PROTOCOL NO. MN-166-NEURONEXT

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE SAFETY, TOLERABILITY AND ACTIVITY OF IBUDILAST (MN-166) IN SUBJECTS WITH PROGRESSIVE MULTIPLE SCLEROSIS

IND Number:	118318
Study Phase:	2
Amendment:	6.0
Lead Principal Investigator:	Robert J. Fox, MD, MS, FAAN Staff Neurologist and Medical Director Mellen Center for MS Cleveland Clinic
Clinical Coordinating Center:	NeuroNEXT Clinical Coordinating Center (CCC) Massachusetts General Hospital
Data Coordinating Center:	NeuroNEXT Data Coordinating Center (DCC) University of Iowa
MediciNova:	Kazuko Matsuda, MD PhD MPH Chief Medical Officer

INVESTIGATOR AGREEMENT

PROTOCOL NN102 SPRINT-MS

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 NeuroNEXT and MediciNova, Inc., in strict confidence and to request the same from his/her staff and the Institutional Review Board. Study documents provided by NeuroNEXT/MediciNova, Inc. will be stored appropriately to ensure their confidentiality. Investigator should not disclose such information to others without authorization, except to the extent necessary to conduct the study.

_____
Investigator Name (Print)

_____ _____
Investigator Signature Date

PROTOCOL SIGNATURE PAGE

Study No: NN102 SPRINT-MS

Principal Investigator Approval:

Signature: _____ Date: _____

Name: Robert Fox, MD, MS

NeuroNEXT Clinical Coordinating Center Approval:

Signature: _____ Date: _____

Name: Merit Cudkowicz, MD

NeuroNEXT Data Coordinating Center Approval:

Signature: _____ Date: _____

Name: Christopher Coffey, PhD

MediciNova Approval:

Signature: _____ Date: _____

Name: Kazuko Matsuda, MD PhD MPH

1. SYNOPSIS

Name of Primary Sponsor: NINDS

Name of Secondary Sponsor: MediciNova Inc.

Name of Investigational Product: ibudilast (MN-166), previously known as AV411. Throughout this synopsis and protocol, the study drug will be referred to as ibudilast or MN-166.

Title of Study: A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability and Activity of Ibudilast (MN-166) in Subjects with Progressive Multiple Sclerosis

Study Center(s): NeuroNEXT clinical sites and Cleveland Clinic

Duration of Study: approximately 36 months	**Phase of Development:** 2

Study Objectives

Primary Objectives:

The primary objectives of the study are:

- to evaluate the activity of ibudilast (MN-166) (100 mg/d) versus placebo at 96 weeks as measured by quantitative magnetic resonance imaging (MRI) analysis for whole brain atrophy using brain parenchymal fraction (BPF).
- to evaluate the safety and tolerability of ibudilast (MN-166) (100 mg/d) versus placebo administered orally in subjects with primary progressive multiple sclerosis (PPMS) and secondary progressive multiple sclerosis (SPMS)

Major Secondary Objectives:

The secondary objectives are to evaluate the activity of ibudilast (MN-166) at 96 weeks versus placebo as measured by:

- Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
- Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
- Retinal nerve fiber layer as measured by Optical coherence tomography (OCT)
- Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm [CLADA]

The additional secondary outcomes are to measure the activity of ibudilast (MN-166) at 96 weeks versus placebo on:

- Inflammatory disease activity, as measured by T1 lesion volume, T2 lesion volume, and annualized relapse rate
- Disability, as measured by Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC)
- Cognitive impairment, as measured by Symbol Digit Modalities Test and the Selective Reminding Test
- Quality of Life as measured by Multiple Sclerosis Impact Scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36)
- Neuropathic pain, as measured by Brief Pain Inventory (BPI)

Tertiary Objectives:

- The first set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at 48 weeks

versus placebo as measured by the primary and secondary imaging outcome measures: whole brain atrophy using brain parenchymal fraction (BPF), diffusion tensor imaging (DTI) in descending pyramidal white matter tracts, magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue, retinal nerve fiber layer as measured by Optical coherence tomography (OCT), and cortical atrophy as measured by cortical longitudinal atrophy detection algorithm (CLADA).

- The second set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at 96 weeks versus placebo as measured by whole-brain gray matter fraction, magnetization transfer ratio (MTR) in gray matter, new T1 lesions since baseline, and new T2 lesions since baseline.

Exploratory Objectives:
The exploratory objectives include evaluation of the pharmacokinetics (PK) of ibudilast (MN-166) using a population PK approach, correlations of cerebrospinal fluid (CSF), and serum biomarkers with imaging and clinical measures of progressive disability, identification of unique phase 2 endpoints, and composite MRI scales (combining BPF, MTR, and DTI).

Rationale:

Despite recent improvements in pharmacotherapy for relapsing remitting multiple sclerosis (RRMS), there are no therapies with demonstrated efficacy in progressive multiple sclerosis (MS) in the absence of relapses. The few studies showing efficacy of anti-inflammatory therapies in progressive forms of MS were likely driven by the anti-inflammatory effect of the therapies. There is great need for a safe, effective, and conveniently-administered therapy for progressive MS without overt inflammation.

Ibudilast (MN-166, AV411) is a small molecule macrophage migration inhibitory factor (MIF)- and phosphodiesterase (PDE) 4, 10 inhibitor drug candidate with demonstrated neuroprotective action in vitro and in vivo. MIF knockout or antibody-neutralization studies have provided neuroprotection validation in certain MS and other neurological animal models. Ibudilast has additionally shown attenuation of glial cell activation in multiple in vitro and in vivo model systems. Hence, these molecular and cellular actions by ibudilast represent a novel pharmacotherapy approach which may provide unmet needs in progressive MS.

The original rationale for the multiple sclerosis indication was the putative anti-inflammatory effects of this drug. Using an autoimmune encephalomyelitis (EAE) model in rats as an experimental model for multiple sclerosis, the severity of acute EAE was significantly ameliorated by prophylactic oral treatment with ibudilast (Fujimoto et al 1999). In another study, ibudilast has been demonstrated to suppress pro-inflammatory cytokines in a dose-dependent manner, such as IL-1β, IL-6, and TNF-α, while increasing the anti-inflammatory cytokine IL-10 in LPS-treated microglial-neuronal co-cultures (Mizuno et al 2004). Indeed, this anti-inflammatory profile combined with potential immunoregulatory action lead to its consideration for utility in multiple sclerosis. Two pilot studies with MS subjects were conducted in Japan. One pilot study was conducted in six patients with MS who had more than 3 relapses per year. Sixty (60) mg of MN-166 was administered orally in three divided doses daily for 12 to 20 months. MN-166 significantly reduced the mean relapse rate by 48%, from 4.0 ± 0.9 before initiation of treatment to 2.1 ± 1.1 after treatment ($p<0.05$). (Sakoda et al 2004) A second pilot trial was conducted to investigate the immunoregulatory effects of MN-166 in patients with MS. In this trial, 12 patients with relapsing-remitting MS were administered 60 mg of MN-166 orally in 3 divided doses daily for 4 weeks. Serum Th1 and Th2 cytokine levels were measured before and after 4 weeks of treatment. After treatment with MN-166, there was a tendency for Th1 cytokine mRNA such as IFN-γ and TNF-α to be downregulated and for Th2 cytokine mRNA such as IL-4 and IL-10 to be upregulated. This study showed that MN-166 induced a shift in the cytokine profile from Th1 towards Th2 and increased the natural killer T cell subset in MS patients (Feng et al 2004).

A previous Phase 2 12-month randomized, double-blind, placebo-controlled trial evaluating MN-166 30 and 60 mg/d vs. placebo followed by a 12-month open-label extension phase was conducted in Central Eastern Europe in RRMS subjects. Two-hundred, ninety-seven subjects were randomized; 95 subjects to 30 mg/d, 99 subjects to 60 mg/d and 103 subjects to placebo. Subjects receiving MN-166 (ibudilast) 60 mg/d exhibited significantly reduced brain atrophy, persistent black hole formation (from gadolinium-enhancing lesions), time to relapse and EDSS progression vs. placebo (Barkhof et al 2010). A dose-response was evident between MN-166 at 30 and 60 mg/d with 60 mg/d demonstrating statistical significance for these endpoints. Inflammation MRI endpoints were not significantly affected, providing the first therapy with evidence of primary neuroprotection independent from a substantial effect on overt inflammation. Subset analyses of secondary progressive multiple sclerosis (SPMS) patients or patients enrolling with EDSS $\geq$ 4.5 show improved EDSS and/or whole brain atrophy outcomes for the 60 mg/d vs. placebo group that were better than the whole population (predominantly RRMS subjects).

