PROTOCOL NO. MN-166-ALS-1202

Amendment 4

A MULTI-CENTER, OPEN-LABEL BIOMARKER STUDY TO EVALUATE MN-166 (IBUDILAST) IN SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

IND Number:	128011
Study Phase:	1b/2a
Sponsor:	Kazuko Matsuda, MD PhD MPH Chief Medical Officer MediciNova, Inc. 4275 Executive Square La Jolla, CA 92037
Investigational Product Name:	MN-166 (ibudilast)
Indication:	Amyotrophic Lateral Sclerosis (ALS)
Principal Investigator:	Nazem Atassi, MD, MMSc Massachusetts General Hospital Neurological Clinical Research Institute (NCRI) 165 Cambridge St, Suite 600 Boston, MA 02114

INVESTIGATOR AGREEMENT

PROTOCOL NUMBER: MN-166-ALS-1202

I have read the foregoing protocol and agree to conduct the study as described herein.

By signing the protocol, the Investigator agrees to keep all information provided by MediciNova, Inc. in strict confidence and to request the same from his/her staff and the Institutional Review Board. Study documents provided by MediciNova, Inc. 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.

Nazem Atassi MD MMSc
Associate Director, Neurological Clinical Research Institute (NCRI)
Massachusetts General Hospital

_____
Investigator Name (Print)

_____ _____
Investigator Signature Date

PROTOCOL SIGNATURE PAGE

Protocol Number: MN-166-ALS-1202

Signature: _____ Date: _____

Nazem Atassi, MD MMSc

MediciNova:

Signature: _____ Date: _____

Kazuko Matsuda, MD PhD MPH

1. SYNOPSIS

Name of Sponsor: MediciNova, Inc.	
Name of Investigational Product: MN-166 (ibudilast)	
Protocol No: MN-166-ALS-1202	
Title of Study: A Multi-Center, Open-Label Biomarker Study to Evaluate MN-166 (ibudilast) in Subjects with Amyotrophic Lateral Sclerosis (ALS)	
Principal Investigator: Nazem Atassi, MD, MMSc	
Study Centers: Massachusetts General Hospital (MGH); South Shore Neurologic Associates Long Island	
Duration of Study Treatment: Screening up to 6 weeks; Treatment for 36 weeks; Follow-up at Week 40 for a total duration of 46 weeks.	**Phase of Development:** 1b/2a

Study Rationale

Amyotrophic lateral sclerosis (ALS) is a fatal, neurodegenerative disease for which there is no cure.

A substantial body of evidence implicates the neuroimmune system and specifically activated microglia in ALS pathophysiology. Activated microglia are detected near the motor neurons of $SOD1^{G93A}$ transgenic mice at 39 days, well before the onset of weakness, and the inflammatory response in these mice correlates with disease progression. Under-expression of mSOD1 in microglia prolonged the post-symptomatic survival of these transgenic mice. With relevance to human disease, activated microglia are increased in postmortem brain and spinal cord tissue from patients with ALS compared to healthy controls, and the number of activated microglia positively correlates with the rate of disease progression.

Ibudilast is a small molecule macrophage migration inhibitory factor (MIF) and phosphodiesterase (PDE) 4,10 inhibitor. Ibudilast has an excellent safety track record and has been marketed for more than 2 decades in Japan for the treatment of post-stroke dizziness. In addition, ibudilast has been shown to reduce microglia activation in multiple in vitro and in vivo model systems. Ibudilast inhibited the release of specific inflammatory mediators and upregulated the release of neurotrophic factors from cultured microglia in a dose-dependent manner. Further, in neuron and microglia co-cultures, ibudilast significantly suppressed neuronal cell death induced by the activation of microglia with lipopolysaccharide (LPS) and interferon (IFN)-gamma. Thus, ibudilast-mediated neuroprotection is thought to be primarily due to the inhibition of microglia/monocyte recruitment, modulation of the inflammatory response, and release of neurotrophic factors.

Numerous PET radioligands have been developed to image activated immune cells by binding to translocator protein (18kDa, TSPO), also called the peripheral benzodiazepine receptor (PBR). TSPO is highly expressed in both activated monocytes and microglia. Older generation TSPO radioligands, such as [^{11}C]PK11195, suffer from poor signal-to-noise ratio in the brain. Our tracer, [^{11}C]-PBR28**,** binds to TSPO with 80times higher specificity, offering a unique opportunity to study *in vivo* neuroinflammation due to activated microglia and monocytes.

The principal investigators of this study were able to successfully synthesize and administer [^{11}C]-PBR28 to over 40 subjects at MGH. We found that [^{11}C]-PBR28 uptake is significantly increased in the motor cortices of ALS patients compared to healthy controls, and that the anatomical distribution of increased [^{11}C]-PBR28 binding corresponds to ALS site of weakness onset (i.e., limb-onset patients have more inflammation in the motor cortex and bulbar-onset patients have more inflammation in the brain stem), and higher levels of inflammation were correlated with pathological reflexes and worse functional status. Thus, [^{11}C]-PBR28 is an ideal biomarker to inform ibudilast target engagement and proof-of-mechanism in patients with ALS.

Study Objectives

Primary Objective

The primary objectives are:

- to measure the impact of MN-166 (ibudilast) on [^{11}C]-PBR28 uptake in the motor cortices and brain stem measured by positron emission tomography (PET) imaging at 24 weeks
- to measure the impact of MN-166 (ibudilast) on several markers of neuro-inflammation measured by blood biomarkers.

Secondary Objectives

The secondary objectives are:

- to evaluate the safety and tolerability of ibudilast over 36 weeks
- to evaluate the effect of ibudilast on ALS clinical outcomes (ALS functional rating scale [ALSFRS-R], slow vital capacity [SVC], strength [measured by HHD- Hand-held dynamometry]) over 36 weeks.

STUDY DESIGN

This is a multi-center, open-label study of MN-166 (ibudilast) in subjects with ALS. To be eligible subjects must meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS. Safety, tolerability, blood, neuro-imaging biomarkers, and clinical outcomes will be collected on all subjects. Subjects will receive study drug for 36 weeks.

The study will consist of a Screening Phase (up to 6 weeks), an Open-Label Treatment Phase (36 weeks) and an Off-Treatment Follow-up Phase (4 Weeks).

Screening Phase (up to 6 weeks prior to Baseline Visit)

During the Screening Phase, eligible ALS subjects will sign an informed consent form and the following screening assessments will be performed: review of inclusion/exclusion criteria: El Escorial ALS Diagnostic criteria, medical history and demographics, ALS diagnosis history, physical and neurological examination, U. Penn upper motor Neuron Burden (UMNB), pulmonary function tests, vital signs including height and weight, blood for safety labs including TSPO affinity test, ECG and review and documentation of concomitant medications and therapies.

Open-Label Treatment Phase (36 weeks)

The Treatment Phase will consist of a Baseline visit and 4 subsequent clinic visits at Weeks 4, 12, 24, and 36. Telephone follow-ups will occur at Weeks 1, 2, 8, 16, 20, 28, and 32.

At the Baseline visit, subjects will return to the clinic and the following assessments will be performed/administered: review of inclusion and exclusion criteria for continued eligibility, vital signs, blood for safety labs and biomarkers, ECG, ALSFRS-R questionnaire, slow vital capacity (SVC),

baseline strength as measured by hand held dynamometry (HHD), and Columbia Suicide Severity Rating Scale (C-SSRS). At this visit, study drug will be dispensed, and adverse events, concomitant medications and therapies will be assessed and documented.

At subsequent visits during the Treatment Phase, similar assessments will be performed.
In addition, a [^{11}C]-PBR28-PET scan will be performed once between the Screening and Baseline visit, and once between the Week 12 and Week 24 visits. The ALSFRS-R, SVC and UMNB will be repeated on the same day as the PET scans.

Off-Treatment Follow-up Phase (4 Weeks post-Treatment Phase)

The follow-up visit will consist of a telephone call to document adverse events and concomitant therapies.

Number of Subjects (Planned):

Approximately 45 subjects are planned to be screened with the goal of enrolling 35 subjects. Subjects enrolled and followed at the South Shore Neurologic Associates Long Island site will come to Massachusetts General Hospital for two PET scans. Five subjects s will be enrolled by flexible inclusion and exclusion criteria and will not have any PET scans.

Study Entry Criteria:

Inclusion Criteria:

1. Subjects must be diagnosed as having possible, probable, probable-laboratory supported, or definite ALS, either sporadic or familial according to modified El Escorial criteria.
2. Age 18 or above, able to provide informed consent, and safely comply with study procedures.
3. Vital capacity (VC) of at least 50% predicted value for gender, height and age at screening visit, or in the opinion of the study physician, able to safely tolerate study procedures. (*Not applicable to flexible arm*)
4. Subject must be able to swallow oral medication at the Baseline Visit and expected to be able to swallow the capsules throughout the course of the study.
5. Subject must not have taken riluzole for at least 30 days, or be on a stable dose of riluzole for at least 30 days, prior to screening (riluzole-naïve participants are permitted in the study). (*Not applicable to flexible arm*)
6. Women must not be able to become pregnant (e.g. post-menopausal, surgically sterile, or using adequate birth control) for the duration of the study and 3 months after study completion.
7. Males should practice contraception for the duration of the study and 3 months after completion.
8. Ability to safely lie flat for 90 min for PET procedures in the opinion of the study physician. (*Not applicable to flexible arm*)
9. High or mixed affinity to bind TSPO protein (Ala/Ala or Ala/Thr) (see Section 7.2.1). (*Not applicable to flexible arm*)
10. Upper motor Neuron Burden (UMNB) Score ≥25 (out of 45) at screening visit. (*Not applicable to flexible arm)*

Exclusion Criteria

1. Abnormal liver function defined as AST and/or ALT > 3 times the upper limit of normal.
2. Renal insufficiency as defined by a serum creatinine > 1.5 times the upper limit of normal.
3. The presence of unstable psychiatric disease, cognitive impairment, or dementia that would impair ability of the participant to provide informed consent, according to PI judgment.

4. Clinically significant unstable medical condition (other than ALS) that would pose a risk to the participant if they were to participate in the study.
5. History of HIV, clinically significant chronic hepatitis, or other active infection.
6. Active inflammatory condition of autoimmune disorder (*Not applicable to flexible arm)*
7. Females lactating or pregnant.
8. Active participation in another ALS clinical trial or exposure to an off-label ALS experimental treatment within 30 days of the Baseline Visit (*Not applicable to flexible arm)*
9. Exposure to immunomodulatory medications within 30 days of the Baseline Visit. (*Not applicable to flexible arm)*
10. Any contraindication to undergo MRI studies such as
    a. History of a cardiac pacemaker or pacemaker wires
    b. Metallic particles in the body
    c. Vascular clips in the head
    d. Prosthetic heart valves
    e. Claustrophobia

    (*Not applicable to flexible arm)*
11. Radiation exposure that exceeds the site's current guidelines (*Not applicable to flexible arm)*
12. EKG finding of QTc prolongation > 450 ms for males and > 470 ms for females at screening or baseline.
13. Not on any prohibited medications: Refer to ***8.3 Prohibited Medications***

Investigational Product, Dosage and Mode of Administration: MN-166 (ibudilast) 10 mg capsules administered orally. 50 mg bid (5 capsules) in the morning and evening will be administered for a total daily dose of 100 mg/d. Study drug dosing may vary based on individual tolerability.

Duration of Treatment: Open-label treatment for 36 weeks

Reference Therapy, Dosage, and Mode of Administration: N/A

Outcome Measures

1. Neuroimaging biomarkers ([^{11}C]-PBR28-PET) including both regions of interest (ROI) and voxel-based analyses;
2. Safety, as measured by AEs and clinically meaningful changes in vital signs, physical examination, and standard clinical laboratory tests;
3. Tolerability, defined as the ability of subjects to complete the entire 36-week treatment portion of study;
4. Clinical outcomes: Measured by ALSFRS-R, SVC, and strength (measured by HHD).
5. Blood biomarkers: including analysis of pro-inflammatory cytokines.

Sample Size Justification

Sample size calculation was based on the primary outcome of the biomarker. The primary outcome is the changes in the ROI in the motor cortex as assessed by [^{11}C]-PBR28-PET. The study PI's previous studies comparing mean PBR28 binding in the motor cortices in limb-onset ALS subjects (1.18 units) with matching healthy volunteers (1.064 units) revealed 0.116 mean difference between the two groups, with 0.08 standard deviation in the ALS group. With 10 people tolerating the drug among 15 participants in this study, the probability is 90 percent that the study will detect a treatment difference at a one-sided 0.1 significance level if the true difference is 0.096 units. The proposed increase in sample size will increase the statistical power to detect changes in PBR28 uptake after MN-166 treatment. This will also provide more safety and tolerability data on MN-166 for up to 50 mg BID in people with ALS.

Table 1: Schedule of Assessments

	Screening Visit[1]	Baseline *** Visit (Day 1)	Week 1, Week 2 Phone call (±3 Days)	Week 4 Visit (±7 Days)	Week 8 Phone call (±3 Days)	Week 12 Visit (±7 Days)	Monthly Phone calls Week 16, Week 20 (±3 Days)	Week 24 Visit (±7 Days)	Monthly Phone calls Week 28, Week 32 (±3 Days)	Week 36 Visit (±7 Days)	Week 40 Phone call (±3 Days)	Final Safety Visit (for early drug discontinuation only)
Informed Consent	X											
Eligibility Criteria	X	X										
El Escorial Criteria	X											
Medical History	X											
Create GUID	X											
Blood sample for TSPO Affinity test[5]	X											
Safety Labs[2]	X	X		X		X		X		X		X
12-lead ECG	X	X		X		X		X		X		X
Vital Signs[3] /Height and weight[4]	X	X		X		X		X		X		X
Physical & Neurological exam	X			X		X		X		X		X
[^{11}C]-PBR28-PET[5]		X*				X**						
MRI Safety Questionnaire[5]	X							X				
ALSFRS-R		X*		X		X		X**		X		X
U Penn Upper Motor Neuron Burden	X*			X		X		X**		X		X
Slow Vital Capacity	X	X*		X		X		X**		X		X
Hand-held Dynamometry		X*		X		X		X		X		X
Columbia Suicide Severity Rating Scale		X*								X		X
Ibudilast Dispensing		X		X		X		X				
Ibudilast Accountability			X	X	X	X	X	X	X	X		X
AE review		X	X	X	X	X	X	X	X	X	X	X
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X
Collection of blood for biobanking		X*[6]		X		X		X[6]		X[6]		

[1] Screening procedures must be completed within 6 weeks prior to Baseline Visit.
[2] Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, Serum pregnancy test (WOCBP).

