HEALEY ALS Platform Trial - Regimen C CNM-Au8

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REGIMEN-SPECIFIC APPENDIX C

FOR CNM-Au8

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TABLE OF CONTENTS

TABLE OF CONTENTS ---- 2

SIGNATURE PAGE ---- **5**

LIST OF ABBREVIATIONS ---- **6**

REGIMEN-SPECIFIC APPENDIX SUMMARY ---- **8**

SCHEDULE OF ACTIVITIES – ---- **10**

1. INTRODUCTION ---- **15**

1.1 CNM-Au8 Background Information ---- *15*

1.2 CNM-Au8 Therapeutic Rationale ---- *17*

1.2.1 Investigational Product Characteristics ---- 17

1.2.2 Preclinical Data Supporting CNM-Au8 in the Treatment of ALS ---- 18

1.2.2.1 In vitro ALS Neuroprotection Models ---- 23

1.2.2.1.1 CNM-Au8 Protects Primary Rodent Motor Neurons from Excitotoxicity ---- 23

1.2.2.1.2 CNM-Au8 Protects Normal iPSC Derived Human Motor Neurons From ALS-Participant-Derived Toxic Astrocytes ---- 25

1.2.2.2 In vivo ALS Neuroprotection Models ---- 26

1.2.3 Summary CNM-Au8 Treatment Rationale for ALS ---- 28

2. OBJECTIVES ---- **29**

2.1 Study Objectives and Endpoints ---- *29*

3. RSA DESIGN ---- **31**

3.1 Scientific Rationale for RSA Design ---- *31*

3.2 End of Participation Definition ---- *31*

3.3 End of Regimen Definition ---- *31*

4. RSA Enrollment ---- **33**

4.1 Number of Study Participants ---- *33*

4.2 Inclusion and Exclusion Criteria ---- *33*

4.2.1 RSA Inclusion Criteria ---- 33

4.2.2 RSA Exclusion Criteria ---- 33

4.3 Treatment Assignment Procedures ---- *33*

5. INVESTIGATIONAL PRODUCT ---- **34**

5.1 Investigational Product Manufacturer ---- *34*

5.2 Labeling, Packaging, and Resupply ---- *34*

5.3 Acquisition, Storage, and Preparation ---- *35*

5.4 Study Medication/Intervention, Administration, Escalation, and Duration ---- *35*
5.5 Dosage Adjustment ---- *36*
5.6 Justification for Dosage ---- *37*
5.6.1. Human Dosing Safety Margins ---- 37
5.6.2 Neuroprotection Pharmacologic Exposure Data ---- 39
5.7 Participant Compliance ---- *40*
5.8 Overdose ---- *40*
5.9 CNM-Au8 Known Potential Risks and Benefits ---- *40*
5.9.1 Known Potential Risks ---- 40
5.9.1.1 Immediate Risks ---- 40
5.9.1.2 Long Range Risks ---- 41
5.9.1.3 Summary of Participant Risk ---- 41
5.9.2 Known Potential Benefits ---- 41
5.10 Regimen-Specific Lab Alerts ---- *42*
6. REGIMEN SCHEDULE ---- 43
6.1 Regimen-Specific Screening Visit ---- *44*
6.2 Baseline Visit ---- *44*
6.3 Week 2 Telephone Visit ---- *45*
6.4 Week 4 and 8 Visits ---- *45*
6.5 Week 12 Telephone Visit ---- *45*
6.6 Week 16 Visit ---- *45*
6.7 Week 20 Telephone Visit ---- *46*
6.8 Week 24 Visit or Early Termination Visit ---- *46*
6.9 Follow-Up Safety Call ---- *46*
6.10 Process for Early Terminations ---- *47*
6.11 Open Label Extension ---- *47*
6.11.1 Week 2 OLE Telephone Visit ---- 48
6.11.2 Week 4 OLE Visit ---- 48
6.11.3 Week 8 OLE Visit ---- 49
6.11.4 Week 12 OLE Telephone Visit ---- 49
6.11.5 Week 16 OLE Visit ---- 49
6.11.6 Week 20 OLE Telephone Visit ---- 50
6.11.7 Week 24 OLE Telephone Visit ---- 50
6.11.8 Week 28 OLE Visit ---- 51
6.11.9 Week 40 OLE Visit ---- **Error! Bookmark not defined.**
6.11.10 Week 52 OLE Visit ---- **Error! Bookmark not defined.**
7 OUTCOME MEASURES AND ASSESSMENTS ---- 52
7.1 Voice Analysis ---- *52*

7.2 ALSAQ-40 ---- *52*

7.3 Center for Neurologic Study Bulbar Function Scale ---- *52*

8 BIOFLUID COLLECTION ---- 54

8.1 Pharmacokinetic (PK) Assessments ---- *54*

8.1.1 Whole Blood PK Assessments of CNM-Au8 ---- 54

8.1.2 Plasma PK Assessments of Riluzole ---- 54

8.2 Pharmacodynamic (PD) Assessments ---- *54*

9 REGIMEN-SPECIFIC STATISTICAL CONSIDERATIONS ---- 56

Appendix I: The ALSAQ-40 ---- 57

Appendix II: The Bulbar Function Scale (CNS-BFS) ---- 66

REFERENCES ---- 68

SIGNATURE PAGE

I have read the attached Regimen-Specific Appendix (RSA) entitled, "REGIMEN C: CNM-Au8" dated October 15, 2021 (Version 5.0) and agree to abide by all described RSA procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable FDA regulations and guidelines identified in 21 CFR Parts 11, 50, 54, and 312, central Institutional Review Board (IRB) guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

By signing the RSA, I agree to keep all information provided in strict confidence and to request the same from my staff. Study documents will be stored appropriately to ensure their confidentiality. I will not disclose such information to others without authorization, except to the extent necessary to conduct the study.

Site Name: _____

Site Investigator: _____

Signed: _____ Date: _____

LIST OF ABBREVIATIONS

Abbreviation	Definition
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire-40
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
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
CNS-BFS	Center for Neurologic Study Bulbar Function Scale
CSF	Cerebrospinal fluid or cerebral spinal fluid
fALS	Familial ALS
FVC	Forced Vital Capacity
HED	Human equivalent dose
HDPE	High density polyethylene
hr	Hour
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IP	Investigational Product
K2EDTA	Dipotassium ethylenediaminetetraacetic acid
kg	Kilogram
m	meter
MAD	Multiple ascending dose
mg	Milligram
mL	Milliliter
MRS	Magnetic Resonance Spectroscopy
mRNA	Messenger Ribonucleic acid (RNA)
MTD	Maximum tolerated dose
NAD+	Oxidized form of nicotinamide adenine dinucleotide
NADH	Reduced form of nicotinamide adenine dinucleotide
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
NaHCO3	Sodium bicarbonate (baking soda)

Abbreviation	Definition
ng	nanogram
nm	nanometer
NOAEL	No observed adverse effect level
OLE	Open Label Extension
PD	Pharmacodynamic
PPP	Pentose phosphate pathway
RSA	Regimen-Specific Appendix
SAE	Serious adverse event
SOD1	Superoxide dismutase
SVC	Slow Vital Capacity
TEM	Transmission electron microscopy
TK	Toxicokinetic
USP	United States Pharmacopeia
VC	Vital Capacity

REGIMEN-SPECIFIC APPENDIX SUMMARY

Regimen-Specific Appendix C
For CNM-Au8

Rationale and RSA Design

CNM-Au8 is a suspension of clean-surfaced, faceted gold nanocrystals whose catalytic activity independently impacts energetic, metabolic, and redox pathways, resulting in significant neuroprotection from oxidative and excitotoxic insults.

CNM-Au8 is a nanocatalyst that is administered orally, penetrates the blood brain barrier, and has a unique mechanism of action involving: (1) the catalytic production of nicotinamide adenine dinucleotide (NAD), a key co-factor in the ATP-generating pathways of all cells, (2) catalytic anti-oxidative activity resulting in cellular protection from oxidative stress, and (3) catalytic stimulation of the pentose phosphate pathway, influencing both anti-oxidative as well as anabolic processes. Importantly, CNM-Au8 demonstrated significant neuroprotection of healthy human motor neurons from cell death induced by toxic ALS-participant derived astrocytes, and of primary rat motor neurons from glutamatergic excitotoxicity. Oral delivery of CNM-Au8 to a mouse model of ALS resulted in the recovery of functional behaviors and significant extension of lifespan.

Given the involvement of cellular bioenergetic failure in the pathogenesis of ALS, the development of a disease modifying therapeutic that addresses the energetic dysregulation underlying the progressive accumulation of oxidative stress and dysregulated RNA processing is a rational therapeutic strategy.

This clinical protocol will test CNM-Au8 at doses of 30 mg and 60 mg versus placebo in a randomized, double-blind manner, to determine if CNM-Au8 can demonstrate sufficient efficacy and safety on accepted clinical and paraclinical outcomes for the treatment of ALS. The investigation of two doses of the study drug is based on counsel from the FDA recommending including a dose finding strategy in the study. The 30 mg and 60 mg doses of CNM-Au8 selected for the study achieve at least comparable AUC exposure in humans as those shown to demonstrate neuroprotection efficacy in nonclinical animals studies, and demonstrated minimal treatment emergent adverse events in the Phase 1 First-In-Human study in healthy volunteers.

Allocation to Treatment Regimens
Participants must first be screened under the Master Protocol before they are randomized to an RSA.

As soon as pre-defined criteria for futility for the RSA are met, or the target number of randomized participants has been reached, enrollment will stop in the RSA.

Number of Planned Participants and Treatment Groups

The number of planned participants for this regimen is approximately160.

There are 2 treatment groups for this regimen, active and placebo. Participants will be randomized in a 3:1 ratio to active treatment or placebo (i.e., 120 active: 40 placebo). Half of the active treatment group will receive CNM-Au8 30mg/day and the other half will receive 60mg/day (i.e., 60 at 30mg/day: 60 at 60mg/day).

Planned Number of Sites

Research participants will be enrolled from approximately 60 centers in the US.

Treatment Duration

The maximum duration of the placebo-controlled treatment portion is 24 weeks.

Follow-up Duration

At the conclusion of the 24-week placebo-controlled Treatment Period of the study, all participants will either schedule a 28-day follow up phone call and end their participation in the regimen or have the option to receive CNM-Au8 in the Open Label Extension (OLE) phase of the study.

In the OLE, CNM-Au8 will be provided by Clene Nanomedicine, until the primary results of the 24 week double-blind portion of the study are available, or Clene or sponsor terminate support or development of CNM-Au8 for ALS.

Total Planned Trial Duration

For participants completing the placebo-controlled Treatment Period of the Study, the planned amount of time for a participant in the trial is up to 34 weeks, or about 8 months. This duration assumes a 6-week screening window, a 24-week placebo-controlled treatment period, and a 4-week safety follow-up period for those participants who do not enter the Open Label Extension. Participants will complete approximately 10 study visits during the placebo-controlled treatment period of the study.

SCHEDULE OF ACTIVITIES – PLACEBO-CONTROLLED PERIOD[14]

As per the Schedule of Activities (SOA) below, visits must occur every 4 weeks and will be alternatively clinic-, phone-, or telemedicine-based, as applicable. There is a maximum 24-week duration of placebo-controlled treatment for a Regimen.