During the 12-month double-blind core phase, more treatment-emergent adverse events (TEAEs) were experienced by subjects in the 60 mg/d group (26 TEAEs) compared to the 30 mg/d (18 TEAEs) or placebo group (12 TEAEs). The most common treatment-emergent AEs were headache (n =3) and nausea (n =2) in the 30 mg/d; nausea (n =4), headache (n =3), vomiting (n =2) and dizziness (n =2) in the 60 mg/d group; and upper respiratory infection (n =5) and headache (n =2) in the placebo group. During the open-label extension phase, five additional treatment-related TEAEs occurred. These TEAEs were night cramps (60 mg/d), hepatotoxity (60 mg/d), depression (60 mg/d), and pruritus (2 subjects in placebo to 60 mg/d).

Over the entire study, a total of 20 SAEs were reported by 19 subjects. Of these 20 SAEs, 12 SAEs were reported by 12 subjects in the core period and nine SAEs were reported by 7 subjects during the extension period. More subjects in the 60 mg/d dose group (10.1%) experienced an SAE compared to the placebo to 30 mg/d (5.8%), placebo to 60 mg/d (3.9%), and 30 mg/d (4.2%) dose groups. Overall the most commonly reported SOCs for the SAEs were in Gastrointestinal Disorders and Injury, Poisoning and Procedural Complications categories. All of the SAEs were considered by the Investigators to be unlikely related or not related to study drug. Eight of the SAEs were considered by the Investigator to be severe or life threatening. No deaths occurred during the study.

A total of 9 subjects discontinued from the study due to a TEAE; two of these subjects had TEAEs that were considered to be treatment-related; hepatic steatosis in the 30 mg/d group and hepatotoxicity in the 60 mg/d group.

The safety of MN-166 from 30 to 80 mg/d has also been evaluated in single and multiple doses up to 14 days has also been evaluated in healthy volunteers and diabetes mellitus Type 1 &2 patients and chronic pain patients (diabetic neuropathy and complex regional pain syndrome). MN-166 100 mg/d has been evaluated in single and multiple doses for up to 8 days (following 6 days of dose-incremental 40, 60 and 80 mg/d dosing at 2 days each) in healthy volunteers and diabetes mellitus Type 1 and 2 patients. A Phase 1b safety study to evaluate MN-166 in methamphetamine-dependent addicts recently completed enrollment. In this study, subjects received 40 mg/d for 1 week and then 100 mg/d for 1 week. Medication overuse headache (MOH) pain patients are currently being enrolled in a trial with dosing at 80 mg/d for 2 months and opioid addicts at 100 mg/d for 3 weeks in another trial. To date, approximately 450 subjects have been dosed with MN-166.

The Phase 2 RRMS trial results suggest that MN-166 is best positioned for primary neuroprotection in MS subjects (Fox 2010) and the dose-response data from this and other controlled neurological trials suggest that doses ≥ 60 mg/d may be ideal.

This Phase 2 trial is designed to generate proof-of-concept evidence evaluating the activity of ibudilast (MN-166) on imaging measures of brain atrophy and tissue integrity, to evaluate the safety and tolerability of 100 mg/d (50 mg b.i.d.) over 96 weeks, and to identify imaging markers for measuring biologic activities of potential therapies in progressive MS.

METHODOLOGY

This is a multicenter, randomized, double-blind, placebo-controlled, parallel-group study designed to evaluate the safety, tolerability and activity of MN-166 administered twice daily over a 96 week period in subjects with primary or secondary progressive multiple sclerosis who are currently untreated with long-term MS disease modifying therapy (DMT) or who are receiving either glatiramer acetate (GA) or interferon beta (IFNβ-1a [Avonex, Rebif] or IFNβ-1b [Betaseron Etavia]) treatment. Study drug will be administered as an adjunct to glatiramer or beta interferon treatment. A total of 250 male and female subjects from 21 to 65 years old, inclusive, are planned to be enrolled into two treatment groups. Randomization of subjects will be stratified by disease status (PPMS or SPMS) and immunomodulating therapy status: current use of immunomodulating therapy or no current use of immunomodulating therapy.

The study will consist of a screening phase (up to 45 days) followed by a treatment phase (96 weeks) and a follow-up visit (1 month post Week 96 visit). Following the screening phase, subjects who continue to meet entry criteria will be randomly assigned to 1 of 2 treatment groups: MN-166 100 mg/d or matching-placebo in a 1:1 ratio. Study drug will be administered either b.i.d. (ie, MN-166 50 mg or placebo taken in the morning and evening) or three times per day, depending on subject's tolerance to ibudilast.

Screening Phase (up to 45 days)

During the screening phase, subjects will be assessed for study eligibility. The following assessments will be performed: medical/multiple sclerosis history including review of prior medications, physical examination including height and body weight, vital signs and an electrocardiogram. Clinical labs (chemistry, hematology, lipid profile, and urinalysis) including a serum pregnancy test for women of child-bearing potential and a serum biomarker sample will be collected. An MRI of the brain will be performed at the Screening Visit (or within 1 week following the Screening Visit). Following the MRI, an optional lumbar puncture (LP) will be performed. Other assessments to be conducted include cognitive tests (Symbol Digit Modalities Test and Selective Reminding Test), Short Form-36 Health Survey (SF-36), Multiple Sclerosis Impact Scale-29 (MSIS-29), EuroQol 5 Dimensions (EQ-5D), Suicide Behaviors Questionnaire-Revised (SBQ-R), Brief Pain Inventory (BPI), Multiple Sclerosis Functional Composite (MSFC), Expanded Disability Status Scale (EDSS), and Optical Coherence Tomography (OCT).

Treatment Phase (96 weeks)

The Baseline Visit must occur within 45 days following the Screening Visit. At the Baseline Visit (Day 1), subjects who have completed all of the screening assessments and continue to meet eligibility criteria will be randomized to one of two treatment groups and will take their first dose of study medication on the evening of the Baseline Visit (Day 1). On the evening of Day 1, all the subjects will take 3 capsule of MN-166. On the morning of Day 2, all subjects will begin a 3 capsule BID (twice daily) dosing regimen through Day 14. Subjects randomized to MN-166 will start at 60 mg/d (30 mg BID) and will remain on 60 mg/d through Day 14. Beginning on Day 15, all subjects will begin a 5 capsule BID regimen; those randomized to MN-166 will therefore be taking 100 mg/d (50 mg BID).

After Day 15, subjects with intolerable side-effects (eg nausea, diarrhea, vertigo) may reduce their dose to either 4 capsules twice a day (80 mg/d for those taking ibudilast) or 3 capsules twice a day (60mg/d for those taking ibudilast). Subjects with intolerable side-effects (eg nausea, diarrhea, vertigo) at the end of Day 14 may continue to taking 3 capsules twice a day at the Investigator's discretion. At the investigator's discretion, the daily dose of ibudilast can be changed between 3 capsules twice a day, 4 capsules twice a day, and 5 capsules twice a day over the first 8 weeks of treatment. At the end of the first 8 weeks of treatment, the subject must maintain their then-current daily dose of study medication (3 capsules twice per day, 4 capsules twice per day, or 5 capsules twice per day) for the duration of the trial. Additionally, at the investigator's discretion, the daily dose of ibudilast may be divided and taken three times per day to help improve tolerability.

Subjects will return to the clinic for follow-up visits on a regular basis at Weeks 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 (see Table 1 Schedule of Assessments).

Subjects who experience a relapse will return to the clinic within three days of notifying the Investigator and will undergo assessments described in Table 2 Schedule of Unplanned Procedures and Assessments).

Subjects who prematurely discontinue study medication will continue to be followed on a semi-annual basis (Table 2 Schedule of Unplanned Procedures and Assessments) until the end of the study (Week 96).

For subjects who are no longer taking study drug and are being followed on a semi-annual basis (Wk 24, 48, 72, 96), adverse events will not be collected post study drug discontinuation. Existing AEs will be followed until the AE resolves or stabilizes.

Follow-up Phase

All subjects who complete the study active on study medication will return for a follow-up safety visit at Week 100 (4 weeks after their last study visit) to assess general health and adverse event status.

Number of Subjects (Planned): The number of randomized subjects planned for this study is 250: 125 in the placebo treatment group and 125 in MN-166 100 mg/d treatment group.