[3] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[4] Height measured at Baseline Visit only.
[5] Assessments not applicable to flexible arm cohort
[6] PBMC Collection to be done. (*Serum Biomarkers to be collected at Baseline, Week 4, 12, 24, 36*). PBMC collection not applicable for flexible arm.
***Off* treatment Assessments will be performed one time only between the Screening and Baseline visits. ALSFRS-R, SVC and UPenn Upper Motor Neuron Burden will be repeated on the same day as the scan for subjects screened and enrolled at South Shore Neurologic Associates (SSNA). For subjects screened and enrolled at Massachusetts General Hospital (MGH), the ALSFRS-R, SVC and UMNB do not need to be repeated on the same day as the pre-treatment MRI/PET scan, if the pre-scan visit differs from the baseline visit. These outcomes can be collected within ± 14 days of the scan for MGH subjects.**
****On* treatment [^{11}C]-PBR28-PET will be performed one time only between the Week 12 and Week 24 visits. ALSFRS-R, SVC and UPenn Upper Motor Neuron Burden will be repeated on the same day as the scan for subjects screened and enrolled at South Shore Neurologic Associates (SSNA). For subjects screened and enrolled at Massachusetts General Hospital (MGH), the ALSFRS-R, SVC and UMNB do not need to be repeated on the same day as the post-treatment MRI/PET scan, if the post-scan visit differs from the week 12 or week 24 visit. These outcomes can be collected within ± 14 days of the scan for MGH subjects.**

*** The baseline visit or Day 1 is the time when study drug is dispensed and the first dose of drug is administered. Safety labs, Vital signs, ECG, and AE review should happen on Day 1.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: Abbreviations and Terms

Abbreviation	Term
AE	adverse event
ALP	alkaline phosphatase
ALS	amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale-revised
ALT (SGPT)	alanine aminotransferase
ANCOVA	analysis of covariance
AST (SGOT)	aspartate aminotransferase
AV	atrioventricular
AUC	Area under the curve
β-hCG	beta-subunit of human chorionic gonadotropin
bid	twice daily
BP	blood pressure
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CGIC	Clinical Global Impression of Change
CIRB	Central Institutional Review Board
CK	creatine kinase
C_{max}	maximum plasma concentration
CMP	comprehensive metabolic panel
CNS	central nervous system
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
CSF	cerebrospinal fluid
C-SSRS	Columbia Suicide Severity Rating Scale
CYP	cytochrome
DBP	Diastolic Blood Pressure
DDI	drug-drug interaction

Abbreviation	Term
DHD	dihydrodiol
dL	deciliter
DM	diabetes mellitus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition-Text Revision
EAE	Experimental autoimmune encephalomyelitis
ECG	electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMG	electromyography
ET	Early Termination
EW	early withdrawal
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GUID	Global Unique Identifier
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HR	heart rate
HV	healthy volunteer
IBU	ibudilast
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ISF	Investigator Site File
ISM	Independent Safety Monitor
L	liter
MMT	Manual Muscle Test

Abbreviation	Term
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
MGH	Massachusetts General Hospital
MIF	macrophage migration inhibitory factor
MIP	maximum inspiratory pressure
MRI	Magnetic Resonance Imaging
NIF	negative inspiratory force
NIV	non-invasive ventilation
NSAID	Non-steroidal anti-inflammatory drug
OLE	Open-label extension
PET	positron emission tomography
PDE	phosphodiesterase
PI	Principal Investigator
PK	pharmacokinetics
PO	per oral
qd	once-daily
QTcF	QT interval corrected for heart rate using Fridericia's formula
ROI	Region of Interest
SA	sinoatrial
SAE	serious adverse event
SAP	statistical analysis plan
SI	sub investigator
SVC	slow vital capacity
SOD	superoxide dismutase
SOP	standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
tid	three times daily
TMF	trial master file
TRAE	treatment-related adverse event
TSPO	translocator protein
ULN	upper limit of normal

Abbreviation	Term
WBC	white blood cells
WHO-DD	World Health Organization Drug Dictionary
WOCBP	Women of Childbearing Potential

4. BACKGROUND AND RATIONALE

4.1. Clinical Features and Epidemiology of ALS

Amyotrophic lateral sclerosis (ALS) is a rare degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles[1]. The incidence of ALS is currently approximately 2/100,000[2]. The lifetime ALS risk is 1 in 600 to 1 in 1000[2]. Even though the incidence of ALS is similar to that of multiple sclerosis, the prevalence is only 4 to 6/100,000 (about 25,000-30,000 subjects in the United States), due to the higher mortality rate. Fifty percent of ALS cases die within three years of onset of symptoms and 90% die within five years[3]. The median age of onset is 55 years[3]. The cause in most cases is unknown[4]. Age and male gender are the only risk factors repeatedly documented in epidemiological studies. No treatment prevents, halts or reverses the disease, although the use of riluzole (FDA-approved therapy for ALS) is associated with a slight prolongation of survival[5]. Another drug that recently received FDA approval for treatment of ALS is Edaravone (Radicava®) and its use is not exclusionary for this study.

4.2. Role of neuro-inflammation in ALS Pathogenesis

Despite decades of focused research, a unifying and well-tested theory of ALS disease pathophysiology remains enigmatic[4]. At the same time, the body of knowledge has expanded dramatically as research tools have improved. Research increasingly implicates neuro-inflammation as one of the major molecular mechanisms leading to neuronal death in ALS.

Preclinical evidence:
Activated microglia are detected near the motor neurons of $SOD1^{G93A}$ transgenic mice at 39 days, well before the onset of weakness, and the inflammatory response in these mice correlates with disease progression[6]. Underexpression of mSOD1 in microglia prolonged the post-symptomatic survival of these transgenic mice[7]. In $SOD1^{G93A}$ ALS mice, there is an influx of inflammatory blood monocytes ($CD14^{+}/CD16^{-}$), which are recruited to the CNS and mimic the appearance of activated microglia[8]. In addition, in $SOD1^{G93A}$ mice, peripheral blood monocytes ($CD14^{+}/CD16^{-}$) become activated, demonstrating a pro-inflammatory gene profile[8]. Furthermore, miR-155, a micro-RNA that acts as an upstream regulator of inflammation, becomes upregulated in peripheral blood monocytes, and in central nervous system tissue in these $SOD1^{G93A}$ mice[8]. Knocking out this gene ameliorates disease in transgenic SOD1 mice, as does reducing its expression with an antisense oligonucleotide[8-10].

Human studies:
With relevance to human disease, activated microglia are increased in postmortem brain and spinal cord tissue from patients with ALS compared to healthy controls, and the number of activated microglia correlates positively with faster rate of disease progression[11]. Studies of cerebrospinal fluid (CSF) show increased immune cells, including monocytes/macrophages, and pro-inflammatory cytokines[12-14].

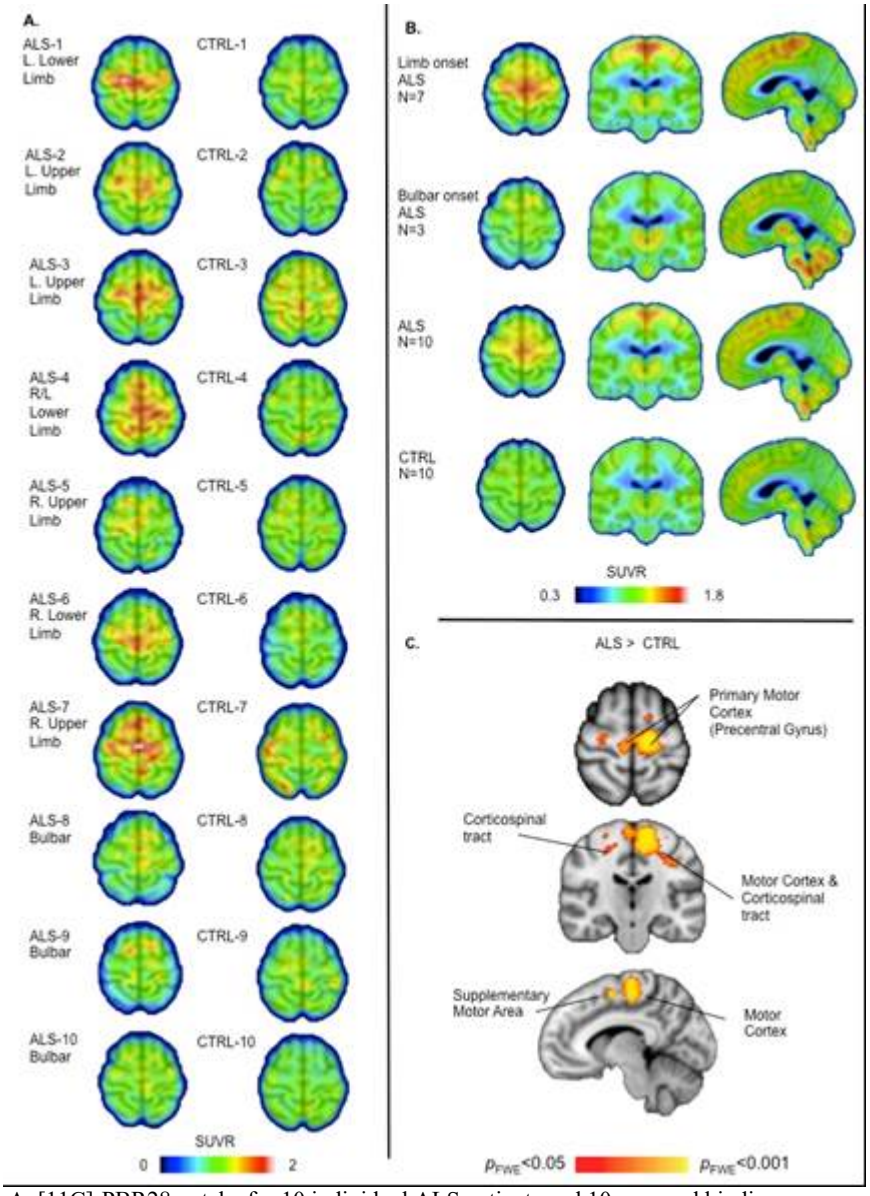
4.3. Biomarkers of neuro-inflammation in ALS

4.3.1. PBR28 PET

[^{11}C]-PBR28 is an investigational PET tracer that binds to the translocator protein (TSPO) expressed on activated microglia and astrocytes. The name of the non-radioactive drug is PBR. N-Acetyl-N-(2-methoxybenzyl)-2-phenoxy-5-pyridinamine. More than 168 individuals received PBR28 (mean dose 2.2mCi/nmol) at MGH and there were no reported tracer-related Adverse Events.

4.3.2. Imaging glial activation in ALS

It was found that PBR28 uptake is significantly increased in the motor cortices in ALS patients compared to healthy controls[15] (Figure 1). We also demonstrated that the anatomical distribution of increased PBR28 uptake corresponds to ALS clinical presentation; patients with limb-onset weakness had higher PBR28 uptake in the motor cortices and patients with bulbar-onset weakness had higher PBR28 uptake in the brainstem[15] (Figure 1). Furthermore, higher PBR28 uptake was strongly correlated with worse functional status measured by ALSFRS-R and more pathological upper motor neuron signs measured by upper motor neuron burden scale (UMNB) [15].



A. [11C]-PBR28 uptake for 10 individual ALS patients and 10 age- and binding affinity- matched healthy controls. B. Mean [11C]-PBR28 binding images for the ALS and control groups, including comparisons between limb- and bulbar-onset patients C. Brain regions that exhibit significantly higher binding in ALS compared to the control group in the voxel-wise whole brain analysis, pFWE<0.05

Figure 1: [^{11}C]PBR28 uptake in ALS patients

4.4. MN-166 (ibudilast)

Ibudilast is a small molecule that crosses the blood-brain barrier after oral administration[16]. Its potential as a neuroprotective agent is based on *in vitro* and *in vivo* evidence of its ability to reduce microglial activation, inhibit microglia-monocyte recruitment to the central nervous system (CNS), and trigger the release of neurotrophic factors. Ibudilast has been marketed for more than 2 decades in Japan for the treatment of post-stroke dizziness and has an excellent safety track record.

Ibudilast reduces active microglia:
Ibudilast inhibits the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) and suppresses mononuclear cell migration to the brain in a dose-dependent fashion[17]. MIF is essential for promoting microglial activation and production of the innate soluble mediators IL-1β, IL-6, tumor necrosis factor (TNF)-α, and inducible nitric oxide (NO) synthase[18]. The *in vivo* suppression of MIF effects inhibits CNS inflammation and reduces the number of active microglia and infiltrating inflammatory macrophages[18]. Ibudilast also reduces microglial activation and protects against white matter damage induced by chronic cerebral ischemia[19]. These data suggest that by inhibiting MIF, ibudilast is a potent suppressor of both microglial recruitment and activation.

Ibudilast modulate neuroinflammation:
In cultures of active microglia, ibudilast inhibits the production of the pro-inflammatory cytokine TNF-α and of NO in a dose-dependent manner[20,21]. Ibudilast also inhibits the production of TNF-α by microglia that are activated in response to viral proteins[22]. In addition, ibudilast reduces microglial TNF-α expression, demyelination on histology, and the development of clinical symptoms in a rodent genetic model of Krabbe's neurodegeneraton[23]. Oral administration of ibudilast reduces the severity of clinical signs of the autoimmune encephalitis (EAE) rodent model of multiple sclerosis in a dose-dependent manner[24]. In a multicenter, double-blind, phase II trial in patients with relapsing multiple sclerosis, ibudilast reduced inflammation measured by two independent MRI outcomes, and showed beneficial clinical effect on disease disability[25]. Based on these promising results, ibudilast is currently under investigation as a disease-modifying agent for multiple sclerosis (NCT01982942).

Ibudilast is neuroprotective:
The anti-inflammatory effects of Ibudilast are neuroprotective in several *in vitro* models. In murine neuron and microglia co-culture experiments, ibudilast suppresses neuronal cell death that is induced by the activation of microglia with lipopolysaccharide (LPS) and interferon (IFN)-γ[21]. These activities were medicated by selective inhibition of certain phosphodiesterases (PDEs)[20,26]. At the same time, ibudilast increases the production of the anti-inflammatory cytokine IL-10, and of neurotrophic factors (nerve growth factor (NGF), glia-derived neurotrophic factor (GDNF), and neurotrophin (NT)-4[21]. Further, ibudilast protects against glutamate excitotoxicity in cultured hippocampal neurons[27]. Ibudilast exerts beneficial effects on glial cell survival as it protects astrocytes against apoptosis[28] and prevents kainate-induced excitotoxicity in cultured oligodendrocytes[29].

In summary, ibudilast reduces active microglia, inhibits neuroinflammation, and has strong neuroprotective properties in several disease models.

4.5. Prior Clinical Experience

4.5.1. Clinical Study Overview

To date, 5 clinical studies have been completed and 2 clinical trials are ongoing in the US. As of May 29, 2015, approximately 537 subjects have received MN-166 and 290 subjects have received placebo.

Table 3: Summary Table of Completed and Ongoing Clinical Trials

Type of Study	Study Identifier	Study Objectives	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Healthy Subjects or Diagnosis of Patients	Number of Enrolled Subjects	Number of MN-166 Subjects	Number of Placebo Subjects	Duration of Treat-ment	Study Status; Type of Report
Multiple Sclerosis Study										
2	MN-166-CL-001	To assess safety, tolerability and efficacy	R (1:1:1), DB, PC	AV411 30 mg/d, 60 mg/d or placebo; oral	Multiple Sclerosis (18 to 55 years old)	297	(291 during entire study)	103 (core period)	Core 12 months; extension 12 mos	Completed; Full
2	NN102-SPRINT-MS	To assess safety and efficacy	Multi-center, R, DB, PC	MN-166 100 mg/d orally or placebo	Progressive multiple sclerosis	255	129	126	96 weeks	Completed; In Progress
Phase 1 Studies										
1	AV411-009	To assess safety, tolerability and PK	R (3:1), DB, PC	On Day 1 subjects received a single dose of AV411 30 mg or pbo; no study drug on Day 2 and on Day 3 subjects received AV411 60 mg/d or pbo for 14 days; oral	Healthy Volunteers (18 to 70 years old)	18 (14 active, 4 pbo)	14	4	2 wk	Completed; Full

Type of Study	Study Identifier	Study Objectives	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Healthy Subjects or Diagnosis of Patients	Number of Enrolled Subjects	Number of MN-166 Subjects	Number of Placebo Subjects	Duration of Treat-ment	Study Status; Type of Report
1	AV411-016	To assess safety, tolerability and PK	R (3:1), DB, PC, single escalating dose	AV411 30 mg, 50 mg, 70 mg, 80 mg, and 100 mg or placebo; oral	Healthy Volunteers (18 to 55 years old)	60 (45 active, 15 pbo)	45	15	Single dose	Completed; Full
1b	AV411-026	To assess safety, tolerability and PK	R (3:1), DB, PC, MD	On Days 1 thr 6, subjs received AV411 or pbo as follows: 40mg x 2d then 60 mg x 2 d, and 80 mg x 2 d. On D 7 -14, subjects received AV411 100 mg/d or pbo; oral	Healthy Volunteers & Diabetes Mellitus (Type 1 and 2) Subjects (18 to 75 years old)	24 (18 active, 6 pbo); 12 HV; 12 DM	18	6	2 wk	Completed; Full
Neuropathic Pain Study										
1b/2a	AV411-010	To assess safety, tolerability, PK and preliminary efficacy	R, DB, PC; 7-day single-blind run-in phase (placebo and active dose) followed by 14 day treatment phase in 2 cohorts	Treatment Phase: Cohort 1: randomized 1:1:1 to AV411 40 mg, 60 mg or pbo. Cohort 2: randomized 2:1 (AV411:pbo) to 60 mg/80 mg/d or pbo; oral	Diab. Periph. Neuropath. Pain (DPN) subjects (18 to 75 years old)	34 (24 active/10 pbo)	34 Run-in 24 Treatment	34 Run-in 10 Treatment	Single and multiple dosing 2 wk (subset ≥3mos)	Completed; Full
Ongoing Studies										
2	MN-166-ALS-1201	To assess safety and efficacy	Single-center, R 2:1, DB,PC with an OLE	MN-166 60 mg/d or placebo	Amyotrophic lateral sclerosis (ALS)	21	~14	~7	52 weeks	Ongoing; unblinded interim safety report

CO=crossover, OLE=open label extension, MD=multiple dose, PC=placebo-controlled, PK=pharmacokinetic, PBO=placebo, R=randomized

Safety and Tolerability Summary of MN-166 (ibudilast)

As of 29 May 2015, approximately 537 subjects have received MN-166 and 290 subjects have received placebo in five completed Phase 1 and Phase 2 clinical trials and two ongoing trials.