Activity (page 1 of 2)	Master Protocol or Regimen-Specific	Master Protocol Screening[1]	Regimen Specific Screening[1]	Baseline	Week 2	Week 4[16, 17]	Week 8[16, 17]	Week 12	Week 16[17]	Week 20	Week 24 or Early Term. Visit[14, 16]	Follow-Up Safety Call[11, 14]
		Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
		-42 to -1 Days	-41 to 0 Day	Day 0	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day 168 ±7	28 days after last dose ±3 days
Written Informed Consent[2]	Master	X	X									
Written Informed Consent - OLE	Master								X			
Inclusion/Exclusion Review	Master	X	X[3]									
ALS & Medical History	Master	X										
Demographics	Master	X										
Physical Examination	Master	X										
Neurological Exam	Master	X										
Vital Signs[4]	Master	X		X		X	X		X		X	
Slow Vital Capacity	Master	X[18]		X			X		X		X	
Home Spirometry	Regimen	X[18]		X			X		X		X	
Muscle Strength Assessment	Master			X			X		X		X	
ALSFRS-R	Master	X		X		X	X	X	X	X	X	
ALSAQ-40	Regimen			X							X	
CNS Bulbar Function Scale	Regimen			X			X		X		X	
12-Lead ECG	Master	X									X	
Clinical Safety Labs[5]	Master	X		X		X	X		X		X	
PK/PD Biomarker Blood Collection[12]	Regimen			X		X	X				X	
Biomarker PD Urine Collection	Regimen			X							X	

Biomarker Blood Collection	Master			X			X		X		X	

Activity (page 2 of 2)	Master Protocol or Regimen-Specific	Master Protocol Screening[1]	Regimen Specific Screening[1]	Baseline	Week 2	Week 4[16, 17]	Week 8[16, 17]	Week 12	Week 16[17]	Week 20	Week 24 or Early Term. Visit[14, 16]	Follow-Up Safety Call[11, 14]
		Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
		-42 to -1 Days	-41 to 0 Day	Day 0	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day 168 ±7	28 days after last dose ±3 days
Biomarker Urine Collection	Master			X			X		X		X	
DNA Collection[7] (optional)	Master			X								
CSF Collection (optional)	Master			X					X[15]			
Concomitant Medication Review	Master	X	X	X	X	X	X	X	X	X	X	
Adverse Event Review[6]	Master	X	X	X	X	X	X	X	X	X	X	X
Columbia-Suicide Severity Rating Scale	Master			X		X	X		X		X	
Install Smartphone App[19]	Regimen			X								
Voice Recording[9]	Regimen			X		X	X		X		X	
Uninstall Smartphone App	Regimen										X	
Assignment to the Regimen	Master	X										
Randomization within the Regimen	Master		X									
Administer/Dispense Investigational product	Master			X[8]			X		X		X[10]	
Study Drug Accountability/Compliance	Master				X[20]	X	X	X[20]	X	X[20]	X	
Exit Questionnaire	Master										X	
Vital Status Determination[13]	Master										X	

[1] Master Protocol Screening procedures must be completed within 42 days to 1 day prior to the Baseline Visit. The Regimen-Specific Screening Visit and Baseline Visit should be combined, if possible.

[2] During the Master Protocol Screening Visit, participants will be consented via the Master Protocol informed consent form (ICF). After a participant is randomized to a regimen, participants will be consented a second time via the regimen-specific ICF.
[3] At the Regimen Specific Screening Visit, participants will have regimen-specific eligibility criteria assessed.
[4] Vital signs include weight, systolic and diastolic pressure, respiratory rate, heart rate and temperature. Height measured at Master Protocol Screening Visit only.
[5] Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function and urinalysis. Serum pregnancy testing will occur in women of child-bearing potential at the Master Protocol Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.
[6] Adverse events that occur after signing the consent form will be recorded.
[7] The DNA sample can be collected after baseline if a baseline sample is not obtained or the sample is not usable.
[8] Administer first dose of investigational product only after Baseline Visit procedures are completed.
[9] In addition to study visits outlined in the SOA, participants will be asked to complete twice weekly voice recordings at home.
[10] Drug will only be dispensed at this visit if the participant continues in the OLE.
[11] Participants will only have a Follow-Up Safety Call at this time if they *do not* continue on in the OLE. Participants who continue into OLE will have a Follow-Up Safety Call after their last dose of study drug during the OLE phase.
[12] Whole blood and plasma will be collected prior to administration of the daily dose at the Baseline and Week 24 visit. Plasma will be collected prior to administration of the daily dose at the Week 4 and Week 8 visits. Refer to the RSA laboratory manual for collection and processing parameters.
[13] Vital status, defined as a determination of date of death or death equivalent or date last known alive, will be determined for each randomized participant at the end of the placebo-controlled portion of their follow-up (generally the Week 24 visit, as indicated). If at that time the participant is alive, his or her vital status should be determined again at the time of the last patient last visit (LPLV) of the placebo-controlled portion of a given regimen. We may also ascertain vital status at later time points by using publicly available data sources as described in section 8.15 of the Master Protocol.
[14] The maximum window between study visits during the placebo-controlled period may not exceed 64 days.
[15] If the CSF collection is unable to be performed for logistical reasons, such as scheduling, at the Week 16 Visit, it may be performed at the Week 24 Visit.
[16] Participants should be instructed to hold the study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.
[17] Visit may be conducted via phone or telemedicine with remote services instead of in-person if this is needed to protect the safety of the participant due to a pandemic, or other reason.
[18] If required due to pandemic-related restrictions, Forced Vital Capacity (FVC) performed by a Pulmonary Function Laboratory evaluator or with a study-approved home spirometer, or sustained phonation using a study approved method may be used for eligibility (Master Protocol Screening ONLY).
[19] Two smartphone apps should be installed on the participant's phone, one to collect the voice recordings and one to collect home spirometry.
[20] Drug accountability will not be done at phone visits. A drug compliance check-in must be held during phone visits to ensure participant is taking drug per dose regimen and to note any report of missed doses.

SCHEDULE OF ACTIVITIES – REGIMEN SPECIFIC OPEN LABEL EXTENSION PHASE (OPTIONAL)

Activity (page 1 of 1)	Master Protocol or Regimen-Specific	Open Label Extension (Optional)[5]								
		Week 2	Week 4[9]	Week 8[9]	Week 12	Week 16[8,9]	Week 20	Week 24	Week 28 and Q12 Weeks[8,9,11]	Follow-Up Safety Call[6]
		Phone	Clinic	Clinic	Phone	Clinic	Phone	Phone	Clinic	Phone
		Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day168 ±3	Day 196 ± 14	28±7 days after last dose
Vital Signs[1]	Master		X	X		X			X	
Slow Vital Capacity	Master		X	X		X			X	
Home Spirometry	Regimen		X	X		X			X	
ALSFRS-R	Master		X	X	X	X	X	X	X	
ALSAQ-40	Regimen								X	
CNS Bulbar Function Scale	Regimen			X		X			X	
Clinical Safety Labs[2]	Master		X	X		X			X	
PK/PD Biomarker Blood Collection[11]	Regimen					X			X[11]	
Biomarker PD Urine Collection[11]	Regimen					X			X[11]	
Biomarker Blood Collection[11]	Master					X			X[11]	
Biomarker Urine Collection[11]	Master					X			X[11]	
Concomitant Medication Review	Master	X	X	X	X	X	X	X	X	
Adverse Event Review[3]	Master	X	X	X	X	X	X	X	X	X[6]
Columbia-Suicide Severity Rating Scale	Master		X	X		X			X	

Administer/Dispense Investigational product	Master			X		X			X	
Drug Accountability/Compliance	Master	X[10]	X	X	X[10]	X	X[10]	X[10]	X	

[1] Vital signs include weight, systolic and diastolic pressure, respiratory rate, heart rate and temperature. Height in cm measured at Master Protocol Screening Visit only.

[2] Clinical safety labs include hematology (CBC with differential), complete chemistry panel, liver function tests, thyroid function and urinalysis. Serum pregnancy testing will occur in women of child-bearing potential at the Master Protocol Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.

[3] Adverse events that occur after signing the consent form will be recorded.

[4] Participants who continue into OLE will have a Follow-Up Safety Call (as described in the body of this RSA) after their last dose of study drug during the OLE phase.

[5] The duration of the OLE is until the primary results of the 24 week double-blind portion of the study are available, or Clene or sponsor terminates support or development of CNM-Au8 for ALS.

[6] Participants who continue into the OLE and then withdraw consent or early terminate will be asked to complete an Early Termination Visit and Follow-Up Safety Call as described in the body of this RSA.

[7] The maximum window between study visits during the OLE may not exceed 64 days for the Week 8 and Week 16 visits; and 96 days for the Week 28, Week 40, and Week 52 visits.

[8] Participants should be instructed to hold the study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.

[9] Visit may be conducted via phone or telemedicine with remote services instead of in-person if this is needed to protect the safety of the participant due to a pandemic or other reasons.

[10] Drug accountability will not be done at phone visits. A drug compliance check-in must be held during phone visits to ensure participant is taking drug per dose regimen and to note any report of missed doses

[11] Blood and urine biomarker collection in OLE phase will only occur at week 16, 28, and 52

1. INTRODUCTION

Regimen C: CNM-Au8

1.1 CNM-Au8 Background Information

Amyotrophic lateral sclerosis (ALS) is a late-onset, fatal neurodegenerative disease affecting motor neurons with an incidence of approximately 1 in 100,000. Most ALS cases are sporadic, with 5–10% of cases being familial ALS (Zarei et al., 2015). Both sporadic and familial ALS are associated with degeneration and loss of cortical and spinal motor neurons, resulting in muscle wasting and weakness, eventually leading to respiratory failure and death. The etiology of ALS remains unknown. Emerging research has discovered interconnecting pathophysiological mechanisms amongst the seemingly disparate genetic mutations, implicated in both familial (fALS) and sporadic (sALS) ALS. Importantly, these emerging data suggest that impairments related to oxidative stress and mitochondrial function appear to be common across many underlying genetic abnormalities. Genetic mutations in superoxide dismutase (SOD1), regulation of RNA processing and metabolism (represented by mutations in FUS, TDP43 and several more genes) all appear to result in both oxidative stress and RNA dysregulation, indicative of an underlying bioenergetic failure in ALS. Bioenergetic dysfunction affects not only motor neurons themselves but also astrocytes and oligodendrocytes that provide crucial energetic support to motor neuron survival. Postmortem analyses of brain tissues from both fALS and sALS participants exhibit evidence of widespread accumulation of oxidative damage to proteins, lipids, and DNA. Transgenic mice expressing mutant SOD1 forms have evidence of nerve and spinal cord tissue damage that recapitulates key aspects of ALS and demonstrates clear evidence of excessive protein and lipid oxidation (D'Amico et al., 2013b). Oxidative stress also leads to the accumulation of protein aggregates such as TDP43 (Bozzo et al., 2017a), which are invariably found in ALS motor neurons, and which indicate failures of protein degradation systems such as autophagy (Blokhuis et al., 2013).

Elevated oxidative stress has been shown to impact the processing of gene transcripts important for motor neuron survival. This observation connecting oxidative stress to RNA processing was demonstrated when paraquat, a well-known inducer of oxidative stress, was shown to induce striking changes in RNA splicing in neuroblastoma cells (Maracchioni et al., 2007). Two of the pre-mRNAs whose splicing was shown to be altered in this study are transcripts of genes known to be important for motor neuron survival, Survival Motor Neuron (SMN) and Apoptotic Peptidase Activating Factor 1 (APAF1) (Maracchioni et al., 2007). The mis-localization of the RNA-binding protein TDP43 in ALS is further evidence of the connection between oxidative stress and RNA dysmetabolism. In general, TDP43 is involved in pre-RNA processing in the nucleus, where it functions in transcriptional control. However, oxidative stress causes the

TDP43 to translocate to the cytoplasm, where it accumulates in stress granules, as protein aggregates. Sequestration of an RNA regulator such as TDP43 in the cytoplasm and away from the nucleus is thought to prevent it from carrying out its normal functions and thereby can lead to dysregulation of RNA processing and transcription. The converse relationship has similarly been shown, namely that mis-regulation of RNA metabolism increases oxidative stress within the CNS. Mutations in RNA regulators FUS and TDP43 cause disruptions in the expression of key nuclear-encoded mitochondrial genes (Zhang et al., 2014) as well as in the expression of energy metabolism regulators such as PGC-1 alpha and oxidative stress reducers such as MnSOD and catalase (Sanchez-Ramos et al., 2011). Therefore, the pathophysiologic disease mechanism of ALS appears to involve a positive feedback cycle of oxidative stress and protein aggregation impacting RNA regulation which in turn leads to a sustained increased oxidative stress disease state, ending in motor neuron death (Bozzo et al., 2017b).