Study Entry Criteria:

Inclusion Criteria:

- Written informed consent is obtained and willing and able to comply with the protocol in the opinion of the Investigator
- Male or female subjects ages 21 to 65, inclusive
- Confirmed diagnosis of SPMS or primary progressive multiple sclerosis (PPMS) according to 2010 International Panel Criteria
- Typical MS lesions on MRI according to Swanton's MRI Criteria (at least one lesion in two or more of the following regions: periventricular, juxtacortical, infratentorial [brainstem/cerebellum], spinal cord)
- EDSS 3.0-6.5, inclusive
- Clinical evidence of disability progression in the preceding two years, as measured by any of the following (excluding progression during clinical relapses):
    - worsening overall EDSS of at least 0.5 points (may be estimated retrospectively but cannot be during a clinical relapse) or
    - 20% worsening in 25-foot walk (25-FW) or

  - 20% worsening in 9-hole peg test (9-HPT) in either hand
- Existing multiple sclerosis pharmacotherapy status may include interferon-beta or glatiramer acetate or none (ie, untreated)
- Females of child-bearing potential must have a negative serum ß-hCG at screening and must be willing to use appropriate contraception (as defined by the investigator) for the duration of study treatment and 30 days after the last dose of study treatment
- Males should practice contraception as follows: condom use and contraception by female partner
- Subject is in good physical health on the basis of medical history, physical examination, and laboratory screening, as defined by the investigator
- Subject is willing and able to comply with the protocol assessments and visits, in the opinion of the study nurse/coordinator and the Investigator.

Exclusion Criteria:

- Progressive neurological disorder other than SPMS or PPMS
- Relapse and/or systemic corticosteroid treatment within 3 months of screening. Inhaled or topical steroids are allowed
- Current use of intermittent systemic corticosteroids (ie, monthly or bimonthly intravenous methylprednisolone)
- Use of oral immunosuppressants (eg, azathioprine, methotrexate, cyclosporine, teriflunomide [Aubagio®]) within 6 months of screening
- Use of mitoxantrone, natalizumab, or IVIg within 6 months of screening, or use of alemtuzumab within the prior 10 years
- Use of fingolimod or dimethyl fumarate [Tecfidera®] within 3 months of screening
- Use of rituximab or other B-cell therapy within 12 months of screening
- Current use of other MS disease-modifying therapies (DMTs) besides glatiramer acetate, IFNβ-1 (any formulation), and the above listed medications
- Current use of cimetidine, cyclosporine, dronedarone, lopinavir, probenecid, quinidine (including Neudexta), ranolazine, rifampin, ritonavir, or tipranavir
- Clinically significant cardiovascular disease, including myocardial infarct within last 6 months, unstable ischemic heart disease, congestive heart failure or angina
- Resting pulse < 50 bpm, SA or AV block (Type II or greater), uncontrolled hypertension, or QTcF > 450 ms
- Clinically significant pulmonary conditions, including severe COPD, fibrosis, or tuberculosis
- Evidence of acute hepatitis, clinically significant chronic hepatitis, or evidence of clinically significant impaired hepatic function through clinical and laboratory evaluation including ALP > 1.5x ULN; ALT or AST > 2x ULN; GGT > 3x ULN
- Immune system disease (other than multiple sclerosis and autoimmune thyroid disease)
- History of stomach or intestinal surgery or any other condition that could interfere with or is judged by the Investigator to interfere with absorption, distribution, metabolism, or excretion of study drug
- Any significant laboratory abnormality which, in the opinion of the Investigator, may put the subject at risk and with the following laboratory abnormalities at screening:
  - Creatinine: females > 0.95 mg/dL; males > 1.17 mg/dL
  - WBCs < 3,000 mm^3
  - Lymphocytes < 800 mm^3
  - Platelets < 90,000 mm^3
- History of malignancy < 5 years prior to signing the informed consent, except for adequately treated basal cell or squamous cell skin cancer or *in situ* cervical cancer
- History of HIV (human immunodeficiency virus), clinically significant chronic hepatitis, or

other active infection
- Subject currently has a clinically significant medical condition (other than MS) including the following: neurological, psychiatric, metabolic, hepatic, renal, hematological, pulmonary, cardiovascular (including uncontrolled hypertension), gastrointestinal, urological disorder, or central nervous system (CNS) infection that would pose a risk to the subject if they were to participate in the study or that might confound the results of the study

  Note: Active medical conditions that are minor or well-controlled are not exclusionary if, in the judgment of the Investigator, they do not affect risk to the subject or the study results. In cases in which the impact of the condition upon risk to the subject or study results is unclear, the Medical Safety Monitor should be consulted
- Subjects with moderate to severe depression as determined by the Beck Depression Inventory-Fast Screen (BDI-FS)
- Subject has a history of alcohol or substance abuse (DSM-IV-TR criteria) within 3 months prior to screening or alcohol or substance dependence (DSM-IV-TR criteria) within 12 months prior to screening. The only exceptions include caffeine or nicotine abuse/dependence
- Subject has poor peripheral venous access that will limit the ability to draw blood as judged by the Investigator
- Subject is currently participating, or has participated in, a study with an investigational or marketed compound or device within 3 months prior to signing the informed consent
- Subject is unable to cooperate with any study procedures, unlikely to adhere to the study procedures and keep appointments, in the opinion of the Investigator, or was planning to relocate during the study
- Subject is unable to undergo MRI imaging because of having an artificial heart valve, metal plate, pin, or other metallic objects (including gun shots or shrapnel) in their body or is unable to complete all the five MRI scans required for this study.
- Subject is unable to lie sufficiently still in an MRI to obtain a high quality MRI image.

Investigational Product, Dosage and Mode of Administration: MN-166 (ibudilast) delayed-release capsules administered orally, twice a day, in the morning and evening for up to a total daily dose of 100 mg/d.

Duration of Treatment: 96 weeks of double-blind treatment.

Reference Therapy, Dosage, and Mode of Administration: Matching-placebo capsules administered orally, twice a day in the morning and evening.

Criteria for Evaluation

Primary Safety Endpoints:

The proportion of subjects in each group with:
- Treatment-emergent adverse events (TEAEs))
- Treatment-emergent serious adverse events (TESAEs)

Primary Tolerability Endpoints:

The proportion of subjects in each group who:
- Discontinue treatment early (early study termination and/or early study drug withdrawal) due to treatment-related AEs or SAEs
- Discontinue treatment early (early study termination and/or early study drug withdrawal) for any reason

Primary Activity Endpoint:

Covariate-adjusted mean rate of change in brain atrophy over 96 weeks as measured by brain parenchymal fraction (BPF).

Major Secondary Activity Endpoints:

The secondary endpoints will be measured at 96 weeks by:

- Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
- Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
- Retinal nerve fiber layer as measured by optical coherence tomography (OCT)
- Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm [CLADA]

Additional secondary endpoints to be measured at 96 weeks are:

- Inflammatory disease activity, as measured by T1 lesion volume, T2 lesion volume, and annualized relapse rate
- Disability, as measured by Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC)
- Quality of Life, as measured by Multiple Sclerosis Impact Scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36)
- Cognitive impairment, as measured by Symbol Digit Modalities Test and the Selective Reminding Test
- Neuropathic pain, as measured by Brief Pain Inventory (BPI).

Tertiary and Exploratory Endpoints: *see Objectives above*.

Pharmacokinetic: Plasma samples for analysis of ibudilast and its metabolite, 6,7-dihydrodiol (DHD), will be collected and population PK analysis will be performed.

Safety Monitoring: After 30 patients have been enrolled for at least 30 days and 60 patients have been enrolled for at least 60 days, the Medical Safety Monitor will review pooled (i.e., blinded to treatment) safety data provided by the Data Coordinating Center. Beginning after approximately month 3 of enrollment start, a National Institute of Neurological Disorders and Stroke (NINDS) Data and Safety Monitoring Board (DSMB) will meet every six months during the study (and more often, if deemed necessary), and will review blinded safety data including adverse events and serious adverse events (SAE). Efficacy data may also be reviewed if warranted, as determined by the DSMB. The DSMB will be empowered to recommend stopping the study due to safety concerns. The group's mandates and membership will be described in the NeuroNEXT DSMB Guideline.

Statistical Methods

Analysis Populations:

The modified Intent-to-Treat (mITT) Population: The primary population for analysis is the mITT, which is defined as all subjects who are randomized and receive at least one dose of study medication and have at least one efficacy assessment in the double-blind phase. Subjects will be analyzed based on the treatment to which they are randomized.

The Per Protocol (PP) Population: The per-protocol population includes all mITT subjects who satisfy the following conditions:

- Have 75% -125% compliance, both limit values inclusive in the double-blind phase
- Have no major protocol deviations, determined by a blinded data review

Endpoint Analysis:

All imaging endpoints will be statistically evaluated using linear mixed models (LMMs: Laird and Ware 1982). LMMs are advantageous for longitudinal clinical trials because they can account for the dependency due to repeated measures with relatively few parameters, which potentially enhances statistical efficiency. Furthermore, LMMs can accommodate incomplete cases (ie, missing data), which is expected in this study due to dropout. LMMs are typically estimated using maximum likelihood methods (Verbeke and Molenberghs 2000) that yield valid inferences with incomplete cases under the widely applicable assumption that the missing data are ignorable (Little and Rubin 2002). For all analyses, the residuals of the fitted statistical models will be examined for evidence of departure from assumptions, such as normality. If assumptions appear to be grossly violated, then transformations of response variables might be considered. Alternatively, generalized LMMs might be used because of their ability to accommodate a wider range of distributional forms (eg, beta distribution). The primary analysis will be conducted using a modified intent-to-treat analysis, which includes all subjects who are randomized and receive at least one dose of study medication in the double-blind phase. Since this is a phase 2 proof of concept study, statistical significance will be determined by any test that exceeds the 0.10 significance level. To account for baseline imbalance due to randomization vagaries, the baseline group means (intercepts) in the statistical analysis will be constrained to be equal. Sensitivity analyses will be conducted using brain atrophy as measured by SIENA and covariate adjustment for covariates with potential impact on atrophy (ie, non-steroidal anti-inflammatory drugs [NSAIDs]) that were unbalanced between treatment groups.

