CLINICAL PROTOCOL
IND No. 167326

An open-label, single ascending dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecally administered PrP-siRNA in adults diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.

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SHORT PROTOCOL TITLE
Safety and pharmacology of PrP-siRNA in prion disease

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Study Intervention Provided by:
Broad Institute of MIT and Harvard

Sponsor of IND/IDE: Broad Institute of MIT and Harvard
Sponsor representative: Eric Vallabh Minikel, PhD

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INVESTIGATOR AGREEMENT

I have read the foregoing protocol **version 4.0** (08/03/2026*)* and agree to conduct the study as described herein.

By signing the protocol, the Investigator agrees to keep all information provided by the Sponsor in strict confidence and to request the same from his/her staff and the Institutional Review Board. Study documents provided by the Sponsor will be stored appropriately to ensure their confidentiality. The Investigator should not disclose such information to others without authorization, except to the extent necessary to conduct the study.

_____ _____

Investigator Signature **Date**

_____

Print Investigator's Name

SIGNATURE PAGE

Study Number: TBD

Protocol Principal Investigator Approval:

Signature: [redacted] Date: _____

Name: Eric Vallabh Minikel, PhD

NeuroNEXT Clinical Coordinating Center Approval:

Signature: [redacted] Date: 08-Sep-2026

Name: Merit Cudkowicz, MD

NeuroNEXT Data Coordinating Center Approval:

Signature: [redacted] Date: _____

Name: Christopher Coffey, PhD

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonization Good Clinical Practice (ICH GCP) and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT) Single Institutional Review Board (sIRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the sIRB before the changes are implemented to the study. In addition, all changes to the consent form will be sIRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
ASO	Antisense Oligonucleotide
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
C-CASA	Columbia Classification Algorithm for Suicide Assessment
CBC	Complete Blood Count
CDE	Common Data Elements
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CJD	Creutzfeldt-Jakob Disease
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
COI	Conflict of Interest
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CSS	Clinical Study Sites
CSSR-S	Columbia Suicide Severity Rating Scale
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCC	Data Coordinating Center
di-siRNA	Divalent Short Interfering RNA
DLT	Dose Limiting Toxicity
DM	Data Management
dpi	Days Post-Infection
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ED_{50}	Median Effective Dose
eGFR	Estimated Glomerular Filtration
ELISA	Enzyme-linked Immunosorbent Assay
exNA	Extended Nucleic Acid
FDA	Food and Drug Administration
FFI	Fatal Familial Insomnia
FSR	Final Study Report
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein

GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GSS	Gerstmann-Straussler-Scheinker Disease
hCG	Human Chorionic Gonadotrophin
Hct	Hematocrit
HgB	Hemoglobin
ICF	Informed Consent Form
ICH	International Council for Harmonization
INR	International Normalization Ratio
IT	Intrathecal
KO	Knockout
LAR	Legally Authorized Representative
LP	Lumbar Puncture
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
MGB	Mass General Brigham
MoCA	Montreal Cognitive Assessment
MRC	Medical Research Council
MRC-PDRS	Medical Research Council Prion Disease Rating Scale
MRI	Magnetic Resonance Imaging
MSM	Medical Safety Monitor
NeuroNEXT	Network for Excellence in Neuroscience Clinical Trials
NfL	Neurofilament
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	No Observed Adverse Effect Level
NPI-Q	Neuropsychiatric Inventory Questionnaire
PD	Pharmacodynamic
PID	Participant ID
PK	Pharmacokinetic
PLT	Platelets
PPI	Protocol Principal Investigator
PrP	Prion Protein
PSC	Protocol Steering Committee
RBC	Red Blood Cell Count
RBW	Red Cell
SAE	Serious Adverse Event
sIRB	Single Institutional Review Board
siRNA	Short Interfering RNA

SOA	Schedule of Activities
SOC	System Organ Class
WBC	White Blood Cell Count
WOCBP	Women of Child-Bearing Potential

SYNOPSIS

Prion disease is a fatal and incurable neurodegenerative disease caused by prion protein (PrP)[1]. PrP is encoded by a chromosomal gene, *PRNP*, and is expressed in the brains of all humans and all mammals. In prion disease, PrP undergoes post-translational misfolding into a self-replicating conformer known as a prion. Prions spread exponentially throughout the brain by recruiting other molecules of PrP, eventually accumulating to a level where neurotoxicity ensues. Subtypes of prion disease known historically by various names including Creutzfeldt-Jakob Disease (CJD), Fatal Familial Insomnia (FFI), and Gertsmann-Straussler-Scheinker Disease (GSS) all share this fundamental molecular mechanism. Prion disease typically presents clinically as a rapidly progressive dementia. 85% of cases are sporadic, apparently due to stochastic protein misfolding events, 15% are genetic, due to gain-of-function mutations in *PRNP*[2], and <1% of cases are acquired through exposure to exogenous prions via dietary or iatrogenic routes[3]. Regardless of etiology, once symptomatic, patients decline rapidly. Overall median survival is 5 months from first symptom[4], but often 3 months are spent in diagnostic odyssey[5]. The median survival from enrollment in previous trials was approximately two months[6,7]. Prion disease has no effective therapy and no standard of care. Those harboring genetic variants that cause the disease may know their risk status decades in advance of symptom onset, yet no measures exist to delay or prevent disease.

Extensive preclinical studies demonstrate that lowering PrP delays the onset and slows the progression of prion disease in diverse animal models[8–16]. While PrP lowering is effective across different disease stages, it is most effective at pre-symptomatic stages. In near-symptomatic or symptomatic mice, the overall survival benefit from ASO treatment was more modest (20% to 70% increase in survival time depending on the treatment timepoint and disease endpoint), and was derived mostly or entirely from slowing of symptomatic progression. Reversal of symptoms already accrued prior to dosing was not observed. In pre-symptomatic mice, chronic ASO treatment lowering PrP by 50% tripled survival time, and this arose mostly from an increase in healthy lifespan as quantified by weight gain, nest-building, and observation of symptoms[13,14]. Therefore, while PrP lowering is expected to be effective even in symptomatic patients, the greatest good that PrP lowering can do is to delay onset in pre-symptomatic at-risk individuals who still have full quality of life.

This therapeutic hypothesis has only just yielded its first clinical test: ION717, an antisense oligonucleotide (ASO) designed to lower PrP, entered a Phase I/IIa clinical trial (NCT06153966) in January 2024. This study reached primary completion date in July 2025 but an update from the sponsor has not yet been announced. There remains an urgent need to develop and test additional modalities capable of lowering PrP.

The primary objective of this trial is to evaluate safety and tolerability of investigational drug PrP-siRNA (referred to elsewhere[15] as 2439-s4 or 2439-exNA) in prion disease and in pre-symptomatic adult participants at genetic risk for prion disease. Secondary objectives are to evaluate the pharmacokinetics and pharmacodynamic impact of PrP-siRNA in these patient populations. In rats[17] and macaques[18], CSF PrP responds to brain PrP lowering, suggesting that measurement of PrP in the CSF may serve as an adequate surrogate of brain target engagement

for PrP-siRNA. Key exploratory endpoints will include measures of disease progression and biomarkers of neurodegeneration.

Study Title

Full title: An open-label, single ascending dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecally administered PrP-siRNA in adults diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.

Short title: Safety and pharmacology of PrP-siRNA in prion disease

Objectives

The primary and secondary objectives of this study are to evaluate the safety, tolerability, pharmacodynamics, and pharmacokinetics of intrathecally (IT) administered PrP-siRNA in prion disease and in pre-symptomatic adults at genetic risk for prion disease. Key exploratory endpoints will examine important measures of disease progression.

The objectives and endpoints are summarized in Table 1.

Table 1. Summary Overview of Objectives and Endpoints for this Clinical Trial. Unless otherwise noted, endpoints apply to all arms.

Ranking	Objective	Endpoint
Primary	To characterize the safety and tolerability of PrP-siRNA in participants diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.	Frequency of adverse events. In addition to adverse events reported by the patient or study partner, we will perform vital signs, physical exams, cognitive assessments, suicide severity assessment, and clinical laboratory assessments, any of which could contribute to the detection of adverse events.
Secondary	To characterize the pharmacokinetics of single dose PrP-siRNA in participants diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease	siRNA concentration in CSF and blood measured by mass spectrometry
	To characterize the pharmacodynamic effects of single doses of PrP-siRNA in participants diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease	Change from baseline CSF PrP concentrations measured by ELISA over time

	To compare change in CSF PrP over time in treated and untreated (Arms 1 and 2) participants diagnosed with symptomatic prion disease	Change from baseline CSF PrP concentrations measured by ELISA
Key Exploratory	To characterize potential measures of disease progression in participants diagnosed with symptomatic prion disease .	Survival MRC-PDRS
	To characterize potential indicators of disease onset or progression in pre-symptomatic participants.	

Study Design

This study consists of three arms:

1. An observational cohort study in symptomatic adults diagnosed with prion disease. No drug treatment will occur in this arm. The study will consist of a screening period, a baseline visit, and 4- and 8-week follow-up visits. Newly diagnosed patients will be offered the opportunity to enroll in this arm at any time when recruitment for Arm 2 is not open. After completing at least a 4-week follow-up visit, participants may switch from Arm 1 to Arm 2 at a later time (if Arm 2 resumes enrollment). At least 10 participants will be enrolled in Arm 1. Because Arm 1 participants may cross over to Arm 2 before completing an 8-week follow-up visit, and because attrition occurs in any clinical trial, this protocol allows for enrollment of up to 15 participants in Arm 1.

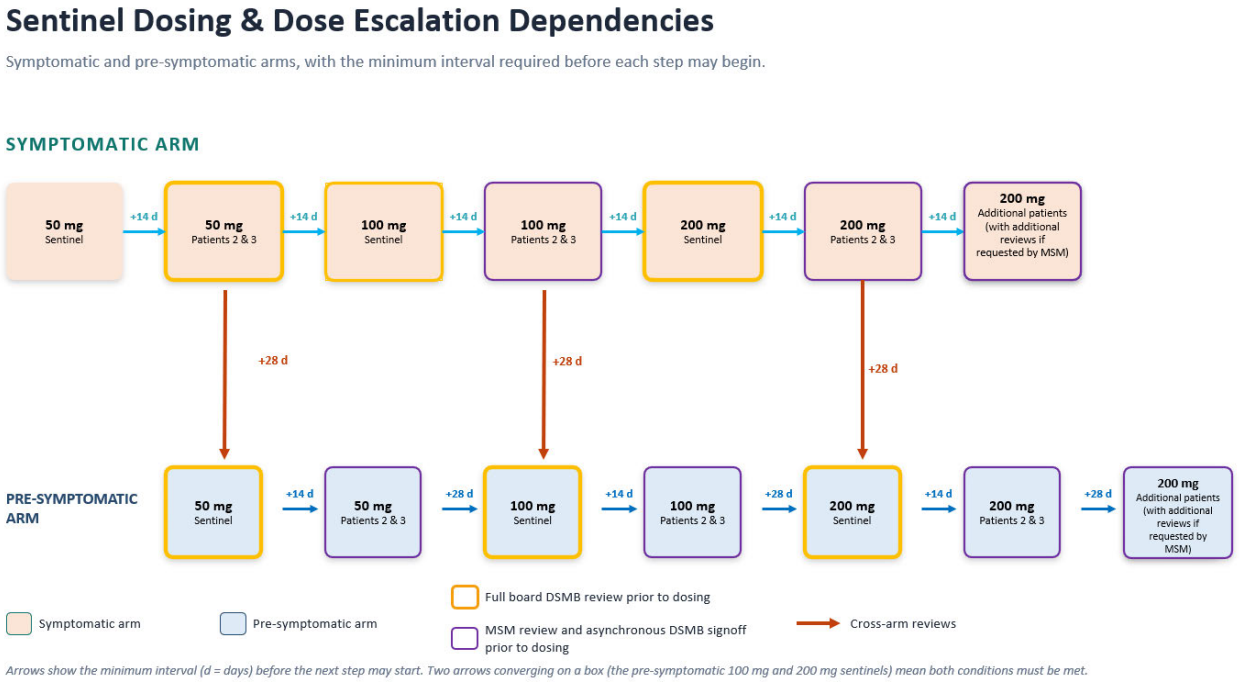
2. A first-in-human, open-label single ascending dose study in symptomatic adults diagnosed with prion disease. The study will consist of a screening period of up to 3 weeks, administration of a single drug dose, and a 24-week post-dose monitoring period. This arm will enroll up to 15 participants.

3. A first-in-human, open-label single ascending dose study in pre-symptomatic adults at genetic risk for prion disease. The study will consist of a screening period of up to 3 weeks, administration of a single drug dose, and a 24-week post-dose monitoring period. This arm will enroll up to 15 participants.

In Arms 2 and 3, cohorts of three participants each will be enrolled sequentially. Within each cohort, each participant will receive a single dose of one of three dose levels: 50, 100, or 200 mg. The first cohort will be dosed at the lowest dose. The dose chosen for each successive cohort will be determined based on reviews conducted by the Medical Safety Monitor (MSM) and the Data and Safety Monitoring Board (DSMB). After each review, the dose can be increased, held, or lowered. A dose can only be increased by one level across subsequent cohorts. The number of participants per dose level is targeted to be 3, 3, and 9 participants, respectively – but may change depending on the MSM and DSMB reviews. Each dose level will be evaluated in Arm 3 only after a minimum of 42 days since the sentinel participant in Arm 2 (symptomatic participants) and 28 days since the first 3 symptomatic participants received that same dose level in Arm 2, with

permission required from the MSM and DSMB to proceed. See Figure 1 for a diagram of DSMB reviews.

Figure 1. Sentinel Dosing & Dose Escalation Dependencies



Sentinel dosing will be employed with each new dose escalation. After a minimum of 14 days of post-dose safety for the first participant at that dose level, the MSM and DSMB will review all information collected for that participant to date and make a decision regarding whether the subsequent two participants in that cohort can be dosed. For Arm 3, this will also include review of all available safety data from Arm 2. The MSM and DSMB may also consider Arm 3 data in any Arm 2 reviews. This sentinel dosing review will be required for the first dose cohort at each level. It is not required for subsequent cohorts at a given dose level, but may be added if the MSM and/or DSMB request that it be added. Assuming the MSM and DSMB gives approval to advance, the second and third participants within a given dose level cohort will be treated at least one day apart. Participants will never be dosed on the same day.

For Arm 2 participants, upon review of a minimum of 14 days of available post-dose safety data from the 2nd and 3rd participant of a dose cohort, and minimum of 29 days of available post-dose safety data from the 1st participant of a dose cohort, the MSM and DSMB will conduct a review of all participants within the cohort to determine whether dosing can proceed as planned or needs to be modified in the next dose level cohort. For later cohorts (where a sentinel dosing review is not needed), the MSM and DSMB review will occur after all three participants have a minimum of 14 days of available post-dose safety data.

For Arm 3 participants, upon review of a minimum of 14 days of available post-dose safety data from the 2nd and 3rd pre-symptomatic participant of a dose cohort, and minimum of 29 days of available post-dose safety data from the 1st pre-symptomatic participant of a dose cohort, the MSM

and DSMB will conduct a review of all participants within the cohort, as well as all available safety data from Arm 2, to determine whether dosing can proceed as planned or needs to be modified in the next dose level cohort. For later cohorts (where a sentinel dosing review is not needed), the MSM and DSMB review will occur after all three participants have a minimum of 14 days of available post-dose safety data.

Interventions and Duration

The investigational drug, named PrP-siRNA, is a divalent siRNA sequence-targeted to bind and degrade the RNA molecule encoding prion protein (PrP), the protein that causes prion disease. Dosing of this siRNA is hypothesized to lower PrP concentration in the brain and cerebrospinal fluid (CSF). The investigational product is administered IT.

After informed consent is obtained, participants will be screened between Day -21 and Day 0.

In Arm 1, participants will undergo lumbar punctures (LPs) to collect CSF and undergo other study activities at baseline (Day 0) and at Weeks 4 and 8 (see Schedule of Activities for details).

In Arms 2 and 3, eligible participants will be admitted to the clinical trial center on Day -1 (if performing the Baseline visit over two days) or Day 0 to confirm continued eligibility and for pre-dose assessments including LP to collect CSF. Participants will then receive a single dose of PrP-siRNA via IT injection. Assessments during the initial admission period include vital signs, physical exam, electrocardiogram (ECG) monitoring, clinical labs, CSF labs and cognitive evaluation. Full neurological examination will be performed post-dose and prior to discharge from the clinical study center. Participants will be discharged on Day 2 and then periodically return to the study center on an outpatient basis at Week 4, 8, 12 and 24 for safety monitoring and study activities per SOA through the 24 week follow up period. Each outpatient study visit will include LP to collect CSF, phlebotomy, vital signs, physical exam, neurological exam, adverse event (AE) review, and suicide severity rating scale.

Sample Size and Population

This study will enroll up to 45 participants, with each arm enrolling up to 15 participants.

Key inclusion criteria for Arms 1 and 2 include: (1) clinically manifested symptoms of prion disease, in the opinion of the investigator; (2) a diagnosis of probable prion disease according to CDC criteria; (3) a positive CSF RT-QuIC or *PRNP* genetic test; (4) no more than moderate functional impairment as quantified by an MRC-PDRS score ≥15; and (5) availability of a study partner to assist with study procedures. Key inclusion criteria for Arm 3 includes: (1) lack of clinically manifested symptoms of prion disease, as demonstrated by an MRC Score of 20, a MoCA score between 24-30 (inclusive)[19], and a neurological exam with no clinically significant

findings, in the opinion of the investigator; and (2) a *PRNP* genetic test indicating the presence of a highly penetrant genetic variant causal for prion disease[20].

Key exclusion criteria for any arm include: (1) pregnancy; (2) contraindication to LP; or (3) recent participation in a different prion disease clinical trial. Full inclusion and exclusion criteria are provided in sections 4.1 and 4.2.

1 STUDY OBJECTIVES

The study objectives are summarized in Table 1 above and are detailed below.

1.1 Primary Objectives

The primary objective is to characterize the safety and tolerability of prion protein short interfering RNA (PrP-siRNA) in adults diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.

The primary endpoint serving this objective is the frequency of adverse events (AEs). Definitions are provided in Section 7 below. Note that in addition to AEs reported by the participant or study partner, we will also perform (as described in section 6.1) vital signs, physical exams, cognitive assessments, suicide severity assessment, and clinical laboratory assessments, any of which could contribute to the detection of AEs.

1.2 Secondary Objectives

Secondary Objective #1: To characterize the pharmacokinetics (PK) of single-dose PrP siRNA in adults diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.

