedures, study monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and Sponsor's requirements. When reviewing data collection procedures, the discussion will also include identification and documentation of source data items.

The Sponsor or its designee will visit clinical sites periodically to monitor study activity to verify that the:

- Data are authentic, accurate, and complete
- Safety and rights of patients are being protected
- Study is conducted in accordance with the currently approved protocol (including study treatment being used in accordance with the protocol)
- All other study agreements, GCP, and all applicable regulatory requirements are being met.

The site Investigator and the head of the medical institution, as applicable, agree to allow the Sponsor and/or designated study monitors direct access to all relevant documents at the clinical site.

11.9.4 Data Collection and Management

Data collection will be conducted via a validated electronic data collection (EDC) system, which will be managed by the Sponsor's biostatistics and data management CRO.

Patient data necessary for analysis and reporting will be entered and/or transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable Sponsor and CRO standards for data cleaning procedures. This is applicable for data recorded on the eCRF as well as for data from other sources (e.g., laboratory, ECG). For data coding (e.g., AEs, medication), internationally recognized and accepted dictionaries will be used. Data will be electronically verified through use of programmed edit checks specified by the clinical team. Discrepancies in the data will be brought to the attention of the clinical team, and investigational site personnel, as applicable. Resolutions to these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

11.9.5 Data Audit and Inspection

To ensure compliance with GCP and regulatory requirements, a member of the Sponsor's quality assurance unit, or designated representative, may conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The site Investigator and institution will be informed of the audit outcome. In addition, inspections by regulatory health authority representatives and/or IRB(s) are possible. The Investigator should notify the Sponsor immediately of any such inspection. The Investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and to provide sufficient time and staff support to review any findings and any discuss any issues. Audits and inspections may occur at any time during or after completion of the study.

11.9.6 Data Archiving

Essential site documents shall be archived safely and securely in such a way that ensures that they are readily available upon regulatory authorities' request. Patient files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice associated with the study. Where the archiving procedures do not meet the minimum timelines required by the Sponsor, alternative

arrangements must be made to ensure the availability of the source documents for the required period. The Investigator/institution shall notify the Sponsor if the archival arrangements change (e.g., relocation or transfer of ownership).

The Investigator site file is not to be destroyed without the Sponsor's written approval.

12 RESPONSIBILITIES, ETHICS, AND LEGAL ASPECTS

12.1 Investigator Responsibilities

12.1.1 Good Clinical Practice

The Investigator will ensure that this study is conducted in full compliance with the principles of the "Declaration of Helsinki" (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), ICH guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study Patient. For studies conducted under a United States IND, the Investigator will ensure that the basic principles of "Good Clinical Practice," as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, Part 50, 1998, and 21 CFR, Part 56, 1998, are adhered to. This study will be conducted in compliance with 21 CFR, Part 320, 1993, "Retention of Bioavailability and Bioequivalence Testing Samples."

12.1.2 Institutional Review Board (IRB)Approval

The Investigator will submit this protocol and any related documents to an IRB, and the Competent Authority (CA) as applicable. Approval from the IRB and the statement of no objection from the CA must be obtained before starting the study, and should be documented in a dated letter/email to the Investigator, clearly identifying the study, the documents reviewed and the date of approval. A list of IRB members must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted. Modifications made to the protocol after receipt of the IRB approval must also be submitted as amendments by the Investigator to the IRB in accordance with local procedures and regulations.

12.1.3 Informed Consent

It is the responsibility of the Investigator or designee to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures. The Investigator or designee must utilize an IRB-approved informed consent form (ICF) for documenting written informed consent. Each informed consent will be appropriately signed and dated by the patient and the person obtaining consent. A patient may enter the study

only if the patient or legal representative voluntarily agrees to sign the informed consent form. A copy of the signed consent form will be provided to the patient.

If informed consent is obtained on the date that Screening study procedures (Visit -1) are performed, the study record or patient's clinical record must clearly show that informed consent was obtained prior to these procedures.

If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (e.g., independent of the Sponsor and the Investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.

The ICF and any other written information provided to patients or the patient's legal representative will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol that necessitates a change to the content of the patient information and/or the written ICF. The Investigator will inform the patient or legal representative in a timely manner and will ask patients to confirm their participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IRB's approval prior to use.