Safety Analysis:

The safety analyses will be conducted using the double-blind safety population, defined as subjects who are randomized and receive at least one dose of study medication in the double-blind phase. Adverse events (AEs), discontinuation due to AEs, and serious adverse events (SAE) will be summarized by presenting, for each treatment group, the number and percentage of subjects with any AE, and AEs by system organ class and preferred term. Adverse events will be further summarized by severity and by relationship to study drug. The summary will be limited to treatment-emergent AEs.

Sample Size Justification:

Estimated required sample for the primary objective was computed from pilot data and relevant literature. The pilot data (considered the control group) consisted of N = 36 relapse-remitting (RR) and secondary progressive (SP) participants with up to 3 annual BPF measures from the same 3T scanner. Unpublished pilot data and a published study (Altmann et al 2008) suggested a reasonable range of percentage difference was 30% to 50%. Also, using a different atrophy metric and only 2 time points, the ibudilast RRMS Phase 2 trial observed a 33%-36% slowing in brain atrophy (Barkhof et al 2010). Based on these assumptions, a sample size of N =125 subjects per treatment arm provides power close to 80% for effects of 33% or larger (assuming a type I error rate of 0.10).

Table 1: Schedule of Assessments

Tests and Evaluations	Screening Visit	Baseline Visit [4]	Week 4 ± 5 days	Week 8 ± 5 days	Week 12 ± 14 days	Week 24 ± 5 days	Week 36 ± 14 days	Week 48 ± 5 days	Week 60 ± 14 days	Week 72 ± 5 days	Week 84 ± 14 days	Week 96 ± 5 days	Week100 follow-up (± 14 days)
Study Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Informed consent	X												
Inclusion/exclusion criteria	X	X											
Medical and MS history	X												
Physical examination	X		X	X	X	X	X	X	X	X	X	X	X
Randomization		X											
Body height	X												
Body weight	X		X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X
Interval history		X	X	X	X	X	X	X	X	X	X	X	X
Adverse event review		X	X	X	X	X	X	X	X	X	X	X	X[8]
Concomitant meds	X	X	X	X	X	X	X	X	X	X	X	X	X
Relapse assessment			X	X	X	X	X	X	X	X	X	X	X
Cognitive test (SRT)[7]	X	X				X		X		X		X	
Brief Pain Inventory (BPI)	X	X	X	X	X	X	X	X	X	X	X	X	
Short Form-36 Health Survey (SF-36)	X	X				X		X		X		X	
Multiple Sclerosis Impact Scale (MSIS-29)	X	X				X		X		X		X	
EuroQol 5 Dimensions (EQ-5D)	X	X				X		X		X		X	
Beck Depression Inventory-Fast Screen (BDI-FS)	X												
Suicide Behaviors Questionnaire-revised (SBQ-R)		X	X	X	X	X	X	X	X	X	X	X	X
Clinical labs (chemistry, hematology, urinalysis)	X	X	X	X	X	X	X	X	X	X	X	X	
Lipid Profile	X							X				X	
Serum samples (biomarkers)	X			X				X				X	
Serum pregnancy test[9]	X		X	X	X	X	X	X	X	X	X	X	
Urine pregnancy test		X											
Plasma for biomarkers and PK	X[10]			X				X				X	
ECG	X		X	X	X	X	X	X	X	X	X	X	
Multiple Sclerosis Functional Composite (MSFC)	X	X				X		X		X		X	
Expanded Disability Status Scale (EDSS)[1]	X	X				X		X		X		X	
Brain MRI	X[5]					X[5]		X[5]		X[5]		X[5]	
Optical Coherence Tomography (OCT)	X					X		X		X		X	
Lumbar Puncture (optional)[3]	X							X				X	
Study Drug Dispensing[6]		X				X		X		X			
Study Drug Accountability			X	X	X	X	X	X	X	X	X	X	

1. EDSS must be performed by a neurologist blinded to treatment assignment.
2. The baseline MRI must be approved by the MRI Reading Center before randomization.
3. If performed, the lumbar puncture must be done after the brain MRI.
4. The Baseline visit must occur within 45 days following the Screening visit.
5. Postpone MRI until 1 month after completion of any unscheduled corticosteroid treatment.
6. Study drug will be taken twice daily in the morning and evening. The first dose will be taken the evening of the baseline visit.
7. Cognitive tests include the Symbol Digit Modalities Test (the only CORE CDE cognitive test) and the Selective Reminding Test.
8. For subjects who are no longer taking study drug and are being followed on a semi-annual basis (Wk 24, 48, 72, 96), AEs will not be collected post study drug discontinuation. Existing AEs will be followed until the AE resolves or stabilizes.
9. If a pregnancy is discovered between regularly scheduled study visits, subjects should return for an unscheduled visit and a pregnancy test should be obtained. Once confirmed, the patient should be discontinued from the study and study drug should be returned.
10. At screening, plasma samples required for biomarkers ONLY. Plasma PK aliquot is omitted.

Table 2: Schedule of Unplanned Procedures and Assessments

Tests and Evaluations	Relapse Evaluation	Early Study Withdrawal	Study Visits after Study Drug Discontinuation[4]
Study Visit Number	**Rel**	**EW**	**6, 8, 10, 12**
Physical examination		X	
Body weight	X	X	
Vital signs	X	X	
Interval history	X	X	
Adverse event review	X	X	
Concomitant meds	X	X	
Relapse Assessment		X	X
Cognitive Test (SRT)		X	X
Brief Pain Inventory (BPI)	X	X	X
Short Form-36 Health Survey (SF-36)	X	X	X
Multiple Sclerosis Impact Scale (MSIS-29)	X	X	X
EQ-5D		X	X
SBQ-R	X	X	X
Clinical labs (chemistry, hematology, urinalysis)	X	X	
Lipid Profile		X	
Serum samples (biomarkers)		X	
ECG		X	
Multiple Sclerosis Functional Composite (MSFC)		X	X
Expanded Disability Status Scale (EDSS)[3]	X	X	X
Brain MRI		X[1]	X
Optical Coherence Tomography (OCT)		X	X
Lumbar Puncture (optional)		X[2]	X

1. Postpone MRI until 1 month after completion of any unscheduled corticosteroid treatment.
2. If performed, lumbar puncture must be done after the brain MRI
3. EDSS must be performed by a neurologist by a blinded to treatment assignment.
4. For subjects who prematurely discontinue study medication, a semi-annual (SA) visit will be conducted at Weeks 24, 48, 72, and 96

2. TABLE OF CONTENTS

1. SYNOPSIS 4
2. TABLE OF CONTENTS 16
3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS 20
4. BACKGROUND AND RATIONALE 25
4.1. Introduction 25
4.2. Ibudilast (MN-166/AV411) background 26
4.3. Investigational Agent 27
4.3.1. Formulation of the Drug to be Studied 28
4.4. Pre-clinical toxicology 28
4.4.1. Pre-clinical study overview 28
4.4.2. Evaluation of organ- and species-specific findings 28
4.4.3. Justification of the proposed human dose of 50 mg bid 31
4.5. Prior Clinical Experience 31
4.5.1. Clinical Study Overview 31
4.6. Ibudilast (MN-166/AV411) safety overview 33
4.6.1. Published safety summary from experience in Japan 33
4.6.2. Safety overview from previous clinical trials 34
4.6.3. Safety summary of Phase 2 MS trial (MN-166-CL-001) 34
4.6.4. Abnormal Laboratory Test Values in the clinical trials 36
4.6.5. Safety Overview for Higher Dosage 37
4.7. Dose rationale 40
4.8. Potential Drug-Drug Interactions 41
5. TRIAL OBJECTIVES AND PURPOSE 43
5.1. Primary Objective 43
5.2. Secondary Objectives 43
5.3. Tertiary Objectives 43
5.4. Exploratory Objectives 44
6. OVERALL STUDY DESIGN AND PLAN: DESCRIPTION 45
6.1. Screening Phase (up to 45 days) 45
6.1.1. Diagnosis at Screening 45
6.1.2. Treatment Phase (96 weeks) 46
6.1.3. Follow-up Phase 47
7. SELECTION AND WITHDRAWAL OF SUBJECTS 48
7.1. Clinical Trial Population 48