The measurement of PrP-siRNA concentration by mass spectrometry in plasma and cerebrospinal fluid (CSF) will permit the study to characterize drug exposure in treated study participants.

Secondary Objective #2: To characterize the pharmacodynamic (PD) effects of single dose PrP-siRNA in adults diagnosed with symptomatic prion disease and in pre-symptomatic adults at genetic risk for prion disease.

This will be accomplished by measurement of PrP concentration in CSF by Enzyme-linked Immunosorbent Assay (ELISA)[17]. Each participant's CSF PrP concentration at each visit will be normalized to their own individual baseline prior to investigational drug administration. PrP will be measured at 4 and 8 weeks for arm 1 (untreated) participants. For arm 2 and 3 (treated) participants, PrP will be measured at 4, 8, 12, and 24 weeks post-dose, spanning the times when it is expected (based on nonclinical studies in mice) that PrP-siRNA target engagement may be maximal (4 weeks post-dose) and when PrP will have returned to within 20% of baseline (24 weeks post-dose).

Secondary Objective #3: To compare change in CSF PrP over time between treated and untreated (Arms 1 and 2) adults diagnosed with symptomatic prion disease.

Our longitudinal study of healthy controls and pre-symptomatic individuals at risk for prion disease has established that individuals have different baseline concentrations of CSF PrP, driven primarily by *PRNP* genotype, but that an individual's CSF PrP is longitudinally stable. In symptomatic individuals, longitudinal data are not yet available, and cross-sectional evidence

suggests that CSF PrP may decline as a function of disease process. The lumbar punctures (LPs) at 4 and 8 weeks post-baseline in Arm 1 will allow determination of whether CSF PrP exhibits longitudinal change post-diagnosis in individuals with symptomatic prion disease. Furthermore, since 4-week post-baseline data will be collected in both arms, individuals in Arm 2 will also be compared to untreated individuals in Arm 1.

1.3 Key Exploratory Objective

The key exploratory objectives are to characterize potential measures of disease progression in symptomatic participants with prion disease treated with PrP-siRNA (Arm 2) and to characterize disease onset or progression towards disease based on imaging and biochemical markers in pre-symptomatic participants (Arm 3). Exploratory endpoints serving this objective will include:

- Survival (time to death or life-extending measures)
- Medical Research Council Prion Disease Rating Scale (MRC-PDRS): A clinical measure of disease progression capturing ability to perform daily activities of life.

2 BACKGROUND

2.1 Rationale

Debilitating and rapidly fatal, prion disease has a profound unmet medical need. Prion disease is a fatal and incurable neurodegenerative disease caused by PrP[1]. PrP is encoded by a chromosomal gene, *PRNP*, and is expressed in the brains of all humans and all mammals. In prion disease, PrP undergoes post-translational misfolding into a self-replicating conformer known as a prion. Prions spread exponentially throughout the brain by recruiting other molecules of PrP, eventually accumulating to a level where neurotoxicity ensues. Subtypes of prion disease known historically by various names including Creutzfeldt-Jakob Disease (CJD), Fatal Familial Insomnia (FFI), and Gertsmann-Straussler-Scheinker Disease (GSS) all share this fundamental molecular mechanism. Prion disease typically presents clinically as a rapidly progressive dementia. 85% of cases are sporadic, apparently due to stochastic protein misfolding events. 15% are genetic, due to gain-of-function mutations in *PRNP*[2], and <1% of cases are acquired through exposure to exogenous prions via dietary or iatrogenic routes[3]. Regardless of etiology, once symptomatic, patients decline rapidly. Overall median survival is 5 months from first symptom[4], but often 3 months are spent in diagnostic odyssey[5]. The median survival from enrollment in previous trials was approximately two months[6,7]. Prion disease has no effective therapy and no standard of care. At-risk individuals may know their genetic status decades in advance of symptom onset, yet at present there are no medications nor lifestyle measures known to delay the onset of the disease. The therapeutic hypothesis of PrP lowering, has been validated extensively in preclinical studies (see below)[11,13,14,21,22]. However, this therapeutic hypothesis has only just yielded its first clinical test: ION717, an antisense oligonucleotide (ASO) designed to lower PrP, entered a Phase I/IIa clinical trial in January 2024. This study reached primary completion date in July 2025 but an update from the sponsor has not yet been announced. There remains an urgent need to develop additional modalities to address, delay, or prevent prion disease.

***PRNP* genotype can identify individuals very likely to develop prion disease, before any pathological changes occur.** The target population for this trial's Arm 3 is pre-symptomatic adults at high genetic risk for prion disease. All genetic cases of prion disease are caused by protein-altering germline DNA variants in the *PRNP* gene, encoding PrP. Although mutations that confer only low lifetime risk have been identified, a subset of mutations are known to confer a very high (estimated >90%) lifetime risk of developing prion disease[2,20]. The high penetrance of these genetic variants has been demonstrated by their segregation with disease in multiple multigenerational families, by their observation as *de novo* variants in certain patients, by survival curves constructed from over one thousand patients, and by their dramatic enrichment in prion disease cases relative to their extreme rarity in the general population[2,20,23]. This protocol is focused on individuals with these high risk mutations. This includes any subtype of prion disease, regardless of clinical syndrome classification as CJD, FFI, GSS, or other. Cross-sectionally, individuals with these high-risk genotypes exhibit normal biomarkers for most of their lives: their CSF RT-QuIC is generally negative, and their concentrations of CSF and plasma NfL and T-tau and other markers of neurodegeneration are indistinguishable from mutation-negative individuals[24,25]. When at-risk individuals do eventually convert to active disease, their RT-QuIC may become positive 1-3 years before symptom onset[25], but even in this period, CSF PrP levels remain unchanged, and any increase in markers of neurodegeneration such as plasma NfL often occurs only in the final months leading up to clinical onset, consistent with the rapidity of disease after onset[26,27].

All subtypes of adult prion disease share a molecular basis and will be included in this trial. The patient population for Arms 1 and 2 is symptomatic participants diagnosed with prion disease. This includes any subtype of prion disease, regardless of genetic, sporadic, or acquired etiology, and regardless of classification as CJD, FFI, GSS, or other. All of these types share PrP toxic gain of function as a root molecular cause. Prion disease strikes almost exclusively in adulthood, with peak risk of sporadic disease occurring in one's 60s, and some genetic subtypes striking more often in one's 50s. Only adult participants (not pediatric) will be included in this trial. Worldwide, the best predictor of observed prion disease case count is the level of investment in national surveillance systems tasked with diagnosis and ascertainment of cases, suggesting that the underlying disease incidence is similar across countries and populations[28]. In the United States (US), prion disease affects people of all racial and ancestry backgrounds, and the proportion of men and women affected is close to 50/50[29]. Any differences in reported incidence are likely attributable mostly to differences in health care access. The prevalence of genetic prion disease is driven mostly by the same three pathogenic mutations (P102L, D178N, and E200K) that have arisen independently through de novo events in dozens of families and in many countries worldwide[2,23]. No single founder mutation or ethnic population explains a large fraction of cases in the US. PrP-siRNA is designed to lower PrP, thus reducing the substrate for misfolding and neurotoxicity. This mechanism is expected to be able to address all forms of prion disease regardless of etiology or subtype.

PrP lowering delays and slows prion disease in animal models. The advent of transgenic and knockout (KO) mice in 1996 provided the first genetic evidence that PrP dosage governs the tempo of prion disease[11,30] (Figure 2A). These models are relevant because prion disease naturally afflicts not only humans but also diverse mammals including deer, elk, sheep, goats, cattle, camels, and mink. It is exceptionally well modeled in mice, where intracerebral

inoculation of prions into naive wild-type animals leads to fatal disease within 5-6 months, with all of the same pathological hallmarks seen in the human disease[31]. Heterozygous KO mice survive prion infection more than twice as long as wild-type mice[11] (Figure 2A), and homozygous KOs are incapable of propagating prions or developing prion disease[8]. Conversely, disease is accelerated in transgenic mice overexpressing PrP[30]. This finding has been replicated in conditional genetic models, where lowering or abolishing PrP expression can respectively delay or prevent development of disease[21,22]. More recently, we showed that chronic administration of an ASO lowering PrP by ~50% can triple survival in prion-infected mice[14] (Figure 2B). This result was obtained as long as therapy is initiated at any time from prior to infection up through 78 days post-infection (dpi), when animals are presymptomatic but neuropathological sequelae including astrogliosis and increase in plasma NfL are emerging[14]. Survival increase in early-treated animals was comprised mostly of an extension of healthy life. In animals treated at the cusp of frank symptom onset, a single dose of ASO was still able to increase survival by 70% (Figure 2C)[14]. Survival is directly responsive to PrP dosage: a low dose of ASO corresponding to 21% knockdown of *Prnp* RNA in cortex led to a significant 15% increase in survival time, but higher doses achieving deeper target engagement increased survival by larger amounts[14]. The efficacy of PrP lowering against prion disease in mice is dose-dependent, with no apparent minimum threshold to effect (Figure 2D). Pharmacologic reduction of PrP in the brain is also dose-dependently reflected in the CSF in rats (Figure 2E), suggesting the utility of CSF PrP by ELISA as a PD biomarker[17].

All of the proof-of-concept ASO studies described above were conducted with experimental rigor and transparency: animal monitoring and euthanasia decisions were taken by blinded technicians, all cause survival was reported as the primary endpoint, and individual-level animal data were publicly released along with source code sufficient to allow third parties to reproduce all of the reported statistics and figures (see https://github.com/ericminikel/prp_lowering/).

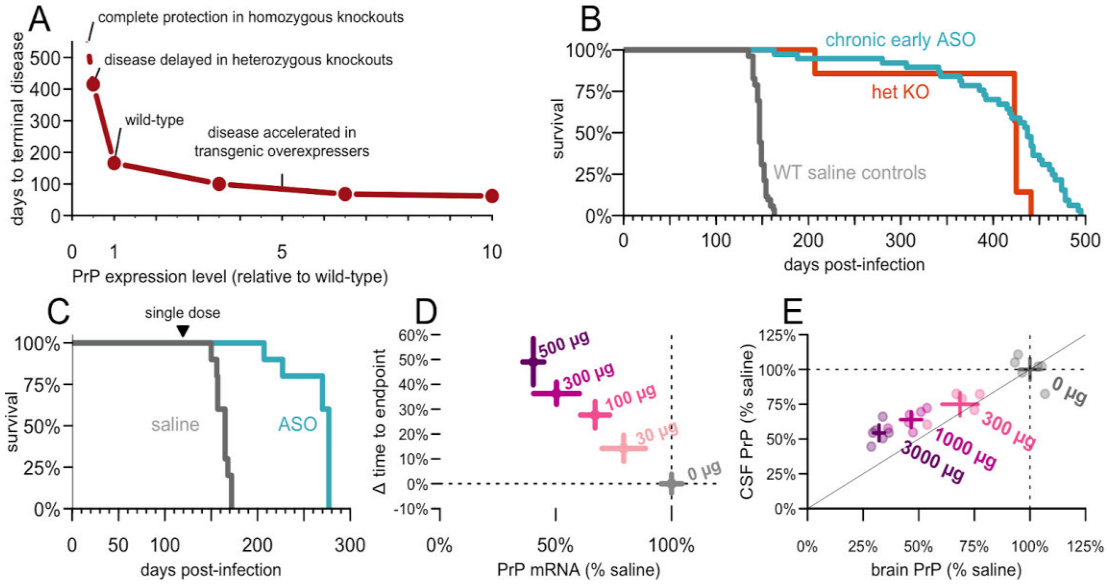


Figure 2. Published Genetic and Pharmacologic Proofs-of-Concept Validating PrP Lowering as a Candidate Therapy in Prion Disease. A) *Effect of PrP expression level on survival time after intracerebral prion inoculation in mice across wild-type, transgenic, and knockout lines.* ***B)***

Impact of chronic early ASO treatment on a symptomatic endpoint mirrors heterozygous knockout in mice. ***C)*** *A single ASO dose near the onset of symptoms delays terminal endpoint in mice.* ***D)*** *Continuous dose-response relationship between PrP and time to symptomatic endpoint in mice.* ***E)*** *CSF PrP mirrors brain PrP knockdown after ASO administration in rats. Panel A adapted from ref.* [30]*, B-D from ref.* [14]*, E from ref.* [17]*.*

Animal model and human genetic data suggest that PrP is non-essential and PrP lowering is well tolerated. Complete (homozygous) PrP KO mice, cows, and goats are all healthy and viable and initially defied efforts to identify any knockout phenotype other than resistance to prion infection[32–35]. It was eventually discovered that homozygous (but not heterozygous) PrP KO mice exhibit a myelin maintenance deficiency in peripheral nerves [but not in the central nervous system (CNS)] leading to a mild peripheral neuropathy[36]. This is apparently due to a lack of signaling of an N-terminal PrP peptide to Adgrg6, a receptor expressed on peripheral Schwann cells but not in the CNS[37]. While this myelin phenotype is histologically evident in aged animals, it is phenotypically subtle. Identifying a difference in grip strength, rotarod performance, or hot plate response is difficult, and some aged (60 week) PrP KO mice but not others exhibit a small but significant difference from wild-type animals, apparently as a function of mouse strain[36]. The histological phenotype has further replicated in PrP KO goats, where no outward phenotypic difference was observed[38]. Acute postnatal PrP lowering has also been observed to be safe in two conditional genetic mouse models[21,22] and with PrP-lowering ASOs[14]. We have identified older humans with naturally occurring heterozygous loss-of-function mutations in *PRNP* who appear to be healthy[2,39]. Moreover, such mutations occur in the population at exactly the frequency expected based on mutation rates. Unlike the vast majority of genes in the human genome, there appears to be no purifying selection against loss-of-function variants in *PRNP*[39]. The fact that no homozygous *PRNP* KO humans have yet been observed is expected based on mutation rates and the number of individuals sequenced, and thus does not constitute evidence that this genotype is not tolerated[39]. Overall, animal and human genetic studies support that PrP lowering itself may be well tolerated in the context of this therapeutic approach.

CSF PrP can report on PrP lowering in brain and serve as a target engagement biomarker. In rats[17] (Figure 2E) and macaques[18], the lowering of brain PrP by ASOs is mirrored in CSF, suggesting that measurement of PrP in the CSF may serve as an adequate surrogate of brain target engagement for PrP-siRNA. In some pre-symptomatic at-risk PRNP mutation carriers, CSF PrP is lower than in healthy controls without mutations[24,25], apparently due to altered metabolism and localization of the mutant protein[40]. Reduced CSF PrP in this population is not related to disease pathology because it is observed regardless of age and presence of pathological biomarkers, and these mutation carriers exhibit extremely stable concentrations of PrP in CSF over up to 6 years, with a within-individual coefficient of variation of just 9% over 6 years of follow-up[25]. Against such a stable long-term baseline in this population, an acute decrease in CSF PrP is expected to be readily detectable and report on drug target engagement.

2.2 Supporting Data

To supplement the evidence for PrP-lowering from genetic KO and ASO experiments, we also sought to validate the therapeutic hypothesis with a divalent siRNA tool compound sharing the same chemical backbone as our clinical candidate. In vitro screening identified oligo 1682 as promising, with 11.7% residual *Prnp* mRNA. In vivo, at 30 days post-dose this compound at 10 nmol (348 µg) yielded 49.4% residual PrP protein in whole brain hemispheres measured by ELISA. This is similar to the potency of PrP-lowering ASO tool compounds we have tested previously. (Note that PrP-siRNA is more potent than this tool compound). We advanced the tool compound into studies in a standard disease model: wild-type mice infected intracerebrally with the RML strain of mouse prions. Divalent siRNA treatment was initiated at different timepoints in two cohorts: 75 dpi, corresponding to a pathological disease stage (evinced by elevated neurofilament and astrogliosis), and at 126 dpi, approximately at the onset of symptoms. 75 dpi animals received chronic treatment every 120 days (q120d) while 126 dpi animals received a single dose. At each dosing timepoint, animals received 348 µg of divalent siRNA split between bilateral doses into CSF via the cerebral ventricle. Control animals received a non-targeting control extended nucleic acid (exNA) compound, saline, or no injection.

In the 75 dpi early chronic study (Figure 3A),1682-exNA animals significantly outlived controls by 2.7-fold (median 442 vs. 165 dpi, P = 0.0002, log-rank test), with disease-attendant weight loss both delayed and slowed (Figure 3B). In the 126 dpi single late dose cohort, 1682-exNA animals lived 3 months longer than controls (median 270 vs. 164 dpi, P = 0.0002, log-rank test, Figure 3C), eventually developing typical prion disease signs and succumbing to weight loss endpoint (Figure 3D). This amounts to a 64% increase in *total* survival time, or a 3.8x increase in *remaining* survival time from the moment of treatment at 126 dpi. The impact of 50% reduction of PrP on disease progression appears generally similar whether achieved by divalent short interfering RNA (di-siRNA), ASO, or heterozygous genetic KO[14,15], consistent with our mechanistic understanding of prion disease as strictly dependent upon PrP expression.