Unifying both themes of RNA dysregulation and oxidative stress is the concept that ALS is a disease of energetic dysmetabolism (Dupuis et al., 2011; Tefera and Borges, 2016; Vandoorne et al., 2018). Motor neurons consume high amounts of energy to function and are therefore exquisitely sensitive to apoptosis if their energetic demands cannot be met. The main energy-producing organelle of the cell, the mitochondria, exhibit dysfunction in ALS on multiple levels. For example, overall there are fewer mitochondria, as measured by mitochondrial DNA, in the spinal cords of both fALS and sALS participants (Wiedemann et al., 2002). Presynaptic mitochondrial swelling in motor neurons has been observed in both ALS participants (Siklos et al., 1996), as well as in a SOD1 transgenic mouse model prior to disease onset (Manfredi and Xu, 2005). Swollen and vacuolarized mitochondria are indicative of impaired mitochondrial function (Siklos et al., 1996), which is corroborated by studies showing that there is decreased activity of the electron transport chain in spinal cord mitochondria of ALS participants (Wiedemann et al., 2002). Because reduced respiration and reduced ATP synthesis preceded clinical symptom onset in SOD1G93A mice (Jung et al., 2002; Mattiazzi et al., 2002; Szelechowski et al., 2018), energetic dysmetabolism appears to play an important role in the etiology of this disease. Overexpression of PGC-1 alpha, which stimulates mitochondrial biogenesis, improved survival, motor neuron function and motor neuron survival in mutant SOD1G93A mice (Zhao et al., 2011), indicating that improving mitochondrial function and/or efficiency may be a successful therapeutic strategy for ALS (Vandoorne et al., 2018).

Energetic dysmetabolism in ALS is further underscored by studies uncovering carbohydrate metabolism abnormalities and ATP deficits in ALS motor neurons (Vandoorne et al., 2018). FDG-PET imaging has demonstrated a correlation between reduced ALS brain glucose uptake and cognitive impairment (Canosa et al., 2016) as well as overall disease severity (Dalakas et al., 1987). Some groups who conducted FDG-PET studies showing reduced glucose uptake in ALS participant brains suggested CNS hypometabolism can be used as an early diagnostic indication

of ALS (Van Laere et al., 2014; Van Weehaeghe et al., 2016). While it is difficult to determine whether lowered glucose utilization detected in imaging studies is due to reduced neuronal catabolism of glucose or due to motor neuron loss, there are further lines of evidence to suggest energetic dysmetabolism in ALS cells. A proteomic study of ALS participant fibroblasts indicated a significant reduction in key enzymes involved in glycolysis (Szelechowski et al., 2018), and expression profiling of sALS participant motor cortexes showed downregulation of glycolytic genes (Lederer et al., 2007). A GWAS analysis involving 12577 cases and 23475 controls identified glycolysis/gluconeogenesis as among the top seven biological pathways represented by the genes identified as associated with ALS (Du et al., 2018). Because glia preferentially utilize glycolysis, these results may indicate a dysfunction of glia in providing energetic support to motor neurons in ALS participants. Energetic stress on motor neurons may increase their levels of oxidative stress, as observed in post-mortem brain samples of ALS participants (Ferrante et al., 1997), and lead to motor neuron death.

1.2 CNM-Au8 Therapeutic Rationale

CNM-Au8 is a concentrated aqueous suspension of clean-surfaced nanocrystals consisting solely of Au atoms organized into clean surfaced crystals of highly faceted, substantially uniform geometrical shapes that act as nanocatalysts, supporting cellular bioenergetic reactions.

CNM-Au8 is supplied for dosing orally in sodium bicarbonate buffered USP purified water. Each mL of CNM-Au8 suspension at 500 μg/mL is estimated to contain between 100 - 500 trillion highly faceted Au nanocrystals. IP will consist of 60 mL high density polyethylene (HDPE) bottles containing CNM-Au8 at concentrations of 250 μg/mL (15 mg) or 500 μg/mL (30 mg), or placebo, administered orally, once daily in the morning, at least 30 minutes prior to food intake. Study participants will consume two (2) bottles of IP each morning for total daily intake of 120 mL equivalent to CNM-Au8 30mg or 60mg, or placebo.

1.2.1 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 2.7×10^3 kDA to 1.3×10^4 kDA. Summary estimates for CNM-Au8 mass, volume, and particle characteristics are described below in Table 1. Each mL of CNM-Au8 suspension at 500 μg/mL is estimated to contain between 100 - 500 trillion highly faceted Au nanocrystals.

Table 1. 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 x 10^2	1.2 x 10^3
Au Nanocrystal Surface Area (nm^2)	3.2 x 10^2	5.3 x 10^2
Au Atoms per Nanocrystal (count)	1.4 x 10^4	6.8 x 10^4
Au Nanocrystal Molecular Weight (kDa)	2.7 x 10^3	1.3 x 10^4
Total Au Nanocrystal Surface Area per mL (cm^2)	3.6 x10^2	1.2 x10^2
Au Nanocrystals per mL CNM-Au8 (count)	1.1 x 10^14	2.3 x 10^13
Au Nanocrystals per 60 mL Dose (count)	3.4 x 10^15	6.8 x 10^14

1.2.2 Preclinical Data Supporting CNM-Au8 in the Treatment of ALS

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

Preclinical characterization of the *in vitro* and *in vivo* properties of CNM-Au8 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 Ca2+ signaling involved in cell cycle control and insulin signaling (Canto et al., 2015). In the Wallerian Degeneration Slow Dominant Mutant Mouse, heightened expression of NMNAT1, resulting in increased NAD+, has been attributed to protect against the axonal degeneration, otherwise observed in these mice (Sasaki et al., 2009).

Quantitative measurement of regional brain area NAD+ concentrations and redox states in 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 people, brain NAD+/NADH redox potential is inversely correlated with age. ^{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 demonstrated a linear decline of NAD+ redox potential with increasing age (Zhu et al., 2015). This age-related decrease in cerebral energy metabolism is believed to be associated with the bioenergetic failure underlying many 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) (Canto et al., 2015; Chua and Tang, 2013a; Villanueva-Paz et al., 2016). 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).

The first demonstration of catalyzed 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 absorbance peak was assessed

while investigating each of the gold nanoparticles at the same 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. CNM-Au8 NADH Catalysis Rates

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

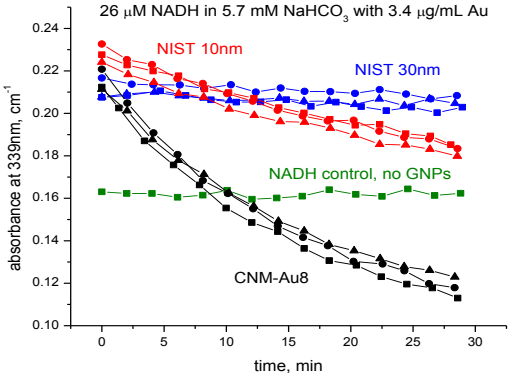
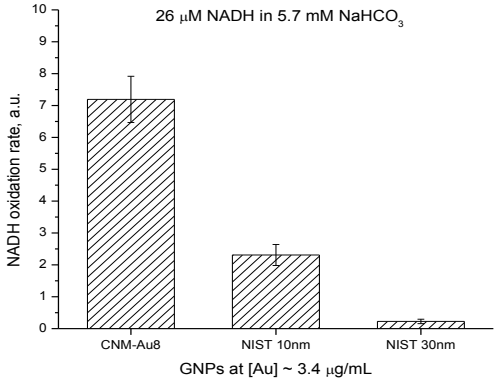


Figure 1B. Relative NADH Oxidation Rates vs. NIST Citrated AuNP



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

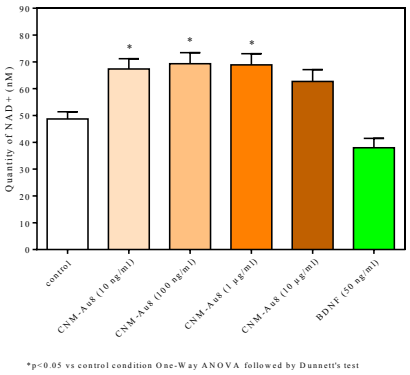
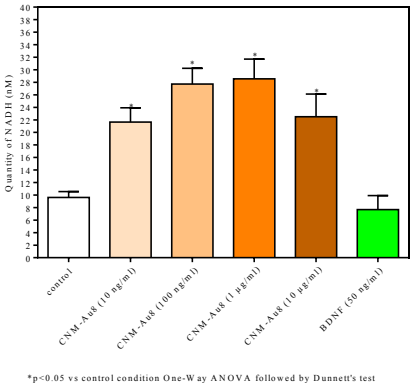


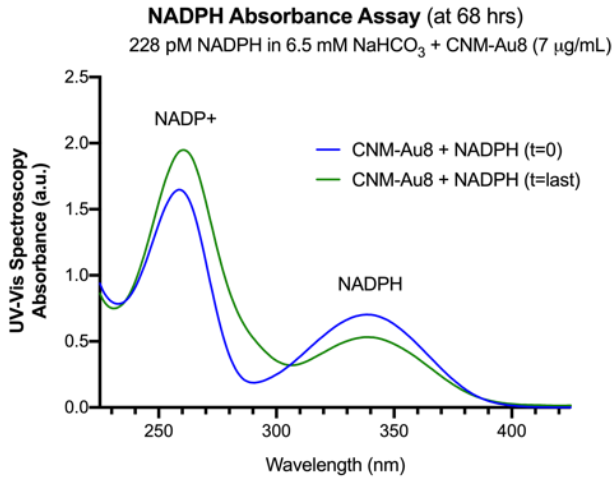
Figure 2B. Effect of CNM-Au8 on NADH Levels



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



Excessive reactive oxygen exposure disrupts cellular redox homeostasis, damages intracellular organelles as well as DNA and proteins, and may also underpin pathophysiologic mechanisms of ALS. 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 . Effects of CNM-Au8 on SOD and ROS Generation

Figure 4A. Effects on SOD Activity Assay

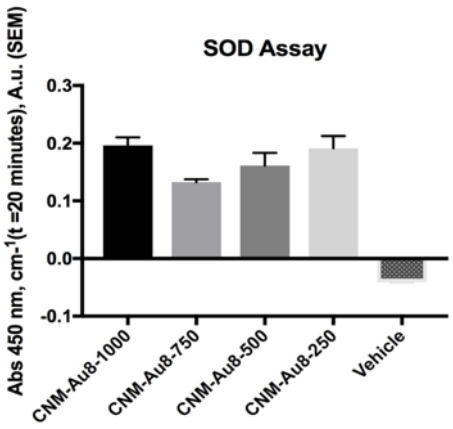


Figure 4B. Effects on ROS Generation in Purified Murine OPC cultures

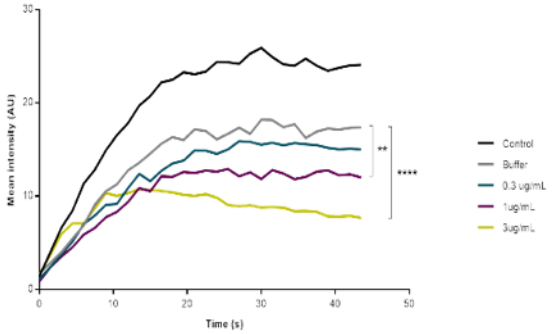
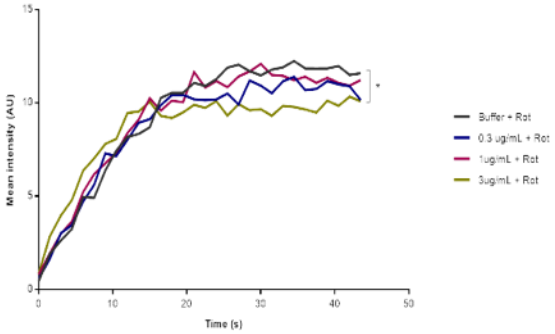