7.2. Inclusion/Exclusion Criteria 48
7.2.1. Inclusion Criteria 48
7.2.2. Exclusion Criteria 49
7.3. Subject Withdrawal/Discontinuation Criteria 51
7.3.1. Follow-up Procedures Upon Discontinuation/Withdrawal 51
8. TREATMENT OF SUBJECTS 53
8.1. Description of Study Drug 53
8.2. Concomitant Medications 53
8.3. Prohibited Medications 53
8.4. Treatment Compliance 53
8.5. Randomization and Blinding 54
8.6. Dosing Guidelines 54
8.6.1. Treatment Phase 54
8.6.2 Dosing Interruption for Abnormal Laboratory Values 54
8.6.3 Subsequent Additional Laboratory Abnormalities 55
8.7. Written Informed Consent 55
8.8. Assessments 55
8.8.1. Assessments by Visit 55
8.8.1.1. Screening 55
8.8.2. Procedures/Assessment Details 66
8.8.2.1. Informed Consent 66
8.8.2.2. Medical History 66
8.8.2.3. Prior/Concomitant Medication Review 66
8.8.2.4. Relapse Assessment 66
8.8.2.5. Physical Examination 67
8.8.2.6. Vital Signs, Height, and Weight 67
8.8.2.7. Electrocardiogram (12-Lead ECG) 67
8.8.2.8. Interval History 67
8.8.2.9. Brain Magnetic Resonance Imaging (MRI) 68
8.8.2.10. Optical Coherence Tomography (OCT) 68
8.8.2.11. Lumbar Puncture (LP) 68
8.8.2.12. Adverse Event (AE) Monitoring 68
8.8.2.13. Laboratory Evaluations 69
8.8.2.14. Instruments and Rating Scales 69
9. STUDY DRUG MATERIALS AND MANAGEMENT 72
9.1. Study Drug 72

9.2. Study Drug Packaging and Labeling 72
9.3. Study Drug Storage 73
9.4. Study Drug Dispensation and Handling 73
9.5. Administration 73
9.6. Study Drug Accountability 73
10. ASSESSMENT OF ACTIVITY 74
10.1. Primary and Secondary Endpoints 74
10.2. Tertiary Endpoints 74
10.3. Exploratory Endpoints 74
11. ASSESSMENT OF SAFETY 76
11.1. Primary Safety Parameters 76
11.2. Definition of Adverse Events 76
11.3. Assessment of Adverse Events 76
11.3.1. Severity Assessment 76
11.3.2. Relationship to Study Drug 77
11.4. Recording Adverse Events 78
11.5. Treatment and Follow-Up of AEs 79
11.6. Serious Adverse Events (SAEs) 79
11.6.1. SAE Reporting Requirements 79
11.6.2. Emergency Treatment Code-Break 81
11.7. Guidance for Overdose 81
11.8. Reporting and Follow-up of Pregnancies 81
11.9. Preplanned Hospitalizations or Procedures 82
11.10. Data and Safety Monitoring Board (DSMB) 82
12. STATISTICS 83
12.1. Data Analysis 83
12.1.1. Analysis Populations 83
12.1.2. Statistical Analysis Plan 83
12.1.3. Sample Size Justification 87
12.1.4. Interim Monitoring Plan 91
12.2. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS 92
12.3. Study Monitoring 93
12.4. Audits and Inspections 94
12.5. Institutional Review Board (IRB) 94
12.6. Study Documentation 94
13. QUALITY CONTROL AND QUALITY ASSURANCE 96

14. ETHICS 97
14.1. Ethics Review 97
14.2. Ethical Conduct of the Study 97
14.3. Written Informed Consent 97
14.4. Confidentiality 97
14.4.1. Confidentiality of Data 97
15. DATA HANDLING AND RECORDKEEPING 98
15.1. Review of Records 98
15.2. Retention of Records 98
16. ADMINISTRATIVE AND REGULATORY DETAILS 99
16.1. Protocol Amendments and Study Termination 99
16.2. Discontinuation of the Study 99
16.3. Compliance with Financial Disclosure Requirements 99
17. REFERENCES 100
18. APPENDICES 103

List of Figures and Tables

Figure 1: Study Design 47
Figure 2: Power as a Function of Single-arm sample size, effect size, and data set 88
Figure 3: Power as a Function of Effect Size and data source for sample size of 125 and alpha = 0.10 89

Table 1: Schedule of Assessments 13
Table 2: Schedule of Unplanned Procedures and Assessments 15

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

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

Abbreviation	Term
5-HT	5-hydroxytryptamine
9-HPT	9-hole peg test
25 FW	25 foot walk
AE	adverse event
AERS	Adverse event reporting system
ALP	alkaline phosphatase
ALT (SGPT)	alanine aminotransferase
AST (SGOT)	aspartate aminotransferase
AV	atrioventricular
AUC	Area under the curve
BDI-FS	Beck Depression Inventory-Fast Screen
β-hCG	beta-subunit of human chorionic gonadotropin
BID	twice daily
BP	blood pressure
BPF	brain parenchymal fraction
BPI	Brief Pain Inventory
BUN	Blood urea nitrogen
CCC	Clinical Coordinating Center
CCF	Cleveland Clinic Foundation
CDE	Common data elements
CEE	Central & Eastern Europe
CFR	Code of Federal Regulations
CIRB	Central Institutional Review Board
CLADA	Cortical Longitudinal Atrophy Detection Algorithm
CLTR	Consistent long term retrieval
C_{max}	Maximum plasma concentration
CMSU	Clinical Materials Service Unit
CNS	central nervous system
COPD	Chronic obstructive pulmonary disease

Abbreviation	Term
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
CSF	cerebrospinal fluid
CSS	Clinical Study Site
CSSPI	Clinical Study Site Principal Investigator
CTCAE	Common terminology criteria for adverse events
CYP	cytochrome
DCC	Data Coordinating Center
DDI	drug-drug interaction
DHD	dihydrodiol
dL	deciliter
DM	Diabetes mellitus
DMT	disease modifying therapy
DR	Delayed recall
DSMB	Data and Safety Monitoring Board
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition-Text Revision
DTI	diffusion tensor imaging
EAE	Experimental acute encephalomyelitis
ECG	electrocardiogram
eCRF	electronic case report form
EDSS	Expanded Disability Status Scale
EQ-5D	EuroQol 5 Dimensions
ET	Early Termination
EW	early withdrawal
FDA	Food and Drug Administration
GA	glatiramer acetate
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GI	gastrointestinal
HED	Human equivalent dose

Abbreviation	Term
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HR	heart rate
HV	Healthy volunteer
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IFNβ	interferon beta
IL	interleukin
IMM	Independent Medical Monitor
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
IXRS	Interactive Voice/Web Response System
L	liter
LD_{50}	Lethal dose of 50%
LMM	linear mixed model
LOCF	last observation carried forward
LP	lumbar puncture
LPS	lipopolysaccharide
LTR	Long term retrieval
LTS	Long term storage
MCP-1	Monocyte chemoattractant protein-1
MCR	Number of correct recognized Multiple Choice items
MedDRA	Medical Dictionary for Regulatory Activities
Mg	milligram
MIF	migration inhibitory factor
mITT	modified intent to treat
MOH	medication overuse headache
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid

Abbreviation	Term
MS	multiple sclerosis
MSDS	Materials safety data sheet
MSFC	Multiple Sclerosis Functional Composite
MSIS-29	Multiple Sclerosis Impact Scale-29
MSM	Medical Safety Monitor
MTR	magnetization transfer ratio
NDA	New Drug Application
NeuroNEXT	Neurological Network for Excellence in Neuroscience Clinical Trials
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	no observed adverse effect level
NONMEM	Non-linear mixed-effect modeling
NSAID	Non-steroidal anti-inflammatory drug
NYSPI	New York State Psychiatric Institute
OCT	Optical coherence tomography
PBMC	Peripheral blood mononuclear cell
PBVC	percent brain volume change
PDE	phosphodiesterase
PK	pharmacokinetics
PO	Per oral
PP	per protocol
(P)PI	(Protocol) Principal Investigator
PPMS	primary progressive multiple sclerosis
PRMS	progressive relapsing multiple sclerosis
QD	once-daily
QTcF	QT interval corrected for heart rate using Fridericia's formula
RNFL	retinal nerve fiber layer
RR	relapsing remitting
RRMS	relapsing remitting multiple sclerosis
SA	sinoatrial
SAE	serious adverse event
SAP	statistical analysis plan