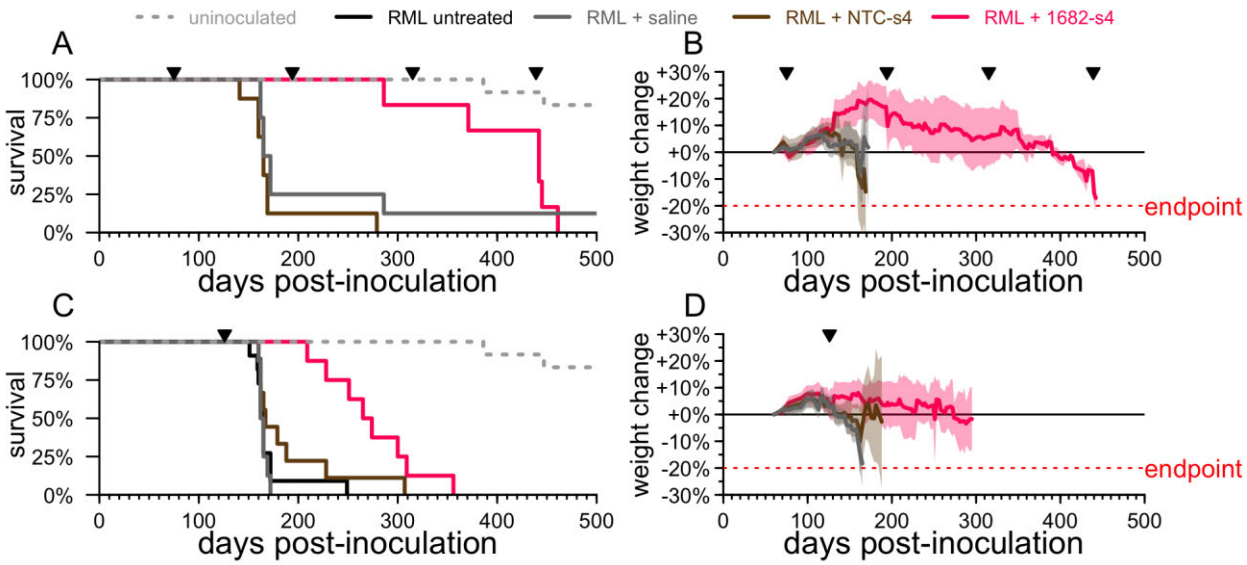


Figure 3. Efficacy of a Divalent siRNA Tool Compound in Mice with Prion Disease. A) *All-cause survival of RML prion-inoculated animals treated with 1682-exNA every 120 days beginning at 75 dpi, triangles indicate doses. N=8 per group.* ***B)*** *Weight change trajectories versus individual baseline in animals from (C), lines indicate means and shaded areas indicate 95%CIs.* ***C)*** *All-cause survival of RML prion-inoculated animals treated with a single dose of 1682-exNA at 126 dpi, triangle indicates dose. N=8-11 per group.* ***D)*** *Weight change trajectories versus individual baseline in animals from (E), lines indicate means and shaded areas indicate 95%CIs.*

Following extensive screening in cell culture and in transgenic mice expressing human PrP, we nominated PrP-siRNA as the lead candidate[15]. We performed a 3-point in vivo dose response experiment in human PrP mice with 5-fold dose increments (7, 35, and 174 µg) versus saline controls, N=8 per group, with harvest at 4 weeks. PrP protein in whole mouse brain hemispheres was dose-dependently reduced (Figure 4A). Six mouse brain regions were dissected for regional qPCR of the *PRNP* transcript. Individual dose-response curves for each scaffold/tail combination in each brain region yielded median effective dose (ED_{50}) for PrP-siRNA ranging from 5 µg in thalamus to 18 µg in cerebellum (Figure 4B).

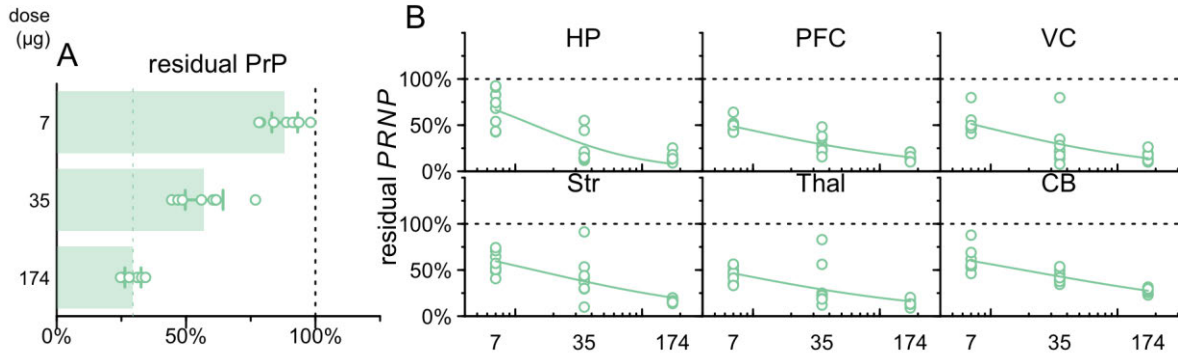


Figure 4. Dose-Response of PrP-siRNA. A) *Whole hemisphere residual PrP by ELISA (x axis) by dose (y axis). Each point is one animal, rectangular bars are means, error bars are 95% confidence intervals.* ***C)*** *Regional PRNP RNA by qPCR (y axis) versus dose (x axis). Each point is one animal. Curves are 4-parameter log-logistic dose-response curves.*

We tested the durability of the effect of PrP-siRNA in two studies. After a 348 µg dose we observed 17% and 40% residual PrP in whole hemispheres at 34 and 120 days post-dose (Figure 5A). After a 174 µg dose we observed 29%, 51%, and 74% residual PrP at 29, 91, and 180 days post-dose (Figure 5B).

We also explored the effect of a loading dose administered 7 days after the initial dose, and of repeat dosing after 90 days (Figure 5C). When the 348 µg dose was given at day 0, 7, and 90, the residual PrP in whole hemisphere at day 120 was 15%. We also evaluated a 52 µg dose which may more realistically correspond to dose levels reachable clinically (see Discussion). Compared to a single dose at day 0, a loading dose regimen (day 0 and 7) for 52 µg provided improved target engagement at day 30 (32% vs. 49% residual PrP). An additional dose at day 90 yielded 41% residual at day 120.

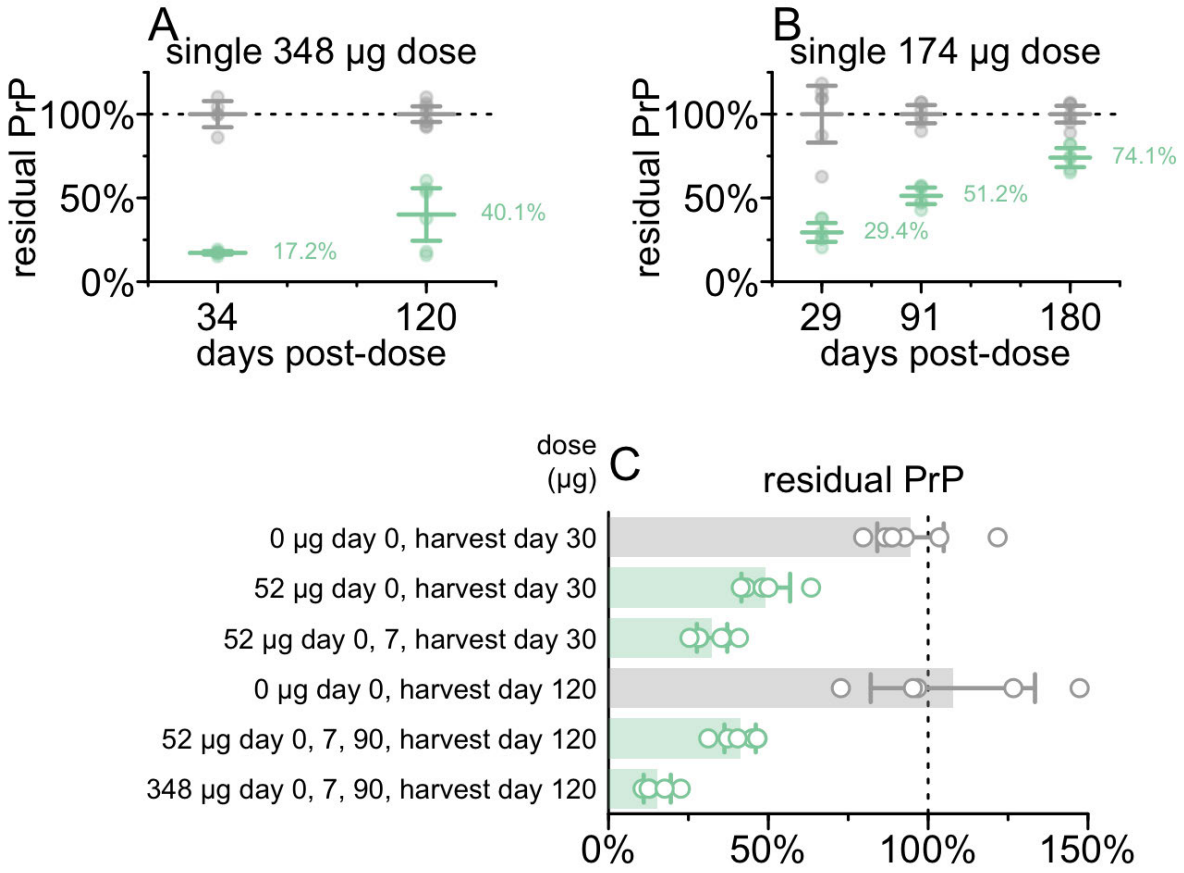


Figure 5. Characterization of lead compound PrP-siRNA. ***A)*** *Durability of effect of a single 348 µg dose in human PrP mice. Whole hemisphere PrP (y axis) versus months post-dose (x axis). Each point is one animal, horizontal line segments are means, error bars are 95% confidence intervals.* ***B)*** *Durability of effect of a single 174 µg dose in human PrP mice. Whole hemisphere PrP (y axis) versus months post-dose (x axis). Each point is one animal, horizontal line segments are means, error bars are 95% confidence intervals.* ***C)*** *Impact of repeat dosing regimens on target engagement in human PrP mice. Whole hemisphere PrP (x axis) for indicated dose levels and regimens (y axis). Each point is one animal, rectangular bars are means, error bars are 95% confidence intervals.*

Note that the durability of PrP-siRNA, with activity out to 4 or 6 months, compares favorably with the median ~2 month survival time of prion disease patients in prior randomized trials[6,7].

2.3 Risk/Benefit Assessment

This drug has never been tested in humans before and thus carries potential risks.

Nonclinical Good Laboratory Practice (GLP) toxicology studies were conducted by intrathecal (IT) administration of PrP-siRNA to rats and dogs, using the same Good Manufacturing Practice (GMP) batch of material that will be used in the clinical trial. In both species, there were no in-life findings, nor any pathology findings deemed adverse up to the maximum dose tested, which was 3 mg in rat and 200 mg in dog. In dogs given 200 mg, gliosis was observed at 24 hours post-dose in four of eight animals but was not associated with any necrosis or degeneration except focally at the injection site in one animal. In dogs given 60 mg, gliosis was observed in 1/8 animals at 24 hours post-dose. Gliosis was not observed in recovery animals at 28 days post-dose. Pathologists classified the findings as non-adverse. In rats, there were no pathological findings at any dose. Scaling based on CSF volume suggests that the maximum doses in these toxicology studies are approximately eight times higher than the maximum dose planned for this clinical trial, and 32 times higher than the starting dose. Nevertheless, no siRNA of this chemical configuration has ever been administered to humans before, and chemical features of this investigational drug such as its tetraethylene glycol linker and its exNA internucleotide linkages could have toxicological properties in humans not anticipated from nonclinical toxicology studies.

PrP-siRNA is not sequence-matched to the PrP gene in rat nor in dog, thus the above studies cannot assess on-target toxicity due to PrP lowering. As detailed in Section 2.1, decades of evidence from PrP KO animals (mice, cattle, goats) and recent evidence from heterozygous KO humans all support the non-essentiality of PrP. Acute postnatal PrP lowering has also been observed safe in two conditional genetic models[21,22] and with PrP-lowering ASOs[14]. Nevertheless, no data from acute PrP lowering in humans are presently available, leaving a potential risk of side effects not anticipated from animal models.

LP and IT drug administration are relatively safe procedures, however, some procedural AEs can occur including back discomfort, headache, bleeding, pain at the LP site, infection, and radicular pain.

There are at present no human data to suggest a direct benefit to participants in this trial. Participants may benefit from the opportunity to contribute to research and generate generalizable knowledge about PrP-siRNA and about prion disease.

3 STUDY DESIGN

This is a first-in-human single ascending dose open label study of an intrathecal divalent siRNA oligonucleotide drug in symptomatic participants diagnosed with prion disease (Arm 2) and in pre-symptomatic adults at genetic risk for prion disease (Arm 3), along with an observational study of participants meeting the same inclusion criteria and not treated with the investigational drug (Arm 1).

This study consists of three arms:

Arm 1. An observational cohort study in symptomatic adults diagnosed with prion disease. No drug treatment will occur in this arm. The study will consist of a screening period, a baseline visit, and 4- and 8-week follow-up visits. Newly diagnosed patients will be offered the

opportunity to enroll in this arm at any time when recruitment for Arm 2 is not open. After completing at least a 4-week follow-up visit, participants may switch from Arm 1 to Arm 2 at a later time (if Arm 2 resumes enrollment). At least 10 participants will be enrolled in Arm 1. Because Arm 1 participants may cross over to Arm 2 before completing an 8-week follow-up visit, and because attrition occurs in any clinical trial, this protocol allows for enrollment of up to 15 participants in Arm 1.

Arm 2. A first-in-human, open-label single ascending dose study in symptomatic adults diagnosed with prion disease. The study will consist of a screening period, administration of a single drug dose, and a 24-week post-dose monitoring period. This arm will enroll up to 15 participants, treated in cohorts of three individuals each.

Arm 3. A first-in-human, open label single ascending dose study in pre-symptomatic adults at genetic risk for prion disease. The study will consist of a screening period, administration of a single drug dose, and a 24-week post-dose monitoring period. This arm will enroll up to 15 participants, treated in cohorts of three individuals each.

Relationship between observational arm (Arm 1) and interventional arm in symptomatic participants (Arm 2). In lieu of a randomized, placebo-controlled trial, we will enroll participants in Arm 2 whenever permitted by our dose escalation and sentinel criteria. When enrollment in Arm 2 is paused for any reason, or is completed, we will enroll participants in Arm 1. If Arm 2 re-opens, Arm 1 participants who still meet inclusion criteria may have the opportunity to transition to Arm 2 (provided they have completed at least a 4-week follow-up visit in Arm 1). Scientifically, Arm 1 will provide a comparator group for longitudinal changes in biomarkers, including CSF PrP, as well as provide useful information on AEs and clinical progression. In particular, this trial relies on lowering of CSF PrP in Arm 2 as a measurement of PD target engagement. Arm 1 will help to control for any changes in CSF PrP that occur as part of the natural history of prion disease.

Relationship between Arm 3 (interventional arm in pre-symptomatic participants) to Arms 1 (observational arm in symptomatic participants) and 2 (interventional arm in symptomatic participants). Each dose level of PrP-siRNA will be evaluated in pre-symptomatic subjects in Arm 3 only after that dose has already been evaluated in symptomatic participants in Arm 2 and the Medical Safety Monitor (MSM) and DSMB have given clearance to continue onward to dose pre-symptomatic subjects. At each dose level, dosing of the sentinel pre-symptomatic patient may occur no sooner than 42 days after dosing of the sentinel symptomatic patient and 28 days after the dosing of the first 3 symptomatic participants at the same dose level (whichever comes later).

Relationship between schedule of activities and study endpoints. As indicated in the Schedule of Activities (SOA), all participants will complete a screening visit and then a baseline visit. Those in Arm 1 will not receive study drug and will be expected to undergo study visits at 4 and 8 weeks after the baseline visit. Those in Arms 2 and 3 will have drug administered during their baseline visit. Hospital admission at the baseline visit, for those in Arms 2 and 3, will allow close monitoring of safety for at least 24 hours following drug administration. AE reporting throughout the study will support the primary objective. Repeat LPs at 4, 8, 12, and 24 weeks post-dose will

allow monitoring of secondary endpoint PD biomarker CSF PrP, which is expected to decrease to its minimum level by 4 weeks post-dose as a result of drug treatment, then rebound gradually as drug washes out and to be within 20% of baseline by end of study at 24 weeks. Each participant's CSF PrP values will be compared to their own individual baseline, defined as the CSF PrP concentration at their baseline visit. The 4-week percentage changes in CSF PrP from individual baseline in symptomatic participants (Arm 2) will be further compared to the 4-week trajectories in an observational study of longitudinal CSF PrP change in participants meeting the same inclusion criteria but not treated with any drug (Arm 1). Blood and CSF collected at each visit will also permit measurement of the secondary endpoint of PK as well as exploratory biomarker endpoints. Clinical and imaging data collected at visits will also support exploratory objectives.

3.1 Scientific Rationale for Study Design

Rationale for intrathecal dosing. PrP-siRNA does not cross the blood-brain barrier. All proof-of-concept studies in animal models were conducted using intracerebroventricular administration into CSF, and nonclinical GLP toxicology studies were performed using IT administration. PK measurements in GLP toxicology animals indicate broad distribution of PrP-siRNA in the brain following IT dosing.

Rationale for single dose. A single dose is supported by the single dose GLP toxicology studies conducted. Target engagement studies in mice suggest that PrP-siRNA exhibits duration of action on par with the typical survival time in prion disease patients, suggesting that single dose pharmacology may occur over clinically relevant timelines.

Rationale for Arm 1 observational study in symptomatic adults. CSF PrP is lower in symptomatic prion disease patients than in patients with non-prion neurological diagnoses, likely due to a disease sink (PrP being incorporated into brain plaques instead of shedding into CSF)[41–45]. At present, the kinetics of CSF PrP decline within the natural history of prion disease is not well understood. There is minimal evidence for longitudinal decline in CSF PrP in symptomatic patients[43], and most historical samples are uninterpretable due to poor control of pre-analytical variables[45]. Arm 1 will therefore allow us to determine whether, and at what rate, CSF PrP declines after prion disease diagnosis, as well as establishing a trajectory of disease-related biomarkers and clinical progression in untreated patients.

Rationale for Arm 2 in symptomatic adults. This arm will allow us to assess the safety and tolerability of PrP-siRNA in symptomatic prion disease participants. Our secondary objective is to characterize PK and PD impact in this same population. Compared with a healthy volunteer study, it is important that PrP-siRNA is studied directly in prion disease patients who have 1) abnormally folded, distributed, and metabolized PrP protein, 2) lower brain and CSF concentrations of PrP, and 3) where PK assessments can reflect the biodistribution of PrP-siRNA within a diseased brain.

Rationale for Arm 3 in pre-symptomatic participants. Arm 3 will allow the study to assess the safety and tolerability of PrP-siRNA in pre-symptomatic subjects at risk for genetic prion disease. This is an important population for the potential application a PrP-lowering drug, in

which prevention of disease and preservation of full quality of life may be evaluated in a future pivotal trial. Our secondary objective is to characterize pharmacokinetics and pharmacodynamic impact in this same population. Compared with a healthy volunteer study, it is important that PrP-siRNA is studied directly in at-risk individuals who have 1) mutant PrP with a different mRNA sequence and PrP amino acid sequence, 2) altered metabolism of mutant PrP resulting in reduced steady-state CSF concentrations of PrP at baseline[24,25]. Compared with a symptomatic patient population (studied in Arms 1 and 2), it is important that we study at-risk individuals who 1) do not yet have clinical symptoms of prion disease, 2) do not yet have confounding changes in CSF PrP due to disease progression, and 3) do have PrP mutations (whereas a majority of symptomatic patients have sporadic disease with two wild-type *PRNP* alleles).