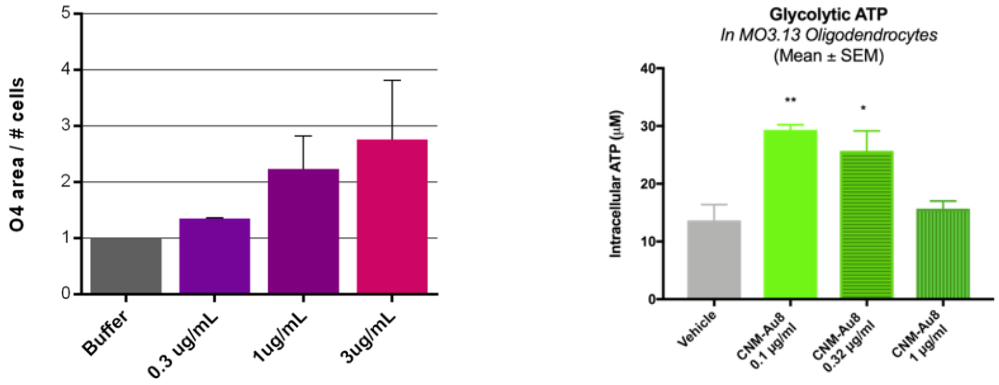
Figure 4C. Effects on ROS Generation in Murine OPC Cultures Plus Rotenone



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 ALS, 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. 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 isolated murine OPCs and b) ATP production in MO3.13 oligodendrocytes (72hr). Statistical analysis performed using one-way ANOVA ($p < 0.05$).

Nonclinical Summary of *In Vitro* and *In Vivo* ALS Neuroprotection Studies

1.2.2.1 *In vitro* ALS Neuroprotection Models

CNM-Au8 exhibits neuroprotective effects in a broad range of neural cell types in *in vitro* and *in vivo* preclinical models of ALS.

1.2.2.1.1 CNM-Au8 Protects Primary Rodent Motor Neurons from Excitotoxicity

Excitotoxicity is the pathological process by which neuronal death occurs as a result of excessive stimulation of receptors at excitatory synapses such as the NMDA receptor. Excitotoxicity has been implicated in acute neurological damage from ischemia and traumatic brain injury and in the chronic neurodegeneration in Huntington's disease. Glutamate excitoxicity is also believed to be a significant contributor to motor neuron death in ALS. Excitotoxic neuronal death via over-activation of NMDA receptors contributes to excessive flux of calcium (Ca2+) into the cell. This triggers a range of responses resulting in cell death, including increased oxidative stress, inappropriate activation of proteases such as calpain, dysregulation of Ca2+-related pathways, mitochondrial damage, and the apoptotic cascade.

Determination of the neuroprotective effects of CNM-Au8 on motor neurons in an in vitro glutamate challenge model was carried out in ventral spinal cord cultures from E14 rat embryos (Martinou et al., 1992). On Day 11 after seeding, cultures were pre-treated with CNM-Au8 or

vehicle. The positive control riluzole was added for 1 hour of pre-treatment before glutamate addition. On Day 13, glutamate was added to the cultures for 20 minutes, followed by treatment of riluzole or CNM-Au8 for another 48 hours before cells were fixed and stained. Cultures were stained with anti-MAP-2 to assess motor neuron survival and neurite lengths, and also with TDP-43 and Hoechst stain to assess extranuclear TDP43. CNM-Au8 dose-dependently protects motor neurons from glutamate-induced cell death (Figure 6A) while also preserving neurite length (Figure 6B). CNM-Au8 also outperformed riluzole on neurite network preservation. In addition, CNM-Au8 treatment prevented the accumulation of cytoplasmic TDP-43, a hallmark of ALS cytotoxicity (Figure 6D).

Figure 6 . Neuroprotection of Motor Neurons from Glutamate Excitotoxicity by CNM-Au8

Figure 6A. Motor Neuron Survival

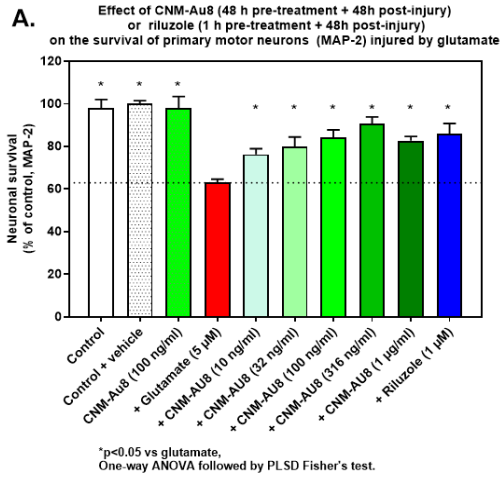


Figure 6B. Motor Neuron Neurtie Network Area

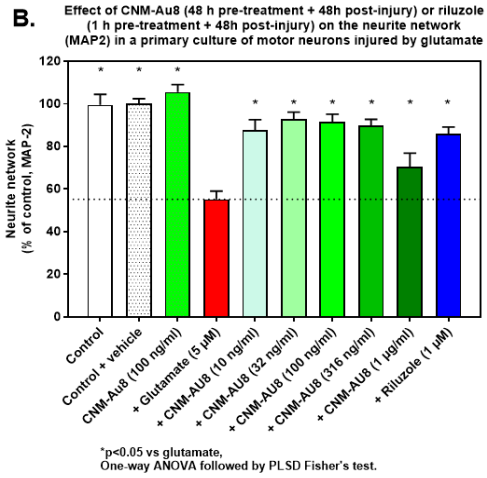


Figure 6B. Effect Of CNM-Au8 on MAP-2 Staining in Rat Hippocampal Neurons Following Glutamate Excitotoxicity Injury

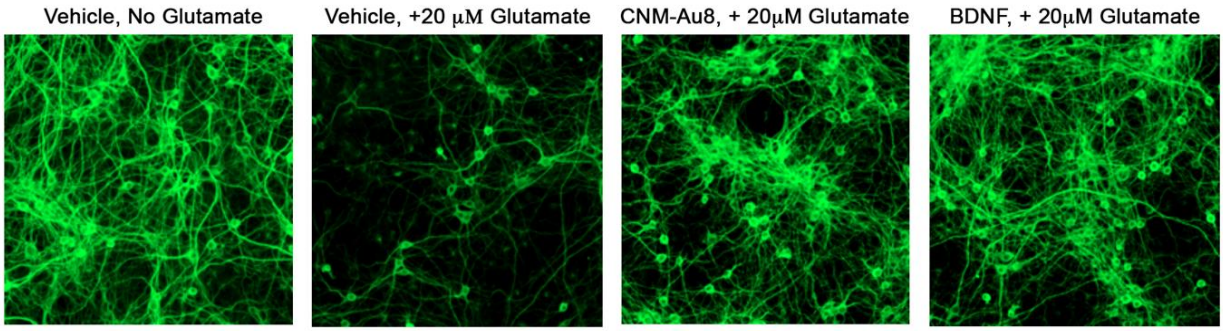
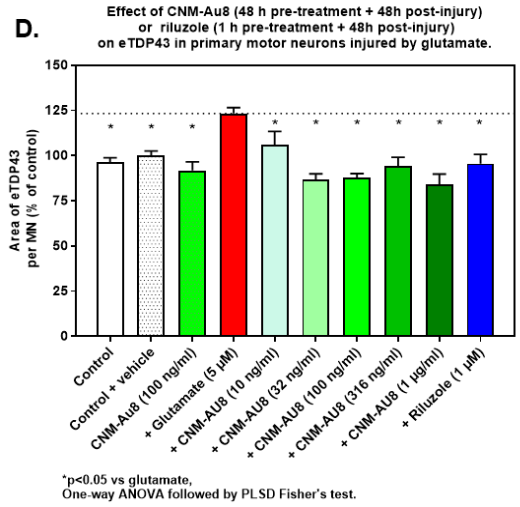


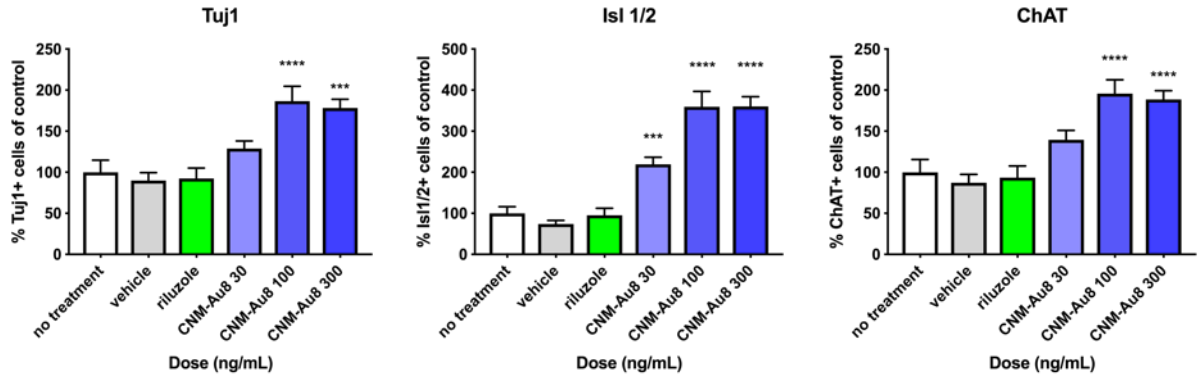
Figure 6D. Reduction in Cytoplasmic TDP43



1.2.2.1.2 CNM-Au8 Protects Normal iPSC Derived Human Motor Neurons From ALS-Participant-Derived Toxic Astrocytes

Astrocytes from both sporadic and familial ALS participants display toxicity against normal human motor neurons (MN) in co-culture (Meyer et al., 2014a). Toxic ALS-participant-derived astrocytes appear to secrete an unidentified toxic factor; and when co-cultured with human MNs, the MNs die (Meyer et al., 2014a). Similar studies have shown that astrocytes from sporadic participants also exhibit toxicity against healthy MNs, likely by similar mechanisms (Haidet-Phillips et al., 2011; Meyer et al., 2014b; Qian et al., 2017). To determine whether CNM-Au8 protects MNs from toxic ALS-participant-derived astrocytes, healthy human iPSC-derived MNs were plated with SOD1A4V astrocytes for 14 days in the presence of various doses of CNM-Au8 or vehicle. Neuroprotection was assessed by quantification of the expression of neuronal markers (Tuj1, Isl1/2, and ChAT) compared to vehicle treated control. Measurement of primary neurite lengths and a neurite network complexity Sholl analysis was also conducted (Sholl, 1953). As shown in Figure 7 below significant neuroprotective dose-dependent effects of CNM-Au8 treatment were consistently observed on survival in the presence of $SOD1^{A4V}$ participant-derived toxic astrocytes.