Abbreviation	Term
SBQ-R	Suicidal Behaviors Questionnaire-Revised
SC	subcutaneous
SDMT	Symbol Digit Modalities Test
SEMI-A	Semi-annual
SF-36	Short Form-36 Health Survey
SIENA	Structural image evaluation, using normalization, of atrophy
SOPs	standard operating procedures
SP	secondary progressive
SPMS	secondary progressive multiple sclerosis
SRT	Selective Reminding Test
STR	Short term retrieval
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TID	three times daily
TK	toxicokinetics
TNF	Tumor necrosis factor
TR	Total recall
TRAE	Treatment-related adverse event
ULN	Upper limit of normal
WBC	White blood cells
WHO-DD	World Health Organization Drug Dictionary

4. BACKGROUND AND RATIONALE

4.1. Introduction

Multiple sclerosis (MS) is a complex autoimmune disease with predominantly unknown etiology currently affects approximately 2.5 million people worldwide. Several pathological processes such as inflammation, demyelination, axonal damage and repair mechanisms contribute in the complex disease manifestation of MS. MS is usually a sporadic disease and is characterized as a variably progressive disease of the nervous system in which the patchy degenerative and inflammatory changes occur within the brain and spinal cord. The degenerative and inflammatory changes are associated with the formation of sclerotic plaques due, in part, to abnormal hardening and fibrosis of the neuronal myelin sheath. The symptoms are diverse, ranging from tremor, nystagmus, paralysis, and disturbances in speech and vision. Symptoms of the disease often occur in early adult life with characteristic exacerbations and remissions.

Relapsing remitting multiple sclerosis (RRMS) is the most common type of the disease, accounting for 65%-85% of patients. Most patients with RRMS eventually progress to the secondary progressive (SPMS) form of the disease. Despite recent improvements in pharmacotherapy for relapsing remitting multiple sclerosis (RRMS), there are no therapies generally considered efficacious in progressive MS in the absence of relapses. The few studies showing efficacy of anti-inflammatory therapies in progressive forms of MS were likely driven by the anti-inflammatory effect of the therapies. Currently, mitoxantrone is the only FDA-approved therapy for secondary progressive MS, but is not commonly used because of its risks of heart injury and blood cancers (ie leukemia). There is a great need for a safe, effective, and conveniently-administered therapy for patients with progressive MS without overt inflammation. Ibudilast (MN-166) may meet these needs.

The Phase 2 trial proposed in this protocol is designed to generate proof-of-concept evidence evaluating the activity of MN-166 on imaging measures of brain atrophy and tissue integrity, to evaluate the safety and tolerability of this dose over 2 years, and to identify imaging markers for measuring biologic activities of potential therapies in progressive MS.

4.2. Ibudilast (MN-166/AV411) background

Ibudilast is an anti-inflammatory/neuroprotective agent that has been in use for over 20 years in Japan and some other Asian countries for the treatment of asthma and cerebrovascular disorders (post-stroke dizziness) with 30mg/d dosage based on attenuating airway hypersensitivity and improving cerebral blood flow, respectively (Kawasaki et al 1992; Fukuyama et al 1993). Ibudilast, in delayed-release capsule format, is marketed as Ketas® (via innovator, Kyorin Pharmaceuticals) or Pinatos® (a generic by Teva/Taisho Pharmaceuticals). While ibudilast has not yet been approved for any conditions outside of Asia, clinical development for neurological conditions, including multiple sclerosis, neuropathic pain, and certain drug addictions, has been undertaken by Avigen Inc. (as AV411) and MediciNova Inc. (as MN-166). (Ledeboer et al 2007a; Ledeboer et al 2007b; Rolan et al 2009, Barkhof et al 2010) These entities merged at the end of 2009 such that all ibudilast assets could be integrated with continued development as MN-166 with MediciNova (San Diego, CA).

Ibudilast distributes well to the CNS and it is a selective inhibitor of certain cyclic nucleotide phosphodiesterases (Gibson et al 2006) and the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) (Cho et al 2010). At clinically-relevant plasma or CNS concentrations, ibudilast selectively inhibits macrophage migration inhibitory factor (MIF) and, secondarily, PDEs-3, 4 and -10. MIF inhibition or knockout has been linked to attenuated disease progression in animal models of MS (Powell et al 2005; Kithcart et al 2010) and attenuates neuronal death and promotes recovery in mouse spinal cord injury (Nishio et al 2009). Another MIF knockout study reveals its role in neurodegeneration in experimental stroke model (Inacio et al 2011). PDE inhibition has likewise shown some neuroprotective actions (Chen et al 2007; Nakamizo et al 2003).

The original rationale for the multiple sclerosis indication was the putative anti-inflammatory effects of this drug. Using autoimmune encephalomyelitis (EAE) model in rats as an experimental model for multiple sclerosis, the severity of acute EAE was significantly ameliorated by prophylactic oral treatment with ibudilast (Fujimoto et al 1999). Ibudilast has been demonstrated to suppress, in a dose-dependent manner, pro-inflammatory cytokines such as IL-1 beta, IL-6, and TNF-alpha, while increasing the anti-inflammatory cytokine IL-10 in LPS-treated primary microglial-neuronal cultures (Mizuno et al 2004). Indeed, this anti-inflammatory profile combined with potential immunoregulatory action lead to its consideration for utility in MS. Two pilot studies with MS subjects were conducted in Japan. One pilot study was conducted in six patients with MS who had more than 3 relapses per year. Sixty (60) mg of MN-166 was administered orally in three divided doses daily for 12 to 20 months. MN- 166 significantly reduced the mean relapse rate by 48%, from 4.0 ± 0.9 before initiation of treatment to 2.1 ± 1.1 after treatment ($p<0.05$). The mean Expanded Disability Status Score (EDSS) was 4.3 ± 2.0 before the treatment and 4.8 ± 2.6 after treatment, but this difference was not significant (Sakoda et al 2004) A second pilot trial was conducted to investigate the immunoregulatory effects of MN-166 in patients with MS. In this trial, 12 patients with relapsing-remitting MS were administered 60 mg of MN-166 orally in 3 divided doses daily for 4 weeks. Serum Th1 and Th2 cytokine levels were measured before and after 4 weeks of treatment. After 4 weeks of treatment with MN-166, TNF-α mRNA decreased in all but one patient and this change was statistically significant. After treatment with MN-166, there was a tendency for Th1 cytokine mRNA such as IFN-γ and TNF-α to be downregulated and for Th2

cytokine mRNA such as IL-4 and IL-10 to be upregulated. The reductions in IFN-γ and TNF-α were statistically significant. This study showed that MN-166 induced a shift in the cytokine profile from Th1 towardsTh2 and increased the natural killer T cell subset in MS patients (Feng et al 2004). More recently, ibudilast has been recognized to have glial attenuating activity in vitro and in vivo. Researchers identified activation of glial cells (astrocytes, microglia, oligodendrocytes) as an important component of the pathogenesis and/or maintenance of multiple sclerosis and neuropathic pain (Sloane et al 2009; Watkins et al 2003; DeLeo and Yezierski 2001). Notably, ibudilast treatment was also shown in a rodent genetic model of Krabbe's neurodegeneration (Twitcher mouse) to reduce demyelination, apoptosis, and pro-inflammatory cytokine expression (Kagitani-Shimono et al 2005). Additional means by which ibudilast may impart neuroprotective action include enhanced release of cell growth factors (NGF, GDNF, NT-4) in glial-neuronal co-cultures (Mizuno et al 2004), and inhibition of MIF which has been linked to neurodegeneration (Inacio et al 2011; Kithcart et al 2010).

4.3. Investigational Agent

Research Codes: MediciNova MN-166 (Avigen AV411)

Non-proprietary Name: ibudilast

Chemical Abstract Service (CAS):

3-Isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine;1-(2-isopropylH-pyrazolo[1,5-a]pyridin-3-yl)-2-methylpropan-1-one 1-Propanone, 2-methyl-1-[2-(1-methylethyl)pyrazolo[1,5-a]pyridin-3-yl]

Other names:

Ketas®, KC-404 (Kyorin Pharmaceutical Co., Ltd.; Japanese innovator)
Pinatos® (Taisho Pharm. Ind., Ltd.; Japanese Generic)
Vichang (Shenzhen Neptunus Pharmaceutical Co., Ltd., Chinese Generic)
CAS Number: 50847-11-5

General Properties

Physical Form: White crystalline powder
Molecular Formula: C14H18N2O
Molecular Weight: 230.3 g/mol
Melting point: 54 - 58°C (Source: Sanyo MSDS and Certificate of Analysis)
Solubility: Very soluble in methanol or chloroform
Freely soluble in acetic anhydride, ethanol, or ether
Soluble in hexane
Very slightly soluble in water
The solubility characteristics of ibudilast are summarized below:

Solubility of ibudilast API (AV411) by Solvent and by pH in Aqueous Solution

Solubility	Solvent
Very soluble	Methanol, ethyl acetate
Freely soluble	Ethanol (95), acetic anhydride, diethyl ether
Soluble	Hexane
Very slightly soluble	Water

pH of aqueous solution (37˚C)	Solubility (µg/mL)
1.2	198
4.0	193
6.8	179
Water	188

(Ethical drugs Quality Data No. 16, 2003, p.149 Society of the Japanese Pharmacopeia)

4.3.1. Formulation of the Drug to be Studied

MN-166 is a extended release pharmaceutical preparation comprising white extended release granules contained in a No.4 capsule. The capsules are white and without logo and contain 10 mg ibudilast plus excipient. The generic drug product utilized is licensed in Japan and manufactured under Japanese CGMP guidelines and distributed as Pinatos®. It is the same product which has been used in Avigen/MediciNova clinical trials for several years.