Rationale for open label design. In this small Phase I clinical trial, our power to characterize common AEs is maximized if all participants are treated with investigational drug. For symptomatic participants, the short survival time in prion disease and the long duration of action of PrP-siRNA renders a double cross-over trial design logistically infeasible. For pre-symptomatic participants, the stable baseline concentration of CSF PrP in this population makes it feasible to assess target engagement (PrP lowering by the siRNA) by comparing each individual to their own pre-treatment baseline, without needing to include a placebo arm.

Rationale for trial duration. In preclinical proof-of-concept studies, PrP lowering by PrP-siRNA was maximal at 1-month post-dose and PrP had begun to rise back towards its baseline by 3-4 months. Even at a dose higher than those tested in this trial, PD activity was limited by 6 months post-dose. Thus, the 24-week trial duration is anticipated to span the PD period of PrP-siRNA and thus when drug-related AEs are most likely to occur. The observational Arm 1 is limited to 8 weeks duration, making participation in this arm less burdensome for patients for whom no experimental drug is being administered.

Rationale for inclusion and exclusion criteria (Arms 1 and 2). Disease-specific molecular diagnosis is essential for ensuring that all participants in this trial have prion disease. Although CDC diagnostic criteria for prion disease[46] permit probable diagnosis based on clinical features plus imaging or other tests, the specificity of these criteria is not perfect as they do not measure the root cause of disease. One study showed that combining MRI with CSF RT-QuIC yields improved sensitivity to detect prion disease cases[47]. However, approximately 7% of non-prion disease cases were classified as MRI positive in that study. For this trial, specificity — strict exclusion of non-prion patients — is paramount to achieving the study objectives. RT-QuIC specificity for prion disease is uniformly high across published studies, ranging from 99-100%[48]. Therefore, in Arms 1 and 2 we further require either CSF RT-QuIC positivity, or the presence of a *PRNP* mutation. The *PRNP* mutation criterion will allow us to include genetic cases whose subtypes are often atypical and can be CSF RT-QuIC negative. Because our objective for Arm 2 is to evaluate PrP-siRNA in symptomatic prion disease participants, we require that participants have symptoms consistent with prion disease in the opinion of the Site Investigator. Among symptomatic patients, we have chosen to select individuals with only mild to moderate functional impairment [Medical Research Council Prion Disease Rating Scale (MRC-PDRS) ≥15], for two reasons. First, our studies in animal models indicate that while PrP lowering can extend survival even late in disease, we have never yet observed reversal of existing symptoms. Thus, in the long term, clinical development of PrP-siRNA should focus on patients with quality

of life remaining to preserve, and therefore, it is in this same population that we must characterize the safety and pharmacology of the investigational drug. Second, PrP protein has a half-life of ~5 days in vivo[49], which means that achieving full target engagement with a drug that lowers PrP at the RNA level will require a few weeks' time. Therefore, we must focus on individuals who are still early enough in the disease course that they are likely to survive long enough for the drug to reach full effect. Patients with lower MRC scores might die of disease progression before AEs and PD impact could be observed, thus subverting the objectives of the trial.

Rationale for inclusion and exclusion criteria (Arm 3). Because our objective is to evaluate PrP-siRNA in pre-symptomatic subjects at genetic risk for prion disease, we require that participants not have any symptoms of prion disease (such patients would be referred to Arms 1 and 2) and we require the presence of a highly penetrant *PRNP* genetic variant — those categorized as having evidence for high penetrance (Mendelian segregation or de novo variants observed) according to Goldman & Vallabh (Table S1)[20]. We have chosen not to include individuals with lower penetrance variants because they are unlikely to develop disease in their lifetime and therefore are not the target population for longer term clinical development of this investigational drug.

3.2 Justification for Dose

Our proposed starting dose for our first in human phase 1 clinical trial for PrP-siRNA in symptomatic and pre-symptomatic prion disease participants is a single dose of 50 mg IT administered. We have chosen a dose that allows reasonably rapid attainment of our phase 1 trial objectives: assessment of the investigational drug's safety, tolerability, or PK and PD profiles.

The route of administration is intrathecal. Oligonucleotide drugs do not cross the blood-brain barrier and are not available in the brain following systemic delivery. Delivery into CSF results in broad, albeit non-uniform, distribution throughout the CNS. This has been demonstrated clinically with ASOs and in nonclinical models for our divalent siRNA. IT administration of the clinical drug product 2349-exNA is consistent with the IT administration performed in animal toxicology studies completed to support its safety in nonclinical test species.

The dosing regimen is single dose. We chose a single dose regimen because our data from dosing humanized mice show that a single dose of drug can last for 3-6 months, which is longer than the typical survival time in prion disease. Thus, a single dose may produce meaningful pharmacology in humans. A single dose is supported by our single dose toxicology studies in rats and dogs. At present, FDA has cleared us only to administer a single dose to participants. Understanding the tolerability and pharmacology of a single dose is a first step towards eventually studying a repeat dosing regimen in this population.

We propose a starting dose of 50 mg, with escalation to 100 mg and 200 mg. In setting a starting dose, we considered the margins both by CSF volume calculation and by local injection concentration (dose formulation concentration) as suggested by communications from FDA[50]. In

Table 2, we compare our proposed dose levels in humans to the no observed adverse effect levels (NOAELs) for rat and dog, using CSF volumes from Emami et al[50,51].

Table 2. Proposed Dose Levels Compared to Toxicology Data.

species	CSF volume (mL)	dose volume (mL)	NOAEL (mg)	proposed doses (mg)	local concentration (mg/mL)	CSF concentration (mg/mL)	margin by local vs. rat	margin by local vs. dog	margin by CSF vs. rat	margin by CSF vs. dog
rat	0.25	0.03	3	–	100.0	12.0				
dog	16	3	200	–	66.7	12.5				
human	130	20	–	50	2.5	0.4	40.0	26.7	31.2	32.5
				100	5.0	0.8	20.0	13.3	15.6	16.3
				200	10.0	1.5	10.0	6.7	7.8	8.1

At the proposed starting dose of 50 mg, we have at least a 25-fold safety margin both by local concentration and by CSF concentration compared to the NOAELs from both species. At the proposed maximum dose of 200 mg, we have at least a 6.7-fold safety margin by all four calculation methods.

Three additional considerations support this choice of dose levels. First, our dose-response studies in humanized mice indicate that 50% PrP lowering is expected to be achieved somewhere within the 50 - 200 mg dose range, while deeper knockdown is expected to correspond to superior slowing of disease progression. Second, our Drug Product is formulated at 10 mg/mL, and 20 mL is a well-established volume for IT drug injections. Thus, 200 mg is an achievable dose level for a single administration. Third, the lower limit of quantification of the endotoxin assay performed on our Drug Substance allows us to establish that doses up to 200 mg still meet FDA endotoxin limits for IT injection.

3.3 Study Completion and End of Study Definitions

A participant is considered to have completed the study if he or she has completed all visits shown for their arm in the SOA.

The primary completion date is defined as completion of the last visit or procedure shown in the SOA for the last participant.

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

For Arms 1 and 2:

1. A diagnosis of probable prion disease according to CDC diagnostic criteria.
2. Positive CSF RT-QuIC, or a positive DNA test for a pathogenic *PRNP* mutation.
3. Symptoms of prion disease in the opinion of the Investigator.
4. Age ≥18 at the time of informed consent.
5. Meeting the criteria for prevention of pregnancy.
6. MRC-PDRS score ≥15 at the time of screening.
7. Provide written informed consent and be able and willing to comply with all study requirements (in the opinion of the Investigator), including travel to the Study Center, procedures, assessments, and visits. If the patient is not capable of providing consent, consent must be given by a legally authorized representative (LAR).
8. Study partner available to assist with study visits, who is sufficiently knowledgeable of the patient's condition to respond to inquiries about the patient for the duration of the trial.

For Arm 3:
1. Positive genetic test for a highly penetrant *PRNP* mutation. These are mutations where either de novo occurrence in prion disease cases or Mendelian segregation in families have been observed, indicating that a large majority of individuals harboring this mutation eventually develop prion disease. The high penetrance of these mutations is further supported by their high frequency in case series relative to their very low frequency in, or total absence from, general population cohorts[2]. See Goldman & Vallabh 2022 [ref.[20]] Table S1 for a review of the literature evidence supporting the penetrance of such mutations.
2. No symptoms of prion disease, as demonstrated by an MRC Score of 20, a MoCA score between 24-30 (inclusive)[19], and a neurological exam with no clinically significant findings, in the opinion of the investigator.
3. Age ≥18 at the time of informed consent.
4. Meeting the criteria for prevention of pregnancy.
5. Provide written informed consent and be able and willing to comply with all study requirements (in the opinion of the Investigator), including travel to the Study Center, procedures, assessments, and visits.

4.2 Exclusion Criteria

For Arms 1, 2, and 3:

1. Neurodegenerative disease such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, frontotemporal dementia, or Huntington's; untreated epilepsy; multiple sclerosis; or any other non-prion neurological condition that in the opinion of the investigator would pose a safety risk to the participant or confound interpretation of data from this trial.
2. Attempted suicide, suicidal ideation with a plan that required hospital admission and/or change in level of care within 12 months prior to Screening. For patients with (i) a suicide ideation score ≥ 4 on the C-SSRS within the last 12 months, or (ii) suicidal behaviors within the last 12 months (as measured by the answer “Yes” on any of the C-SSRS Suicidal Behavior Items), a risk assessment should be done by an appropriately qualified mental health professional (e.g., a Psychiatrist or licensed Clinical Psychologist). Any individual deemed by the Investigator

to be at significant risk of suicide, major depressive episode, psychosis, confusional state or violent behavior are excluded.
3. Platelet count < 50,000/mm3 , INR>1.5, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 3x upper limit of normal, estimated glomerular filtration (eGFR) of <60 mL/min/1.73m^2 at screening, or any other clinically significant laboratory abnormality at Screening that, in the opinion of the Investigator, would render a patient unsuitable for inclusion. The Investigator may choose to repeat a laboratory test that they suspect to have failed due to a technical failure, at their own discretion.
4. Clinically significant vital sign or electrocardiogram (ECG) abnormality at Screening (including heart rate < 45 bpm; SBP < 90 mmHg; confirmed BP readings > 170/105 mmHg).
5. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1.
6. Known active infection with hepatitis C, hepatitis B or HIV diagnosed by initial serological testing and confirmed with RNA testing, or prior treatment for hepatitis C. Testing for these infections is not included in the screening process; only prior knowledge of these infections, for example, in the patient's medical record, will be considered. Patients positive by serology, but negative by RNA may be deemed eligible by the Investigator in consultation with the MSM.
7. Malignancy within 5 years, except adequately treated non-melanoma skin cancer, carcinoma in situ successfully treated, or low-risk localized prostate cancer managed with active surveillance/watchful waiting and considered clinically stable in the opinion of the investigator. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be deemed eligible by the Investigator in consultation with the Medical Safety Monitor (MSM).
8. Participation in a different clinical trial of experimental (approved or investigational) therapy for prion disease within 4 months prior to Screening.
9. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator.10. Previous treatment with an oligonucleotide (including siRNA) within 4 months of screening. (This does not apply to mRNA vaccines, including COVID-19 vaccines.)
11. History of bleeding diathesis or coagulopathy, or current or anticipated use of warfarin, apixaban or other anti-coagulant or anti-platelet medication that cannot be safely held for the appropriate time prior to LP.
12. Any contraindication to repeated LPs, including bleeding disorders, brain or spinal diseases or deformities. Examples include but are not limited to mass lesions, obstructive hydrocephalus, ventriculoperitoneal shunt, Chiari malformations, significant spondyloarthropathy and spinal stenosis, significant connective tissue disorders.
13. Any contraindication or unwillingness to undergo MRI, e.g., metal implants, claustrophobia, agitation, or motor symptoms that precludes scans) or MRI or CT results that suggest a different diagnosis or that trial participation would be unsafe.
14. Have any other condition, which, in the opinion of the Investigator would make the patient unsuitable for inclusion or could interfere with the patient participating in or completing the study.
15. Patients unwilling to comply with the contraceptive requirements during the study period. (See section 6.2.4)
16. Female patient is breastfeeding, pregnant or planning a pregnancy.

4.3 Participant Study Enrollment Procedures

4.3.1 Participant Recruitment and Retention

Postings will be placed on Prion Registry (prionregistry.org) and with participating patient advocacy organizations such as Prion Alliance and CJD Foundation.

Participants may also be recruited from among Site Investigators' own patients. Special care will be taken to ensure patients are aware that their participation is completely voluntary and that their decision to participate will not affect their care. If the investigator has identified a patient as a potential candidate for the study, the investigator will either present the patient with a recruitment letter or offer the patient the opportunity to meet with a member of the study team. If the patient approaches the study team, then a study team member will discuss recruitment with the participant and their family member at the end of a scheduled clinic visit. The research coordinator will ask the patient if they would like to be contacted to hear about potential clinical trials. The coordinator will collect the patient's contact information and add it to a shared database. After the patient has been deemed potentially eligible to participate in this study by a doctor, a study team member will contact the participant and explain study in further detail and if the participant is interested, potentially complete a telephone prescreening.

Finally, participants who have undergone screening for other protocols in the Site Investigator's center and indicated that they are open to future contact about other study participation opportunities on telephone prescreens and consent forms may be contacted about participating in this trial.

4.3.2 Screening Documentation

Data related to eligibility or reasons for ineligibility for participants who have consented to participate will be maintained in the Electronic Data Capture System.

4.3.3 Informed Consent

Written informed consent will be obtained from each study participant or their LAR before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The consent form approved by the Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT) Single Review Board (sIRB) will be signed by the participant or LAR with the date of that signature indicated. The investigator will keep the original consent form(s), and a copy will be given to the participant. It will also be explained to the participant that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

Informed consent will be obtained by a physician investigator or a licensed nurse practitioner (NP) who are trained on the study and listed on delegation logs as being authorized to obtain consent. This responsible study staff member will be involved in explaining the study and will play a role in the consent process, answering any questions in conjunction with the appropriate study team member and ensuring that the process is conducted correctly. All participants will be offered the opportunity to discuss participation with a licensed physician Investigator and the participant's decision to accept or decline this opportunity will be documented in the research file. Participants will be consented under a consent form specific to the Arm they are enrolling in. Participants who crossover from Arm 1 to Arm 2 will be reconsented with an Arm 2 ICF prior to initiation of any Arm 2-specific activities.

Some participants in this study may lack the decisional capacity to provide informed consent due to cognitive impairment. After reviewing the consent form in full, participants will be asked to independently fill out an Informed Consent worksheet to assess their decisional capacity. This will evaluate the participant's understanding of the relevant information, appreciation of how the information applies to their situation, ability to reason with information to assess risks/benefits, and ability to express a clear choice regarding study participation. If the participant does not get all questions correct on the first attempt, the qualified study team member, or outside party, will review the details of the study again with the participant. The participant will then be given another opportunity to fill out the consent worksheet. If the participant does not answer all questions correctly on the second try, they will be considered unable to consent and therefore, for these individuals, consent will be obtained from a LAR. The relationship of the representative to the participant will be documented and kept for study records. The list of eligible LARs, in order of preference, is as follows:

1. Court appointed guardian with specific authority to consent to participation in research or authority to make health care decisions for a class of diagnostic and therapeutic decisions inclusive of the proposed research
2. Health care proxy/person with durable power of attorney with specific authority for making health care decisions inclusive of the proposed research
3. Spouse, adult child, or adult sibling. All must be (18+)

If the individual objects to participation, s/he will not be enrolled.

4.3.4 Treatment

This is an open-label study. All participants in Arms 2 and 3 will receive PrP-siRNA. Participants in Arm 1 will not receive study drug.

Symptomatic participants screened when Arm 2 is open to enrollment will be enrolled in Arm 2. Enrollment will be first come, first served, meaning that each eligible screened participant will be offered the next available spot in Arm 2. As detailed in section 5.1.5, for the first cohort treated at each of the three dose levels, there will be one sentinel participant, followed by a 14-day waiting period prior to dosing participants number 2 and 3. Two participants can never be dosed on the same day. Dose escalation may only occur after 28 days of follow-up data on participant 1, and 14 days on participants 2 and 3. Thus, if participants have already screened in

and been enrolled for all 3 spaces at the current dose level, there exists a minimum of 31 days before dose escalation will be possible.

Whenever Arm 2 is closed to enrollment, screened symptomatic participants will be offered a chance to enroll in Arm 1. Participants in Arm 1 may cross over to Arm 2 after their 4-week follow-up visit, if they still meet study inclusion criteria. If Arm 2 enrollment is paused because all spots at the current dose level have already been enrolled, then eligible participants may be offered the opportunity to enroll in Arm 1 as long as there is time and willingness to complete a 4-week follow-up visit. If Arm 2 enrollment is re-opened by the time the 4-week follow-up visit arrives, then an Arm 1 participant's 4-week follow-up visit may also serve as their baseline and drug administration visit for Arm 2.

Pre-symptomatic participants screened when Arm 3 is open to enrollment will be enrolled in Arm 3. Enrollment will be first come, first served, meaning that each eligible screened participant will be offered the next available spot in Arm 3. As detailed in section 5.1.5, for the first cohort treated at each of the three dose levels, there will be one sentinel participant, followed by a 14-day waiting period prior to dosing participants number 2 and 3. Two participants can never be dosed on the same day. Dose escalation may only occur after 28 days of follow-up data on participant 1, and 14 days on participants 2 and 3. Additionally, Arm 3 dosing is tied to Arm 2 dosing. Before the first participant of a given dose cohort in Arm 3 may be dosed, the MSM and DSMB will review at least 28d of safety data from participants 2 and 3 in the same dose cohort in Arm 2 and at least 42d of safety data from the same dose level sentinel participant in Arm 2. See Figure 1 for a diagram of DSMB reviews.

4.4 Participant Withdrawal Criteria

Participants are free to withdraw from participation in the study at any time upon request. Participants who withdraw will not be replaced.

5 STUDY INTERVENTION/INVESTIGATIONAL AGENT

5.1 Investigational Agent/Interventions, Administration, and Duration

5.1.1 Blinding

The dose administered in Arms 2 and 3 is unblinded. There is no blinding in this open label study.

5.1.2 Setting

Investigational drug administration will take place at the clinical study center. Participants will be admitted to the hospital for approximately 24 hours for post-dose monitoring.