Figure 7. CNM-Au8 Protects Normal iPSC Derived Human Motor Neurons From ALS-Participant-Derived Toxic Astrocytes



1.2.2.2 *In vivo* ALS Neuroprotection Models

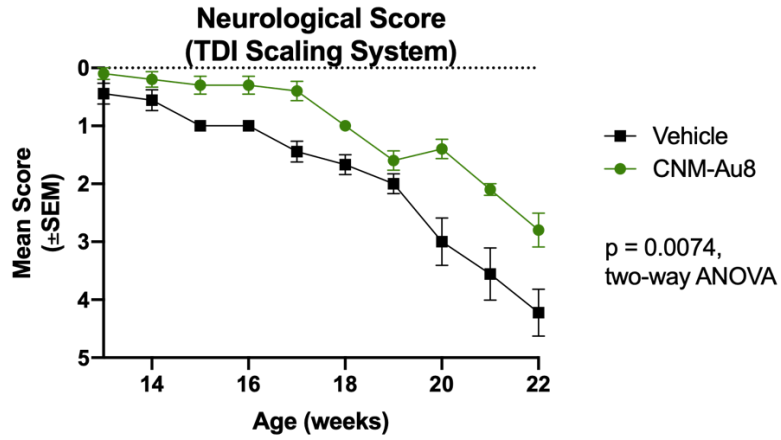
To demonstrate the efficacy of CNM-Au8 treatment in an in vivo model of ALS, two studies were conducted in separate SOD1^{G93A} mouse model strains. These included the rapidly progressive SOD1^{G93A} transgenic line on a mixed SJL/C57BL6 background (Heiman-Patterson et al., 2005) and the more slowly progressive SOD1^{G93A} transgenic line on a congenic C57Bl/6 background (Lutz, 2018) with lifespans of ~157 days compared to ~129 days, respectively. The study using rapidly progressing SOD1^{G93A} animals showed only minor improvement in clinical onset ($p=0.13$, Mantel-Cox test), as well as significant lack of brainstem atrophy ($p<0.05$, unpaired t-test) in the CNM-Au8-treated group (N=15 animals per group;); all other functional measures were not significant (data not shown). In the study of the slower progressing SOD1^{G93A} (N=20 female mice, 10 per group), four-week-old female SOD1 mice were randomly assigned one of two groups with each group balanced for weight.

Beginning at four weeks of age, mice were provided either CNM-Au8 or vehicle ad libitum in their drinking water. Beginning at Week 8 of age each mouse was tested weekly for strength and motor coordination utilizing several measures including the triple horizontal bar hang time test, static rod orientation test, inverted screen hang time test, weight hold test, and home cage wheel activity (average speed) in addition to standard ALS clinical scoring (Deacon, 2013a, 2013b; Hatzipetros et al., 2015). The CNM-Au8-treated group significantly outperforms the vehicle treated group at virtually all timepoints across these tests (Figure 8). In addition, survival benefits have been observed through Week 22 of the study (Figure 9).

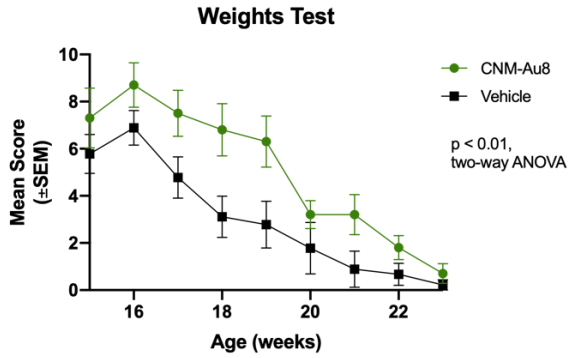
Taken together, these preliminary data from an ongoing study in a transgenic ALS mouse model demonstrate preservation of motor function following chronic treatment with CNM-Au8.

Figure 8. Locomotor Functional Efficacy of CNM-Au8 in an SOD1^{G93A} (C57Bl/6 congenic strain) Murine Study

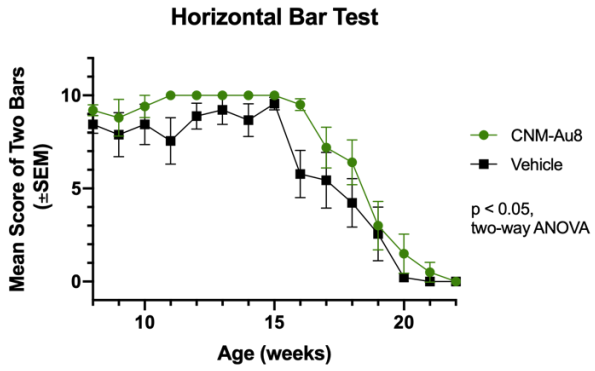
A. ALS TDI Neuro Score



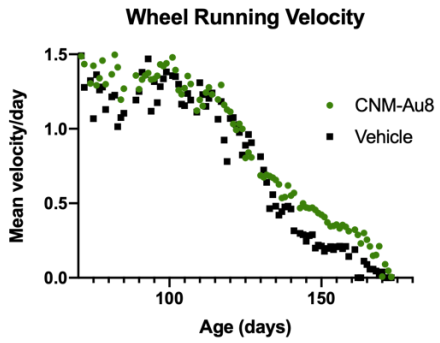
B. Weights Hold Test



C. Horizontal Bar Test



D. Home Wheel Velocity



E. Home Wheel Velocity By Period

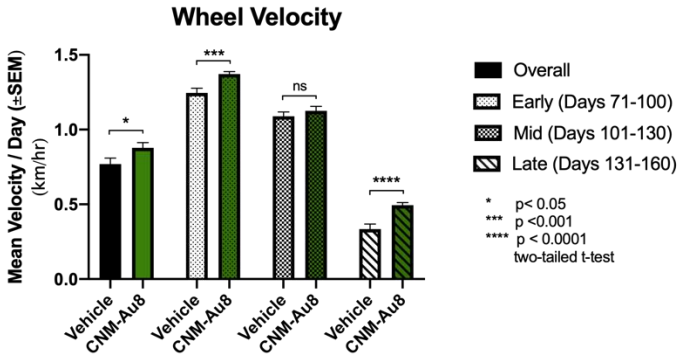
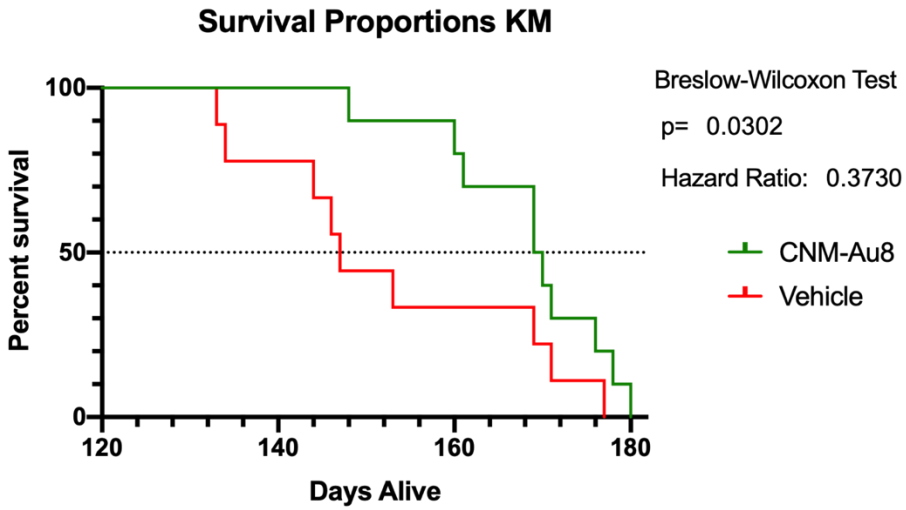


Figure 9. Effect of CNM-Au8 on Survival in a Murine SOD1^{G93A} Model



1.2.3 Summary CNM-Au8 Treatment Rationale for ALS

CNM-Au8-treated motor neurons are protected against cell death by glutamate excitotoxicity in a dose-dependent manner in a primary rat neural-glial co-culture where cytoplasmic levels of TDP-43 are significantly reduced. Aberrant cytoplasmic localization and aggregation of TDP-43 has been observed in over 90% of cases of ALS in humans (Neumann et al. 2006). Further, results from these multiple iPSC human motor neuron studies derived from sporadic, familial, and healthy lines indicate that CNM-Au8 is neuroprotective to MNs challenged with multiple different ALS disease related stressors, namely, excitotoxic and toxic astrocytic stress. Results from the SOD1^{G93A}-overexpression mouse study demonstrate significant improvements in locomotor performance of CNM-Au8-treated mice with a significant survival benefit.
In summary, CNM-Au8 has demonstrated neuroprotection across a range of neuronal cell types, antioxidant activity to counteract the accumulation of ROS in ALS, and preservation of motor function in an ALS mouse model. CNM-Au8 therefore represents an important therapeutic candidate for ALS.

2. OBJECTIVES

2.1 Study Objectives and Endpoints

Primary Efficacy Objective:
To evaluate the efficacy CNM-Au8 as compared to placebo on ALS disease progression.

Secondary Efficacy Objective:

- To evaluate the effect of CNM-Au8 on selected secondary measures of disease progression, including survival.

Safety Objective:

- To evaluate the safety of CNM-Au8 for ALS.

Exploratory Efficacy Objective:

- To evaluate the effect of CNM-Au8 on selected biomarkers and endpoints.

Primary Efficacy Endpoint:
Change in disease severity as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) total score using a Bayesian repeated measures model that accounts for loss to follow-up due to mortality.

Secondary Efficacy Endpoints:

- Change in respiratory function as assessed by slow vital capacity (SVC).
- Change in muscle strength as measured isometrically using hand-held dynamometry (HHD) and grip strength.
- Survival.

Safety Endpoints:

- Treatment-emergent adverse and serious adverse events.
- Changes in laboratory values and treatment-emergent and clinically significant laboratory abnormalities.
- Changes in ECG parameters and treatment-emergent and clinically significant ECG abnormalities.
- Treatment-emergent suicidal ideation and suicidal behavior.

Exploratory Efficacy Endpoints:

- Changes in quantitative voice characteristics.
- Difference in the proportion of patients experiencing a ≥ 6-point decline in the ALSFRS-R between active treatment and placebo from Baseline to Week 24.
- Changes in biofluid biomarkers of neurodegeneration.

- Changes in patient reported outcomes.
- Change in respiratory function as assessed by home spirometry.

3. RSA DESIGN

This study is a multi-center, randomized, placebo-controlled trial, testing two active doses of CNM-Au8 (30 mg, 60 mg), given orally daily versus color-matched placebo. Participants will be randomized 3:1 active (CNM-Au8): placebo. Participants randomized to active will be equally allocated between the CNM-Au8 30 mg and 60 mg doses.

3.1 Scientific Rationale for RSA Design

The comparator for this study is placebo. Placebo will consist of a sodium bicarbonate solution in USP water, color matched to active CNM-Au8. Placebo was chosen as the comparator because there are no highly effective, disease-modifying therapies currently available for people with ALS. Both riluzole and edaravone, the only two therapies currently approved for ALS, are available to participants as concomitant therapy during this study. In this manner, participants are not sacrificing any potential benefit from currently approved therapies to participate in this study.

3.2 End of Participation Definition

A participant is considered to have ended his or her participation in the placebo-controlled period of the Regimen if they:

- Complete planned placebo-controlled period visits, as described in the SOA, including participants on or off study drug
- Early terminate from the study and complete the Early Termination Visit and Follow-Up Phone call as described in Section 6.1.11
- Withdraw consent to continue participation in the study, or are lost to follow-up

.