12.1.4 Adherence to Protocol

The Investigator may not modify or alter the procedures described in this protocol and is required to strictly adherence to all specifications laid down in this protocol for all aspects of study conduct.

Neither the Sponsor nor the Investigator will implement protocol amendments or modifications to the study protocol without agreement by both parties. However, the Investigator or the Sponsor may implement a deviation from, or a change to the protocol to eliminate an immediate hazard to patients without prior IRB/Sponsor approval. In this case, the implemented deviation or change, the reasons for it, and if appropriate, the proposed protocol amendment should be submitted to the IRB/head of medical institution and Sponsor as soon as possible.

Any deviations from the protocol must be explained and documented by the Investigator.

12.1.5 Confidentiality

The Investigator must assure that patients' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only patient initials and an identification code (i.e., not names) should be recorded on any form submitted to the Sponsor and IRB. The Investigator must keep a patient log showing codes, names, and addresses for all patients screened and for all patients enrolled in the study.

12.1.6 Study Files and Retention of Records

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 separate categories (although not limited to) the following: (1) Investigator's study file, and (2) Patient clinical source documents.

The Investigator's study file will contain the protocol/amendments, CRF and query forms, IRB approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

Patient clinical source documents would include (although is not limited to) the following: patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, electroencephalogram (EEG), X-ray, MRI, pathology and special assessment reports, consultant letters, screening and enrollment log, etc.

All clinical study documents must be retained by the Investigator until at least two years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region (i.e., United States, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or, until two years after the IND is discontinued and regulatory authorities have been notified. The Investigator must notify Sponsor prior to destroying any clinical study records.

Should the Investigator wish to move study records to another location, arrangements must be made to store these in sealed containers so that they can be returned sealed to the Investigator in

case of a regulatory audit. Where source documents are required for the continued care of the Patient, appropriate copies should be made for storage outside of the site.

12.1.7 Case Report Forms

For each Patient who receives study drug, a CRF must be completed and signed (electronically signed if eCRF) by the Principal Investigator or sub-Investigator within a reasonable period after data collection. This also applies to records for those patients who fail to complete the study. If a patient withdraws from the study, the reason must be noted on the eCRF. If a patient is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

12.1.8 Drug Accountability

The Investigator or designee (i.e., pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgment of receipt of each shipment of study product (quantity and condition) and Patient dispensing records and returned or destroyed study product. Dispensing records will document quantities received and quantities dispensed to Patients, including lot/batch number, date dispensed, Patient identifier number, Patient initials, and the initials of the person dispensing or witnessing dispensing of the study medication.

At study initiation, the monitor will evaluate the site's SOP for study drug disposal/destruction in order to ensure that it complies with study requirements. Following blinded study drug reconciliation by the monitor, the study site will be instructed by the Sponsor to return any unused study drug supplies returned by patients including empty containers to the investigational product central supply depot for storage or destruction.

12.1.9 Inspections

The Investigator will provide access to source documents and all study records for this study to appropriately qualified personnel from the Sponsor or its representatives, and to regulatory authority inspectors.

12.2 Sponsor Responsibilities

12.2.1 Study Materials and Instructions

It is the Sponsor's responsibility to ensure that the Investigator is provided with the documents and other study materials necessary to conduct the study. Examples of those materials include, but are not limited to: protocol, Investigator's Brochure, study drug, eCRF, SAE collection forms, logs, etc. The Sponsor will also provide training and oversight through site and medical monitoring.

12.2.2 Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study patients, will be made by Sponsor-initiated amendment. IRB approval must be obtained before changes can be implemented except for non-substantial amendments (e.g., changes in study staff or contact details or minor changes in the packaging or labeling of the investigational product) as described below.

Administrative or logistical minor changes will require a non-substantial amendment. Non-substantial amendments will be approved (signed) by the Investigator(s) and will be recorded and filed by the Investigator/Sponsor and the Institutional Review Board (IRB) and the Competent Authority (CA) may or may not be notified per local regulatory guidelines. The implementation of a non-substantial amendment can be done without notification or approval to the appropriate IRB or CA.

The following amendments will be regarded as non-substantial:

- Change in timing of the samples;
- Changes in assay-type and/or institution where an assay will be performed, provided that validated assays will be used;
- Editorial changes to the patient information sheets;
- Determination of additional parameters in already collected materials, which agree with the study objectives and do not provide prognostic or genetic information;
- Other statistical analyses than described in the protocol.