5.1.3 Dosing Instructions

The injection site will be lumbar IT, between L2 to L5 unless the study Investigator's judgment determines that a different lumbar location is necessary. Non-lumbar dosing will not be permitted. The individual performing the LP (principal investigator or experienced nurse practitioner) will disinfect the skin according to institutional practices and may provide local anesthesia by injecting subcutaneous lidocaine, or similar, as deemed appropriate by the interventionalist. Upon insertion of an atraumatic spinal needle, they will collect up to 20 mL of CSF. A minimum of 5 mL CSF must be collected, otherwise the IT injection should not proceed. The syringe received from the Research Pharmacy containing the investigational drug (20 mL volume) will then be attached to the spinal needle, and the drug product will be slowly infused over the course of approximately 1-3 minutes.

Predose CSF collection of up to 20mL prior to injection of 20mL of investigation product ensures that the net administration procedure is isovolumetric. The CSF collected will be processed and stored for analysis.

For participants in Arm 1, only the predose CSF collection will occur; no drug will be administered.

5.1.4 Dosing Frequency

Each participant in Arm 2 will receive only a single dose of drug.

5.1.5 Sentinel Dose and Dose Escalation Scheme

In Arms 2 and 3, cohorts of three participants each will be dosed sequentially. Within each cohort, each participant will receive a single dose of one of three dose level: 50, 100, or 200 mg. The first cohort will be dosed at the lowest dose (50 mg). The dose chosen for each successive cohort will be determined based on reviews conducted by the MSM and DSMB. After each review, the dose can be increased, held, or lowered. A dose can only be increased by one level across subsequent cohorts. The number of participants per dose level is targeted to be 3, 3, and 9 participants respectively, but may change depending on MSM and DSMB reviews.

Sentinel dosing will be employed with each new dose escalation, with the first participant at each dose level serving as a sentinel. After observing a minimum of 14 days of post-dose safety data for the first participant at that dose level, the MSM and DSMB will review all information collected for that participant to date and make a decision regarding whether the subsequent two participants in the cohort can be dosed with PrP-siRNA. Even after the 14-day sentinel period has passed, doses should never be administered to more than one patient on the same day. This sentinel dosing will be required for the first cohort at each dose level. It is not required for subsequent cohorts at a given dose level, but may be added if the MSM and/or DSMB request that it be added.

Dose escalation for Arm 3 will be tied to dose escalation in Arm 2. Each dose level will be studied in Arm 3 only once 28 days have passed since the 3rd patient was dosed at that level in the

symptomatic Arm 2. (Note that timelines within Arm 2 mean that this will also be at least 42 days after the sentinel at that dose in Arm 2). In addition, the first participant in this Arm at each dose level will serve as a separate sentinel for the pre-symptomatic population. After observing a minimum of 14 days of post-dose safety data for the sentinel participant at that dose level, the MSM and DSMB will review all information collected for that participant (and arm 2 treated participants) to date and make a decision regarding whether the subsequent two participants in that cohort can be dosed with PrP-siRNA. Even after the 14-day sentinel period has passed, doses should never be administered to more than one participant on the same day. This sentinel dosing will be required for the first cohort at each dose level. It is not required for subsequent cohorts at a given dose level, but may be added if the MSM and/or DSMB request that it be added.

Assuming the MSM and DSMB gives approval to advance, the second and third participants within a given dose level cohort will be treated. Upon review of a minimum of 14 days of available post-dose safety data from the 2nd and 3rd patient of a dose cohort, and minimum of 29 days of available post-dose safety data from the 1st patient of a dose group, the MSM and DSMB will conduct a review of all participants within the cohort to determine whether dosing can proceed as planned or needs to be modified in the next dose level cohort. For Arm 3, this review also requires review of available safety data from Arm 2 to determine if escalation is permitted. For later cohorts (where a sentinel dosing review is not needed), the MSM and DSMB review will occur after all three pre-symptomatic participants have a minimum of 14 days of available post-dose safety data.

If participants withdraw from the trial or fail to make follow-up visits, it is possible that full AE data will not be available for the initial sentinel participant or all participants at a dose level. If this occurs, it will be at the MSM and DSMB's discretion to determine future dosing decisions and/or the need for an additional sentinel participant based on the available data.

5.2 Handling of Investigational Agent/Interventions

The drug product is formulated at 10 mg/mL in 5.3 mL vials with guaranteed 5 mL extractable volume, thus, a 50 mg dose can be obtained from each vial. Vials will be shipped as needed from a GMP storage facility to each participating hospital's Research Pharmacy. The starting dose is 50 mg, and we plan to escalate to doses of 100 mg and 200 mg (see dose escalation scheme below). Regardless of the dose level in milligrams, the dose volume is 20 mL. Thus, for the 50 mg and 100 mg doses, vials of drug product will be diluted in Elliotts B (Lukare Medical) at the Research Pharmacy. Regardless of dose level, the Research Pharmacy will provide a 20 mL syringe containing the indicated amount of drug to the investigator.

Because the investigational drug is administered IT, sterility and maintenance of low endotoxin are essential. Syringes will be filled and dilutions performed in sterile aseptic conditions by experienced staff. The amount of Drug Product and diluent to be mixed will be according to the following table:

Table 3. Volumes of Drug Product and diluent to be used in formulation of administered doses.

Dose level	Drug Product	Diluent	Total Volume
50 mg	5 mL	15 mL	20 mL
100 mg	10 mL	10 mL	20 mL
200 mg	20 mL	—	20 mL

The study drug solution will be administered to the participant within no more than 4 hours at room temperature after preparation and storage in the syringe.

In accordance with local regulatory requirements, the investigator, designated site staff, or head of the medical institution (where applicable) must document the amount of investigational product dispensed and/or administered to study participants, the amount received from the site research pharmacy, and the amount destroyed upon completion of the study. An investigator is responsible for ensuring that product accountability records are maintained throughout the course of the study. The inventory will include details of vials received and dispensed to participants, batch, and ID numbers. All unused vials must be kept until the site receives written instruction from the monitor to destroy the remaining investigational agent/device or to return it to the distributor or manufacturer. An accounting must be made of any drug deliberately or accidentally destroyed. Discrepancies between the amount of drug product received and dispensed drug must be reconciled.

5.2.1 Prohibited Medications/Interventions

PrP-siRNA is not an inhibitor, substrate, or potentiator of any known enzymes or transporters. A drug-drug interaction study revealed no evidence of any interactions with sufficient potency that a median effective concentration could be identified. Anti-coagulant / anti-platelet drugs (other than low-dose aspirin) are prohibited because they would increase the risk of AEs related to the LP. If an individual in the study has a reason to begin treatment with one of these drugs, they will be asked to discontinue the follow-up LPs, but they will be asked to continue repeated study visits if possible, to gather safety, blood biomarker and neurocognitive assessment data.

6 CLINICAL AND LABORATORY EVALUATIONS/STUDY PROCEDURES

6.1 Schedule of Activities

SCHEDULE OF ACTIVITIES – ARM 1

Activity	Pre- Screening	Screening	Baseline	Week 4	Week 8 or Early Term. Visit	Optional Safety Visit[11]	Optional Remote Follow-up Visit[12]
	Phone	Clinic	Clinical Study Center	Clinic	Clinic	Clinic	Phone/ Teleconference
		Day -21 to 0[1]	Day 0-1	Day 28 ±7	Day 56 ±7		
Written Informed Consent[2]		X					
Pre-Screen Questionnaire	X						
Inclusion/Exclusion Review		X	X				
Neurocognitive History/Medical	X[16]	X					
Demographics		X					
Physical Examination/Review of Systems		X	X[7]	X	X		
Neurological Examination/Prion Disease Checklist		X	X[8]	X	X		
Vital Signs[3]		X	X	X	X		
12-Lead ECG							
Clinical Safety Labs[4,6]		X	X	X	X		
Blood or urine collection for pregnancy testing (for WOCBP)		X	X[15]				
Concomitant Medication Review	X	X	X	X	X		X
Assess and Document Adverse Events[5]		X[14]	X	X	X	X	X
Columbia-Suicide Severity Rating Scale		X		X	X		X
Neuropsychiatric Inventory Questionnaire		X	X[9]	X	X		X
Montreal Cognitive Assessment		X	X[10]	X	X		X
Blood Biomarker Collection			X	X	X		
DNA Collection			X				
CSF Collection			X	X	X		
Brain MRI		X[13]			X		
MRC-PDRS	X	X	X	X	X		X
Exit Questionnaire					X		X

[1] Screening procedures must be completed within 21 days to 0 days prior to the Baseline Visit. Participants will not undergo screening for this study until they have already received a clinical diagnosis. Turnaround time for diagnostic tests such as CSF RT-QuIC is not included in the interval between screening and baseline/drug administration.
[2] During the Screening Visit, participants will be consented via the informed consent form (ICF).
[3] Vital signs include systolic and diastolic blood pressure, pulse rate /minute, respiratory rate/minute, temperature, and weight will be assessed at the visit. Height will be measured and recorded at the screening visit only. Weight will be measured and recorded at most once per day.

[4] Clinical safety labs include INR, complete blood count (CBC) with differential, comprehensive metabolic panel, CSF cell count and differential, CSF glucose and CSF total protein. Blood safety labs will be performed at screening and all subsequent visits. Serum pregnancy testing will occur in women of child-bearing potential at the Screening Visit.
[5] Adverse events that occur after signing the consent form will be recorded.
[6] CSF safety labs include CSF glucose and CSF total protein. CSF safety labs will be performed only at those visits where CSF is collected.
[7] Physical examinations/Review of Systems do not need to occur at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[8] Neurological examinations/Prion Disease Checklist do not need to occur at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[9] Neuropsychiatric Inventory Questionnaire does not need to be administered at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[10] Montreal Cognitive Assessment does not need to be administered at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[11] Optional Safety Visits may occur for suspected adverse events.
[12] If a participant is unable or unwilling to attend an in-person visit, the visit may be conducted by telephone at the discretion of the Site Investigator or licensed staff.
[13] If an MRI cannot be obtained or is uninterpretable (for ex. due scheduling constraints or patient movement artifact), eligibility may be supported by alternative brain imaging (prior MRI or CT) performed within the last 3 months, provided that the imaging results are available to the study team for review. If no prior imaging is already available, a brain CT scan may be performed during screening as an alternative to MRI to ensure there are no current safety concerns.
[14] Only AEs related to study procedures will be collected at the Screening Visit.
[15] Urine pregnancy will occur in women of child-bearing potential (WOCBP) at the Baseline visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.
[16]Medical history not performed at pre-screening.

SCHEDULE OF ACTIVITIES – ARMS 2 AND 3

Activity	Pre-Screening	Screening	Baseline	Week 1	Week 2	Week 4	Week 8	Week 12	Week 24 or Early Term. Visit	Optional Safety Visit[24]	Remote Follow-up Visit [25]
	Phone	Clinic	Clinical Study Center	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Phone/ Teleconference
		Day -21 to 0[1]	Day 0-1	Day 7 ±3	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±7	Day 168 ±3		
Written Informed Consent[2]		X									
Pre-Screen Questionnaire	X										
Inclusion/Exclusion Review		X	X[28]								
Neurocognitive/Medical History	X[31]	X									
Demographics		X									
Physical Examination/Review of Systems		X	X[6,20]	X	X	X	X	X	X		
Neurological Examination/Prion Disease Checklist[32]		X	X[7,21]	X	X	X	X	X	X		
Vital Signs[3]		X	X[8]	X	X	X	X	X	X		
12-Lead ECG		X	X[9]								
Clinical Safety Labs[4,19]		X	X[10]	X	X	X	X	X	X		
Blood or urine collection for pregnancy testing (for WOCBP)		X	X[29]								
Clinical Study Center Admission			X[11]								
Concomitant Medication Review	X	X	X[27]	X	X	X	X	X	X		X
Assess and Document Adverse Events[5]		X[28]	X[12]	X	X	X	X	X	X	X	X
Columbia-Suicide Severity Rating Scale		X		X	X	X	X	X	X		X
Neuropsychiatric Inventory Questionnaire		X	X[13,22]			X	X	X	X		X
Montreal Cognitive Assessment		X	X[14,23]			X	X	X	X		X
Blood Biomarker/PK Collection			X[15]			X	X	X	X		
DNA Collection			X[16]								
CSF Collection			X[17]			X	X	X	X		
Brain MRI		X[26]					X				
MRC-PDRS	X	X	X[18]			X	X	X	X		X

Activity	Pre-Screening	Screening	Baseline	Week 1	Week 2	Week 4	Week 8	Week 12	Week 24 or Early Term. Visit	Optional Safety Visit[24]	Remote Follow-up Visit [25]
	Phone	Clinic	Clinical Study Center	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Phone/ Teleconference
		Day -21 to 0[1]	Day 0-1	Day 7 ±3	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±7	Day 168 ±3		
Administer/Dispense Study Drug			X[30]								
Study Drug Accountability/Compliance			X								
Exit Questionnaire									X		X

[1] Screening procedures must be completed within 21 days to 0 days prior to the Baseline Visit. Participants will not undergo screening for this study until they have already received a clinical diagnosis. Turnaround time for diagnostic tests such as CSF RT-QuIC is not included in the interval between screening and baseline/drug administration.
[2] During the Screening Visit, participants will be consented via the informed consent form (ICF).
[3] Vital signs include systolic and diastolic blood pressure, pulse rate /minute, respiratory rate/minute, temperature, and weight will be assessed at the visit. Height will be measured and recorded at the screening visit only. Weight will be measured and recorded at most once per day.
[4] Clinical safety labs include INR, complete blood count (CBC) with differential, comprehensive metabolic panel. Blood safety labs will be performed at screening and all subsequent visits. Serum pregnancy testing will occur in women of child-bearing potential at the Screening Visit.
[5] Adverse events that occur after signing the consent form will be recorded. Between consent and dosing including the Screening Visit, only adverse events related to study procedures will be collected.
[6] Physical Examination/Review of Systems should occur pre-infusion and 1hr (+/- 0.5hrs) post-infusion of study drug at the Baseline visit.
[7] Neurological Examination/Prion Disease Checklist should occur pre-infusion at the Baseline Visit. Neurological examination should also occur 1hr (+/- 0.5hrs) post-infusion of study drug at the Baseline visit. Prion Disease Checklist may be omitted for pre-symptomatic participants at the Baseline visit.
[8] Vital signs should occur pre-infusion and 0hr (up to +0.5hr), 1hr (+/- 0.5hrs), 4hrs (+/- 2hrs) and 24hrs (+/- 6hours) post-infusion of study drug at the Baseline visit.
[9] 12-Lead ECG should occur 1hr (+/- 0.5hrs) post-infusion of study drug at the Baseline visit.
[10] Clinical safety labs should occur pre-infusion and 24hrs (+/- 6rs) post-infusion of study drug at the Baseline visit.
[11] Clinical Study Center Admission should occur prior to study drug administration at the Baseline visit.
[12] Adverse Event Assessment and Documentation should occur 1hr (+/- 0.5hrs) and 24hrs (+/- 6hrs) post-infusion of study drug at the Baseline visit.
[13] Neuropsychiatric Inventory Questionnaire should occur pre-infusion of study drug at the Baseline visit.
[14] Montreal Cognitive Assessment should occur pre-infusion of study drug at the Baseline visit.
[15] Blood biomarker/PK sample collection should occur pre-infusion. Also, 4 hours (+/- 30mins) and 24hrs (+/- 1hr) post-infusion of study drug at the Baseline visit.
[16] DNA collection should occur pre-infusion of study drug.
[17] CSF collection will occur prior to study drug administration at the Baseline visit. (seeding (CSF RT-QuIC), neurodegeneration (CSF NfL, CSF T-tau, CSF beta-synuclein, plasma brain-derived tau, plasma NfL)
[18] MRC-PDRS should occur pre-infusion of study drug at the Baseline visit.
[19] CSF safety labs include CSF glucose and CSF total protein. CSF safety labs will be performed only at those visits where CSF is collected.
[20] Physical examinations/Review of Systems do not need to occur at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[21] Neurological examinations/Prion Disease Checklist do not need to occur at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.

[22] Neuropsychiatric Inventory Questionnaire does not need to be administered at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[23] Montreal Cognitive Assessment does not need to be administered at the Baseline Visit if the Screening Visit occurs within 5 calendar days of the Baseline Visit.
[24] Optional Safety Visits may occur for suspected adverse events.
[25] If a participant is unable or unwilling to attend an in-person visit, the visit may be conducted by telephone at the discretion of the Site Investigator or licensed staff.
[26] If an MRI cannot be obtained or is uninterpretable (for ex. due scheduling constraints or patient movement artifact), eligibility may be supported by alternative brain imaging (prior MRI or CT) performed within the last 3 months, provided that the imaging results are available to the study team for review. If no prior imaging is already available, a brain CT scan may be performed during screening as an alternative to MRI to ensure there are no current safety concerns.[27] Concomitant Medication Review should occur prior to study drug administration at the Baseline visit.[28] Inclusion/Exclusion review should occur prior to study drug administration at the Baseline visit.
[29] Urine pregnancy will occur in women of child-bearing potential at the Baseline visit prior to study drug administration and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.
[30] Collections and assessments should be timed from the start of the infusion at the Baseline Visit.
[31]Medical history not performed at pre-screening.
[32]Prion Disease checklist may be omitted for pre-symptomatic participants unless there is suspicion of disease onset.

Study Schedule

This section describes the trial procedures that will occur for Arm 1 and 2. All participants will sign the ICF prior to undergoing any study tests or procedures.

Study Visit Recommendations:

1. Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit – Day 0.
2. Outcomes for individual participants should be measured by the same evaluator throughout their participation. Evaluators must be qualified, trained, certified, and delegated. Training documentation must be filed in the Investigator Site File (ISF).

If a participant is unable or unwilling to complete an assessment in full, any partial data obtained should be recorded, along with any documentation of the reason the assessment was incomplete.

Pre-Screening

Participants will be pre-screened over the phone or via videoconference by study staff to determine eligibility for screening and to ensure that the participant is safe to undergo an LP and other study assessments. These procedures include:

- Obtain prion disease diagnosis history (Arms 1 and 2) or genetic testing history (Arm 3)
- Obtain family history, medical history, and neurocognitive history
- Perform MRC-PDRS
- Review and document concomitant medications and therapies
- Discuss willingness and desire to participate in a clinical trial and understanding of the difference between treatment and research

This activity is necessary for determining whether the prospective participant should be scheduled for a screening visit.