If a participant initiates open-label study drug in the OLE period, he or she is considered to have completed his or her participation in the OLE period of the Regimen if they choose to discontinue participation or if all planned OLE period visits, including the last visit or the last scheduled procedure shown in the SOA, have been completed.

3.3 End of Regimen Definition

The end of the placebo-controlled period in a Regimen occurs when all randomized participants have completed their participation in the placebo-controlled period as defined in section 3.2.

The end of the OLE period in a Regimen occurs when all participants who initiated open-label study drug in the OLE period have completed their participation in the OLE period as defined in section 3.2.

4. RSA ENROLLMENT

4.1 Number of Study Participants

Approximately one hundred-sixty (160) participants will be randomized for this Regimen.

4.2 Inclusion and Exclusion Criteria

In order to be randomized to an RSA, participants must meet the Master Protocol eligibility criteria. In addition, participants meeting all of the following inclusion and exclusion criteria will be allowed to enroll in this Regimen:

4.2.1 RSA Inclusion Criteria

Per the Master Protocol.

4.2.2 RSA Exclusion Criteria

1. History of allergy to gold, gold salts, or colloidal gold preparations.

4.3 Treatment Assignment Procedures

Each participant who meets all eligibility criteria for the RSA will be randomized to receive either CNM-Au8 or placebo for approximately 24-weeks of double-blind treatment. An additional period of open-label treatment may be offered to participants following completion of the placebo-controlled portion of the study.

5. INVESTIGATIONAL PRODUCT

5.1 Investigational Product Manufacturer

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 2. CNM-Au8 Investigational Product Dosing Administration

Description	CNM-Au8 30 mg	CNM-Au8 60 mg	Matched Placebo
Total Daily Dosage	30 mg	60mg	NA
Concentration	250 μg/mL	500 μg/mL	NA
Volume per Bottle	60 mL	60 mL	60 mL
Bottles per Day	2	2	2
Daily Volume	120 mL	120 mL	120 mL
Route of Administration	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)

The investigational product components and quality standards are described in Table 6 below.

Table 3. CNM-Au8 Components and Quality Standards

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

5.2 Labeling, Packaging, and Resupply

All investigational drug products 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
- Unique number/code

5.3 Acquisition, Storage, and Preparation

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 regimen industry partner 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 IP via the interactive web response system (IWRS) and will use the IP only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the IP must be properly documented according to the Sponsor's agreed and specified procedures.

All IP must 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.

5.4 Study Medication/Intervention, Administration, Escalation, and Duration

Participants will either receive CNM-Au8 30 mg, 60 mg, or color-matched placebo in the study. The Investigational Product (IP) will be self-administered by participants who will be directed to take the IP each day in the morning at approximately the same time, at home at least thirty (30) minutes prior to 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 Site Investigator's team and to participants. There are no visible differences between CNM-Au8 30 mg, 60 mg, and placebo dosing units.

Participants may take the study drug by mouth or through a gastric tube. Participants taking study drug by gastric tube should be directed to first flush the tube with approximately 20-30 mL of distilled water, administer study drug, and then flush the tube post-study drug administration with another 20-30 mL of distilled water.

Based on the supply of the IP provided, the maximum window between in-clinic study visits during the placebo-controlled period may not exceed 64 days. The maximum window between in-clinic study visits during the Open Label Extension period may not exceed may not exceed 64 days for the first 16 weeks, and may not exceed 96 days for the duration of the OLE study period.

5.5 Drug Returns and Destruction

At each in person visit the steps outlined in Manual of Procedures must be followed for study drug accountability and compliance, as well as study drug return and destruction.

Prior to study drug destruction, all used and unused IP requires a second accountability verification to be completed by a different study team member, and both verifications should be documented on the study destruction logs. No study drug may be destroyed on-site until written approval is provided by the study monitoring team. Sites should follow their local drug destruction policies.

5.5 Dosage Adjustment

No dosage adjustments are anticipated during the placebo-controlled period of the study. If participants have difficulty tolerating IP, the Investigator may permit the participants to reduce the daily dose by one (1) bottle. Potential tolerability issues may include gastrointestinal disturbances, headache, somnolence, or other treatment emergent adverse events are not otherwise explained.

Down titration of IP to one (1) bottle daily will only be allowed to address tolerability issues and only with Medical Monitor approval. After a participant has been down titrated to, he/she may be allowed to retry two (2) bottle dose (re-challenge), after a discussion with and approval by the Site Investigator and Medical Monitor. Any dosing changes must be appropriately documented including start date(s) and re-challenge date(s). If the participant cannot tolerate the re-challenge, he/she may remain at the lower dose of one (1) bottle daily. Drug holidays (e.g., treatment-free periods) are not permitted.

During the Open Label Extension, participants will maintain their current blinded dose. Participants previously randomized to placebo during the placebo-controlled period of the study will be re-randomized to receive CNM-Au8 30 mg or 60 mg for the Open Label Extension. All dosing assignments will remain blinded throughout the Open Label Extension. IP tolerability issues encountered during the Open Label Extension will be managed on a case-by-case basis by the Site Investigator in coordination with the Medical Monitor.

5.6 Justification for Dosage

Dosage selection has been made based on human safety margins from nonclinical toxicology studies and the exposure data from pharmacology studies that demonstrated neuroprotection benefits.

5.6.1. Human Dosing Safety Margins

Based upon the maximum doses (mg/kg/day) evaluated in the nonclinical GLP repeat-dose 21-day toxicokinetic studies, the completed first-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 4. Summary of CNM-Au8 Conversion of Animal 21-Day Repeat Doses To Human Equivalent Dose (HED) Based On Body Surface Area (mg/m2) 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(0-24) (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 the Table 3 below.

Table 5. 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 4.

Table 6. 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

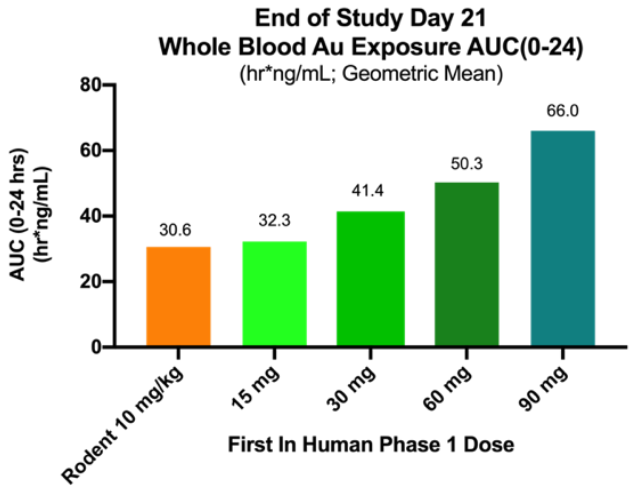
Species (Study)	NOAEL Chronic Dosing (mg/kg/day)	Animal End of Study AUC(0-24) (ng*hr/mL)[a]	Safety Margin Based on Chronic Animal End-of-Study/ Human 21-Day AUC(0-24) Ratio			
			Human 15 mg AUC (32.3 ng*hr/mL)	Human 30 mg AUC (41.4 ng*hr/mL)	Human 60 mg AUC (50.3 ng*hr/mL)	Human 90 mg AUC (66.0 ng*hr/mL)
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

5.6.2 Neuroprotection Pharmacologic Exposure Data

Based on the blood gold exposure observed in rodent toxicokinetic (TK) studies where significant remyelination and neuroprotection benefits were observed in preclinical neuroprotection models at mean AUC value of 30.6 (hr*ng/mL), which suggests a minimum human equivalent exposure to achieve a positive bioenergetic response to CNM-Au8 of 15 – 30 mg. Increased AUC exposure of 50.3 (hr*ng/mL) was observed at the CNM-Au8 60 mg dose.

Figure 10. 21-Day AUC Exposure Ranges Between Rodent Toxicokinetic Studies, Preclinical Efficacy Models, and First-In-Human Dosing



In conclusion, 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 dosage chosen for this study in participants with ALS will be CNM-Au8 30 mg/day and 60 mg/day.

5.7 Participant Compliance

Participants will be requested to return any unused IP including empty packaging and used bottles at each study visit. Treatment compliance will be assessed at in clinic study visits through bottle counts and will be documented and summarized by a drug-dispensing log for each participant. During phone visits, drug compliance check-in will be held to ensure participant is taking drug per dose regimen and to note any report of missed doses. Overall treatment compliance with IP intake for the per protocol analysis set should be between 80% and 120% of the planned dose and will be assessed at specified study visits. In the event participants are not compliant within this range, discontinuation may be considered by the Site Investigator in consultation with the Medical Monitor or designee.

The date of dispensing the IP to the participant will be documented in the eCRF. Changes in IP dosing for tolerability issues will also be documented in the eCRF.

If a dose of IP is missed, the participant 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.

5.8 Overdose

In the event of overdose, study staff should monitor the study participant and provide supportive care as needed. The SI should also contact the Medical Monitor within twenty-four (24) hours. No prior experience has been documented regarding overdosage in humans.

5.9 Prohibited Medications

There are no prohibited medications for this regimen.

5.10 CNM-Au8 Known Potential Risks and Benefits

5.10.1 Known Potential Risks

5.10.1.1 Immediate Risks

- There has been limited human exposure to clean-surfaced Au nanocrystals. There could be unexpected adverse effects from 24 weeks of exposure to 30 mg or 60 mg of CNM-Au8. These risks have been mitigated by an extensive preclinical toxicology program in multiple

species demonstrating No-Adverse-Effect-Levels (NOAEL) at the highest doses studied (up to 10 mg/kg/day in canines; 40 mg/kg/day in rats). Therefore, no Maximum Tolerated Dose (MTD) could be established in these nonclinical studies. In addition, single and multiple ascending dose studies have been performed in healthy human volunteers, which showed no significant adverse events (SAEs) or safety issues. CNM-Au8 was well tolerated at the doses studied up to 90 mg per day over twenty-one days of consecutive dosing.

- There have been no clinical trials of CNM-Au8 in humans with ALS. There is a risk that CNM-Au8 may not be effective and a very small risk that CNM-Au8 could make participants worse. These risks have been mitigated by a robust preclinical program, demonstrating that CNM-Au8 is effective in multiple models of protection from neurodegeneration.

5.10.1.2 Long Range Risks

There has been limited human exposure to CNM-Au8. There could be unexpected adverse effects from chronic exposure to 30 mg or 60 mg of CNM-Au8. Based on rodent and canine toxicokinetic data, when taken orally with daily administration, CNM-Au8 can be expected to be absorbed, and eliminated, relatively slowly with increasing blood and tissue concentrations accumulating over time.

The risks of long-term exposure to CNM-Au8 are mitigated by toxicology studies with chronic dosing, demonstrating no significant toxicities or maximum-tolerated dose.

5.10.1.3 Summary of Participant Risk

Given the potential benefits of CNM-Au8 demonstrated in pre-clinical models of ALS, the safety of CNM-Au8 demonstrated in preclinical toxicology studies and Phase 1 clinical trials in healthy controls, overall, the benefit-risk of CNM-Au8 is positive for participants with ALS.

5.10.2 Known Potential Benefits

As described previously, in preclinical models of ALS, CNM-Au8 has demonstrated efficacy in several relevant models of neurodegeneration. CNM-Au8 has been shown 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. These data suggest that

CNM-Au8 could improve participant functioning and/or prolong life in participants with ALS. The safety of CNM-Au8, at the doses to be tested in this study have been shown to be safe and well tolerated in preclinical toxicology and Phase 1 clinical trials in healthy controls.