A total of 124 subjects with multiple sclerosis have received MN-166 30 mg/day and 60 mg/day for up to 12 months and 167 have received MN-166 30 mg/day and 60 mg/day for up to 24 months.

The safety of MN-166 from 30 to 80 mg/day in single and multiple doses up to 14 days has been evaluated in 77 subjects (68 healthy volunteers, 9 diabetes mellitus subjects). Of these 77 subjects, MN-166 100 mg/day has been evaluated as a single dose in 9 healthy volunteers and multiple doses for up to 8 days in 9 healthy volunteers and 9 diabetes mellitus patients. Additionally, MN-166 has been evaluated in 34 subjects with neuropathic pain.

The results of the completed Phase 2 randomized double-blind relapsing-remitting multiple sclerosis (RRMS) study suggest that MN-166 at daily doses up to and including 60 mg/day appear to be generally safe and well-tolerated. A smaller number of subjects have received MN-166 at 80 mg/day and 100mg/day; the adverse event profile appears to be similar to that of the lower doses. The adverse events that appear to be drug-related based on the available data are headache, nausea, vomiting, dyspepsia and hyperhidrosis. In the multiple sclerosis study, there was a slight dose related increase in the percent of subjects with headaches and gastrointestinal AEs. The incidence of nausea demonstrated a dose-related increase with the greatest incidence in the 60 mg/day group compared to the 30 mg/day, and placebo groups although the number of subjects experiencing nausea was small. Similarly, the incidence of vomiting followed the same pattern. MN-166 also appeared to cause transient changes in laboratory values particularly AST, ALT and GGT which, in most cases, seemed to resolve over time. These adverse events appear to be consistent with the more commonly reported adverse drug reactions reported in the Ketas® package insert. MN-166 does not appear to cause significant changes in blood pressure, heart rate or ECGs. No new adverse events appear to occur with long-term exposure.

Ongoing Studies

There are currently two ongoing Phase 2 studies to evaluate MN-166 for the treatment of amyotrophic lateral sclerosis (ALS) and the treatment of multiple sclerosis (MS).

Amyotrophic Lateral Sclerosis (ALS) Study

The ALS study is currently being conducted under IND 118318 and has enrolled approximately 21 patients. An unblinded interim analysis was conducted by an

independent Safety Monitor and as of 01 April 2015, a total of 61 adverse events have been reported in 21 subjects (64% of these were considered mild and 36% were considered moderate). Of the 61 AEs, only 2 were determined to be "possibly related". All others AEs were deemed to be either "unrelated" or "unlikely related". Adverse events were evenly distributed among the two treatment groups. No clinically significant abnormalities were noted on ECG and there were no clinically significant laboratory abnormalities. The recommendation was to continue the trial as planned.

As per the recommendation of the Division of Neurology Products, this proposed IND is being filed to conduct future ALS studies.

Multiple Sclerosis (MS) Study

As of an interim analysis cutoff date of 05/04/2015, a total of 645 adverse events (AEs) have been reported (307 in treatment group A, 338 in treatment group B) in 178 subjects. No significant trend appears for the overall distribution of AEs across the treatment groups. When examined by system organ class (SOC), there is a trend towards significantly more AEs in the “Infections” SOC with treatment A. However, this trend does not appear to be due to any particular difference for any MedDRA preferred term within this SOC.

Of the 645 AEs, a total of 196 (30%) have been determined to be at least possibly related to treatment (59 in treatment group A, 137 in treatment group B). When restricted only to the subset of AEs determined to be at least possibly related to treatment, there is a trend towards significantly more related AEs with treatment B (31% of subjects on treatment A with at least one related AE vs. 50% of subjects on treatment B; RR = 0.62 w/ 95% CI: 0.44, 0.87). The data suggests that this is primarily due to a trend towards significantly more related AEs in the “Gastrointestinal” & “Nervous System” SOCs with treatment B – with the overall observed differences primarily due a significant increase in study-related “Nausea” (8% of subjects on treatment A vs. 21% of subjects on treatment B; RR = 0.37 w/ 95% CI: 0.18, 0.76) and a marginally significant increase in study-related “Headache” (3% of subjects on treatment A vs. 10% of subjects on treatment B; RR = 0.34 w/ 95% CI: 0.11, 1.02) on treatment B.

Other Clinical Studies

There have been 6 additional studies conducted with MN-166 under Investigator-Initiated INDs for drug and alcohol addiction, and medication overuse headache.

4.6. Study Dose Rationale

The findings from the efficacy endpoints from the previous Phase 2 RRMS trial (MN-166-CL-001) indicated that patients receiving 60 mg/d MN-166 exhibited significantly reduced brain volume loss, reduced progression to persistent black hole (PBH) formation from gadolinium-enhancing lesions and extended time to first relapse vs. patients treated with placebo[25] (see table below). A dose-response effect was evident between the 30 and 60 mg/d regimens, with 60 mg/d showing statistical significance for all efficacy measures. These study results suggest that MN-166 is best positioned for primary neuroprotection in MS patients[30] and the dose-response data reported here and combined with animal PK-PD and preliminary clinical efficacy outcomes in other trials suggest that doses of 60 mg/d up to 100 mg/d be suitable to demonstrate neuroprotective efficacy of MN-166.

A summary of daily dose vs. efficacy results across some of the sponsor's patient trials are presented in the table below.

Table 4: Dose versus Efficacy Results in RRMS study

Study	**Indication**	**Endpoint**	**Dose** (mg/d)	**Significance** ($p \leq 0.05$)
Pilot study Ibudilast for MS	RRMS	Mean relapse rate	60	+
MN-166-CL-001	RRMS (small subset of SPMS)	Reduced Brain atrophy	30 60	- +
		Reduced PBH formation	30 60	- +
		Time to first relapse	30 60	– +
AV411-OWA	Opioid Analgesia Addiction	McGill pain survey, Subjective Opioid Withdrawal Scale	40 80	Not available +

Note: A 60 mg/d MN-166 regimen yields steady-state plasma conc. ~75 ng/mL and estimated brain conc. ~225 ng/mL. Correlates for 80 and 100 mg/d regimens are proportionally higher.

For the first clinical trial with ALS subjects (MN-166-ALS-1201), 60 mg/d MN-166 was selected for the following reasons: 1) 60 mg/d was administered to patients in the RRMS trial and found to be generally safe and well-tolerated; 2) the safety and tolerability of MN-166 given as an adjuvant to riluzole had not been previously evaluated and, 3) MN-166 and riluzole each have shown hepatotoxicity on their own and it was uncertain whether co-administration of the drugs would result in an additive hepatotoxic effect. For these reasons, it was decided that a more conservative dose regimen was to be implemented. Recently, however, the safety and tolerability of MN-166 were evaluated in the ongoing progressive MS and ALS trials with recommendations from the independent safety monitors to continue the dosing regimens and the trials, overall. In April 2015, an interim

safety analysis was conducted by an Independent Safety Monitor to evaluate the safety results of the first 21 subjects (n =14 ibudilast; n =7 placebo) who had completed 3 months of study drug treatment in the MN-166-ALS-1201 study. It was reported that there was no trends or signs of increased hepatotoxicity in these subjects who were administered MN-166 with riluzole. Moreover, no trends or signs of increased incidence of adverse events or laboratory value changes were reported from the interim safety analysis of the ongoing Phase 2 progressive MS trial utilizing up to 100 mg/day.

Collectively, the safety and efficacy findings from the completed and ongoing trials make a compelling case for administering up to 100 mg/d MN-166 to ALS patients in this study.

5. TRIAL OBJECTIVES AND PURPOSE

5.1. Primary Objectives

The primary objectives are:

- to measure the impact of MN-166 (ibudilast) on [^{11}C]-PBR28 uptake in the motor cortices and brain stem measured by positron emission tomography (PET) imaging at 24 weeks
- to measure the impact of MN-166 (ibudilast) on several markers of neuro-inflammation measured by blood biomarkers.

5.2. Secondary Objectives

The secondary objectives are:

- to evaluate the safety and tolerability of ibudilast over 36 weeks
- to evaluate the effect of ibudilast on ALS clinical outcomes [ALS functional rating scale (ALSFRS-R), slow vital capacity (SVC), strength (measured by HHD- Hand-held dynamometry)] over 36 weeks.

6. OVERALL STUDY DESIGN AND PLAN: DESCRIPTION

This is a multi-center, open-label study with a 6-week Screening Phase, 36-week Treatment Phase, and a Follow-up Phase 4 week after the last dose. Approximately 45 subjects are expected to be screened and 35 subjects are planned to be enrolled. Subjects enrolled and followed in South Shore Neurologic Associates Long Island will come to Massachusetts General Hospital for two PET scans. Five subjectss will be enrolled by flexible inclusion and exclusion criteria and will not have any PET scans. MN-166 (ibudilast) 50 mg will be administered orally twice a day.

All study visit windows (±7 days) after baseline are consecutive calendar days and are calculated from the day the subject starts study treatment (the day of the Baseline Visit). The study phases are described below and displayed in Figure 2 and the Schedule of Assessments is displayed in Table 1.

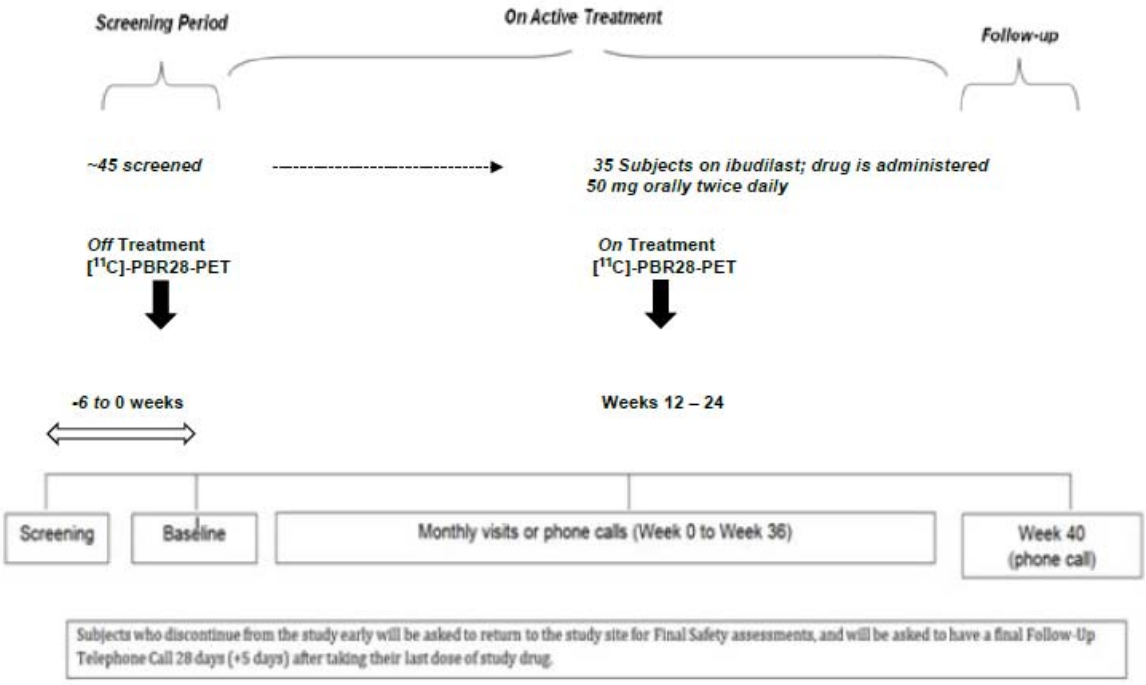


Figure 2: Study Design

6.1. Screening Phase (up to 6 weeks prior to Baseline Visit)

During the Screening Phase, a total of up to 6 weeks will be allowed to complete the screening assessments.

Subjects will be approached for study participation and upon signing an informed consent form, the following assessments will be performed: review of inclusion/exclusion criteria: medical history including review of prior and current medications, physical and neurologic examination, height, body weight, and vital signs (sitting). An EMG report will be documented and an ALSFRS-revised questionnaire will be administered. Safety labs (chemistry, hematology, and serum pregnancy test), 12-lead ECG, slow vital capacity and a manual muscle strength test will be conducted; an ALSAQ-5 will be administered.

Detailed information on permitted and excluded concomitant medications is provided in Section 8.2 and Section 8.3 of the protocol.

6.1.1. Diagnosis at Screening

Subjects must have a diagnosis of ALS as defined by the El Escorial-Revised research diagnostic criteria for ALS (clinically definite, clinically probable, probable-laboratory supported).

6.1.2. Open-Label Treatment Phase (36 weeks)

During the Open-label Treatment Phase, subjects will return to the clinic at Baseline, and at Week 4, Week 12, Week 24, and Week 36 (end of study drug dosing). Telephone follow-ups will occur at Weeks 1, 2, 8, 16, 20, 28, and 32 (see Table 1 Schedule of Assessments).

At the Baseline Visit, subjects who have completed all of the screening assessments and continue to meet eligibility criteria will have their baseline assessments performed.

Subjects will take their first dose of study medication (30 mg MN-166) on the evening of the Baseline Visit (Day 1). On the morning of Day 2, all subjects will begin a 3 capsule BID dosing regimen and will remain on 60 mg/d through Day 14. Beginning on Day 15, all subjects will increase dosing to 5 capsules BID regimen. After Day 15, subjects who experience intolerable side-effects (e.g., nausea, diarrhea, vomiting) may reduce their dose to either 4 capsules twice a day (80 mg/d) or 3 capsules twice a day (60 mg/d) in discussion with the site investigator. The daily dose of ibudilast may be divided and taken three times per day if needed to improve tolerability. At the end of the first 8 weeks of treatment, the subject must maintain their then-current daily dose of study medication (6 capsules per day, 8 capsules per day, or 10 capsules per day).

Subject with intolerable side-effects (e.g., nausea, diarrhea, vomiting) may discontinue the study drug at any time at the investigator's discretion.

6.1.3. Off-Treatment Follow-up Phase (4 Weeks post Treatment Phase)

All subjects who complete the study will receive a follow-up phone call at Week 40 to collect adverse events and concomitant therapies. Subjects who stop study medication for any reason prior to the end of treatment period (week 36) will also have a Final Safety Visit 4 weeks after the last MN-166 dose.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1. Clinical Trial Population

The population for this trial will include approximately 35 male and female subjects at least 18 years old with a diagnosis of ALS.