Arm 1
Screening Visit

The following procedures will be performed in-person, after informed consent is obtained, to determine the participant's eligibility and are listed in preferred order to be completed:

- Obtain written informed consent
- Assess inclusion and exclusion criteria
- Obtain medical history
- Obtain neurocognitive history
- Obtain demographics
- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist
- Measure vital signs including height and weight
- Collect blood samples for clinical screening assessments
- Collect blood or urine for pregnancy testing (for WOCBP only)
- Review and document concomitant medications
- Assess and document AEs (only AEs related to study procedures are collected at the Screening Visit)
- Administer the C-SSRS screening questionnaire
- Administer NPI-Q screening questionnaire
- Administer MoCA screening assessment
- Administer MRC-PDRS screening assessment
- Brain MRI or CT scan

Screen Failures

Any participant who signs the consent form will be considered enrolled in the trial. If a participant fails screening, *at a minimum* the following information is captured and entered into the electronic data capture system:

- Demographics
- Reason for screen failure
- Any eligibility criteria that were assessed prior to when the research participant failed to meet an inclusion criterion or met an exclusion criterion.

Safety labs required for the site investigator to determine eligibility, can be repeated during the screening period, if necessary.

If a participant is re-screened for eligibility after the initial screening window ends, they must complete a full Screening Visit, including informed consent, as outlined in section 6.1.

Baseline Visit

This visit will take place in-person after the Screening Visit. The following procedures will be performed and are listed in preferred order to be completed:

- Assess inclusion and exclusion criteria
- Perform physical examination/review of systems*
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers
- Collect blood for DNA
- Collect blood or urine for pregnancy testing (for WOCBP only)
- Perform LP for clinical safety labs and CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer NPI-Q baseline questionnaire*
- Administer MoCA baseline assessment*
- Administer MRC-PDRS baseline assessment

***The following assessments do not need to occur at the Baseline Visit if the Baseline Visit occurs within 5 calendar days of the Screening Visit:**

- Physical examination/Review of systems
- Neurological examination/Prion Disease checklist
- NPI-Q
- MoCA

Week 4 Visit

This visit will take place in-person 28 ± 7 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs

- Administer the C-SSRS questionnaire
- Administer MRC-PDRS assessment
- Administer NPI-Q questionnaire
- Administer MoCA assessment

Week 8 Visit or Early Termination

This visit will take place in-person 56 ± 7 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire
- Administer NPI-Q questionnaire
- Administer MoCA assessment
- Administer MRC-PDRS assessment
- Brain MRI

Administer exit questionnaire**The exit questionnaire will only be administered to participants leaving the trial entirely.

Early Termination Visit

Participants who terminate their participation in the study early will be asked to be seen for an in-person Early Termination Visit.

Arms 2 and 3

Screening Visit

The following procedures will be performed in-person, after informed consent is obtained, to determine the participant's eligibility and are listed in preferred order to be completed:

- Obtain written informed consent
- Assess inclusion and exclusion criteria
- Obtain medical history and neurocognitive history
- Obtain demographics
- Perform physical examination/review of systems

- Perform neurological examination/prion disease checklist*
- Measure vital signs including height and weight
- Perform 12-lead ECG
- Collect blood samples for clinical screening assessments
- Collect blood or urine pregnancy testing (for WOCBP only)
- Review and document concomitant medications
- Assess and document AEs (only AE's related to study procedures are collected at the Screening Visit)
- Administer the C-SSRS screening questionnaire
- Administer NPI-Q screening questionnaire
- Administer MoCA screening assessment
- Administer MRC-PDRS screening assessment
- Brian MRI or CT scan

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Baseline Visit

This visit will take place in-person after the Screening Visit. Collections and assessments should be timed from the start of the infusion at the Baseline Visit. The following procedures will be performed and are listed in preferred order to be completed:

- Assess inclusion and exclusion criteria (pre-infusion)
- Perform physical examination/review of systems (pre-infusion)*
- Perform neurological examination/prion disease checklist** (pre-infusion)*
- Collect vital signs (pre-infusion)
- Collect blood samples for Clinical Safety Labs (pre-infusion)
- Collect blood for PK/biomarkers (pre-infusion)
- Collect blood for DNA (pre-infusion)
- Collect blood or urine for pregnancy testing (for WOCBP) (pre-infusion)
- Review and document concomitant medicationsAdminister NPI-Q baseline questionnaire (pre-infusion)*
- Administer MoCA baseline assessment (pre-infusion)*
- Administer MRC-PDRS baseline assessment (pre-infusion)
- Perform admission to clinical study center (pre-infusion)
- Perform LP for CSF collection (pre-infusion)
- Administer investigational product
- Study drug accountability and compliance
- Collect vital signs (0hr (+0.5hr), 1hr (+/- 0.5hrs), 4hrs (+/- 2hrs) and 24hrs (+/- 6hrs) post-infusion)
- Assess and document AEs (1hr (+/- 0.5hrs) and 24hrs (+ 6hrs) post-infusion)

- Perform physical examination/review of systems (1hr (+/- 0.5hrs) post-infusion)
- Perform neurological examination (1hr (+/- 0.5hrs) post-infusion)
- Perform 12-lead ECG (1hr (+ 0.5hrs) post-infusion)
- Collect blood for PK/biomarkers (4 hours (+/- 30mins) and 24hrs (+/- 1hr) post-infusion)
  Collect blood samples for Clinical Safety Labs (24hrs (+/- 6hours) post-infusion)

*The following assessments do not need to occur at the Baseline Visit, if the Baseline Visit occurs within 5 calendar days of the Screening Visit:

- **Physical examination/review of systems (pre-infusion only)**
- **Neurological examination/Prion Disease checklist (pre-infusion only)**
- **NPI-Q**
- **MoCA**

**Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 1 Visit

This visit will take place in-person 7 ± 3 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 2 Visit

This visit will take place in-person 14 ± 3 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 4 Visit

This visit will take place in-person 28 ± 7 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers/potential PK samples
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire
- Administer NPI-Q questionnaire
- Administer MoCA assessment
- Administer MRC-PDRS assessment

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 8 Visit

This visit will take place in-person 56 ± 7 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers/potential PK samples
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire
- Administer NPI-Q questionnaire
- Administer MoCA assessment
- Administer MRC-PDRS assessment
- Brain MRI

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 12

This visit will take place in-person 84 ± 3 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers/potential PK samples
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire
- Administer NPI-Q questionnaire
- Administer MoCA assessment
- Administer MRC-PDRS assessment

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

Week 24 Visit or Early Termination

This visit will take place in-person 168 ± 3 days after the Baseline Visit. The following procedures will be performed and are listed in the preferred order to be completed:

- Perform physical examination/review of systems
- Perform neurological examination/prion disease checklist*
- Collect vital signs
- Collect blood samples for Clinical Safety Labs
- Collect blood for biomarkers/potential PK samples
- Perform LP for CSF collection
- Review and document concomitant medications
- Assess and document AEs
- Administer the C-SSRS questionnaire
- Administer NPI-Q questionnaire
- Administer MoCA assessment
- Administer MRC-PDRS assessment
- Administer exit questionnaire**

*Prion Disease Checklist may be omitted for Arm 3 participants unless there is suspicion of disease onset

** The exit questionnaire will only be administered to participants leaving the trial entirely.

The below sections define each of these activities and explain the justification for their inclusion in the study.

6.1.1 Written Informed Consent

Participants will be given adequate time to become familiar with the study and ask questions. An investigator or qualified staff member will meet with the potential participant and their LAR to review the details of the study using the ICF as a guide. This discussion will include all the required elements of informed consent, including the purpose of the research, the procedures to be followed, the risks and discomforts, the potential benefit of contributing to generalizable knowledge, and alternative procedures to study participation. Their questions will be answered to their satisfaction. The participant will be provided with adequate time to reflect on the potential benefits and risks and possible discomforts of participation, and to make an informed decision. A trained study investigator or licensed NP will then obtain written informed consent from each participant prior to the initiation of any study procedures. All potential participants will be given the opportunity to speak with the Site Investigator per current sIRB policies should a non-Physician investigator or licensed NP be involved in obtaining informed consent.

This activity is necessary for enrollment into the study.

6.1.2 Demographics

Sex, age, and self-reported race and ethnicity will be collected.

This activity is necessary for trial record keeping and mandatory reporting to NIH.

6.1.3 Neurocognitive & Medical History

A delegated licensed professional will take a detailed medical history, particularly regarding neurological and cognitive symptoms and the diagnosis of prion disease.

This activity is necessary for determining whether the participant meets inclusion/exclusion criteria.

6.1.4 Inclusion/Exclusion Review

A member of the study team will interview the participant (and study partner, for Arms 1 and 2) about each item in the inclusion/exclusion list in order to assist in determining eligibility.

This activity is necessary for determining whether the participant meets inclusion/exclusion criteria.

6.1.5 Vital Signs

Vital signs will be obtained after the participant has been in a seated or lying down position for a minimum of five minutes. Vital signs, including systolic and diastolic blood pressure, pulse rate /minute, respiratory rate/minute, temperature, and weight will be assessed at each visit. Height will be measured and recorded at the first visit only.

This activity is necessary for determining whether the participant meets inclusion/exclusion criteria and for safety monitoring.

6.1.6 Physical & Neurological Examination

Physical and neurological exams will be performed by the Site Investigator or other licensed clinical co-investigator. The following systems will be examined; general appearance, head, eyes, ears, nose, throat, neck, chest, heart, abdomen, extremities, edema, peripheral vascular, skin and appendages, musculoskeletal, and back. Neurological examination will occur at all study visits and will consist of assessment of mental status, cranial nerves, motor and somatosensory functions, reflexes, cerebellar function, and gait. In Arms 1 and 2, a prion disease checklist will also be administered at each exam to capture prion-related symptoms.

This activity is necessary for safety monitoring.

6.1.7 Adverse Event Review

A delegated licensed medical professional will interview the participant and their study partner about any AEs experienced since the last visit and review the status or resolution of any previously reported AEs. AEs that occur after signing the consent form will be recorded.

This activity is necessary for the study's primary objective of determining safety and tolerability.

6.1.8 Concomitant Medication Review

A delegated licensed medical professional will interview the participant and their study partner about the participant's concomitant medications.

This activity is necessary for assessing inclusion/exclusion criteria and for safety monitoring.

6.1.9 Columbia-Suicide Severity Rating Scale (C-SSRS)

The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA)[52]. The C-CASA was developed to assist in coding suicidality data accumulated during the conduct of clinical trials of

antidepressant drugs. The Columbia Suicide Severity Rating Scale (C-SSRS) will be utilized in this trial. The C-SSRS is a rater/clinician-administered tool that involves a series of questions designed to assess severity and intensity of suicidal ideation, types of suicidal behaviors, and lethality of suicide attempts. This scale will be performed at all visits except for the Baseline visit. The full version C-SSRS (lifetime, and within the last 6 months) will be administered at the screening visit. At subsequent visits, we will assess suicidality occurring since the previous visit.

This activity is necessary for assessing inclusion/exclusion criteria and for the study's primary objective of determining safety and tolerability.

6.1.10 Phlebotomy

Blood will be collected by standard phlebotomy at each visit. Approximately 40 mL will be obtained and processed for plasma, serum, buffy coat and, if applicable, DNA.

This activity is necessary for confirming eligibility per the inclusion/exclusion criteria, for performing clinical safety labs (see below) that inform the study's primary objective of evaluating safety and tolerability, for secondary endpoints and exploratory endpoints.

6.1.11 DNA Collection

At the time of phlebotomy during the screening visit, a tube of blood will be obtained for DNA extraction.

This activity is necessary for secondary and exploratory endpoints; genotype information may also be used in stratification analyses related to any of the study's endpoints.

6.1.12 CSF Collection

The injection site will be lumbar IT, between L2 and L5 unless the study Investigator's judgment determines that a different lumbar location is necessary. Non-lumbar dosing will not be permitted. The individual performing the LP (principal investigator or experienced nurse practitioner) will disinfect the skin according to institutional practices and may provide local anesthesia by injecting subcutaneous lidocaine, or similar, as deemed appropriate by the interventionalist. Upon insertion of an atraumatic spinal needle, they will collect up to 20 mL of CSF. In the case of the day of dosing in Arm 2, a minimum of 5 mL CSF collection is also necessary to ensure isovolumetric drug administration – otherwise the IT injection should not proceed. The CSF collected will be processed, stored, and analyzed for drug concentration (PK), PrP concentration (PD biomarker), and markers of prion disease and neurodegeneration. LPs may, at site PI's discretion, be performed by hospital Interventional Radiation (IR) departments at visits after baseline. IR may not perform the LP at the baseline visit. If the LP cannot be performed at baseline for any reason, the participant will be excluded from the study. Since LPs will be performed in a seated position, opening pressure will not be measured. If there is reason for concern about intracranial pressure in the investigator, safety monitor, and/or DSMB's opinion based on relevant AEs, then subsequent LPs may be performed in lateral decubitus (LD)

with an opening pressure measurement on a specific patient-by-patient basis, justified as part of the workup for those AEs.

This activity is necessary for the study's PK and PD secondary endpoints and for its exploratory endpoints.

6.1.13 Clinical Safety Labs

The following safety labs will be conducted, with rationale as provided for each:

Blood safety labs, to be conducted at each blood draw:

- INR. Coagulation in the normal range is essential in order for a LP to be safe.
- Complete Blood Count (CBC) with Differential. Although never observed for divalent siRNA, some other ASOs have been associated with thrombocytopenia. The tests included in a CBC with differential are:
    - White Blood Cell Count (WBC)
    - Nucleated Red Blood Cell Count (optional)
    - Red Blood Cell Count (RBC)
    - Hemoglobin (Hgb)
    - Hematocrit (Hct)
    - Mean Corpuscular Volume (MCV)
    - Platelets (PLT)
    - Red Blood Cell Distribution Width – Coefficient of Variation (RDW)
    - Neutrophil/Segmented % or Absolute Neutrophil Count
    - Lymphocytes % or Absolute Lymphocyte Count
    - Monocytes % or Absolute Monocyte Count
    - Eosinophils % or Absolute Eosinophils Count
    - Basophil % or Absolute Basophils Count
    - Immature Granulocyte % or Absolute Immature Granulocyte
- Serum human chorionic gonadotrophin (hCG) for WOCBP (collected only at the Screening Visit)
- Comprehensive Metabolic Panel (CMP). Oligonucleotide drugs are generally metabolized through liver and kidney. If liver enzymes become elevated or kidney function declines, this could be indicative of drug toxicity. The tests required for a CMP are:
    - Protein, Total
    - Albumin
    - Bilirubin, Total
    - Bilirubin, Direct
    - Alkaline Phosphatase
    - AST (SGOT)
    - ALT (SGPT)
    - Glucose
    - Blood Urea Nitrogen (BUN)
    - Creatinine
    - eGFR

- Sodium
- Potassium
- Chloride
- Calcium
- Carbon Dioxide

Urine safety labs

- Urine hCG pregnancy testing for WOCBP (collected at the Baseline Visit, and as necessary throughout course of study)

CSF safety labs, to be conducted at each CSF collection:

- CSF Cell Count and Differential: An increase in CSF cell count could be indicative of immune reaction or blood/brain barrier compromise.
- CSF Glucose. CSF glucose could be indicative of changes in brain metabolism.
- CSF Total Protein. CSF total protein increase could be indicative of toxicity for an IT drug or progression of underlying disease.

This activity is necessary for determining eligibility for LP (INR) and for the study's primary objective of determining safety and tolerability (all tests).

6.1.14 12-Lead ECG Monitoring

The heart's electrical activity will be recorded from 12 electrodes placed on the skin at screening and immediately after dosing for participants in Arm 2. Cardiac arrhythmias are common drug side effects, and the ECG will allow monitoring for these after drug administration. Toxicokinetic studies indicate drug concentrations peak 0.5 - 4.0 hours post-dose and drop rapidly in the 24 hours post-dose, meaning that any cardiac side effects (though none were observed in toxicology studies) would be most likely to occur soon after dosing. ECGs will be reviewed by the Site Investigator or a licensed medical professional actively working on the trial. If there are no concerns, the Site Investigator or licensed medical professional can sign the ECG and store in the participants binder. If the ECG is abnormal, a cardiologist may be consulted for additional follow-up and evaluation.

This activity is necessary for the study's primary objective of determining safety and tolerability.

6.1.15 Clinical Study Center Admission

Admission to the clinical study center for at least 24 hours after dosing will allow careful monitoring of AEs in the period immediately following drug administration in Arm 2.

This activity is necessary for the study's primary objective of evaluating safety and tolerability.

6.1.16 Brain MRI or CT Scan

A multi-sequence 3T MRI scan will be conducted using the UCSF-Quest Diagnostics Partnership Dementia Care Pathway protocol (https://memory.ucsf.edu/sites/memory.ucsf.edu/files/wysiwyg/UCSFQuest_Protocol_v1.0.pdf), with recommended adjustment for rapidly progressive dementia. This will consist of: 1) Localizer and Calibration/Reference Scan (if necessary); 2) Sagittal 3D T1 MPRAGE/IR-SPGR (angled to midline); 3) Axial 2D T2* GRE (angled to AC/PC); 4) Axial 2D T2 turbo-FLAIR (angled to AC/PC, matches scan 3); 5) Axial 2D turbo/fast spin echo (angled to AC/PC, matches scan 3); 6) Axial 2D ***B2000*** Diffusion Weighted Imaging (angled to AC/PC); 7) Coronal 2D ***B2000*** Diffusion Weighted Imaging (angled to AC/PC).

Brain imaging must be completed as part of screening to confirm eligibility for enrollment. If an MRI can not be obtained or is uninterpretable (for ex. due scheduling constraints or patient movement artifact), eligibility may be supported by alternative brain imaging (prior MRI or CT) performed within the last 3 months, provided that the imaging results are available to the study team for review. If no prior imaging is already available, a brain CT scan may be performed during screening as an alternative to MRI to ensure there are no current safety concerns.

This activity is necessary for the study's primary objective of evaluating safety and tolerability as well as for the exploratory objective of evaluating disease progression.

6.1.17 MRC Prion Disease Rating Scale (MRC-PDRS)

The MRC-PDRS, or MRC Scale for short, measures cognitive function and ability to conduct activities of daily living. Because prion disease is very rapidly progressive and severe, other scales suffer from floor effects when rating the progression of prion disease patients. The MRC Scale was developed specifically to provide dynamic range throughout the progression of prion disease up to death[53].

For Arms 1 and 2, the minimum score of 15 for the inclusion criteria ensures that the patient's disease is not yet too advanced to enroll. While the domains first affected by prion disease will vary from patient to patient, as a general rule, a patient with a score of ≥15 is one who can still walk, eat, use the bathroom, and speak in full sentences.