4.4. Pre-clinical toxicology

4.4.1. Pre-clinical study overview

The following provides an overview of the toxicity findings in the various species studied. In each case, the dose at which a toxic effect is seen in the animal is related to the Human Equivalent Dose (HED). For more detail on the results of the pre-clinical findings, consult the Investigator's Brochure.

4.4.2. Evaluation of organ- and species-specific findings

The acute oral toxicity established in the rat, dog and monkey, was only evident with human equivalent dosing (HED) regimens (and generally plasma ibudilast exposures) >25-fold a single high-dose human oral administration in ongoing or planned trials (eg, 50 mg in a 50 mg BID dosing regimen). Specific organ toxicity with ibudilast treatment has not been clearly defined in acute toxicity studies in multiple species as organ histopathology was performed only in a GLP rat study wherein some unscheduled-sacrifice rats at the highest-dose (400 mg/kg PO) exhibited abnormal findings including discoloration in the glandular and non-glandular stomach, myocardial degeneration and hemorrhage in a few rats, sublingual salivary gland degeneration and inflammation, and thymic lymphocyte necrosis and splenic lymphocyte depletion.

All adverse findings of behavioral or pathological (clinical pathology or histopathology) nature in repeat-dose animal toxicology studies were either markedly or completely recovered in dedicated recovery groups. Other findings including inappetance and body weight gain, and/or serum chemistry changes were similarly reversed in recovery groups. At necropsy, occasional

macroscopic organ changes were observed. There was no clear accumulation of toxicity or presentation of new, consistent, toxicity profiles with repeat daily dosing after 2-4 week durations.

Some proximal renal tubular degeneration and necrosis that in all cases was reversible was seen in rats orally administered ibudilast QD in studies ranging in duration from 2 weeks to 6 months given doses many multiples of the proposed human exposure. In all cases, no abnormal changes in serum chemistry or urinalysis markers of renal function were seen. Some corollary with serum GGT elevation, not considered a renal toxicity marker, was observed.

Specifically, in a two-week dose escalation study in rats given up to 225 mg/kg orally for the final week, oral rat dose levels wherein the proximal tubule effect was identified were ≥ 125 mg/kg (human equivalent dosing (HED) margin ≥ 13-fold a 100 mg/d clinical regimen, and with end-of-study peak plasma ibudilast concentrations exceeding that correlating with the 50 mg BID regimen.

In a 28-day oral dose study in rats given up to 600 mg/kg daily the lowest dose given that resulted in tubular toxicity corresponded to a 42-fold human equivalent dose (HED) and the key PK parameters of C_{max} and AUC at end-of-study exceeded that corresponding to steady 100 mg/d clinical regimens for C_{max}, but with daily AUC below the clinical correlate.

In a 6-month study of oral daily dosing of ibudilast up to 400 mg/kg where renal tubular regeneration rather than degeneration or necrosis was observed, the HED margin was 26-fold and end-of-study peak plasma concentration exceeded a human 100 mg/d correlate.

Interestingly, in a 16-month oral toxicology study that included doses up to 200 mg/kg in male and female rats, no renal tubule abnormalities were noted.

In conclusion, the high dose/exposure renal tubule effects seen only in the rat, were not more evident (eg, greater magnitude and/or observed at lower doses) in chronic dosing conditions compared to 2-4 week dosing periods, and 'new' toxicity was not clearly evident in dog, rat, or monkey studies of 13-week to 39-week duration, or even in 2-year rats studies, that were not apparent in the shorter sub-chronic studies.

The renal toxicity seen in rats dosed orally was *not* observed following subcutaneous dosing of ibudilast, even with plasma ibudilast and DHD exposures similar to that observed with oral dosing. Moreover, adverse renal pathology was *not* observed in oral dosing in a 2-week rabbit toxicology study *nor* in 2-, 4-, or 39-week toxicology studies in cynomolgus monkeys– and was, likewise, not observed with SC dosing of dogs over 2-, 4-, and 13-week durations with HED and plasma ibudilast TK parameters exceeding that correlating with human 50 mg BID regimens [C_{max} 116 ng/ml, $AUC_{0\text{-}24h}$ 1613 ng*hr/ml, study AV411-026].

The plasma metabolite (6,7-DHD) : ibudilast ratios in various toxicology species and studies were consistently $\underline{\geq}$ that observed in humans thus contributing to safety confidence and margin.

The potential toxicity of major metabolites, and impurities within the drug substance itself, or it's degradation products has been evaluated at least acutely and shown to have low intrinsic toxicity (LD_{50}>600 mg/kg in rodents). This provides a toxicity safety margins >400-fold compared to a human dose from a mg/kg perspective.

Inconsistent, but noted in some treatment group monkeys was cytoplasmic alteration in the adrenal cortex at oral ibudilast dosing ≥ 100 mg/kg/d (HED margin >22-fold 100 mg/d clinical regimen) in a 28-day study but not in a 39-week chronic toxicity, even at daily oral doses of 150-200 mg/kg.

Hyperplasia of the rat non-glandular stomach, an organ with *no* human correlate, was observed in one but not in a repeat subchronic and in chronic rat – the clinical significance cannot be determined.

Mammary lobular hyperplasia of mild-moderate nature was observed in a dose independent fashion in the majority of female rats receiving ibudilast at 50-400 mg/kg in the 26-week oral rat study, but not in other toxicology studies (including the 16-month oral rat toxicity study at doses up to 200 mg/kg): it was not evident in recovery animals. Likewise, thyroid follicular cell hypertrophy of a minimal magnitude was observed in two oral rat toxicology studies (2-week study 7613-105 and 26-week study 1123-017) in several animals at the highest doses (>125-200 mg/kg in study 7613-105, 250-400 mg/kg in study 1123-017) and with plasma ibudilast exposures exceeding human 50 mg BID correlates. It was reversible and was not adversely noted in other oral rat or other animal studies.

In the 39-week monkey study, minimal or mild lymphoid follicle formation in the bone marrow and minimal or mild germinal center formation in the thymus was noted in some of the males and females at all ibudilast dose levels. A reviewing pathologist noted it as a test article autoimmune response. While it was not generally observed in vehicle controls, the findings were not dose-related in terms of frequency nor severity. Such potential indicators of autoimmunity were not called out in other secondary lymphoid organs. Notably, such findings were not highlighted over dosing durations and in species in which an autoimmune action might be expected to present including: 4-week dog or monkey studies, a 13-week dog study, 26-week or 16-month rat toxicology studies – and at doses and plasma exposures covering similar or greater ranges than the 39-week monkey study.

Hepatocellular hypertrophy was a common finding in ibudilast-treated animals in the oral rat toxicology studies and correlated with microsomal metabolizing enzyme induction and reduced plasma ibudilast levels over time (see Sanftner et al 2009). Such liver changes and enzyme induction was not so evident with SC dosing of rats, rabbits, or dogs nor orally-dosed monkeys.

Ibudilast treatment has been carefully studied in cardiovascular, pulmonary, CNS, carcinogenicity (via a feed approach in rats), genetic, antigenicity or dependence studies across multiple species and high doses/exposures/concentrations. Results are summarized in the Investigator's Brochure.

4.4.3. Justification of the proposed human dose of 50 mg bid

There is a margin of safety, as determined from the oral toxicity studies, for the proposed clinical use of ibudilast dosing regimens up to at least 100 mg/d (eg, 50 mg BID; ~1.5 mg/kg/d). This was determined in multiple species from both acute and chronic dosing studies and ranged from 5-11-fold by human equivalent dose (HED) extrapolation from the oral dosing studies, (higher in shorter-term studies), and 2.6-3.7-fold for the 13-week SC rat and dogs studies.

The plasma ibudilast (and DHD) C_{max} and AUC toxicokinetic correlates for NOAELs in the ≥ 13-week (and shorter) studies in rats and dogs are generally > human 100 mg/d steady-state PK parameters. The orally-dosed subchronic and chronic monkey studies tended to have lower plasma ibudilast levels, likely due to high metabolism and low oral bioavailability, but still in the context of very high oral doses and hence drug exposures via a relevant route.