Subjects who do not meet all of the inclusion criteria or who meet any of the exclusion criteria will not be eligible to receive study drug.

7.2. Inclusion/Exclusion Criteria

7.2.1. Inclusion Criteria

Subjects must meet all of the following criteria to be included in this study:

1. Subjects must be diagnosed as having possible, probable, probable-laboratory supported, or definite ALS, either sporadic or familial according to modified El Escorial criteria.
2. Age 18 or above, able to provide informed consent, and safely comply with study procedures.
3. Vital capacity (VC) of at least 50% predicted value for gender, height and age at screening visit, or in the opinion of the study physician, able to safely tolerate study procedures. (*Not applicable to flexible arm*)
4. Subject must be able to swallow oral medication at the Baseline Visit and expected to be able to swallow the capsules throughout the course of the study.
5. Subject must not have taken riluzole for at least 30 days, or be on a stable dose of riluzole for at least 30 days, prior to screening (riluzole-naïve participants are permitted in the study). (*Not applicable to flexible arm*)
6. Women must not be able to become pregnant (e.g. postmenopausal, surgically sterile, or using adequate birth control) for the duration of the study and 3 months after study completion.
7. Males should practice contraception for the duration of the study and 3 months after completion.
8. Ability to safely lie flat for 90 min for PET procedures in the opinion of the study physician. (*Not applicable to flexible arm*)
9. High or mixed affinity to bind TSPO protein (Ala/Ala or Ala/Thr) (Details below). (*Not applicable to flexible arm*)
10. Upper motor Neuron Burden (UMNB) Score ≥ 25 at screening visit. (*Not applicable to flexible arm*).

TSPO affinity test*:* venous blood will be drawn from all participants at Screening in order to have them genotyped for the Ala147Thr TSPO polymorphism in the *TSPO* gene (rs6971). About 10% of humans show low binding affinity to PBR28[31]. A recent study has demonstrated that the rs6971 polymorphism predicts PBR28 binding affinity in human platelets[31]. Since the low-affinity binder phenotype is consistent across all tissues within the same person, testing for the Ala147Thr polymorphism can be performed to predict low affinity for PBR28 in all organs, including the brain[32]. **High or Mixed affinity binders**

(Ala/Ala or Ala/Thr) will be considered eligible, whereas the low affinity binders (Thr/Thr) will be considered ineligible for the study.

7.2.2. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from this study:

1. Abnormal liver function defined as AST and/or ALT > 3 times the upper limit of normal.

2. Renal insufficiency as defined by a serum creatinine > 1.5 times the upper limit of normal.

3. The presence of unstable psychiatric disease, cognitive impairment, or dementia that would impair ability of the participant to provide informed consent, according to PI judgment.

4. Clinically significant unstable medical condition (other than ALS) that would pose a risk to the participant if they were to participate in the study.

5. History of HIV, clinically significant chronic hepatitis, or other active infection.
6. Active inflammatory condition or autoimmune disorder (*Not applicable to flexible arm*)
7. Females must not be lactating or pregnant.
8. Active participation in another ALS clinical trial or exposure to an off-label ALS experimental treatment within 30 days of the Baseline Visit (*Not applicable to flexible arm*)
9. Exposure to immunomodulatory medications within 30 days of the Baseline Visit. (*Not applicable to flexible arm*)
10. Any contraindication to undergo MRI studies such as
    a. History of a cardiac pacemaker or pacemaker wires
    b. Metallic particles in the body
    c. Vascular clips in the head
    d. Prosthetic heart valves
    e. Claustrophobia
    (*Not applicable to flexible arm*)
11. Radiation exposure that exceeds the site's current guidelines (*Not applicable to flexible arm*)
12. EKG finding of QTc prolongation > 450 ms for males and > 470 ms for females at screening or baseline.
13. Not on any prohibitive medications.: Refer to ***Section 8.3 Prohibited Medications***

Contraception requirements/pregnancy testing
For the purposes of this study, women of child bearing potential are defined as all women who are capable of becoming pregnant, unless they meet one of the following criteria:

- 12 months postmenopausal

- Post-hysterectomy
- Surgically sterile

If a female subject does not meet these criteria and is considered of child bearing potential they will have a stat serum pregnancy test performed during any visit at which a PET/MR scan or lumbar puncture is done, immediately prior to the PET/MR scan, before administration of the radiopharmaceutical. Subjects with a positive test will be excluded.

7.2.3. Subject Withdrawal/Discontinuation Criteria

Subjects may request to be withdrawn from the study at any time for any reason.

The Investigator may interrupt the treatment of any subject whose health or well-being may be compromised by continuation in the study. The following instances require subjects to be withdrawn from the study:

- Subject fails to adequately comply with the dosing, evaluations, or other requirements of the study at the discretion of Investigator;
- Subjects who have adverse events that require discontinuation of study medication;
- Subjects who, in the opinion of the Investigator, should be discontinued for their well-being;
- Subjects who are no longer able to understand task instructions or to perform tests adequately;
- Subject becomes pregnant during the study. See Section 11.7 for reporting requirements and follow-up of the pregnancy.

If a subject withdraws or is removed from the study for any reason, the reason and date of discontinuation of study medication should be recorded in the appropriate section of the Case Report Form (CRF). At the time of study discontinuation, the subject should return any unused study drug and will be asked to return to the study site for a final safety visit.

This study may be prematurely terminated if, in the opinion of the PI, there is sufficient reasonable cause.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data are not sufficiently complete and/or evaluable.

The study sponsors reserve the right to discontinue the study at any time for medical or administrative reasons.

Subjects who wish to discontinue from study may be replaced.

7.2.4. Follow-up Procedures Upon Discontinuation/Withdrawal

An early termination CRF page should be completed for every subject who received study medication whether or not the subject completed the study. The reason for discontinuation should be indicated on the CRF. Any AEs that are present at the time of discontinuation/ withdrawal should be followed in accordance with the safety requirements outlined in Section 11.

8. TREATMENT OF SUBJECTS

8.1. Description of Study Drug

MN-166 will be provided in 10 mg capsules in polyethylene bottles and will be stored at room temperature. Study drug should be taken with food.

8.2. Concomitant Medications

Concomitant medications required for the treatment of symptoms and signs of amyotrophic lateral sclerosis are permitted except as excluded below.

Concomitant medications for treatment of adverse events may be allowed. Subjects will be instructed to contact a member of the study staff prior to taking any medication.

8.3. Prohibited Medications

8.3.1. Prohibited drugs prior to and during the study

The following medications are **prohibited** prior to and during study participation:

- Systemic corticosteroid treatment within 30 days prior to Baseline (inhaled or topical steroids are allowed).
    - A single course of systemic corticosteroid treatment will be allowed and a course of steroids along with antibiotics to treat intercurrent sinobronchitis will be allowed
- Current use of intermittent systemic corticosteroids (i.e., monthly or bimonthly intravenous methylprednisolone)
- Oral immunosuppressants (e.g. azathioprine, methotrexate, cyclosporine, teriflunomide [Aubagio®]) within 30 days of Baseline

8.3.2. Prohibited drugs during the study

The following medications are **prohibited** as newly prescribed drugs during study participation (i.e., after Screening visit):

- cimetidine, cyclosporine, dronedarone, lopinavir, probenecid, quinidine (with the exception of Nuedexta, which will be allowed with more frequent ECG monitoring), ranolazine, rifampin, ritonavir, or tipranavir.

Additionally, subjects should washout from benzodiazepines and non-steroidal anti-inflammatory medications one week prior to PET scan as these compounds would affect the results of the scan.

8.3.3. Prohibited drug for "flexible arm" subjects

The following medications are prohibited prior to and during study participation for the flexible arm subjects:

- cimetidine, cyclosporine, dronedarone, lopinavir, probenecid, quinidine (with the exception of Nuedexta, which will be allowed with more frequent ECG monitoring), ranolazine, rifampin, ritonavir, tipranavir, retigabine, and mexiletine.

8.4. Treatment Compliance

Compliance will be monitored closely at each visit. Subjects will be instructed to bring all unused study medication with them to each visit. Compliance will be assessed by counting capsules and dividing the actual number of doses taken (per capsule count) by the number of doses the subject should have taken within a visit period and multiplying by 100. All subjects will be reminded of the importance of strict compliance with taking study medication for the effectiveness of treatment and for the successful outcome of the study. Subjects who miss more than 25% of scheduled doses over the course of the study or take more than 125% of the scheduled doses will be considered noncompliant and may be discontinued from the study per investigator's judgment.

8.5. Randomization and Blinding

Subjects will not be randomized in this study. All subjects will receive MN-166 (ibudilast).

8.6. Dosing Guidelines

8.6.1. Treatment Phase

Capsules containing MN-166 (10 mg) will be used. At the Baseline Visit (Day 1), subjects will be instructed to take 3 capsules of study medication in the evening. Although the study drug can be taken in a fasted or fed state, subjects will be instructed to take study medication with food or within an hour of eating to improve gastrointestinal tolerability. Starting from Day 2, subjects will be instructed to take 3 capsules of study medication twice daily during the first 14 days and 5 capsules twice a day thereafter (or as directed by Investigator) once in the morning (e.g., between approximately 6-9 AM) and once in the evening (e.g., approximately 12 h later, which would be approximately 6-9 PM), by mouth. The investigator may also choose to administer the total daily dose of study medication on a three-times a day regimen to help reduce side effects.

If a subject forgets to take their AM or PM study drug dose at the assigned time (between 6-9 AM and PM), subjects will be allowed to take their medication up to 12 PM or 12 AM, respectively. Beyond this time, subjects will be instructed to skip the dose and take the next dose at their regularly scheduled time.

8.6.2. Dose Adjustments

Any dosage adjustment, including the reason for and dates of adjustment, will be documented in the CRF for each subject requiring this manipulation. The PIs or licensed physician Sub-Investigator may reduce the dosage of study drug or discontinue the study drug in its entirety for AEs thought to be related to the study drug or for other reason during the trial (the reason for, and dates of suspension or dose reduction must be documented). If the AE is mild or moderate, the dosage may be reduced until the event improves. The PIs may then choose to resume the higher dosage or maintain the subject at a reduced dosage. If the event is serious or life threatening, and deemed to be definitely drug related, the study drug will be discontinued immediately. Study subjects must remain off the study drug permanently. Subjects may not resume study drug. All AEs will be followed to resolution.

8.6.3. Dose Interruptions

Laboratory tests should be repeated within two weeks after any of the following laboratory values are met:

- AST or ALT or ALP or T.bil >3x upper limit of normal (ULN)
- GGT $\geq$ 4 x ULN
- Cre > 1.5 x ULN
- White blood count <2500/mm^3
- Platelet count <75,000/ mm^3

If, after repeat testing, the laboratory value is still outside the above limits, then the subject should stop study medication. While dosing is withheld, subjects will continue tests and assessments according to the schedule defined in the protocol (and may also undergo additional assessments to evaluate the laboratory abnormality as per the Investigator's standard practice). In addition, subjects must have the abnormal laboratory result rechecked at least every 2 weeks (rechecks will be run at the local laboratory) until resolution or stabilization of the laboratory value.

After laboratory values return within normal limits, resumption of study treatment is to be considered on a case by case basis and must be discussed with the Independent Safety Monitor.

8.6.4. Subsequent Additional Laboratory Abnormalities

Subjects who subsequently develop the same abnormal laboratory value at any other time must permanently discontinue dosing with study treatment (i.e., only one dosing interruption is allowed for the same lab abnormality). However, subjects who subsequently experience a different laboratory abnormality can have study treatment withheld again for a different laboratory abnormality. However, only two dosing interruptions are allowed for each subject. Any subject who experiences a third abnormal laboratory tests as defined in the section above (Section 8.6.2) must permanently discontinue study treatment.

8.7. Informed Consent

Only patients capable of providing informed consent will be enrolled. Each subject is required to provide written informed consent prior to undergoing any study procedures. However, some patients may be incapable of physically signing the Consent Form due to their disease. If the person retains the ability to understand the concepts of the study and to evaluate the risk and benefit of being in the study when it is explained verbally and the individual is able to indicate approval or disapproval to study enrollment, they may be enrolled. The consent form will document the method used to communicate with the prospective subject and the specific means by which the prospective subject communicated agreement to participate in the study. An impartial third party will witness the entire consent process and sign the consent document. A copy of the signed and dated informed consent (in a language in which the subject is fluent) is required to be given to the subject. If a subject withdraws consent, data collected up to the time of discontinuation will be used to evaluate study results.

8.8. Assessments

The Schedule of Assessments is presented in Table 1.

Each visit during the Treatment Phase will have a window of ±3 days.

Clinical and laboratory evaluations will be performed by qualified study staff at Massachusetts general Hospital and South Shore Neurologic Associates Long Island. All clinical and laboratory evaluations, procedures related to inclusion/exclusion criteria, or performed during treatment must be reviewed, initialed and dated by the Principal Investigator or appropriate designee listed on Form FDA 1572.

8.8.1. Study Assessments by Visit

The following is a summary of assessments by study visit.

8.8.1.1. Screening

Study Visit 1 (up to 6 weeks)

The following procedures will be performed to determine the subject's eligibility for the study:

- Obtain written informed consent from subject
- Assess inclusion and exclusion criteria
- Assess El Escorial ALS Diagnostic criteria
- Obtain medical history and demographics
- Obtain ALS diagnosis history
- Perform physical and neurological examination
- Perform U. Penn upper motor Neuron Burden (UMNB)
- Measure vital signs including height and weight

- Perform Pulmonary Function Tests
- Collect blood for labs including TSPO affinity test
- Perform ECG
- Review and document concomitant medications and therapies
- MRI Safety Questionnaire

8.8.1.2. Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum*, the following information should be captured and entered in the Electronic Data Capture (EDC) System:

- Demographics
- Inclusion and Exclusion criteria
- Reason for screen failure
- Screening labs results

8.8.1.3. Baseline Visit (Day 1)

This visit will take place within 6 weeks of the Screening Visit. The following procedures will be performed:

- Review inclusion and exclusion criteria
- Measure vital signs
- Collect blood for safety labs
- Perform ECG
- Collect blood samples for biobanking
- Administer ALSFRS-R questionnaire Perform Pulmonary Function Tests
- Measure baseline strength (HHD)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Baseline
- Dispense study medication for the following 4 weeks
- Review and document concomitant medications and therapies
- Assess and document AEs
- Create GUID

***Pre-* treatment neuroimaging study (MRI/PET)** will take place after TSPO affinity test results are available. This may occur on any day between release of TSPO test results and Baseline, or on the same day as the Baseline Visit. This first neuroimaging study will be performed before any treatment is administered. ALSFRS-R, U Penn Motor Neuron Burden (UMN-B) and SVC will be repeated on the same day as the scan for subjects screened and enrolled at South Shore Neurologic Associates (SSNA). For subjects screened and enrolled at Massachusetts General Hospital (MGH), the ALSFRS-R, SVC and UMNB do not need to be repeated on the same day as the pre-treatment MRI/PET scan, if the pre-

scan visit differs from the baseline visit. These outcomes can be collected within ± 14 days of the scan for MGH subjects.

8.8.1.4. Study Visits (Week 4, Week 12, Week 24, and Week 36)

The following procedures will be performed at these visits:

- Perform neurological examination
- Measure vital signs
- Perform ECG
- Collect blood for safety labs
- Collect blood samples for biobanking
- Administer ALSFRS-R questionnaire
- Perform U. Penn upper motor Neuron Burden (UMN-B)
- Perform Pulmonary Function Tests
- Measure baseline strength (HHD)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Baseline
- Dispense study medication
- Review and document concomitant medications and therapies
- Assess and document AEs
- Check study drug compliance

***Post*-treatment neuroimaging study (MRI/PET)** will be performed one time only between the Week 12 and Week 24 visits. It can be done on the same day of the Week 24 visit. ALSFRS-R, SVC and UMN-B will be repeated on the same day as the scan for subjects screened and enrolled at South Shore Neurologic Associates (SSNA). For subjects screened and enrolled at Massachusetts General Hospital, the ALSFRS-R, SVC and UMNB do not need to be repeated on the same day as the post-treatment MRI/PET scan, if the post-scan visit differs from either the week 12 and week 24 visit. These outcomes can be collected within ± 14 days of the scan for MGH subjects.