For Arm 3, all pre-symptomatic participants must obtain a score of 20 (the maximum possible score), indicating no functional impairment. Any individual with a score <20 has functional impairment and is not eligible to enroll in Arm 3 (they may be screened for the symptomatic arms of this trial, Arm 1 or Arm 2).

This activity is necessary for assessing inclusion/exclusion criteria and for the study's exploratory objective of evaluating disease progression.

6.1.18 Cognitive / Behavioral / Functional Assessment

This assessment will consist of two measures as defined below.

Montreal Cognitive Assessment: MoCA is a commonly utilized questionnaire in clinical trials and research settings to measure levels of cognitive impairment. The MoCA measures five areas of cognitive function: orientation, visuospatial, attention and calculation, recall, and language. The MoCA version 8.3 will be used and will take approximately 10 minutes to complete.

Neuropsychiatric Inventory Questionnaire (NPI-Q): is a short informant questionnaire, which can be completed in an estimated 5 minutes by a pre-symptomatic patient or a symptomatic patient's study partner[54].

This activity is necessary for the study's exploratory objective of evaluating disease progression.

6.1.19 Administer Investigational Drug

Details of investigational drug administration are provided in section 5.1.3 above.

This activity is necessary for all study primary, secondary, and exploratory objectives.

6.1.20 Exit Questionnaire

This qualitative interview will gather information on participant and, when applicable, study partner perceptions of disease progression and experience of clinical trial participation.

This activity is necessary for the study's exploratory objective of evaluating disease progression.

6.2 Timing of Study Activities

6.2.1 Timing of Activities and Study Visits

All screening activities may occur concurrent with one another, and all will be completed prior to drug treatment and after obtaining informed consent.

Allowable time windows for conducting follow-up visits are shown in the SOA. If participants are unable to complete a visit within the indicated time window, study staff will do their best to accommodate a visit as close to the window as possible.

In the event that a participant is unable or unwilling to continue with in-person study visits, telephone/remote follow-up visits may be conducted at the discretion of the Site Investigator or licensed staff member. These visits should capture safety and study assessments that can be reliably obtained remotely. All information collected during these telephone follow-up visits must be documented in accordance with the protocol and the site's local policies. A clinic visit must only be performed remotely if the participant is unable or unwilling to attend in person. If a Week 4 visit is performed remotely, this may constitute a major deviation. Any other visit performed remotely should be recorded as a minor deviation.

6.2.2 On Study/Off-Intervention Evaluations

Because this is a single-dose study, there is no such thing as discontinuation of study drug. Individuals who have received drug will be expected to complete follow-up visits until/unless they choose to voluntarily withdraw from the study. Participants who choose to discontinue LPs will be encouraged to complete other aspects of remaining study visits to enable safety monitoring.

6.2.3 Contraceptive Requirements

Females of child-bearing potential must be willing to use a highly effective method of contraception from 30 days before dose administration until 6 months after dose administration or until study completion.

Birth control methods which are considered highly effective include bilateral tubal occlusion, placement of an intrauterine device or intrauterine hormone-release system, surgical sterilization of male partner, established use of oral, implantable, injectable, or transdermal hormonal methods of contraception.

A female of child-bearing potential includes individuals who have experienced menarche and who are not postmenopausal or permanently sterilized. A post-menopausal state is defined as the absence of menses for 12 months without an alternative medical cause.

Males with partners of child-bearing potential must be surgically sterilized or agree to use a condom throughout study participation.

6.2.4 Pregnancy

A serum human chorionic gonadotropin (hCG) test will be required at screening in women of child-bearing potential. A urine hCG test will be required at the Baseline Visit and as necessary during the study in women of child-bearing potential. Pregnancy testing is only repeated if there is a concern for pregnancy. Participants will not be eligible for the study if the pregnancy test is positive.

Pregnancy occurring within 12 months after the final dose of IP (single dose) must be reported to the study team as soon as possible after Investigator awareness.

Follow-up for pregnancies in any participant is required. The pregnancy will be followed for outcome of the parent and child (including any premature terminations), and outcome information will be documented in the study records when available.

If the Investigator becomes aware of a pregnancy in the partner of a study participant who has received IP, this must also be reported to the study team as soon as possible after Investigator

awareness. Partner pregnancy does not necessarily preclude continued study participation for the enrolled participant; however, reporting and follow-up are required. Follow-up information will be collected when the partner provides consent. Participants who are capable of becoming pregnant will be advised not to attempt pregnancy for at least 12 months after their last dose of the study drug and should consult with their healthcare provider before attempting to become pregnant.

6.2.5 Protocol Deviations

A protocol deviation is any noncompliance with the current clinical trial protocol. The noncompliance may be on the part of the participant, the PI or Co-Is, or the study staff. A major protocol deviation is a deviation that may impact subject safety, affect the integrity of study data, and/or affect subjects' willingness to participate in the study. Any other type of deviation will be classified as minor. As a result of deviations, corrective and preventative actions will be developed by the PI and implemented promptly. All deviations from the protocol must be addressed in the participant's source documents. Protocol deviations will be sent to the sIRB per their guidelines and entered in the Protocol Deviations Log for the study.

6.2.6 Arms 1 and 2: Documentation of Prion Disease

Prion disease will be determined by CDC diagnostic criteria and must include either CSF RT-QuIC performed by the National Prion Disease Pathology Surveillance Center, and/or presence of a pathogenic or likely pathogenic variant in *PRNP* upon laboratory sequencing. Results will be recorded in the participant's medical record.

6.2.7 Arm 3: Documentation of Genetic Risk of Prion Disease, and Lack of Prion Disease Symptoms

Genetic risk for prion disease will be determined based on PRNP gene sequencing indicating the presence of a genetic variant with evidence for high penetrance according to Goldman & Vallabh[20]. Participants must provide a genetic testing report to confirm eligibility. The absence of symptomatic prion disease will be determined by an MRC Score of 20, a MoCA score between 24-30 (inclusive)[19], and a neurological exam with no clinically significant findings in the opinion of the investigator. Results will be recorded in the participant's medical record.

6.2.8 Medical History

A medical history will be taken at the screening visit by study staff.

6.2.9 Treatment History

No effective therapeutic for prion disease exists; thus, no prior treatment is scientifically relevant to this study. Nevertheless, any treatment history, for example symptomatic or palliative treatment, will be included in the medical history collected by study staff at screening.

6.2.10 Concomitant Medications/Treatments

All concomitant medications, dietary supplements and/or other treatments should be documented.

6.2.11 Protocol Amendments and Study Termination

All revisions and/or amendments to this protocol must be approved in writing by the Sponsor and the sIRB. The Investigator will not make any changes to the conduct of the study or the protocol without first obtaining written approval from the Sponsor and the sIRB, except where necessary to eliminate an apparent immediate hazard to a study participant. The study team will determine whether reconsent of participants is required after a protocol amendment.

The Sponsor reserves the right to discontinue the study at a clinical study site(s) for safety or administrative reasons at any time. Should the study be terminated and/or the clinical study site closed for any reason, all investigational agents pertaining to the study must be returned to the Sponsor or its representative and all documentation will remain stored per policy.

6.2.12 Laboratory Evaluations

The Site Investigator will review laboratory test results and determine whether any qualify as AEs; if so, they will be classified, and actions taken if indicated, per the instructions in section 7.2.

6.3 Details of Laboratory Endpoints

6.3.1 Pharmacokinetics

The investigational drug will be quantified in CSF and plasma using a mass spectrometry-based assay developed and validated atSouthern Research.

6.3.2 Pharmacodynamic Biomarker

CSF PrP will be measured using an ELISA-based method developed at the Broad Institute[17] as well as by a different ELISA method validated atMRI Global.

PrP is highly susceptible to plastic adsorption, and if CSF samples are not handled properly, PrP can exhibit high (100-fold) pre-analytical variability from sample to sample. To mitigate this issue and render PrP reliably quantifiable, we have established a protocol which meticulously specifies the early addition of detergent immediately after sample collection, to prevent plastic adsorption, together with use of uniform tubes and uniform pipetting and aliquoting practices. CSF samples must be aliquoted and frozen within two hours of collection. See the Laboratory Procedures Manual for details. This protocol was established in our natural history study of presymptomatic individuals at risk for prion disease, in which pre-analytical variability was well-controlled, and we observed longitudinal stability of CSF PrP concentration in the absence of drug treatment[25].

CSF for this PD endpoint will be collected and processed according to this same protocol. Studies using PrP-lowering antisense oligonucleotides in rats and cynomolgus macaques have established that CSF PrP reflects pharmacologic lowering of brain PrP[17,18]. Thus, we expect that

lowering of brain PrP by PrP-siRNA should be quantifiable by serial measurement of PrP in CSF. Because each individual has a different baseline level of CSF PrP, each individual will be compared their own pre-drug baseline, see statistical plan for details.

6.3.3 CSF RT-QuIC

CSF RT-QuIC is a seeded amplification assay that monitors fibrillization of recombinant PrP in a wellplate to determine whether CSF contains prion "seeds." The readout is binary (positive/negative). This is part of the standard diagnostic pipeline for prion disease and will be ordered as part of routine clinical care through the National Prion Disease Pathology Surveillance Center; reported results will inform eligibility for this trial. Additional RT-QuIC analyses will be performed to support the study's exploratory objective of evaluating disease progression.

6.3.4 Biomarkers of Neurodegeneration

CSF NfL, T-tau, beta-synuclein, plasma NfL, brain-derived tau, and GFAP will be quantified using Quanterix Single Molecule Immunoassay (Simoa) and/or ProteinSimple Ella assays. These will support the study's exploratory objective of evaluating disease progression.

6.3.5 DNA Analysis

PRNP genotype impacts the rate of disease progression and biomarker change in prion disease[55], and other variants genome-wide are reported to impact disease as well[56], so DNA sequencing will be performed to support exploratory endpoints and to enable exploratory post-hoc analyses of the primary and secondary endpoints stratified by *PRNP* genotype.

7 MANAGEMENT OF ADVERSE EVENTS

The AE definitions and reporting procedures provided in this protocol comply with all applicable FDA regulations and International Council for Harmonization (ICH) guidelines. The Site Investigator will carefully monitor each participant throughout the study for possible AEs. All AEs will be documented on case report forms (CRFs) designed specifically for this purpose. It is important to report all AEs, whether serious or non-serious.

FDA and sIRB requirements for reporting AEs will be followed. Participants will be monitored for AEs from the time they provide informed consent until 24 weeks following the one-time administration of study drug. AEs that occur between consent and administration of study drug will be reported only if related to study procedures.

7.1 Definitions of Adverse Events, Suspected Adverse Drug Reactions & Serious Adverse Events

An AE is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device, whether or not considered related to the drug product or device.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there may be a causal relationship to the medicinal product.

Examples of AEs include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), Stable chronic conditions (i.e., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity would be considered as worsened and therefore would be recorded as AEs. Events that are identified as being related to prion disease should be reported as AEs if their condition is worse than at the time of the Baseline Visit. The worsening condition should be marked as an exacerbation of a pre-existing condition on the AE CRF.

AEs are generally detected in two ways:

- **Clinical Symptoms:** reported by the participant or signs detected on examination.

- **Ancillary Tests:** Abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the non-safety related measurements: the results of which are not being captured as AEs). Only clinically significant laboratory abnormalities, such as those that require intervention, should be classified as AEs.

An unexpected AE is any AE for which the specificity or severity is not consistent with the current Investigators Brochure or package insert or described in the protocol. An unexpected, suspected ADR is any unexpected AE that, in the opinion of the Site Investigator or Sponsor, in consultation with the MSM, there is a reasonable possibility that the IP caused the event.

7.2 Classification of Adverse Events

The different grades of AEs are defined as follows:

- **Grade 1:** Mild
- **Grade 2:** Moderate
- **Grade 3:** Severe*
- **Grade 4:** Life-Threatening*
- **Grade 5:** Death*

*Grades 3-5 will be considered Serious Adverse Events.

The relationship of the AE to the investigational product is specified by the Site Investigator based on temporal relationship and his/her clinical judgment, using the following definitions:

- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, the event is consistent with the natural history of the disease, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

- **Possibly Related:** There is reasonable temporal relationship to study intervention and the event could plausibly be explained by the intervention and other factors, e.g., history of disease, clinical state, concurrent conditions, or concomitant medications.

- **Definitely Related:** There is a clear temporal relationship to study intervention and the event cannot be reasonably explained by other factors like the participants history of disease, clinical state, concurrent conditions, or concomitant medications.

7.3 Assessment and Recording of Adverse Events

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 coding system for AE recording. AEs reported using CTCAE will be coded into Medical Dictionary for Regulatory Activities (MedDRA) terms by the Data Coordinating Center (DCC).

At each visit, the participant will be asked "Have you had any problems or symptoms since your last visit?" in order to determine the occurrence of AE.. If the participant reports an AE, the Site Investigator will report:

- Type of event
- Date of onset and resolution (duration)
- Severity (according to (CTCAE)
- Seriousness (does the event meet the above definition for an SAE?)
- Causality, relation to investigational product, study procedure, or disease
- Action taken regarding investigational product
- Outcome

7.4 Reporting of Adverse Events

Safety monitoring will include careful assessment and appropriate reporting of AEs by the clinical study sites (CSS). Each clinical study Site Investigator and appropriate delegated team members (co-investigators, research nurse, clinical trial coordinator) are responsible for identifying and reporting AEs through the DCC Online Adverse Event Reporting System. The system maintains audit trails and stores data (and data updated) and communication related to all AEs reported in the study. Site Investigators are also responsible for determining the relationship of the AE to the investigational agent/study procedures. Site Investigators are also responsible for complying with the NeuroNEXT sIRB's reporting requirements for all safety reports, and to fulfill all reporting requirements of their local institutions. Copies of each report and documentation of sIRB notification and receipt will be kept in the Study Regulatory File.

All clinical AEs are recorded on the Adverse Event CRF and kept in the participant's study binder. AEs must be entered into the online Adverse Event Reporting System as soon as possible, preferably within 24 hours. Adverse event reporting is critical for the information being provided to the Medical Safety Monitor and other safety reviewers as decisions are made regarding continued dosing in the study. AEs should be entered into the online Adverse Event Reporting System as soon as possible following the site learning of a new AE or receiving an update on an existing AE.

7.4.1 Serious Adverse Events

A serious adverse event (SAE) is defined as an AE that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
   - This serious criterion applies if the study participant, in the view of the Site Investigator or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
   - Hospitalization for an elective procedure (such as elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.
4. Results in persistent or significant disability or incapacity.
   - This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the participant's ability to conduct normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the participant (whether the participant is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical AE may meet criteria for "seriousness" but is not an *adverse* experience and will, therefore, not be considered an SAE. An example of this would include a social admission (participant admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

A serious, suspected ADR is an SAE that, in the opinion of the Site Investigator or Sponsor, suggests a reasonable possibility that the IP caused the event.

SAEs must be reported to the NeuroNEXT DCC **within 24 hours** of the site learning of the SAE. When an SAE is entered by a Site Investigator, the DCC will notify the MSM. The MSM will review the SAE report and may request further information if necessary. If the outcome of the SAE is death, the MSM may determine that the SAE requires expedited reporting to the FDA. The DCC will work with the CCC and Sponsor to prepare a MedWatch (or CIOMS) safety report for submission to the FDA. The MSM may choose to consult the DSMB chair in making a determination and will notify the DSMB chair if warranted. In the event of a participant death, the DSMB will be immediately notified, regardless of attribution of timing.

7.5. Medical Safety Monitor (MSM)

A qualified MSM will be appointed for this study and confirmed as acceptable by the DSMB. The MSM for this study will have three primary responsibilities:

1. The MSM will be responsible for contemporaneous assessment of all SAEs, and all AEs reported with a grade/intensity of three or greater. The MSM will work closely with the DCC using the Online Adverse Event Reporting System to review all reported SAEs in near real time and evaluate them to identify the need for timely intervention and/or reporting. For any reported SAEs (or AEs reported with a grade/intensity of three or greater), an automatic email will be sent to the MSM to prompt a review of the event for determination of whether the event is unanticipated and/or whether it is related to the study drug. With the assistance of the coordinators at the DCC, the MSM has the option of requesting additional information about any SAE. The MSM will complete a form for each review, and this information will be entered into the online data entry system. For the purposes of this study, an SAE is considered to be treatment-related if the attribution is possible or definite. An unexpected SAE is any SAE for which the specificity or severity is not consistent with the current IB. An unexpected and treatment-related SAE is an unexpected SAE that, in the opinion of the MSM, has a reasonable probability of having been caused by the IP. In such instances, the SAE requires expedited reporting to the FDA. The DCC and CCC will work with the IND holder to prepare a MedWatch safety report for submission to the FDA. If deemed necessary by the MSM dosing may be halted while causality and expectedness are evaluated to determine whether dosing should resume.
2. After a minimum of 14 days of post-dose safety for the first participant at each of the three dose levels, the MSM will review all information collected for that participant to date and decide whether the subsequent two participants in that cohort can be dosed. This sentinel dosing review will be required for the first dose cohort at each dose level. It is not required for subsequent cohorts at a given dose level, but may be added if the MSM and/or DSMB requested that it be added.
3. Assuming the MSM gives approval to advance, the second and third participants within a given dose level cohort will be treated. The MSM and DSMB will conduct a review of a minimum of 14 days of available post-dose safety data from the second and third participants of a dose cohort, and a minimum of 29 days available post-dose safety data from the first participant of a dose cohort to determine whether dosing can proceed as planned or needs to be modified in the next dose level cohort. For pre-symptomatic participants, this review will also include a review of all available safety data from Arm 2. For later cohorts (where a sentinel dosing review is not needed), the MSM review will

occur after all three participants have a minimum of 14 days of available post-dose safety data.

7.6. Data and Safety Monitoring Board (DSMB)

The monitoring of study participant safety and data quality will follow the National Institute of Neurological Disorders and Stroke (NINDS) Guidelines for Monitoring in Clinical Trials. A DSMB appointed by the NIH/NINDS will meet at regular intervals (as determined by NINDS) to review data provided by the study statistician. The DSMB will monitor rates of AEs, protocol compliance, and overall performance of the trial.

Additionally, the DSMB will be part of the review process for dosing decisions in the study. The DSMB will join with the MSM in review of sentinel participants at each dose to approve further dosing within a dosing cohort, and will be part of the approval process for planned dosing increases.

7.7. Stopping Criteria

There is no formal, pre-specified stopping criteria for this study. Rather, the consequences of AEs will be determined according to the following contingency table:

Grade	AE Grade	FDA Level	Impact on Study Cohort
1	Mild	AE	No impact.
2	Moderate	AE	No impact.
3	Severe	SAE	MSM performs immediate safety assessment.
4	Life-threatening	SAE	MSM performs immediate safety assessment.
5	Death	SAE	MSM performs immediate safety assessment.