5.11 Regimen-Specific Lab Alerts

Site Investigators and the Medical Monitor will receive an alert from the Central Lab for certain lab changes. (See below.) These alerts are informative only, and they do not mean a participant must reduce drug dosage or discontinue study drug. These alerts indicate that the Site Investigator and Medical Monitor should follow up with additional testing and management as clinically indicated. Discontinuation of any participant on the basis of abnormal laboratory findings will be at the discretion of the Site Investigator.

Chemistry Panel Test Name	**Outlier Criteria Warranting Further Investigation**
ALT and AST	>3x ULN
Creatinine	>1.5 x Baseline
Hematology Panel Test Name	**Outlier Criteria Warranting Further Investigation**
Platelet count	<75,000/mm^3

6. REGIMEN SCHEDULE

In addition to procedures in the Master Protocol, the following regimen-specific procedures will be conducted during the study:

- Home Spirometry
    - Note: Home spirometry should be collected within the visit window but will occur while the participant is not in the clinic (at home or other remote location)e.
- ALSAQ-40
- CNS Bulbar Function Scale
- Voice recording
- Smartphone installation and removal
- Whole blood collection for PK and PD analyses
- Plasma collection for PK and PD analyses
- Urine collection for PD analyses

Modifications to Regimen Schedule

Designated visits in the Schedule of Activities (i.e., Week 4, Week 8, and Week 16) may be conducted via telemedicine (or phone if telemedicine is not available) with remote services instead of in-person if needed to protect the safety of the participant due to a pandemic or other reason. If a planned in-clinic visit is conducted via telemedicine (or phone if telemedicine is not available) with remote services, only selected procedures will be performed. Instructions on how to document missed procedures are included in the MOP.

In addition to the procedures in the Master Protocol that should be conducted during the phone or telemedicine and remote visits, the following regimen-specific procedures should be completed:

- Home Spirometry (Week 8 and 16 only)
- Voice Recording
- CNS Bulbar Function Scale (Week 8 and 16 only)

Details on collection of the CNS Bulbar Function Scale, dispensing IP during remote visits, and documenting participants' willingness to participate in OLE are described in the MOP.

Blood samples for PK and PD analyses are **not** collected during the remote visits by the home health agency and this should be recorded as such in the applicable source documentation and EDC.

6.1 Regimen-Specific Screening Visit

This visit will take place in-person after the Master Protocol randomization to a regimen. There are no regimen-specific procedures for this visit. For this regimen, the Regimen-Specific Screening Visit and Baseline Visit should be combined, if possible.

Participants may be required to reconsent to the regimen if new procedures or information is added in the future. Should a participant need to reconsent, this should occur during the participant's next in-person visit. If the participant's next in-clinic visit is conducted remotely, reconsent may also be completed remotely using the following procedures:

1. The site staff sends copy of the informed consent form to the participant.
2. The participant reads through the consent form but does not sign.
3. The Site Investigator, or other study staff member approved and delegated to obtain informed consent, contacts the participant and reviews the informed consent form with the participant.
4. The participant signs the informed consent form and returns the original signed consent form back to the site.
5. Once received at the site, the individual who consented the participant signs the informed consent form.

6.2 Baseline Visit

This visit will take place on Day 0. The following procedures will be performed for the regimen schedule:

- ALSAQ-40
- CNS Bulbar Function Scale
- Install smartphone app
- Home Spirometry
- Voice recording
- Whole blood collection for PK and PD analyses
- Plasma collection for PK and PD analyses
- Urine collection for PD analyses
- Dispense IP
- Administer first dose of IP in clinic *after* all Baseline procedures have been completed
- Remind participant to bring in investigational product to the next visit

6.3 Week 2 Telephone Visit

This visit will take place 14 ± 3 days after the Baseline Visit via telephone. No regimen-specific procedures will be performed at this visit.

6.4 Week 4 and 8 Visits

Participants should be instructed to hold study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.

These visits will take place on Days 28 ± 7 and 56 ± 7 days, respectively. The following procedures will be performed for the regimen schedule:

- Home Spirometry [Week 8 Only]
- CNS Bulbar Function Scale [Week 8 only]
- Voice recording
- Plasma collection for PK analyses
- Dispense IP (Week 8 only)
- Remind participant to bring in investigational product to the next visit

6.5 Week 12 Telephone Visit

This visit will take place 84 ± 3 days after the Baseline Visit via telephone. No regimen-specific procedures will be performed at this visit.

6.6 Week 16 Visit

This visit will take place on Day 112 ± 7 days. The following procedures will be performed for the regimen schedule:

- Home Spirometry
- ALSAQ-40
- CNS Bulbar Function Scale
- Voice Recording
- Dispense IP
- Document participant's willingness to participate in the OLE
    - If OLE consent is not obtained at Week 16, it may be obtained at Week 24.

- Remind participant to bring in investigational product to the next visit

6.7 Week 20 Telephone Visit

This visit will take place 140 ± 3 days after the Baseline Visit via telephone. No regimen-specific procedures will be performed at this visit.

6.8 Week 24 Visit or Early Termination Visit

Participants should be instructed to hold study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.

This visit will take place on Day 168 ± 7 days. The following procedures will be performed for the regimen schedule:

- Home Spirometry
- ALSAQ-40
- CNS Bulbar Function Scale
- Voice recording
- Uninstall smartphone app
- Whole blood collection for PK and PD analyses
- Plasma collection for PD analyses
- Urine collection for PD analyses
- Dispense IP [See note below.]
- Remind participant to bring investigational product to the next visit (only if continuing in OLE)

Note: Drug is only dispensed at this visit if the participant is continuing in the Open Label Extension.

6.9 Follow-Up Safety Call

Participants will have a Follow-Up Safety Call 28±3 days after their last dose of study drug. Only those participants NOT continuing on in the Open Label Extension will have the Follow-Up Safety Call at the end of their participation in the placebo-controlled portion of the trial. The following procedures will be performed:

- Assess and document AEs

6.10 Process for Early Terminations

Participants who early terminate from the study and do not complete the protocol per ITT will be asked to be seen for an in-person Early Termination Visit and complete a Follow-Up Safety Call. If a participant is not able to be seen in-person, safety assessments and others that can be conducted remotely should be performed.

The in-person Early Termination Visit should be scheduled as soon as possible after a participant early terminates. If the participant early terminates or withdraws consent during the placebo-controlled portion of the Regimen, all assessments that are collected at the Week 24 in-clinic visit should be conducted. If the participant early terminates or withdraws consent during the OLE phase, all assessments that are collected at the OLE Week 52 in-clinic visit should be conducted. The Follow-Up Safety Call should be completed approximately 28 days after the last dose of study drug.

If the Early Termination Visit occurs approximately 28±3 days after the last dose of study drug, the information for the Follow-Up Safety Call can be collected during the Early Termination Visit, and a separate Follow-Up Safety Call does not need to be completed. If the in-person Early Termination Visit does not occur within 28±3 days of the last dose of study drug, the Follow-Up Safety Call should occur approximately 28 days after the last dose of study drug and the Early Termination Visit will be completed after the Follow-Up Safety Call.

If a participant decides to discontinue study drug, but will complete the protocol, an in-person Early Termination Visit and Follow-Up Safety Call is not necessary.

6.11 Open Label Extension

Participants who have completed the placebo-controlled portion of the trial on drug. will be eligible to continue in the Open Label Extension (OLE) as outlined in the SOA. Participants will first be asked about their desire to continue in the OLE at the Regimen-Specific Screening Visit. They will also be asked to *re-confirm* whether they want to continue in the OLE at the Week 16 Visit of the placebo-controlled period. The OLE, CNM-Au8 will be provided by Clene Nanomedicine, until the primary results of the 24 week double-blind portion of the study are available, or Clene or sponsor terminate support or development of CNM-Au8 for ALS.

Modifications to OLE Schedule

Designated visits in the Schedule of Activities for the OLE (i.e. Week 4, Week 8, Week 16, Week 28, and Week 40) may be conducted via telemedicine (or phone if telemedicine is not available) with remote services instead of in-person if needed to protect the safety of the

participant due to a pandemic or other reason. If a planned in-clinic visit is conducted via telemedicine (or phone if telemedicine is not available) with remote services, only selected procedures will be performed. Instructions on how to document missed procedures are included in the Manual of Procedures.

In addition to the procedures in the Master Protocol that should be conducted during the phone or telemedicine and remote visits, the following regimen-specific procedures should be completed:

- Home Spirometry
- CNS Bulbar Function Scale (Not done at OLE Week 4).

Blood and urine samples for PK and PD analysis are **not** collected during the remote visits by the home health agency, and this should be recorded as such in the applicable source documentation and EDC.

6.11.1 Week 2 OLE Telephone Visit

This visit will take place via telephone 14 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Drug Compliance Check-In
- Remind participant to bring investigational product to the next visit

6.11.2 Week 4 OLE Visit

This visit will take place in-person 28 ± 10 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect vital signs including weight
- Perform SVC
- Collect Home Spirometry
- Administer ALSFRS-R questionnaire
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire

- Perform investigational product compliance
- Remind participant to bring investigational product to the next visit

6.11.3 Week 8 OLE Visit

This visit will take place in-person at 56 ± 7 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect vital signs including weight
- Perform SVC
- Collect Home Spirometry
- Administer ALSFRS-R questionnaire
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Dispense investigational product to participant
- Perform investigational product compliance
- Remind participant to bring investigational product to the next visit

6.11.4 Week 12 OLE Telephone Visit

This visit will take place via telephone at 84 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Drug Compliance Check-In
- Remind participant to bring investigational product to the next visit

6.11.5 Week 16 OLE Visit

Participants should be instructed to hold study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.

This visit will take place in-person at 112 ± 7 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect vital signs including weight
- Perform SVC
- Collect Home Spirometry
- Administer ALSFRS-R questionnaire
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Collect urine sample biomarker analyses
- Collect blood sample for biomarker analyses
- Whole blood PK and PD collection
- Plasma PD collection
- PD urine collection
- Dispense investigational product to participant
- Perform investigational product compliance
- Remind participant to bring investigational product to the next visit

6.11.6 Week 20 OLE Telephone Visit

This visit will take place via telephone at 140 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Drug Compliance Check-In
- Remind participant to bring investigational product to the next visit

6.11.7 Week 24 OLE Telephone Visit

This visit will take place via telephone at 168 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications

- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Drug Compliance Check-In
- Remind participant to bring investigational product to the next visit

6.11.8 Week 28 and Q12 Week OLE Visits

Participants should be instructed to hold study drug on the day of the study visit. Study drug should not be taken until after study visit procedures are complete.

The Week 28 OLE Visit will take place in-person 196 ± 14 days after the Week 24 Visit of the placebo-controlled portion of the trial. The procedures listed in the SoA should be performed and participants should be provided with 8 weeks of drug and instructions for dosing. Following the Week 28 OLE Visit, visit will occur every 12 weeks ± 14 days.

7 OUTCOME MEASURES AND ASSESSMENTS

For all assessments listed below, please refer to the Manual of Procedures for detailed instructions.

7.1 Voice Analysis

In addition to the scheduled in clinic voice recordings, voice samples will be collected twice per week, using an app installed on either an android or iOS based smartphone. The app characterizes ambient noise, then asks patients to perform a set of speaking tasks: reading sentences -- 5 fixed and 5 chosen at random from a large sentence bank-- repeating a consonant-vowel sequence, producing a sustained phonation, and counting on a single breath. Voice signals are uploaded to a HIPAA-compliant web server, where an AI-based analysis identifies relevant vocal attributes. Quality control (QC) of individual samples will occur by evaluation of voice records by trained personnel.

The voice analysis app is only available in English, therefore participants who do not speak English should not complete the voice recording. Caregivers cannot provide language assistance when the participant is completing the voice recording.