8.8.1.5. Study Phone Calls (Week 1, Week 2, Week 8, Week 16, Week 20, Week 28, Week 32, and Week 40)

The following assessments will be performed at the study phone calls:

- Check study drug compliance
- Review and document concomitant medications and therapies
- Assess and document AEs

8.8.1.6. Final Safety Visit

Subjects who withdraw consent will be asked to come in for a Final Safety Visit and will be asked to have a final Follow-Up Telephone Call (+7 days, but no earlier than 28 days, after subject's last dose of study drug).

The following will be performed at the Final Safety Visit:

- Vital signs
- ECG
- Neurological exam
- Blood samples for safety labs
- ALSFRS-R questionnaire
- U. Penn upper motor Neuron Burden (UMNB)
- Pulmonary Function Tests
- HHD
- Columbia Suicide Severity Rating Scale (C-SSRS) – Since last visit
- Review and document concomitant medications and therapies
- Assess and document AEs
- Check study drug compliance
- Perform study drug accountability and collect all unused study drug and empty containers

The following procedures will be performed via telephone 28 (+7) days after the Final Safety Visit:

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Document AEs

8.8.1.7. Follow-up

After the Week 40 Follow-up Telephone Interview, subjects may be contacted every three months for up to two years, or until consent is withdrawn, in order to obtain vital status and clinical information including the ALSFRS-R. Additionally, two years of post-study access to subjects' medical records will be requested to allow for review in combination with study data in order to investigate any correlation between imaging data collected during this study and subjects' clinical outcomes.

8.8.2. Procedures/Assessment Details

8.8.2.1. Informed Consent

The Principal Investigator or a qualified designee (e.g., a licensed, qualified medical practitioner such as a physician's assistant or a nurse practitioner) listed on Form FDA 1572 will explain the study to the subject, answer all of the subject's questions, and obtain written informed consent before performing any study-related procedure. Informed

Consent should be conducted in accordance with local requirements. Subjects should be able to verbally describe the benefits and risks associated with this study and what other treatment alternatives are available (as described in the consent form). Only subjects who provide informed consent, as assessed and documented by the Investigator, will be enrolled.

8.8.2.2. Medical History

A medical history obtained by the PI or qualified designee as listed on the Form FDA 1572.

8.8.2.3. Prior/Concomitant Medication Review

Site study staff will record all medications taken for ALS and all current medication within 1 month prior to screening visit in the CRF. Also, the following parameters will be recorded for all concomitant medications: drug name, route of administration, total daily dose, unit, frequency, start/stop dates, indication, and whether the medication was started after last dose of study medication. The concomitant medications will subsequently be coded using the World Health Organization Drug Dictionary (WHO-DD).

8.8.2.4. Physical/Neurological Examination

The physical exams must be performed by the PI or qualified designee (physician, physician's assistant or nurse practitioner) listed on the Form FDA 1572. Clinically significant changes from the signing of the informed consent form (ICF) should be captured as AEs in the CRF.

A complete physical examination includes the following assessments: general appearance, head, eyes, ears/nose/throat, neck, lymph nodes, skin, lungs, heart, abdomen, and musculoskeletal. If the subject is discontinued for any reason, every attempt should be made to perform a final physical and neurological examination.

8.8.2.5. Vital Signs, Height, and Weight

Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, temperature and weight will be assessed at specified visits and will be recorded in the CRFs. Height will be measured and recorded at the Screening Visit only.

Clinically significant changes, as defined by the PI, from the signing of the ICF should be captured as AEs in the CRF.

8.8.2.6. Electrocardiogram (12-Lead ECG)

All subjects will have standard resting 12-lead ECGs performed and interpreted. Subjects are to be supine for at least 5 minutes prior to ECG assessments. The time the ECG is performed will be recorded (using a 24-h clock).

The PI or a qualified designee listed on Form Food and Drug Administration (FDA) 1572 must review, initial, and date the report, which must be filed in the subject's study chart.

Clinically significant findings from the screening report must be captured in the medical history. Any clinically significant changes compared with screening must be captured as an AE in the CRF.

8.8.2.7. Neuroimaging

Subjects will undergo Magnetic Resonance Imaging (MRI) / PBR28 Positron Emission Tomography (PET) twice during the course of the study. The goal of these scans is to measure activated microglia in study participants before treatment and after several weeks of treatment. The first scan will occur between the Screening and the Baseline visits (*off* treatment), after subject eligibility has been confirmed by TSPO affinity binding assay. The second scan will occur between the Week 12 and Week 24 visits (*on* treatment). Neuroimaging will be performed at the MGH Martinos Center for Biomedical Imaging. Subjects will be asked to lie still in a supine position for the duration of the study, which will take approximately 90 min.

Each PET scan will include one administration of [^{11}C]-PBR28 (up to 15mCi, which is equivalent to ~3.7 mSv), injected intravenously with a slow bolus over a 30s period. The catheter will be flashed post-injection of with 0.9% saline solution. Dynamic data will be collected over approximately 90 minutes in list mode, and framed post-collection.

8.8.2.8. Pulmonary Function Test

Pulmonary Function Testing includes Slow Vital Capacity (SVC).

Slow Vital Capacity (SVC): The vital capacity (VC) (percent of predicted normal) will be determined, using the upright slow VC method. The SVC can be measured using conventional spirometers that have had a calibration check prior to subject testing. A printout from the spirometer of all VC trials will be retained. Three SVC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest SVC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF.

8.8.2.9. The Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised

ALSFRS-R is a quickly administered (5 minutes) ordinal rating scale (ratings 0 to 4) used to determine subjects' assessment of their capability and independence in 12 functional activities. All 12 activities are relevant in ALS. Initial validity was established by documenting that in ALS patients, change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival. The test-retest reliability is greater than 0.88 for all test items. The advantages of the ALSFRS-R are that the categories are relevant to ALS, it is a sensitive and reliable tool for assessing activities of daily living function in those with ALS, and it is quickly administered. With appropriate training, the ALSFRS-R can be administered with high

inter-rater reliability and test-retest reliability. The ALSFRS-R can be administered by phone with good inter-rater and test-retest reliability. The equivalency of phone versus in-person testing, and the equivalency of study subject versus caregiver responses have also recently been established. Therefore, if necessary, the ALSFRS-R may be given to the study subject over the phone. All ALSFRS-R evaluators must be NEALS certified.

8.8.2.10. Upper Motor Neuron-Burden (UMN-B)

The Penn Upper Motor Neuron-Burden (UMN-B) is the total number of pathological UMN signs on examination including pathologically brisk biceps, supinator, triceps, finger, knee and ankle reflexes, and extensor plantar responses assessed bilaterally and brisk facial and jaw jerks (9). The scale is a combination of Ashworth, Reflexes, and Pseudobulbar Affect scale (Range score: 0 to 32).

The UMN also includes scoring of the Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report scale that assesses pseudobulbar affect (PBA) by measuring the perceived frequency of PBA episodes (laughing or crying) and the Ashworth Spasticity Scale, a standard measure for spasticity that has been used in numerous ALS clinical trials to assess spasticity due to upper motor neuron dysfunction in ALS. Data is generated from the clinical exam and scored from 1 to 5, the lowest score indicating normal tone and the highest extreme spasticity.

8.8.2.11. Blood Biomarkers

Subjects will also be asked to provide blood samples for biomarker analysis per Schedule of Assessments. These blood samples will be stored in a sample repository. These samples will be used for biomarker research. All samples will be labeled with a code. The code will not include any identifiable information. Any analysis performed on these samples is for research purposes only. Using a bar coding and scanner system, each sample aliquot will be catalogued for future reference. In the electronic data capture system, sample number and location will be cross-referenced to the clinical information. All samples will be linked to GUID and may be stored using the Global Unique Identifier (GUID) to identify the sample.

Unused samples will remain in the biorepository for future research. There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decayed or otherwise unfit for analysis. Subjects have the option of declining participation in this portion of the study at any time by withdrawing their consent to have their sample used. However, it will not be possible to destroy samples that may have already been used.

8.8.2.12. Strength Measurement (HHD)

Changes in strength will be measured using HHD (hand-held dynamometry), a non-invasive device that allows measurements of isometric strength in muscle groups of the arms and legs. It has been directly validated against maximum voluntary isometric contraction (MVIC) in ALS patients, and shown to change at a similar rate with variability

that is only slightly greater than MVIC. The MVIC has proven useful as an outcome measure in natural history studies and clinical trials in ALS and is a valid and reliable measure of disease progression. For both upper and lower extremity muscles, correlations between MVIC and HHD measurements ranged between 0.84 and 0.92, with test–retest variability that was extremely similar as well. The only time at which correlation between HHD and MVIC broke down was at extremely high strength levels, an area not likely to be a problem in an ALS clinical trial. HHD equipment is inexpensive and it takes less than 5 min to complete a test of both upper and lower extremities.

8.8.2.13. Adverse Event (AE) Monitoring

The PI or a qualified designee listed on Form FDA 1572 must assess the severity and relationship to study medication for all AEs (see Section 11.2).

All observed or volunteered AEs regardless of treatment group or suspected causal relationship to the investigational product(s) will be recorded on the AE page(s) of the CRF.

The PI, sub-investigator (SI) and members of the clinical research team are responsible for identifying adverse events and reporting them to RN coordinator.

For all AEs, the Investigator must pursue and obtain information adequate to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE (see Section 11.2) requiring immediate notification to the Sponsor or its designated representative.

For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. The Investigator is required to assess causality and indicate that assessment on the CRF. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the Investigator, and Sponsor concurs with that assessment.

Adverse events (serious and non-serious) including all Suspected Unexpected Serious Adverse Reactions (SUSARs) should be recorded on the CRF from the date of informed consent until the end of their participation in the study (i.e., the subject has discontinued or completed the follow-up visit).

8.8.2.14. Laboratory Evaluations

Laboratory evaluations will include the tests listed in Appendix 1. Samples will be sent to the local laboratory listed on Form FDA 1572.

Laboratory test results as they are reported will be tabulated per subject by date, time, test name, test result and reviewed by PI, SI, RN coordinator daily; if a clinically significant laboratory result is identified upon reporting, it will be reviewed with the PI or SI immediately.

9. STUDY DRUG MATERIALS AND MANAGEMENT

9.1. Study Drug

The investigational product is MN-166 (ibudilast) Study drug information is provided in Table 5.

Table 5: Study Drug Information

Investigational Drug:	MN-166
Formulation:	10 mg capsules
Frequency:	50 mg bid (total daily dose of 100 mg). Study drug dosing may be varied based on individual tolerability.
Storage Conditions:	Store at room temperature
Packaging Description:	Polyethylene bottles

9.2. Study Drug Packaging and Labeling

At a minimum, the following information will be included on each bottle:

- Name of Sponsor
- Study number/Acronym/IND number
- Route of administration
- Quantity of dosage unit
- Directions for use
- Storage conditions
- Space for information to be completed by Investigator/designee:
  - Name and telephone number of Investigator
  - Dispensing date
  - Subject number
- Statement "Caution: New Drug – Limited by federal law to investigational use"
- Statement: "Keep out of reach of children"

9.3. Study Drug Storage

The study drug MN-166 should be stored at room temperature (preferably 18-23^{0}C, but 15-25^{0}C is acceptable).

9.4. Study Drug Administration

The study drug will be dispensed by appropriately qualified site study staff as indicated on the delegation of authority log. Subjects will self-administer the study drug orally at home following the directions given to them in the clinic. The subject will be instructed to return all unused study drug to the clinical trial site at each visit. The subject will be given a drug administration diary to complete and have available for telephone and clinic visits. Subject

drug diary information will be transcribed into eCRF from the subject's diary at each monthly telephone or clinic visit. If the subject loses a diary he will be advised to keep drug administration data on a separate piece of paper until he had a replacement diary.

9.5. Study Drug Accountability

Investigational clinical supplies must be received by the PI or a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the Investigator and/or designated assistants have access. Clinical supplies are to be dispensed only in accordance with the protocol.

The PI or designee is responsible for keeping accurate records of the clinical supplies received from the Sponsor or designee, the amount dispensed to and returned by the subjects, and the amount remaining at the conclusion of the study. At the end of the study, all clinical supplies must be returned to the Sponsor, or designee, after confirmation with the CRA (Clinical Research Associate) or destroyed at the clinical site. Study drug will not be destroyed until written documentation is received from the study sponsor or designee. Proper documentation of the destruction of study drug must be provided by the site.

The following information is to be included in each subject's CRF: visit medication dispensed, dosing start/stop dates, dosage level, number of tablets dispensed and number of tablets returned.

10. ASSESSMENTS OF SAFETY AND CLINICAL ENDPOINT RESPONSIVENESS

10.1. Primary Endpoints

- Neuroimaging biomarkers ([^{11}C]-PBR28-PET) including both regions of interest (ROI) and voxel-based analyses.
- Blood biomarkers: including analysis of pro-inflammatory cytokines.

10.2. Secondary Endpoints

Safety Endpoints

Safety will be assessed by the proportion of subjects with the following events:

- clinical and laboratory treatment emergent adverse events (TEAEs)
  - stratified by severity
  - stratified by persistence over time
  - stratified by relationship to study drug
- discontinuations due to TEAEs
- treatment emergent serious adverse events (TESAEs)

Safety (relationship and severity) and tolerability will further be assessed by statistical and clinical review of AEs, laboratory values, ECGs, physical examinations, vital signs and weight.

Clinical Efficacy Endpoints

Clinical outcomes will be measured by ALSFRS-R, SVC, and strength (measured by HHD).

11. ADVERSE EVENTS

11.1. Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study subject administered a medicinal (investigational) product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. Adverse events may include the onset of a new illness and the exacerbation of pre-existing conditions.

Other untoward events occurring in the framework of a clinical study are also to be recorded as AEs (e.g., those occurring during treatment-free periods, including screening or post-treatment follow-up periods), in association with study-related procedures and assessments.

In this study, symptoms of progression/worsening of ALS, including 'normal' progression, will be recorded as adverse events. The following measures of disease progression will not be recorded as adverse events even if they worsen: ALSFRS-R. These measures will be recorded and analyzed separately.

11.2. Assessment of Adverse Events

The PI or an authorized physician will assess all AEs for severity, relationship with study medication, and whether it meets the criteria for classification as a SAE, requiring immediate notification to the Sponsor or designee (see Section 11.5). These assessments will be made in accordance with the standard ratings detailed in the following sections.

11.2.1. Severity Assessment

The severity of AEs will be determined as described in Table 6.

Table 6: Adverse Events Severity Definitions

Mild Grade 1	Ordinarily transient symptoms that do not influence performance of subject's daily activities. Treatment is not ordinarily indicated.
Moderate Grade 2	Marked symptoms sufficient to make the subject uncomfortable. Moderate influence on performance of subject's daily activities. Treatment may be necessary.
Severe Grade 3	Symptoms cause considerable discomfort. Substantial influence on subject's daily activities. May be unable to continue in the study and treatment may be necessary.

Life-threatening Grade 4	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required hospitalization probable.
Death Grade 5	Death.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the event should be noted for that day. Any change in severity of signs and symptoms over a number of days will be captured by recording a new AE, with the amended severity grade and the date (and time, if known) of the change.

11.2.2. Relationship to Study Drug

One of the following categories in Table 7 should be selected based on medical judgment, considering the definitions below and all contributing factors.