As shown above, if a severe, non-serious AE or an SAE is observed, the Investigator must report the SAE to the MSM via the Online Adverse Event Reporting System within 24 hours. For each review described above, the MSM and DSMB will apply reasonable clinical judgment to determine whether dosing may proceed in additional participants or concerns are sufficient to satisfy a study stopping criteria.

Note that prion disease is a rapidly progressive, debilitating, and fatal disorder. It is expected based on the natural history of the disease that all participants in this trial will experience SAEs. The presence of SAEs, up to and including death, is not itself a reason for stopping. The decision of whether to stop will be made by the MSM and DSMB.

During periods when dosing is halted, or in the event that it is determined that dosing may not resume, participants who have already received their single dose of drug should continue to make follow-up visits for AE monitoring as well as to fulfill secondary and exploratory study objectives.

8 STATISTICAL CONSIDERATIONS

Statistical analysis will be carried out by the NeuroNEXT Data Coordinating Center. The statistical plan is summarized in the following sections of this trial protocol and described in greater detail in the Statistical Analysis Plan.

8.1 Data Analysis

Unless specified otherwise, analyses will include all participants. Post-hoc analyses may stratify by *PRNP* genotype.

8.1.1. Primary Analysis

The primary endpoint is safety and tolerability, as measured by AEs. This will be reported descriptively. A table showing the percentage of participants reporting any type of AE and the overall count of AEs by arm, dose level, and severity category will be tallied and presented in tabular form. Tables will be provided overall, and by system organ class (SOC) and preferred term (PT). No statistical test will be conducted.

8.1.2. Secondary Analyses

8.1.2.1. Pharmacokinetics (PK) of Single Dose PrP-siRNA (Arms 2 and 3 only)

One secondary endpoint is PK – drug concentration in CSF and blood. This will be reported descriptively; no statistical test will be conducted. For arm, timepoint, dose level, and biofluid (CSF vs. plasma), the mean and 95% confidence interval of the drug concentration in μg/mL will be reported.

8.1.2.2. Pharmacodynamic (PD) Effects of Single Doses of PrP-siRNA (Arms 2 and 3 only)

Another secondary endpoint is PD – CSF PrP concentration. Each individual's baseline CSF PrP concentration will be defined as that taken at the baseline / drug administration visit (Day 0). For this endpoint, each individual's CSF PrP concentration at each LP will be normalized to their individual concentration at baseline – i.e., normalized CSF PrP at each subsequent timepoint will be expressed as a percentage of the individual's own baseline level. This normalized value is termed residual CSF PrP. Normalized values will be reported for each arm, timepoint, and dose level, descriptively by displaying means and 95% confidence intervals of PrP concentration (expressed as a percentage of individual baseline). For Arm 2, no formal statistical test will be conducted. For Arm 3, the PD impact of PrP-siRNA can be assessed in pre-symptomatic participants by comparing pre- and post-dose CSF PrP concentrations.

8.1.2.3. Compare Change in PrP Over Time Between Treated and Untreated (Arms 1 and 2) in Participants with Symptomatic Prion Disease

Our longitudinal study of healthy controls and healthy asymptomatic individuals at risk for prion disease has established that individuals have different baseline concentrations of CSF PrP, driving primarily by *PRNP* genotype, but that an individual's CSF PrP is longitudinally stable. In symptomatic individuals, longitudinal data are not yet available, and cross-sectional evidence

suggests that CSF PrP may decline as a function of disease process. For this reason, individuals in Arm 2 will also be compared to untreated individuals in Arm 1.

For Arms 1 and 2, a rank test will be used at the 4-week timepoint to compare the distribution of residual PrP levels in Arm 2 versus Arm 1. This will be assessed overall, and within each dose level of Arm 2 versus Arm 1. For each individual, each CSF PrP concentration measurement will be normalized to individual pre-drug baseline to obtain a percentage residual. This percentage will then be compared across the groups, grouping by arm, timepoint, and dose level. For weeks 8, 12, and 24, where assessments are not required in Arm 1, no statistical test will be performed.

8.1.3. Key Exploratory Analyses

8.1.3.1. Survival (Time to Death or Initiation Life-Extending Measures)

One key secondary endpoint is to assess survival (defined as time to death or initiation of life-extending measures) in Arm 2. Kaplan-Meier curves will be plotted for each dose level. No formal statistical test will be conducted. Participants will be deemed to have an "event" at their time of death or on the date that life-extending measures were first initiated. Participants who do not meet either criteria will be censored at their last date of follow-up.

8.1.3.2. Medical Research Council Prion Disease Rating Scale (MRC-PDRS)

Another key secondary endpoint is the assessment of the MRC-PDRS over time in Arm 2. This will be reported descriptively by displaying means and standard deviations of the MRC-PDRS by timepoint and dose level. No statistical test will be conducted.

8.1.4. Additional Exploratory Analyses

Additional exploratory analyses are also planned but will not be included as part of the Final Study Report (FSR). Exploratory analyses for this study will include, but are not limited to, the following:

- Brain magnetic resonance imaging (MRI)
- Fluid biomarkers of prion seeding (CSF RT-QuIC)
- Fluid biomarkers of neurodegeneration
    - CSF neurofilament (NfL)
    - CSF T-tau
    - CSF beta-synuclein
    - Plasma brain-derived tau
    - Plasma NfL
    - Plasma glial fibrillary acidic protein (GFAP)

8.1.5. Missing Data

Missing data may occur, for example, if one or more participants pass away before the 4-week timepoint. For the primary endpoint of AEs, all AEs will be reported. For the secondary endpoints of CSF PrP and drug concentration, missing values due to absent CSF and blood samples will be omitted from summary descriptive statistics for secondary objectives 1 and 2.

However, missing values will be accounted for in the rank test utilized for addressing secondary objective 3. See the Statistical Analysis Plan for details.

8.2. Sample Size Justification

8.2.1. Proposed Sample Size

We propose to include up to 15 participants in Arm 1 (untreated).

We propose to include 15 participants each in Arms 2 and 3. In Arms 2 and 3, dose level cohorts of three participants each will be enrolled sequentially. If dose escalation proceeds as planned, the number of participants per dose level will be 3, 3, and 9 participants, respectively – but may change depending on the MSM reviews. Sentinel dosing will be employed as described in section 5.1.5. If the MSM and DSMB do not determine that there is a need to halt dosing or halt escalation, then dose escalation is permitted. Depending on the outcomes at each dose level, the final number of individuals may differ from that denoted above.

Preliminary power analysis

For assessing sample size, we considered the ability of the study to identify an SAE serious enough to warrant the cessation of dosing, referred subsequently as a dose limiting toxicity (DLT), at the chosen dose in Arms 2 and 3. Three dose levels will be enrolled sequentially, with each participant receiving a single dose of either 50, 100, or 200 mg. If dose escalation proceeds as planned, the number of participants per dose level will be 3, 3, and 9 participants. However, to account for other possible situations, we consider the probability of observing a DLT at the maximum dose level across samples sizes ranging from 3, 6, 9, 12, and 15. We also consider scenarios where the true (unknown) rate of observing a DLT at the highest dose is equal to 5%, 10%, 20%, or 30%.

The table below displays the probability of observing at least one DLT under each combination of sample size and true rate of observing a DLT at the highest dose. Within the range of most plausible sample sizes for the chosen dose, the table demonstrates that the study has adequate probability of observing a DLT when the true underling rate is greater than or equal to 10%. A higher chance of observing a DLT under various conditions could be achieved by increasing the sample size for Arm 2. However, when weighted against the costs and complexity of a larger study, the team feels that the proposed sample size provides the right balance with respect to selecting the correct dose for further study.

Table 8.2.2.1. Probability of Observing ≥1 Dose Limiting Toxicity at Chosen Dose by Sample Size & True Probability of Observing a DLT

N	Probability of Observing DLT			
	5%	10%	20%	30%
3	14%	27%	49%	66%
6	26%	47%	74%	88%
9	37%	61%	87%	96%
12	46%	72%	93%	99%
15	54%	79%	96%	>99%

9 DATA COLLECTION and SITE MONITORING

9.1 Data Management

Site personnel will collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data. The DCC or designee will monitor clinical sites to ensure compliance with data management requirements and Good Clinical Practices. The DCC is responsible for developing, testing, and managing clinical data management activities, as required, at the clinical study sites, the CCC, and at the DCC.

The general National Institute for Neurological Disorders and Stroke (NINDS) Common Data Elements (CDE) will be used to construct data collection forms. All study data will be collected via systems created in collaboration with the DCC and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

9.1.1 Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical study site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. NeuroNEXT sIRB approval for the protocol must be on file at the DCC before enrollment can occur at a clinical study site.

The DCC will use a system of coded identifiers to protect participant confidentiality. When the participant is registered to participate in the study using the DCC-provided web-based registration, the system will assign a participant ID number. The participant ID is the 3-digit site ID plus the unique participant number. To confirm the correct participant ID, the data entry system will require a second entry of the unique participant ID and compare for consistency. In this fashion, no personal identifiers will be accessible to the DCC, and the data will be collected on the correctly identified participant.

9.1.2 Data Entry

Data entry will occur at the enrolling clinical study sites. Data quality assurance and analyses will be performed by the DCC. The DCC, located at the University of Iowa, will coordinate all data and statistical services for the study, as well as on-site and/or remote monitoring for all participating clinical study sites.

Data collection for this study will be accomplished with online electronic CRFs. Using encrypted communication links, online forms will be developed that contain the requisite data fields.

9.2 Role of Data Management

Data Management (DM) is the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with NeuroNEXT policies and applicable Sponsor and regulatory requirements. The DCC will instruct site personnel to collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data.

The DCC is responsible for all aspects of clinical data management and for properly instructing key study personnel (including the CCC, the CSS, and DCC staff) on how to collect, transcribe, correct, and transmit the data onto CRFs or other data collection forms and logs.

The DCC is responsible for establishing procedures to ensure that clinical data management activities occur as required at the CCC, the CSS, and at the DCC.

9.3 Quality Assurance

By signing this protocol, the Sponsor and Investigator agree to be responsible for implementing and maintaining quality control and quality assurance systems with written standard operating procedures to ensure that studies are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

9.3.1 Development of Monitoring Plan

Onsite and remote monitoring visits will be conducted by BNI monitors according to a pre-defined monitoring plan. The monitoring plan will detail the frequency of on-site visits and remote review of source documentation and CRFs, the study data to be monitored, the review of any regulatory files, drug and supplies accountability (if applicable), documentation of remote reviews and on-site visits, and the resolution process for data errors that are discovered during visits and remote reviews. All participating clinical study sites will be monitored at least once after a study initiation visit and all sites will have a close-out visit. Two remote monitoring visits are anticipated for each clinical study site per year. All participant data will be monitored for inclusion and exclusion criteria, informed consent procedures, and AEs. A certain percentage of data is also monitored/source data verified against the data entered into the study database. The monitoring plan will include flexibility to revise the frequency of visits or data monitored depending on clinical study site or study related issues.

9.3.2 Site Monitoring Visits

On-site monitoring visits will be conducted by BNI monitors according to a pre-defined monitoring plan. The goal of on-site monitoring is to analyze (review) the data as it is collected, to check the validity and integrity of the data, to verify source documentation, to ensure protection of human participants, and to ensure protocol compliance with federal regulations. During the monitoring visit, the monitor assesses the overall status of the study, staff, and facilities to determine whether the study is being conducted per protocol and in compliance with regulatory requirements. The monitor also conducts a CRF review that includes checks of all AE documentation, verifies the presence of all critical correspondence and records related to

investigational products and clinical supplies (if applicable), and determines if protocol violations have occurred and are documented properly. After the monitoring visit, the monitor documents the results of the monitoring visit and completes a post-visit monitoring letter that conveys any issues discovered during the visit and the need for data corrections, if appropriate. Drug and supplies accountability may also be monitored during the site visit. The DCC will work closely with the CCC to monitor and document drug distribution from the manufacturers to the clinical study sites (CSS). Each CSS will be provided with a drug accountability log which will be reviewed by the DCC monitors and reconciled with distribution logs. At the study closeout visit, the monitors confirm that appropriate data have been reviewed, source documentation has been verified, and all required documents are present in the Study Regulatory File.

9.3.3 Remote Monitoring Visits/Review

DCC monitors and data managers will also perform remote review of source documentation, CRFs and investigational product accountability to achieve the goals described in 9.3.2. Remote review involves a range of data management procedures (queries, edit checks, review of missing data, etc.), as well as more traditional monitoring procedure, conducted remotely through the exchange and review of source documentation and CRFs. DCC monitors will request access to source documentation and CRFs through the following methods:

1. Remote review of electronic medical records
2. Site upload of source documentation and CRFs to a secure portal hosted and managed by the DCC.
3. Site upload of source documentation and CRFs to a secure portal hosted and managed by the site.

DCC monitors will contact site staff as early as possible in the course of a trial to arrange remote access to source documentation and CRFs. Sites are required to comply with DCC requests for remote access because, in most cases, on-site monitoring may not be possible throughout the trial.

Additionally, the DCC will conduct remote reviews of investigational product accountability and storage conditions after an initial on-site visit to each site's pharmacy. DCC monitors will request accountability records and temperature logs from CSS coordinators or study pharmacists in order to complete these remote reviews.

9.3.4 Laboratory Data Flow

When a blood or urine sample has been obtained, a member of the study team will send the sample (PID, site ID, and protocol ID numbers will be used) to the hospital's local laboratory. Results will be sent via a secure system with no individual-identifying information on the report. The laboratory will electronically communicate the test results to the respective CSS in a secure manner.

9.3.5 Laboratory Data Flow

Safety Monitoring Labs: The DCC will provide laboratories with online forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. When a blood or urine sample has been obtained, a member of the study team will

send the sample (participant ID, site ID, and protocol ID numbers will be used) to the MGH central laboratory. Results will be sent via a secure system to MGH central laboratory with no individual-identifying information on the report. The laboratory will electronically communicate the test results to the respective CSS in a secure manner. The laboratory will also transfer test results electronically to the DCC.

9.4 Data Monitoring

All aspects of the study will be monitored by qualified individuals designated by the sponsor. Regulatory and data monitoring will be conducted according to Good Clinical Practice and applicable government regulations. The investigator agrees to allow monitors access to the Regulatory files, clinical supplies, dispensing and storage areas, and to the clinical files of the study participants, including electronic medical records, and, if requested, agrees to assist the monitors.

A separate report detailing protocol compliance will also be available periodically from the DCC for review by the Protocol PI, who will provide feedback to individual sites as needed. The Protocol Steering Committee (PSC) will advise the Protocol PI as to whether the protocol or informed consent document requires revision based on these reports.

10 HUMAN PARTICIPANTS

The Mass General Brigham (MGB) IRB is the IRB of record for the study and serves as the single IRB for this multicenter trial. Documented approval from the sIRB will be obtained for all participating sites prior to clinical trial start, according to ICH GCP, local laws, regulations, and organization. When necessary, an extension, amendment, or renewal of the sIRB approval must be obtained.

Evidence of training in responsible conduct of research shall be on file for each study Site Investigator and any co-investigators.

10.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the sIRB responsible for oversight of the study. A signed consent form, approved by the sIRB, will be obtained from the participant. For Arms 1 and 2, a LAR must sign the consent form for participants who cannot provide consent for themselves; additionally, the participant's assent, if applicable, must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. Because Arm 3 recruits pre-symptomatic participants without neurological impairment, all individuals must be able to provide consent for themselves. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, or legal guardian (if applicable), and this fact will be documented in the participant’s record.

10.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the clinical study site will be identified only by the study specific Participant Identification Number

(PID) to maintain participant confidentiality. All records will be kept in a secure location. All computer entry and networking programs will be done using study specific PIDs only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by sIRB, the FDA, the Sponsor, or the Sponsor's designee.

10.2.1 Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by sIRB, the Sponsor, FDA, or other government agencies as part of their duties to ensure that research participants are protected. If the study is terminated or suspended, the PI will promptly inform study participants, the sIRB, and sponsor and provide the reason(s) for the termination or temporary suspension.

11 FUTURE USE OF STORED SPECIMENS AND DATA

Specimens and data collected in this study will be used in future research both on prion disease and on other diseases. All participants will be asked to consent into Prion Biobank (MGB IRB 2025P000879) and a record of this consent status will be stored in the study database. Specimens will be shipped to Eric Minikel's laboratory at the Broad Institute for analysis, storage, and future research purposes including studies conducted both on-site at the Broad Institute and studies conducted with outside collaborators. Outside collaborators will include both non-profit and for-profit entities. Specimens will also be shipped directly from sites to MRI Global and Southern Research, contract research organizations tasked with running bioanalytical assays for this study. De-identified individual participant data may also be shared with collaborators and will be published and made available in a public repository accessible on the internet. Results of any genetic testing performed on these samples will not be disclosed to participants.

In general, identifying information will be removed from samples and data sent to collaborators or deposited in repositories, with each individual linked to a de-identified code. The key connecting these codes to identifying information will be stored on one or more encrypted computers. Identifying information will, however, be shared with the National Prion Disease Pathology Surveillance Center (NPDPSC) in Cleveland, Ohio, in order to link brain samples from any participants who undergo autopsy at NPDPSC to their data in this trial. Data and samples from NPDPSC may then further be shared with companies involved in analyzing the

tissue, but this sharing will be done using only de-identified codes and not identifiable information; only NPDPSC will have access to identifiable information.

Participants who withdraw from the study may choose to request that their demographic and clinical data and any unused sample be destroyed. In the event of such a request, we will destroy the samples and data that we can, however, some information may be retained for regulatory and reporting purposes. In addition, any data that have already been posted to repositories will not be removed, and samples that have already been distributed to approved researchers will not be retrieved.

12 STUDY RECORDS RETENTION

Records will be kept for a minimum of 7 years following study closeout and in accordance with institutional policy.

13 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies of the NeuroNEXT Network and procedures developed by the NeuroNEXT Data Sharing and Publication Committees. Any presentation, abstract, or manuscript will be made available for review by the NeuroNEXT DCC, CCC, sponsor, and the NINDS prior to submission.

14 CONFLICT OF INTEREST POLICY

Any actual conflict of interest (COI) of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed in accordance with their institution's policies. If a COI is disclosed, a COI management plan from the individuals' institution will be requested by the study team and will be reviewed by the NeuroNEXT COI committee.

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