7.2 ALSAQ-40

The Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 (ALSAQ-40) is a patient self-report health status patient-reported outcome. The ALSAQ-40 consists of forty questions that are specifically used to measure the subjective well-being of patients with ALS and motor neuron disease.

Participants will be handed the questionnaire and asked to write their answers themselves. Caregivers can also help, if needed.

7.3 Center for Neurologic Study Bulbar Function Scale

The Center for Neurologic Study Bulbar Function Scale (CNS-BFS) is a patient self-report scale that has been developed for use as an endpoint in clinical trials and as a clinical measure for evaluating and following ALS patients. The CNS-BFS consists of three domains (swallowing, speech, and salivation), which are assessed with a 21-question, self-report questionnaire.

Participants will be handed the questionnaire and asked to write their answers themselves. Caregivers can also help, if needed.

Instructions on administering the questionnaire during a phone or telemedicine visit will be included in the MOP.

7.4 Home Spirometry

Remote/home-based forced vital capacity will be measured with the MIR Spirobank Smart spirometer. Instructions for use will be provided to the participant. The participant will perform the vital capacity maneuver at home (or other remote location) with real time video coaching (or phone coaching, if video is not available) by the evaluator. Three to five vital capacity maneuvers will be performed, consistent with the manner vital capacity is obtained in clinic.

8 BIOFLUID COLLECTION

8.1 Pharmacokinetic (PK) Assessments

8.1.1 Whole Blood PK Assessments of CNM-Au8

Samples for the measurement of whole blood concentrations of Au will be collected before (pre-dose) administration of investigational product per the Schedule of Assessments. PK collection and processing parameters are specified in the RSA laboratory manual.

PK analyses of CNM-Au8 will be specified in a separate PK analysis plan.

PK blood samples will be shipped to and analyzed under the responsibility of the Clene Nanomedicine, Inc.'s bioanalytical laboratory at:

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

8.1.2 Plasma PK Assessments of Riluzole

Samples for the measurement of plasma concentrations of riluzole will be collected before (pre-dose) administration of both riluzole and the IP per the Schedule of Assessments. Riluzole plasma PK collection and processing parameters are specified in the laboratory manual.

Population PK analyses of riluzole will be specified in the separate PK analysis plan. The first forty (40) participants taking riluzole and randomized to Regimen C (CNM-Au8) to reach the Week 8 visit will be evaluated by the DSMB, or unblinded DSMB PK designee, to determine whether there are any changes in riluzole population PK parameters following treatment with CNM-Au8 versus placebo at the Week 4 and Week 8 timepoints. The unblinded riluzole population PK analyses and designee will be implemented as per the DSMB charter.

Riluzole PK plasma samples will be shipped to and analyzed under the responsibility of Covance Central Laboratory Services LP.

8.2 Pharmacodynamic (PD) Assessments

Plasma, whole blood, and urine samples for pharmacodynamic measurements will be collected before (pre-dose) administration of the investigational product per the Schedule of Assessments. PD collection and processing parameters are specified in the laboratory manual.

PD plasma, whole blood, and urine samples will be shipped to the Clene Nanomedicine, Inc.'s bioanalytical laboratory at the address noted above for long-term storage. PD analyses will be conducted with external vendors and specified in a separate PD analysis plan.

9 REGIMEN-SPECIFIC STATISTICAL CONSIDERATIONS

9.1 Deviations from the Default Master Protocol Trial Design

The statistical design for this regimen will be in accordance with the default statistical design described in Appendix I of the master protocol with only one deviation. This regimen will not include interim analyses for early success.

9.2 Regimen Specific Operating Characteristics

Clinical trial simulation is used to quantify operating characteristics for this regimen (refer to the regimen SAP for further details).

9.3 Sharing of Controls from other Regimens

The primary analysis of this regimen will include sharing of all controls from the other regimens. This is justified by the minor differences in inclusion/exclusion criteria of the RSA, such that there are no expected systematic differences in the primary endpoint between the controls across regimens.

APPENDIX I: THE ALSAQ-40

ALSAQ-40

Please complete this questionnaire as soon as possible. If you have any difficulties filling in this questionnaire by yourself, please have someone help you. However it is **your** responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks.** There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please check the box that best describes your own experiences or feelings.**

Please answer every question even though some may seem very similar to others, or may not seem relevant to you.

All the information you provide is **confidential.**

The following statements all refer to difficulties that you may have had **during the last 2 weeks.**
Please indicate, by checking the appropriate box, how often the following statements have been true for you.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot walk at all
please check **Always/cannot walk at all.**

How often during the last 2 weeks have the following been true?

*Please check **one box** for each question.*

	Never	Rarely	Some-times	Often	Always or cannot walk at all
1. I have found it difficult to walk short distances, e.g. around the house.	☐	☐	☐	☐	☐
2. I have fallen over while walking.	☐	☐	☐	☐	☐
3. I have stumbled or tripped while walking.	☐	☐	☐	☐	☐
4. I have lost my balance while walking.	☐	☐	☐	☐	☐
5. I have had to concentrate while walking.	☐	☐	☐	☐	☐

*Please make sure that you have checked **one box for each question** before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you are not able to perform the activity at all please check **Always/cannot at all**

How often during the last 2 weeks have the following been true?

Please check ***one box*** *for each question*

	Never	Rarely	Some-times	Often	Always or cannot do at all
6. Walking had worn me out.	☐	☐	☐	☐	☐
7. I have had pains in my legs while walking.	☐	☐	☐	☐	☐
8. I have found it difficult to go up and down the stairs.	☐	☐	☐	☐	☐
9. I have found it difficult to stand up.	☐	☐	☐	☐	☐
10. I have found it difficult to move from sitting in a chair to standing upright.	☐	☐	☐	☐	☐

Please make sure that you have checked ***one box for each question*** *before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check ***one box*** *for each question*

	Never	Rarely	Some-times	Often	Always or cannot do at all
11. I have had difficulty using my arms and hands.	☐	☐	☐	☐	☐
12. I have found turning and moving in bed difficult.	☐	☐	☐	☐	☐
13. I have had difficulty picking things up.	☐	☐	☐	☐	☐
14. I have had difficulty holding books or newspapers, or turning pages.	☐	☐	☐	☐	☐
15. I have had difficulty writing clearly.	☐	☐	☐	☐	☐

Please make sure that you have checked ***one box for each question*** *before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check ***one box*** *for each question*

	Never	Rarely	Some-times	Often	Always or cannot do at all
16. I have found it difficult to do jobs around the house.	☐	☐	☐	☐	☐
17. I have found it difficult to feed myself.	☐	☐	☐	☐	☐
18. I have had difficulty combing my hair or brushing and/or flossing my teeth.	☐	☐	☐	☐	☐
19. I have had difficulty getting dressed.	☐	☐	☐	☐	☐
20. I have had difficulty washing at the bathroom sink.	☐	☐	☐	☐	☐

Please make sure that you have checked ***one box for each question*** *before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check ***one box*** *for each question*

	Never	Rarely	Some-times	Often	Always or cannot do at all
21. I have had difficulty swallowing.	☐	☐	☐	☐	☐
22. I have had difficulty eating solid food.	☐	☐	☐	☐	☐
23. I have had difficulty drinking liquids.	☐	☐	☐	☐	☐
24. I have had difficulty participating in conversations.	☐	☐	☐	☐	☐
25. I have felt that my speech has not been easy to understand.	☐	☐	☐	☐	☐

Please make sure that you have checked ***one box for each question*** *before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check ***one box*** *for each question*

	Never	Rarely	Some-times	Often	Always or cannot do at all
26. I have stuttered or slurred my speech.	☐	☐	☐	☐	☐
27. I have had to talk very slowly.	☐	☐	☐	☐	☐
28. I have talked less than I used to do.	☐	☐	☐	☐	☐
29. I have been frustrated with my speech.	☐	☐	☐	☐	☐
30. I have felt self-conscious about my speech.	☐	☐	☐	☐	☐

Please make sure that you have checked ***one box for each question*** *before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

How often during the last 2 weeks have the following been true?

*Please check **one box** for each question*

	Never	Rarely	Some-times	Often	Always
31. I have felt lonely.	☐	☐	☐	☐	☐
32. I have been bored.	☐	☐	☐	☐	☐
33. I have felt embarrassed in social situations.	☐	☐	☐	☐	☐
34. I have felt hopeless about the future.	☐	☐	☐	☐	☐
35. I have worried that I am a burden to other people.	☐	☐	☐	☐	☐

*Please make sure that you have checked **one box for each question** before going on to the next page.*

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

How often during the last 2 weeks have the following been true?

*Please check **one box** for each question*

	Never	Rarely	Some-times	Often	Always
36. I have wondered why I keep going.	☐	☐	☐	☐	☐
37. I have felt angry because of the disease.	☐	☐	☐	☐	☐
38. I have felt depressed.	☐	☐	☐	☐	☐
39. I have worried about how the disease will affect me in the future.	☐	☐	☐	☐	☐
40. I have felt as if I have lost my independence	☐	☐	☐	☐	☐

*Please make sure that you have checked one **box for each question.***

Thank you for completing this questionnaire.

APPENDIX II: THE BULBAR FUNCTION SCALE (CNS-BFS)

BULBAR FUNCTION SCALE (CNS-BFS)						
SIALORRHEA	**Does Not Apply (1)**	**Applies Rarely (2)**	**Applies Occasionally (3)**	**Applies Frequently (4)**	**Applies Most of the Time (5)**	
1. Excessive saliva is a concern to me.	❍	❍	❍	❍	❍	
2. I take medication to control drooling.	❍	❍	❍	❍	❍	
3. Saliva causes me to gag or choke.	❍	❍	❍	❍	❍	
4. Drooling causes me to be frustrated or embarrassed.	❍	❍	❍	❍	❍	
5. In the morning I notice saliva on my pillow.	❍	❍	❍	❍	❍	
6. My mouth needs to be dabbed to prevent drooling.	❍	❍	❍	❍	❍	
7. My secretions are not manageable.	❍	❍	❍	❍	❍	
				TOTAL Sialorrhea Score: _____		
SPEECH	**Does Not Apply (1)**	**Applies Rarely (2)**	**Applies Occasionally (3)**	**Applies Frequently (4)**	**Applies Most of the Time (5)**	**Unable to Communicate by Speaking (6)**
1. My speech is difficult to understand.	❍	❍	❍	❍	❍	❍
2. To be understood I repeat myself.	❍	❍	❍	❍	❍	❍
3. People who understand me tell other people what I said.	❍	❍	❍	❍	❍	❍
4. To communicate I write things down or use devices such as a computer.	❍	❍	❍	❍	❍	❍
5. I am talking less because it takes so much effort to speak.	❍	❍	❍	❍	❍	❍

6. My speech is slower than usual.	❍	❍	❍	❍	❍	❍
7. It is hard for people to hear me.	❍	❍	❍	❍	❍	❍
				TOTAL Speech Score: _____		
SWALLOWING	**Does Not Apply (1)**	**Applies Rarely (2)**	**Applies Occasionally (3)**	**Applies Frequently (4)**	**Applies Most of the Time (5)**	
☐ Feeding tube is in place						
1. Swallowing is a problem.	❍	❍	❍	❍	❍	
2. Cutting my food makes it easier to chew and swallow.	❍	❍	❍	❍	❍	
3. To get food down I have switched to a soft diet.	❍	❍	❍	❍	❍	
4. After swallowing I gag or choke.	❍	❍	❍	❍	❍	
5. It takes longer to eat.	❍	❍	❍	❍	❍	
6. My weight is dropping because I can't eat normally.	❍	❍	❍	❍	❍	
7. Food gets stuck in my throat.	❍	❍	❍	❍	❍	
				TOTAL Swallowing Score: _____		
				OVERALL SCORE: _____		

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