Table 7: Adverse Event Causality Definitions

Related	A clinical event, including laboratory test abnormality, occurs in a plausible time relationship to treatment administration, and which cannot be explained by concurrent disease or other medications or chemicals. The response to withdrawal of the treatment (dechallenge[a]) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge[b] procedure if necessary.
Probably related	A clinical event, including laboratory test abnormality, occurs within a reasonable time sequence to administration of the treatment, unlikely to be attributed to concurrent disease or other medications or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
Possibly related	A clinical event, including laboratory test abnormality, occurs within a reasonable time sequence to administration of the treatment, but which could also be explained by concurrent disease or other medications or chemicals. Information on treatment withdrawal may be lacking or unclear.
Unlikely to be related	A clinical event, including laboratory test abnormality, occurs with a temporal relationship to treatment administration that makes a causal relationship improbable, and in which other medications, chemicals, or underlying disease provide plausible explanations.
Unrelated	A clinical event, including laboratory test abnormality, occurs with little or no temporal relationship with treatment administration. May have negative dechallenge and rechallenge information. Typically explained by extraneous factors (eg, concomitant disease, environmental factors, or other medications or chemicals).

[a] Dechallenge is when a medication suspected of causing an AE is discontinued. If the symptoms of the AE disappear partially or completely, within a reasonable time from medication discontinuation, this is termed a positive dechallenge. If the symptoms continue despite withdrawal of the medication, this is termed a

negative dechallenge. Note that there are exceptions when an AE does not disappear upon discontinuation of the medication, yet medication-relatedness clearly exists (e.g., as in bone marrow suppression, fixed medication eruptions, or tardive dyskinesia).

[b] Rechallenge is when a medication suspected of causing an AE in a specific subject in the past is readministered to that subject. If the AE recurs upon exposure, this is termed a positive rechallenge. If the AE does not recur, this is termed a negative rechallenge.

11.3. Recording Adverse Events

Adverse events should be collected and recorded for each subject from the date the informed consent form (ICF) was signed until the end of their participation in the study, (ie, the subject has discontinued or completed the study) through the follow-up visit. Only those AEs that occurred while on study drug that have not been resolved will be followed until resolution or stabilization.

Following the end of the subject's participation in the study, the PI or an authorized delegate should report SAEs "spontaneously" if considered at least possibly related to study medication.

Adverse events may be volunteered spontaneously by the study subject, or discovered by the study staff during physical examinations or by asking an open, non-leading question such as, "How have you been feeling since you were last asked?" All AEs and any required remedial action will be recorded in the subject's source documentation and transcribed onto the appropriate CRF page for the study period indicated. The nature of AE, date (and time, if known) of AE onset, date (and time, if known) of AE outcome to date, severity, and action taken of the AE will be documented together with the PIs or an authorized physician's assessment of the seriousness of the AE and causal relationship to study medication and/or study procedure (at the time of assessment).

All AEs should be recorded individually in the study subject's own words (verbatim) unless, in the opinion of the PI or an authorized physician, the AEs constitute components of a recognized condition, disease, or syndrome. In the latter case, the condition, disease, or syndrome should be named rather than each individual symptom. The AEs will subsequently be coded using the MedDRA.

Throughout the study, safety will be monitored closely for unexpected or significant adverse effects by the Principal Investigator. Any unanticipated problems involving risks to subjects or others including adverse events will be reported to the PHRC in accordance with PHRC unanticipated problems reporting guidelines. The Principal Investigator will confirm that study staff is appropriately trained in study procedures. The Principal Investigator will delegate tasks based on appropriate staff qualifications. The Principal Investigator will carefully monitor each subject throughout the study for possible AEs. AEs, SAEs and all safety-related events will be reported in accordance with IRB guidelines.

11.4. Treatment and Follow-Up of AEs

Appropriate measures should be taken to treat AEs as necessary, and the response of the study subject should be monitored and recorded. Clinical, laboratory, and diagnostic measures should be obtained as needed, and the results of which should be recorded in the subject's source documentation and transcribed onto the appropriate CRF page.

All SAEs will be followed until resolution, stabilization of the condition, the event is otherwise explained, or the subject is lost to follow-up.

11.5. Serious Adverse Events (SAEs)

An AE is considered serious if it meets one or more of the following criteria:

- Results in death
- Is life-threatening (i.e., a subject is at immediate risk of death at the time of the event, not an event where occurrence in a more severe form might have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Is another important medical event (see below)

Important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or in a physician's office, blood dyscrasias or seizures that do not result in inpatient hospitalization, and the development of drug dependency or drug abuse. A distinction should be drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria above. For example, a mild degree of gastrointestinal bleeding requiring an overnight hospitalization for monitoring purposes would be considered an SAE, but is not necessarily severe. Similarly, an AE that is severe in intensity is not necessarily an SAE. For example, alopecia may be assessed as severe in intensity, but would probably not be considered an SAE.

11.5.1. SAE Reporting Requirements

The PI or an authorized delegate is responsible for faxing the requested SAE information to the Sponsor or designee within 24 hours or as soon as possible after learning of the event. Following the end of the subject's participation in the study, the PI or an authorized delegate should report SAEs "spontaneously" to the Sponsor if considered at least possibly related to study medication (MN-166).

Notification should be made by fax or email a completed SAE Report Form to the Sponsor. Study sites in the US should fax or email a completed SAE Report Form to fax number 858-404-0048 or safetyreports@medicinova.com**.** As a minimum requirement, the initial notification should provide the following information:

- Study number
- Subject number
- Gender
- Date of birth
- PI's name and full study site address
- Details of SAE
- Criterion/criteria for classification as "serious"
- Study medication name, or code if blinded, and treatment start date
- Date of SAE onset
- Causality assessment (if sufficient information is available to make this classification).

Initial reports of SAEs must be followed later with detailed descriptions, including clear photocopies of other documents as necessary (e.g., hospital reports, consultant reports, autopsy reports, etc.), with the study subject's personal identifiers removed. All relevant information obtained by the PI or an authorized delegate through review of these documents will be recorded on the AE eCRF page and/or a new SAE Report Form and faxed to the Sponsor or designee within 24 hours of receipt of the information. If a new SAE Report Form is faxed, the PI must sign and date the form. The Sponsor may also request additional information on the SAE, which the PI or an authorized delegate must fax to the Sponsor or designee within 24 hours of the request using a new SAE Report Form, bearing the PI's signature and date.

Any AE fulfilling the criteria for expedited reporting will be reported by the Sponsor to regulatory authorities and Investigators and IEC(s) in accordance with the Sponsor's standard operating procedures (SOPs) and local regulatory requirements.

The PI should report all Investigational New Drug Application safety alerts received from the Sponsor to the local IRB.

11.6. Guidance for Overdose

There is no clinical experience with MN-166 overdose in humans and there is no available specific antidote to the effects of MN-166. Standard symptomatic support measures should be used in the case of excessive pharmacological effects or overdose.

11.7. Reporting and Follow-up of Pregnancies

If any study subject or subject's partner becomes pregnant after receiving the first dose of study medication (MN-166 or placebo) and until the follow-up period specified in the protocol, the PI or an authorized delegate should submit a Pregnancy Report Form to the sponsor within 24 hours of the PI or an authorized delegate first becoming aware of the pregnancy. If a pregnancy is to be terminated, the anticipated date of termination should also be provided in the "Additional Information/Comments" field of the Pregnancy Report Form. If a maternal SAE is reported for the study subject during the initial notification of pregnancy, a separate SAE Report Form should also be completed and submitted to the sponsor within 24 hours of the PI or an authorized delegate first becoming aware of the SAE.

Subjects who become pregnant while in the study should be followed for the duration of their pregnancy. If the pregnancy is discovered between regularly scheduled study visits, subjects should return for an unscheduled visit to return their study medication. A quantitative β-hCG should be obtained and subjects should be encouraged to return for follow-up visits. If follow-up visits are not possible, then the principal investigator should collect information about the pregnancy such as spontaneous or elective termination, details of birth, and presence or absence of birth defects, congenital abnormalities, or maternal and newborn complications.

The Sponsor will request that the PI follow the progress of the study subject's pregnancy with the doctor medically responsible for the pregnancy. A new Pregnancy Report Form should be submitted within 24 hours of the PI or an authorized delegate first becoming aware of any new information.

If additional information on the outcome of the pregnancy and/or the details of the birth/delivery is received "spontaneously" by the study site, the PI or authorized delegate should also submit a Pregnancy Report Form within 24 hours of becoming aware of the information. If the outcome of the pregnancy is reported as premature birth, or as elective termination due to a medical reason or as spontaneous or accidental miscarriage, the details of the outcome should be described in the "Additional Information/Comments" field of the Pregnancy Report Form. The pregnancy outcome will generally be reported as a follow-up report.

Complete an SAE Report Form if the delivery outcome meets the criteria for a SAE (e.g., congenital anomaly/birth defect, stillbirth, some other sickness, etc.). The SAE Report Form should be completed with the study subject's details (e.g., subject number, initials, date of birth, investigational product information, etc.) and the details of the fetal SAE and maternal complications should be described in the "Narrative" field of the SAE Report Form.

If a pregnancy is reported for the study subject's partner, the sponsor will provide instructions on how to collect pregnancy information in accordance with local requirements.

11.8. Preplanned Hospitalizations or Procedures

During the study, if a subject has a hospitalization or procedure (e.g., elective surgery) that was scheduled prior to the subject entering the study (i.e., before the subject signed the ICF) for an event/condition that occurred before the study, the hospitalization is considered a therapeutic intervention and not the result of an SAE. However, if the event/condition worsens during the study, it must be reported as an AE or SAE (if the event/condition results in a serious outcome such as prolongation of hospitalization.)

12. STATISTICAL CONSIDERATIONS

The Statistical Analysis Plan (SAP) will provide details on the statistical methods planned for this study.

12.1. Sample Size Justification

Sample size calculation was based on the primary outcome of the biomarker. The primary outcome is the changes in the ROI in the motor cortex as assessed by [^{11}C]-PBR29-PET. Our previous studies comparing mean PBR28 binding in the motor cortices in limb-onset ALS subjects (1.18 units) with matching healthy volunteers (1.064 units) revealed 0.116 mean difference between the two groups, with 0.08 standard deviation in the ALS group. With 10 people tolerated the drug among 15 participants in this study, the probability is 90 percent that the study will detect a treatment difference at a one-sided 0.1 significance level if the true difference is 0.096 units. The proposed increase in sample size will increase the statistical power to detect changes in PBR28 uptake after MN-166 treatment. This will also provide more safety and tolerability data on MN-166 for up to 50 mg BID in people with ALS.

12.2. Safety Analysis

The incidence of treatment-emergent AEs (TEAEs, defined as AEs occurring from the time of first dose through 7 days after the last dose of study medication), SAEs and AEs leading to discontinuations will be summarized by treatment group. Incidence of TEAEs will also be summarized by severity (mild, moderate, or severe), as well as by relationship to treatment (not related, possibly related, or probably related) and by seriousness.

Changes from baseline in laboratory values will be summarized. Incidence of out-of-normal-range values and markedly abnormal change from baseline in laboratory safety test variables will be tabulated.

Changes in vital signs from baseline will be summarized.

12.3. Direct Access To Source Data/Documents

By signing this protocol, the PI agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (GCP); and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

The PI also agrees to allow monitoring, audits, and regulatory agency inspection of study-related documents and procedures and provide for direct access to all study-related source data and documents.

The PI shall prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable federal, state, and local laws, rules, and regulations; and, for each subject participating in the study, provide all data, and upon completion or termination of the clinical study submit any other reports to the Sponsor, or its designee, as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Study documentation will be promptly and fully disclosed to the Sponsor, or its designee, by the PI upon request and shall also be made available at the PI's site upon request for inspection, copying, review, and audit at reasonable times by representatives of the Sponsor or any regulatory agencies. The PI agrees to promptly take any reasonable steps that are requested by the Sponsor, or its designee, as a result of an audit to address deficiencies in the study documentation and worksheets/CRFs.

The PI will promptly inform the Sponsor or its designee of any regulatory agency inspection conducted for this study.

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on this Sponsor's studies. The PI will immediately disclose in writing to the Sponsor or its designee if any person who is involved in conducting the study is debarred, or if any proceeding for debarment is pending or, to the best of the Investigator's knowledge, threatened.

12.4. Study Monitoring

Monitoring will include on-site monitoring to assure that the investigation is conducted according to protocol, to protect subject rights and safety, and to confirm data integrity and quality.

This study will be monitored through all phases of study conduct by the Sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of GCP. On-site review of CRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each subject. Investigators will be required to store all source documents.

12.5. Audits and Inspections

The PI and appropriate personnel may be periodically requested to attend meetings organized by the Sponsor or its designee to assure acceptable protocol execution. The study may be subject to audit by the Sponsor/designee or by regulatory authorities. If such an audit occurs, the PI must agree to allow access to required subject records. By signing this protocol, the Investigator grants permission to personnel from the Sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring and auditing of all appropriate study documentation, as well as on-site review of the procedures

employed in CRF generation, where clinically appropriate. The PI has to inform the Sponsor if he/she is approached for a regulatory audit.

12.6. Institutional Review Board (IRB)

Before initiation of the study, the PI must obtain approval of the research protocol, informed consent form (ICF), and any advertisement for subject recruitment from the IRB complying with the provisions specified in the Code of Federal Regulations (CFR) 21 Part 56 and applicable government regulations. A copy of written IRB approval of the protocol, ICF, and advertising (if applicable) must be provided to the Sponsor or their designee prior to initiation of the study.

12.7. Study Documentation

By signing a copy of Form FDA 1572, the Investigator acknowledges that he/she has received a copy of the investigational drug brochure on MN-166 and assures the Sponsor that he/she will comply with the protocol and the provisions stated in Form FDA 1572. No changes in this protocol can be made without the Sponsor's written approval.

The Investigator will supply the Sponsor with the following:

1. Original, signed Form FDA 1572
2. Curricula vitae for all Investigators listed on Form FDA 1572
3. Copy of the Investigator's medical licensure/medical registration number
4. Signed protocol signature page
5. Signed IB signature page
6. Financial disclosure forms for all study staff listed on the FDA 1572.

13. QUALITY CONTROL AND QUALITY ASSURANCE

By signing this protocol, the Sponsor and Clinical Study Site Principal Investigator agree to be responsible for implementing and maintaining quality control and quality assurance systems with written standard operating procedures (SOPs) reviewed and approved by the sponsor to ensure that the study is 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.

14. ETHICS

14.1. Ethics Review

Documented approval from the IRB will be obtained for all participating centers prior to clinical trial start, according to ICH (International Conference on Harmonisation) GCP, local laws, regulations and organization. When necessary, an extension, amendment or renewal of the CIRB approval must be obtained.

14.2. Ethical Conduct of the Study

The procedures set out in this clinical trial protocol pertaining to the conduct, evaluation, and documentation of this clinical trial, are designed to ensure that the Sponsor and Principal Investigator abide by Good Clinical Practice Guidelines (GCP in the appropriate current version). The clinical trial will also be carried out in accordance with applicable local law(s) and regulation(s). This may include an inspection by representatives from MediciNova Inc. and/or Regulatory Authority representatives at any time. The PI must agree to the inspection of clinical trial-related records by MediciNova, Inc. representatives, and must allow representatives direct access to source documents.

14.3. Written Informed Consent

An information and consent form will be provided to the subject. The process of obtaining informed consent must be in accordance with applicable regulatory requirements, and must adhere to GCP and ethical principles in the Declaration of Helsinki. Written informed consent must be obtained and documented before any clinical trial-specific procedure takes place. Participation in the clinical trial and date of informed consent given by the subject must be documented in the subject files.

14.4. Confidentiality

14.4.1. Confidentiality of Data

By signing this protocol, the Investigator affirms to the Sponsor that information furnished to the Investigator by the Sponsor will be maintained in confidence and such information will be divulged to the IRB or similar or expert committee; affiliated institution; and employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees.

15. DATA HANDLING AND RECORDKEEPING

15.1. Review of Records

The results from Screening and data collected during the study will be recorded in the subject's CRF, which will be designed and provided by the sponsor or a designee. The Investigator will review all CRFs. The CRFs will be signed by the PI or a sub-Investigator who is listed on the Form FDA 1572 if the PI is unavailable. In order to maintain confidentiality, the subject will be identified only by his/her subject number and initials.