12.2.3 Insurance

The Sponsor maintains clinical trial insurance coverage for this study in accordance with the laws and regulations of the country in which the study is performed.

12.2.4 Premature Study Termination

The Sponsor has the right to prematurely close this study or, if applicable, individual segments of the study, including but not limited to: treatment arms, study sites, titration steps, and study procedures; if at any time the risk-benefit profile of the investigational product, center conduct, or specific study treatment segment becomes unacceptable due to:

- Safety findings from this study (e.g., SAEs)
- Procedural issues or protocol violations from a study site that potentially affects patient safety
- Safety findings from other parallel studies in different patient populations
- Results from animal toxicology studies (e.g., toxicity, teratogenicity, carcinogenicity, or reproduction toxicity).

12.3 Joint Investigator/Sponsor Responsibilities

12.3.1 Access to Information for Monitoring

In accordance with International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines, the study monitor must have direct access to the Investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs at regular intervals throughout the study, to verify adherence to the protocol, and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to any patient records needed to verify the entries on the CRFs. The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

12.3.2 Study Discontinuation

Both the Sponsor and the Investigator reserve the right to terminate the study at any time for safety or medical reasons. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authorities, IRBs and HRECs. In terminating the study, the Sponsor and the Investigator will assure that adequate consideration is given to the protection of the Patients' interests.

12.3.3 Study Report and Publications

After conclusion of the study, Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met:

- The results of the study in their entirety have been publicly disclosed by or with the consent of Sponsor in an abstract, manuscript, or presentation form; or
- The study has been completed for at least 2 years.

No such communication, presentation, or publication will include Sponsor's confidential information.

Any information, inventions, or discoveries (whether patentable or not), innovations, suggestions, ideas, and reports, made or developed by the Investigator(s) as a result of conducting this study shall be promptly disclosed to the Sponsor and shall be the sole property of the Sponsor. The Investigator agrees, upon the Sponsor's request and at the Sponsor's expense, to execute such documents and to take such other actions, as the Sponsor deems necessary or appropriate to obtain patents in the Sponsor's name covering any of the foregoing.

The Investigator will submit any proposed publication or presentation along with the respective scientific journal or presentation forum to the Sponsor at least 30 days prior to submission of the publication or presentation. The Investigator will comply with Sponsor's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days in order to obtain patent protection if deemed necessary.

13 PROTOCOL AMENDMENTS

Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB.

Amendment to version 1.0 of the Protocol dated 12-February-2019 occurred on 06-March-2019 (version 2.0). Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB/HREC. These changes were implemented based off FDA comments and feedback issued upon their review of the clinical protocol.

Amendment to version 2.0 of the protocol dated 06-March-2019 occurred on 29-March-2019 (version 2.1). Summary of changes were noted alongside changes associated with version 2.0. These changes were implemented to clarify that randomization will not occur, rather, a treatment assignment will be assigned to each subject who qualifies for participation.

Amendment to version 2.1 of the protocol dated 29-March-2019 occurred on 21-May-2019 resulting in version 3.0 of the protocol. Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB. Minor modifications were made to the imaging plan, food restriction language added surrounding drug administration, and updates to exploratory endpoints. A window was incorporated for specific Baseline Visit assessments.

Amendment to version 3.0 of the protocol dated 21-May-2019 occurred on 08-July-2019 resulting in version 4.0 of the protocol. Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB. Additional assessments and exploratory outcomes were added surrounding patient mobility. Medical Monitor contact information was updated and minor changes were included throughout.

Amendment to version 4.0 of the protocol dated 08-Jul-2019 occurred on 07-Aug-2019 resulting in version 5.0 of the protocol. Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB. Changes under this amendment

include anxiolytic administration for all subjects undergoing ^{31}P-MRS assessments, modifications to exploratory and safety endpoints, and clarifying language surrounding AE documentation.

Amendment to version 5.0 of the protocol dated 09-Aug-2019 occurred on 09-September-2020 resulting in version 6.0 of the protocol. Summary of changes will be noted in a supplemental document submitted with the amended protocol to the appropriate IRB. Changes under this amendment include modifications to the collection volume of PD samples, reporting of safety analytes and allowance for participants to remain on study drug beyond Week 12 in the event of site logistical issues or safety concerns surrounding the COVID-19 pandemic and allowing for the inclusion of up to 15 participants per cohort to be enrolled.

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