15.2. Retention of Records

The PI must arrange for retention of study records at the site for at least two years after the New Drug Application (NDA) is approved or Investigational New Drug (IND) is withdrawn, as required by the US Food and Drug Administration (FDA) regulations, or in accordance with local and/or national requirements, whichever is longer. The PI should take measures to prevent accidental or premature destruction of these documents. Documents cannot be destroyed without written Sponsor authorization. The Sponsor will inform the PI when the destruction of documents is permitted.

All data collected as part of this study will be entered into an EDC maintained by the Massachusetts General Hospital (MGH) Neurological Clinical Research Institute (NCRI). This platform facilitates:

1. Capture of clinical and research data from neurologic patients for individual projects in a structured and secure system;
2. Aggregating and sharing uniform, deidentified and/or anonymized datasets for secondary analyses.

15.3. Role of Data Management

Data Management (DM) is responsible for 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 applicable Sponsor and regulatory requirements. Site personnel will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track and record clinical research data. DM is responsible for developing, testing, and managing clinical data management activities.

15.4. Data Entry and Checks

The site personnel are instructed to enter information into the EDC. Data capture is the responsibility of the staff at the site under the supervision of the PIs. During the study, the PIs must maintain complete and accurate documentation for the study. The EDC provide password protection. An edit checking and data clarification process will be put in place to

ensure accuracy of the data. Logic and range checks as well as more sophisticated rules may be built into the eCRFs to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing or not calculated correctly.

15.5. Data Lock Process

The platform will have the ability to lock the project-specific visits to prevent any modification of data once the project is closed. Once this option is activated, every user will have Read-Only access to the data.

15.6. Data handling and record keeping

The PIs are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained.

15.7. Publications

The PIs will be responsible for publications of results from this trial. Responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

16. ADMINISTRATIVE AND REGULATORY DETAILS

16.1. Protocol Amendments and Study Termination

All revisions and/or amendments to this protocol must be approved in writing from the Sponsor and the IRB, except where necessary to eliminate an apparent immediate hazard to a study subject.

16.2. Discontinuation of the Study

The Sponsor reserves the right to discontinue the study at site(s) for safety or administrative reasons at any time. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the Sponsor or its representative.

16.3. Compliance with Financial Disclosure Requirements

By signing this protocol, the PI agrees to provide to the Sponsor accurate financial information to allow the Sponsor to submit complete and accurate certification and disclosure statements as required by the US FDA regulations (21 CFR Part 54). The PI further agrees to provide this information on a Financial Disclosure/Certification Form that is provided by MediciNova, Inc. The Investigator will update this information if there are any relevant changes during the conduct of the study and for one year after completion of the study. This requirement also extends to sub-Investigators. The PI also consents to the transmission of this information to MediciNova, Inc. for these purposes.

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18. APPENDICES

Appendix 1: Laboratory Safety Tests for MN-166

Blood Chemistry Tests
aspartate aminotransferase (AST)
alanine aminotransferase (ALT)
albumin
alkaline phosphatase
bicarbonate
blood urea nitrogen
calcium
chloride
creatinine
gamma-glutamyl transferase
potassium
sodium
total bilirubin[a]
total protein
glucose
creatinine kinase
Endocrine Tests
serum beta-human chorionic gonadotropin (for females of childbearing potential)
urine beta-human chorionic gonadotropin
Hematology Tests
white blood cell count
white blood cell differential
eosinophilic leukocyte count
basophilic leukocyte count
neutrophil count
lymphocyte count
monocyte count
platelet count
hemoglobin
blood hematocrit
red blood cell count
red cell distribution width
red blood cell indices:
mean corpuscular volume
mean corpuscular hemoglobin concentration
mean corpuscular hemoglobin

[a] Bilirubin will be fractionated (direct serum bilirubin test/indirect serum bilirubin test) if elevated 2.0 times the upper limit of the normal range.

Appendix 2: El Escorial World Federation of Neurology Criteria for the Diagnosis of ALS

Information obtained from the web site: **www.wfnals.org**.

The diagnosis of Amyotrophic Lateral Sclerosis [ALS] requires:

A - The presence of:

(A:1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,

(A:2) evidence of upper motor neuron (UMN) degeneration by clinical examination, and

(A:3) progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination, together with

B - The absence of:

(B:1) electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and

(B:2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

CLINICAL STUDIES IN THE DIAGNOSIS OF ALS

A careful history, physical and neurological examination must search for clinical evidence of UMN and LMN signs in four regions [brainstem, cervical, thoracic, or lumbosacral spinal cord] (see Table 1) of the central nervous system [CNS]. Ancillary tests should be reasonably applied, as clinically indicated, to exclude other disease processes. These should include electrodiagnostic, neurophysiological, neuroimaging and clinical laboratory studies. Clinical evidence of LMN and UMN degeneration is required for the diagnosis of ALS. The clinical diagnosis of ALS, without pathological confirmation, may be categorized into various levels of certainty by clinical assessment alone depending on the presence of UMN and LMN signs together in the same topographical anatomic region in either the brainstem [bulbar cranial motor neurons], cervical, thoracic, or lumbosacral spinal cord [anterior horn motor neurons]. The terms Clinical Definite ALS and Clinically Probable ALS are used to describe these categories of clinical diagnostic certainty on clinical criteria alone:

A. Clinically Definite ALS is defined on clinical evidence alone by the presence of UMN, as well as LMN signs, in three regions.

B. Clinically Probable ALS is defined on clinical evidence alone by UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.

C. Clinically Probable ALS - Laboratory-supported is defined when clinical signs of UMN and LMN dysfunction are in only one region, or when UMN signs alone are present in one region, and LMN signs defined by EMG criteria are present in at least two limbs, with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.

D. Clinically Possible ALS is defined when clinical signs of UMN and LMN dysfunction are found together in only one region or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of Clinically Probable - Laboratory-supported ALS cannot be proven by evidence on clinical grounds in conjunction with electrodiagnostic, neurophysiologic, neuroimaging or clinical laboratory studies. Other diagnoses must have been excluded to accept a diagnosis of Clinically Possible ALS.

Table 1

	Brainstem	**Cervical**	**Thoracic**	**Lumbosacral**
Lower motor neuron signs weakness, atrophy, fasciculations	jaw, face, palate, tongue, larynx	neck, arm, hand, diaphragm	back, abdomen	back, abdomen, leg, foot
Upper motor neuron signs pathologic spread of reflexes, clonus, etc.	clonic jaw gag reflex exaggerated snout reflex pseudobulbar features forced yawning pathologic DTRs spastic tone	clonic DTRs Hoffman reflex pathologic DTRs spastic tone preserved reflex in weak wasted limb	loss of superficial abdominal reflexes pathologic DTRs spastic tone	clonic DTRs - extensor plantar response pathologic DTRs spastic tone preserved reflex in weak wasted limb

Appendix 3: ALS Functional Rating Scale-Revised (ALSFRS-R)

QUESTIONS: **SCORE:**

1. Speech
4 = Normal speech processes
3 = Detectable speech disturbances
2 = Intelligible with repeating
1 = Speech combined with nonvocal communication
0 = Loss of useful speech

2. Salivation
4 = Normal
3 = Slight but definite excess of saliva in mouth; may have nighttime drooling
2 = Moderately excessive saliva; may have minimal drooling
1 = Marked excess of saliva with some drooling
0 = Marked drooling; requires constant tissue or handkerchief

3. Swallowing
4 = Normal eating habits
3 = Early eating problems – occasional choking
2 = Dietary consistency changes
1 = Needs supplemental tube feeding
0 = NPO (exclusively parenteral or enteral feeding)

4. Handwriting
4 = Normal
3 = Slow or sloppy; all words are legible
2 = Not all words are legible
1 = No words are legible but can still grip a pen
0 = Unable to grip pen

5a. Cutting Food and Handling Utensils (patients without gastrostomy)
4 = Normal
3 = Somewhat slow and clumsy, but no help needed
2 = Can cut most foods, although clumsy and slow; some help needed
1 = Food must be cut by someone, but can still feed slowly
0 = Needs to be fed

5b. Cutting Food and Handling Utensils (alternate scale for patients with gastrostomy)
4 = Normal
3 = Clumsy, but able to perform all manipulations independently
2 = Some help needed with closures and fasteners

1 = Provides minimal assistance to caregivers
0 = Unable to perform any aspect of task

6. Dressing and Hygiene ☐
4 = Normal function
3 = Independent, can complete self-care with effort or decreased efficiency
2 = Intermittent assistance or substitute methods
1 = Needs attendant for self-care
0 = Total dependence

7. Turning in Bed and Adjusting Bed Clothes ☐
4 = Normal function
3 = Somewhat slow and clumsy, but no help needed
2 = Can turn alone, or adjust sheets, but with great difficulty
1 = Can initiate, but not turn or adjust sheets alone
0 = Helpless

☐

8. Walking
4 = Normal
3 = Early ambulation difficulties
2 = Walks with assistance
1 = Nonambulatory functional movement only
0 = No purposeful leg movement

9. Climbing Stairs ☐
4 = Normal
3 = Slow
2 = Mild unsteadiness or fatigue
1 = Needs assistance
0 = Cannot do

R-1. Dyspnea
4 = None
3 = Occurs when walking
2 = Occurs with one or more of the following: eating, bathing, dressing
1 = Occurs at rest, difficulty breathing when either sitting or lying
0 = Significant difficulty, considering using mechanical respiratory support

R-2 Orthopnea ☐
4 = None
3 = Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2 = Needs extra pillow in order to sleep (more than two)
1 = Can only sleep sitting up
0 = Unable to sleep without mechanical assistance

R-3 Respiratory Insufficiency ☐
4 = None
3 = Intermittent use of BiPAP
2 = Continuous use of BiPAP during the night
1 = Continuous use of BiPAP during the night and day
0 = Invasive mechanical ventilation by intubation or tracheostomy

Evaluator's Initials: _____ _____ _____

Appendix 4: Columbia-Suicide Severity Rating Scale (C-SSRS)

Baseline (Version 1/14/09)

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:
This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*
For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.

Lifetime: Time He/She Felt Most Suicidal

1. Wish to be Dead
Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.
Have you wished you were dead or wished you could go to sleep and not wake up?
If yes, describe:

Yes ☐ **No** ☐

2. Non-Specific Active Suicidal Thoughts
General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan.
Have you actually had any thoughts of killing yourself?
If yes, describe:

Yes ☐ **No** ☐

3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."*
Have you been thinking about how you might do this?
If yes, describe:

Yes ☐ **No** ☐

4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan
Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*
Have you had these thoughts and had some intention of acting on them?
If yes, describe:

Yes ☐ **No** ☐

5. Active Suicidal Ideation with Specific Plan and Intent
Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.
Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?
If yes, describe:

Yes ☐ **No** ☐

INTENSITY OF IDEATION

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal.

Most Severe Ideation: _____ _____

Type # (1-5) ***Description of Ideation***

Most Severe

Frequency
How many times have you had these thoughts?
(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day

____

Duration
When you have the thoughts, how long do they last?
(1) Fleeting - few seconds or minutes
(2) Less than 1 hour/some of the time
(3) 1-4 hours/a lot of time
(4) 4-8 hours/most of day
(5) More than 8 hours/persistent or continuous

____

Controllability
Could/can you stop thinking about killing yourself or wanting to die if you want to?
(1) Easily able to control thoughts
(2) Can control thoughts with little difficulty
(3) Can control thoughts with some difficulty
(4) Can control thoughts with a lot of difficulty
(5) Unable to control thoughts
(0) Does not attempt to control thoughts

____

Deterrents
Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?
(1) Deterrents definitely stopped you from attempting suicide
(2) Deterrents probably stopped you
(3) Uncertain that deterrents stopped you
(4) Deterrents most likely did not stop you
(5) Deterrents definitely did not stop you
(0) Does not apply

____

Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain. (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

 C-SSRS—Baseline (Version 1/14/09)

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes No** ☐ ☐

Suicidal Behavior: Suicidal behavior was present during the assessment period?			**Yes** ☐ **No** ☐
Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____

 C-SSRS—Baseline (Version 1/14/09)

Since Last Visit (Version 1/14/09)

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.

Since Last Visit

1. Wish to be Dead
Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.
Have you wished you were dead or wished you could go to sleep and not wake up?
If yes, describe:

Yes ☐ **No** ☐

2. Non-Specific Active Suicidal Thoughts
General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period.
Have you actually had any thoughts of killing yourself?
If yes, describe:

Yes ☐ **No** ☐

3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."*
Have you been thinking about how you might do this?
If yes, describe:

Yes ☐ **No** ☐

4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan
Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*
Have you had these thoughts and had some intention of acting on them?
If yes, describe:

Yes ☐ **No** ☐

5. Active Suicidal Ideation with Specific Plan and Intent
Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.
Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?
If yes, describe:

Yes ☐ **No** ☐

INTENSITY OF IDEATION

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe).

Most Severe Ideation: _____ _____

Type # (1-5) ***Description of Ideation***

Most Severe

Frequency
How many times have you had these thoughts?
(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day

____

Duration
When you have the thoughts, how long do they last?
(1) Fleeting - few seconds or minutes
(2) Less than 1 hour/some of the time
(3) 1-4 hours/a lot of time
(4) 4-8 hours/most of day
(5) More than 8 hours/persistent or continuous

____

Controllability
Could/can you stop thinking about killing yourself or wanting to die if you want to?
(1) Easily able to control thoughts
(2) Can control thoughts with little difficulty
(3) Can control thoughts with some difficulty
(4) Can control thoughts with a lot of difficulty
(5) Unable to control thoughts
(0) Does not attempt to control thoughts

____

Deterrents
Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?
(1) Deterrents definitely stopped you from attempting suicide
(2) Deterrents probably stopped you
(3) Uncertain that deterrents stopped you
(4) Deterrents most likely did not stop you
(5) Deterrents definitely did not stop you
(0) Does not apply

____

Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

 C-SSRS—Since Last Visit (Version 1/14/09)

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	Since Last Visit
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of interrupted _____
Aborted or Self-Interrupted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of aborted or self-interrupted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes No** ☐ ☐ Total # of preparatory acts _____
Suicide: Death by suicide occurred since last assessment.	**Yes No** ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____

Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____

 C-SSRS—Since Last Visit - Clinical (Version 1/14/09) Page 2 of 2

Appendix 5: The Penn Upper Motor Neuron-Burden (UMN-B)

Center for Neurologic Study – Lability Scale (CNS-LS)

INSTRUCTIONS
The purpose of this questionnaire is to help us better understand your neurologic problems. Please read each statement, and using the scale below, determine the degree to which it has applied to you **DURING THE PAST WEEK**. Circle the appropriate answer, or if you need help in marking your responses, tell the interviewer the number of the best response. Please choose only one response for each item.

Please select the number that describes the degree to which each item has applied to you DURING THE PAST WEEK.					
	Does not Apply 1	Rarely Applies 2	Occasionally Applies 3	Frequently Applies 4	Applies Most of the Time 5
1. There are times when I feel fine 1 minute, and then I'll become tearful the next over something small or for no reason at all.	○	○	○	○	○
2. Others have told me that I seem to become amused very easily or that I seem to become amused about things that aren't funny.	○	○	○	○	○
3. I find myself crying very easily.	○	○	○	○	○
4. I find that even when I try to control my laughter, I am often unable to do so.	○	○	○	○	○
5. There are times when I won't be thinking of anything happy or funny at all, but then I'll suddenly be overcome by funny or happy thoughts.	○	○	○	○	○
6. I find that even when I try to control my crying, I am often unable to do so.	○	○	○	○	○
7. I find that I am easily overcome by laughter.	○	○	○	○	○

Total: _____

Appendix 6: Modified Ashworth Spasticity Scale

ASHWORTH SPASTICITY SCALE

Key:

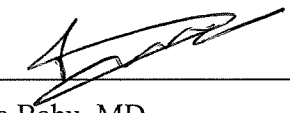
1. No increase in muscle tone
2. Slight increase in tone giving a "catch" when affected part is moved in flexion or extension
3. More marked increase in tone but affected part is easily flexed.
4. Considerable increase in tone; passive movement difficult.
5. Affected part is rigid in flexion or extension

Limb	**Score**
Right Arm	
Left Arm	
Right Leg	
Left Leg	

STATISTICAL ANALYSIS PLAN (SAP)

Title	A multi-Center, open-label biomarker study to evaluate MN-166 (Ibudilast) in Subjects with Amyotrophic Lateral Sclerosis (ALS)
Regulatory Sponsor	Suma Babu, MD
Current Protocol Version	4.0
Current Protocol Date	12 December 2017
Statistical Analysis Plan Version	1.0
Statistical Analysis Plan Date	21 Jun 2019

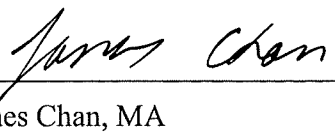
SAP APPROVAL SIGNATURES



06/21/2019

Suma Babu, MD
Principal Investigator and Sponsor

Date



6/21/2019

James Chan, MA
MGH Biostatistician

Study Biostatistician

Date

SAP REVISION HISTORY

Version	Date	Description of Changes
1.0	21Jun2019	Initial version

Table of Contents

1. Introduction

This statistical analysis plan (SAP) defines the outcome measure and analysis samples and specifies the planned analyses of data from the phase Ib/IIa open label trial of MN-166 (Ibudilast) in Amyotrophic Lateral Sclerosis (ALS). The SAP supplements the clinical protocol. Please refer to the clinical protocol for details on the rationale for the intervention, eligibility criteria, conduct of the trial, clinical assessments and the timing of their use in the trial, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. In case of discrepancies between the SAP and the clinical protocol concerning matters of data analysis, the SAP is authoritative. On all other matters, the clinical protocol is authoritative.

2. Study Design

2.1 Overview

This is a multi-center, open label Ib/IIa phase study to evaluate Ibudilast in subjects with ALS. Participants go through 6 weeks of screening and 36 weeks of treatment followed by 4 weeks of off-treatment follow-up.

2.2 Study Objectives

The primary objectives of the study are to measure Ibudilast uptake in motor cortices and brain stem as measured by positron emission tomography (PET) imaging at 12-24 weeks and impact of Ibudilast on biofluid biomarkers of neuro-inflammation over 36 weeks.

The secondary objectives of the study are to evaluate safety and tolerability and the effect of Ibudilast on ALS clinical outcomes such as the revised ALS function rating scale (ALSFRS-R), slow vital capacity (SVC), and strength measured by hand held dynamometry (HHD) over 36 weeks

2.3 Study Populations

Individuals eligible for trial participation are men or women aged 18 or above who are diagnosed as having possible, probable, probable-laboratory supported, or definite ALS, either sporadic or

familial according to the El Escorial Criteria and have a vital capacity of more than 50% of predicted normal. Detailed inclusion and exclusion criteria are specified in the clinical protocol.

Participants will be enrolled at MGH and the South Shore Neurologic Associates Long Island Site. Up to five participants are expected to be enrolled in the study by flexible inclusion and exclusion criteria and not have PET imaging done. This is referred to as the flexible arm.

2.4 Schedule of Assessments

Off-treatment PET imaging will be performed at baseline and on-treatment PET imaging will be performed once between weeks 12 and 24. Blood samples for biomarkers will be collected at baseline and weeks 4, 12, 24, and 36. ALSFRS-R, SVC, and HHD will be collected at baseline and weeks 4, 12, 24, and 36 (SVC will have an additional collection at screening to assess inclusion criteria). Full schedule of assessments is specified in the clinical protocol.

3. General Statistical Considerations

3.1 Statistical Software

Statistical analyses will be performed using SAS (SAS Institute, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria). PET analyses will be done using Freesurfer v6.0 (https://surfer.nmr.mgh.harvard.edu) and FSL Stats.

3.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, safety outcomes, tolerability, and efficacy outcomes. Summary statistics for continuous variables will include the number of subjects, the mean, median, standard deviation, and range. For categorical data, summaries will include counts and percentages.

Biomarkers and clinical outcomes will be summarized longitudinally from baseline to week 36 with means and standard deviations at each timepoint.

3.3 Precision

Results will be reported to 3 significant figures. Percentages will be reported to 0.1 percentage points. P-values will be reported to two digits when greater than or equal to 0.095, to three digits when greater than or equal to 0.00095 and less than 0.095, and as <0.001 for all smaller values.

4. Analysis Samples

The following analysis samples will be used for testing endpoints:

- Per-protocol (PP) sample: Participants who are eligible, randomized, initiate at least the first Ibudilast dose and successfully titrated up to and maintained the full Ibudilast dose of 50mg BID. Flexible arm participants will not be included. This sample will be used to analyze PET, blood biomarker, safety and tolerability endpoints.
- Modified Intent-to-treat (mITT) sample: Participants who are eligible, randomized, initiate at least the first Ibudilast dose, and complete both pre and post-treatment PET scans. Observations made after premature permanent discontinuation or permanent dose reduction of Ibudilast are included in this sample, should such participants remain on study. Flexible arm participants will not be included. This sample will be used to analyze PET, blood biomarker, safety and tolerability endpoints.
- Intent-to-treat (ITT) sample: Participants who are eligible, randomized, and initiate at least the first Ibudilast dose. Observations made after premature permanent discontinuation of Ibudilast are included in this sample, should such participants remain on study. Flexible arm participants will not be included. This sample will be used to analyze blood biomarker, safety and tolerability endpoints.
- Safety (ST) sample: Participants who are eligible, randomized, and initiate at least the first Ibudilast dose. Observations made after premature permanent discontinuation of Ibudilast are included in this sample, should such participants remain on study. Flexible arm participants will be included. This sample will be used to analyze safety and tolerability endpoints.

5. Study Endpoints

5.1 PET Endpoint

The ratio of standardized uptake value (SUVR) will be the only imaging endpoint.

5.2 Biofluid Biomarker Endpoints

MIF (Macrophage migration inhibitory factor), TNF-alpha, Neurofilament light will be used as blood biomarkers for inflammation. All blood biomarkers will be presented as picograms/milliliter (pg/mL).

5.3 Safety Endpoints

To describe safety, clinical and laboratory treatment emergent adverse events (TEAEs) will be summarized overall and stratified by severity, persistence over time and relationship to study drug. Kaplan Meier plots of survival will be presented.

5.4 Tolerability Endpoints

Tolerability will be summarized as the proportion of participants who are tolerant of study drug. Reported proportions will use as their denominator all participants in the ST sample.

5.5 Efficacy Endpoints

- ALSFRS-R Total Score
- SVC as a percent of predicted normal volume
- HHD of the following standardized megascores: (a) upper limbs: proximal (bilateral shoulder and elbow flexion), distal (bilateral wrist extension and first dorsal interossei), (b) lower limbs: proximal (bilateral hip flexion and knee extension) and distal (bilateral ankle dorsiflexion)

6. Measurement Definitions

6.1 PET Measures

Glial activation will be estimated in eligible participants in the regular arm by combined magnetic resonance positron emission tomography (MR-PET) using the [11C]-PBR28 radioligand. Per protocol, individuals who are homozygous for the T/T allele of the Ala147Thr TSPO polymorphism (rs6971) are excluded from the regular arm, since this genotype is associated with low affinity binding for [11C]-PBR28. Glial activation will be quantified as a mean standardized uptake value (SUV) using PET images acquired from 60 to 90 minutes post-injection of approximately 430 MBq [11C]-PBR28. FreeSurfer v6.0 (https://surfer.nmr.mgh.harvard.edu) will be employed to circumscribe a region of interest (ROI) defined anatomically as bilateral grey and white matter of primary motor cortices. SUV of the ROI will be normalized to the SUV of the whole brain tissue and occipital lobes (pseudoreference region)(Albrecht DS et al, J Nucl Med, 2018) and expressed as a SUV ratio (SUVR) separately to control for variability in the global [11C]-PBR28 PET signal. An independent neuroimaging rater, who is blinded to the clinical data will assess for quality control of the PBR28-PET images and SUVR values.

6.2 Blood biomarkers

Blood biomarkers of inflammation and neurodegeneration, including TNF-alpha, MIF (Macrophage migration inhibitory factor) and Neurofilament light will be measured pre- and post-treatment. Prior studies have suggested that by inhibiting MIF, Ibudilast is a potent suppressor of both microglial recruitment and activation in a dose dependent fashion (Cho Y et al, 2010; Cox GM et al 2013). Further, in neuron and microglia co-cultures, Ibudilast significantly suppressed neuronal cell death induced by the activation of microglia and hence exerting a neuroprotective action (Mizuno T et al, 2004). Neuofilament light is a marker of neuronal cell death and axon loss,

shown to be a promising biomarker in ALS, which correlates with disease activity. Measuring NFl is an indirect measure of Ibudilast's effect on neuroprotection in ALS and is hypothesized to be inversely correlated with disease progression markers such as ALSFRS-R and SVC, as well as markers of glial activation on imaging and blood biofluids. TNF-alpha is a marker of neuroinflammation, shown to be elevated in ALS in in vitro and in vivo models and Ibudilast has shown inhibitory action of production of TNF-alpha in a dose-dependent manner (Suzumura A et al, 1999). All these biomarkers will be measures in pg/ml.

6.3 Clinical Progression Measures

6.3.1 ALSFRS-R

The ALSFRS-R (Cedarbaum et al. 1999) is a 12-item clinician-completed interview scale for assessing participants' function in four domains: bulbar, fine motor, gross motor, and respiratory. Each item is scored from 0 to 4 with higher scores indicating greater function. The ALSFRS-R total score is the sum of all items (range 0 to 48).

6.3.2 SVC

Slow vital capacity (SVC) is the maximum volume of air that can be slowly exhaled after slow, maximal inhalation. Trained technicians coach participants through 3 to 5 maneuvers using an EasyOne Plus spirometer (ndd Medical Technologies, Inc., Andover, MA). Assessments will be analyzed if at least two acceptable maneuvers are recorded. The maximum volume expired is converted to percent of predicted normal. Normal values are calculated based on sex, age, and height using equations published by Knudsen et al. (1983). Higher values indicate better pulmonary function.

6.3.3 Handheld dynamometry

Handheld dynamometry (HHD) will be used by trained technicians to estimate isometric strength using a MicroFET2 HHD (Hoggan Scientific, Salt Lake City, UT). Nine upper and lower extremity muscles or muscle groups will be examined: shoulder flexion, elbow flexion, wrist extension, first dorsal interosseous contraction, hip flexion, knee extension, and ankle dorsiflexion. Each muscle or muscle group will be measured twice or three times bilaterally. The average of the two highest measurements will be analyzed. To calculate megascores, the mean and standard deviation of each muscle or muscle group, without regard to laterality, will be calculated from the baseline assessment of all participants in the ITT sample. Maneuvers that cannot be completed by a

participant due to weakness will be scored as zero kg. Strength estimates of each bilateral muscle or muscle group will be converted to Z scores by subtracting the relevant mean and dividing by the relevant standard deviation. Z scores for will be averaged into the standardized megascores:(a) upper limbs: proximal (bilateral shoulder and elbow flexion), distal (bilateral wrist extension and first dorsal interossei), (b) lower limbs: proximal (bilateral hip flexion and knee extension) and distal (bilateral ankle dorsiflexion)

6.4 Safety and Tolerability Endpoints

6.4.1 Safety

TEAEs will be reported for AEs that are possibly or definitely related to study drug and occur in more than 5% of subjects or lead to early drug discontinuation. Discontinuations due to TEAEs, treatment emergent serious adverse events, all SAEs and study deaths, treatment emergent laboratory abnormalities judged to be clinically significant by assay, and mean change from baseline and percent change from baseline in weight, systolic blood pressure, and diastolic blood pressure will also be summarized to describe safety. Reported proportions will use as their denominator all participants in the ST sample and AEs will be classified by MedDRA system organ and preferred term.

6.4.2 Tolerability

Participants will be judged tolerant of Ibudilast if they complete all 36-weeks of treatment and remain on study drug and free from any possibly or definitely drug related AEs leading to permanent study drug discontinuation to the week 36.

7 Power Calculations

Sample size calculation was based on the primary outcome of the biomarker. The primary outcome is the changes in the ROI in the motor cortex as assessed by [11C]-PBR28-PET. The study PI's previous studies comparing mean PBR28 binding in the motor cortices in limb-onset ALS subjects (1.18 units) with matching healthy volunteers (1.064 units) revealed 0.116 mean difference between the two groups, with 0.08 standard deviation in the ALS group. With 10 people tolerating the drug among 15 participants in this study, the probability is 90 percent that the study will detect a treatment difference at a one-sided 0.1 significance level if the true difference is 0.096 units. The proposed increase in sample size will increase the statistical power to detect changes in PBR28

uptake after Ibudilast treatment. This will also provide smore safety and tolerability data on Ibudilast for up to 50mg BID in people with ALS.

8 Participant Characterization

Each analysis sample will be summarized overall for the following characteristics: age, sex, race, ethnicity, El Escorial diagnosis, years since ALS symptom onset, delay between symptom onset and ALS diagnosis, site of ALS symptom onset, use of Riluzole at baseline, and baseline levels of [PET score(s)], [biomarker(s)], ALSFRS-R, SVC, and HHD standardized megascores.

9 Statistical Analyses

9.1 PET Analysis

Primary analysis will be on changes from baseline for [11C]PBR-28 ROI SUVR at 12-24 weeks using the mITT sample. ROI SUVR in the pre- and post- treatment groups will be reported as Median (range) values. Change SUVR scores post-treatment will be tested using Wilcoxon signed rank test to assess if there was a significant change from baseline.

Subjects scan quality will be assessed by an independent imaging expert rater, who is blinded to the clinical data. All poor-quality scans, defined by incomplete scans (less than 20 minutes of PET data collected or incomplete anatomical FOV affecting reliable PET attenuation correction) and excessive motion degradation, will be excluded from the study.

9.2 Biofluid Biomarkers Analysis

Primary analyses will be on changes from baseline for biofluid biomarkers (TNF-alpha, MIF, and NF-l) at week 36 using the ITT sample. Biofluid biomarker concentrations for pre- and post-treatment groups will be reported as median (range) values. Change scores post-treatment will be tested using a Wilcoxon signed rank test to assess if there was a significant change from baseline. Only subjects with complete baseline and week 36 biomarker data will be included. Secondary analysis will repeat the primary analysis using the PP sample.

Exploratory analysis will look at the 36-week trajectory using mixed effects models with a fixed effect for time (weeks since baseline), and a random slope and intercept for each subject with an unstructured covariance will be used for this analysis using the ITT sample. Further exploratory

analyses will use baseline SUVR as a covariate to the mixed effects models of change over 36 weeks to assess the predictive value of SUVR in biofluid biomarker trajectory.

9.3 Clinical Outcomes

Change in clinical outcomes measures (ALSFRS-R, SVC and HHD-megascores) will be assessed over the 36 weeks of the study using mixed effects models with a fixed effect for time (weeks since baseline), and a random slope and intercept for each subject with an unstructured covariance will be used for this analysis on the ITT sample.

Exploratory analyses will use Pearson correlation coefficient to correlate the a) 12-24 week change in PBR28- SUVR in the primary motor cortices and b) 36-week change in biofluid biomarkers (TNF-alpha, MIF, and NF-l) with changes in clinical outcome measures over 36 weeks.

9.4 Safety Outcomes

Safety and tolerability endpoints will be reported for the Ibudilast ST sample and described as counts and proportions.

9.5 Notes on Analyses

Alpha of 0.05 will be split between PET and biomarker outcomes with 0.025 used for SUVR and 0.025 split evenly between the 3 biomarkers. Non-primary analyses will not be corrected for multiple testing.

10 References

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