4.5. Prior Clinical Experience

4.5.1. Clinical Study Overview

To date, 8 clinical studies have been completed in Central Eastern Europe, Australia and US and 4 clinical trials are ongoing (in US). A total of 461 subjects have been known to be exposed to ibudilast (MN-166/AV411) with no SAEs clearly linked to the drug. A summary of the completed and ongoing MediciNova (and Avigen) and collaborative Investigator-initiated trials is as follows:

Summary of Completed and Ongoing Clinical Trials with MN-166

Type of Study	Study Identifier	Study Objective(s)	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status
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 placebo. On Day 2 subjects received no study medication and on Day 3 subjects commenced dosing with AV411 30 mg or placebo BID for 14 days; oral	18 (14 active, 4 pbo)	Healthy Volunteers 18 to 70 years old	2 wks	Completed
1	AV411-016	To assess safety, tolerability and PK	R (3:1), DB, PC, single escalating dose	30 mg, 50 mg, 70 mg, 80 mg, and 100 mg or placebo; oral	60 (45 active, 15 pbo)	Healthy Volunteers 18 to 55 years old	Single dose except 80 mg group who received dose in both fed and fasted state	Completed
1b	AV411-026	To assess safety, tolerability and PK	R (3:1), DB, PC, MD	On Days 1 through 6, subjects received AV411 (or matching placebo) as follows: 20 mg BID x 2 days, then 30 mg BID x 2 days, and then 40 mg BID x 2 days. On Days 7 through 14, subjects were administered AV411 50 mg BID or matching placebo; oral	24 (18 active, 6 pbo)- 12 HV; 12 DM	Healthy Volunteers & Diabetes Mellitus (Type 1 and 2) Subjects 18 to 75 years old	2 wks	Completed

Type of Study	Study Identifier	Study Objective(s)	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status
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 40 mg, 60 mg or pbo. Cohort 2: randomized 2:1 (active:pbo) to 60 mg/ 80 mg or pbo.	34 (24 active/10 pbo)	Diab. Periph. Neuropath. Pain (DPN) subjects 18 to 75 years old	Single and multiple dosing 2 wks	Completed
1b/2a	AV411-OWA (Investigator IND 102,942)	To assess safety, tolerability and preliminary efficacy to reduce opioid withdrawal syndrome and prolong the analgesic effect of oxycodone	R (1:1:1), DB, PC	20 mg BID x 14 days, 40 mg BID x 14 days, pbo; oral	44 (22 active/22 pbo	Heroin addicts 21 to 45 years old	Multiple dose 2 wks	Completed
2	MN166-CL-001	To assess safety, tolerability and efficacy	R, DB, PC followed by an OLE	10 mg TID, 20 mg TID, Pbo for 12 mos, then MN-166 for 12 mo; oral	297 (194 active/103 pbo)	Multiple Sclerosis 18 to 55 years old	Core 12 months; extension 12 months	Completed
1b	UCLA Meth. (Investigator IND 108,996)	To assess safety, tolerability, PK and methamphetamine interaction	R, DB, PC, Within-subject CO	20 mg BID, 50 mg BID x 7days, pbo; oral	11	Methamphetamine addicts 18 to 55 years old	2 wks	Completed
2a/2	IBU-002 (Investigator-sponsored)	To determine the effect of ibudilast in patients with medication over use headaches.	R, DB,PC	40 mg BID, pbo; oral	34 15 active/19 pbo)	chronic pain - Medication Overuse Headache 18 years and older	8 wks	Completed
2a	AV411 SA (Columbia/ NYSPI)	To evaluate the ability of AV411 to dose-dependently alter the reinforcing, analgesic, subjective, performance, and physiological effects of oxycodone	R, PC, CO	50 mg BID, placebo; oral	Planned 24	Opioid and heroin dependent users 21 to 55 years old	3 wks	Ongoing
2a	UCLA Alcohol abuse	To assess safety, tolerability in subjects with alcohol abuse	R, PC, CO	50 mg BID, placebo; oral	Planned 24	Alcohol abuse 18-55 yo	7 days session x 2	Ongoing
2	UCLA Meth Ph2	To evaluate efficacy for craving, withdraw symptom in Meth addict	R, DB,PC	50 mg BID, placebo; oral	Planned 140	Meth addicts 18-55 yo	12 weeks	Ongoing

Type of Study	Study Identifier	Study Objective(s)	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status
2	SPRINT-MS	To evaluate activity by measure brain volume by MRI	R,DB,PO	Up to 50 mg BID, placebo; oral	Planned 250	PPMS, SPMS 21 to 645 yo	96 weeks	Ongoing

CO=crossover; DB=double-blind; DM=diabetes mellitus; HV=healthy volunteers; MD=multiple dose; OLE=open-label extension; PC=placebo controlled, R=randomized.

The safety of ibudilast from 30 to 80 mg/d has been evaluated in single and multiple doses up to 14 days in healthy volunteers and diabetes mellitus Type 1 & 2 patients, chronic pain patients (diabetic neuropathy and complex regional pain syndrome) and opioid addict subjects. The dosage of 100 mg/d has been evaluated in single and multiple doses for up to 8 days (following 6 days of dose-incremental 40, 60 and 80 mg/d dosing at 2 days each) in healthy volunteers (HV) and diabetes mellitus (DM) Type 1 & 2 patients. In a phase 2 study MS trial, subjects received MN-166 30 or 60mg/d up to 2 years (96 weeks). An ongoing Phase 1b safety study to evaluate MN-166 in methamphetamine-dependent addicts recently completed enrollment. In this study, subjects received MN-166 40mg/d for 1 week and then 100 mg/d for 1 week. Other ongoing trials include dosage of 100 mg/d for 3 weeks to opioid addicts (with Columbia University/NYSPI expert investigators) and dosing for 8 weeks at 80 mg/d in chronic medication overuse headache (MOH) pain at the University of Adelaide with a prior AV411 investigator.

Notably, all of the trials listed in the table except for the first two have included all concurrent concomitant medications with ibudilast dosing at 80 or 100 mg/d. While pharmacokinetic drug-drug interactions have not been carefully assessed (or are in progress), there are no clear pharmacologic or safety interactions noted to date. Also, pharmacokinetics have been closely monitored in almost all of the trials and from the data of Study MN-166/AV411, the PK has generally been shown to be dose-proportional both within and between studies. Based on prior trials, steady state plasma C_{max} and $AUC_{0\text{-}24h}$ levels at the highest dose (50 mg BID; 100 mg/d) are anticipated to be approximately 116 ng/ml (C_{max}) and 1613 ng*hr/ml ($AUC_{0\text{-}24h}$) (study AV411-026 HV group).

4.6. Ibudilast (MN-166/AV411) safety overview

4.6.1. Published safety summary from experience in Japan

The largest accumulation of clinical safety experience with ibudilast is from the approval of Ketas® for asthma and post-stroke dizziness. Dosing was usually 20 to 30 mg/d (included uses up to 40 mg/d) and based on chronic administration. In the package insert, the most common adverse events were anorexia (0.6%), nausea (0.6%), increased liver enzymes (ALT 0.4%, AST 0.3%, and GTP 0.4%). A summary table of frequently observed AEs (from Ketas® package insert) is below.

Type of AEs	Frequency	
	0.1% to < 5%	**< 0.1%**
Hypersensitivity	Rash	Itching
CNS	Dizziness, Headache	Tremor, insomnia, sleepiness apathy
Gastrointestinal	Anorexia, Nausea, Vomiting Abdominal pain, Dyspepsia	Feeling of enlarged abdomen, diarrhea, gastric ulcer
Cardiovascular		Palpitation, orthostatic hypotension, hot flushes
Hematologic		Anemia, leukopenia
Haptic	Elevation of AST, ALT, ALP, GGT	Elevation of total bilirubin
Others		Malaise, tinnitus, facial edema, floating feeling, taste abnormality

4.6.2. Safety overview from previous clinical trials

In the previous Phase 1 and 2 clinical studies, 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/d group compared to the 30 mg/d, 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. Most of the reported TRAEs were mild to moderate in severity. One subject in the MS trial had a severe TRAE (hepatic steatosis) and the detail of this case is described further. One subject in the opioid addiction trial had severe insomnia which occurred while the subject was on placebo. Another subject in the opioid addiction trial had severe muscle aches while taking placebo.

4.6.3. Safety summary of Phase 2 MS trial (MN-166-CL-001)

In a Phase 2 trial conducted in CEE in RRMS patients, subjects were treated with either placebo or MN-166, 30 or 60 mg/d up to two years. Enrolled subjects received study drug for 12 months in the “core period” and if eligible, continued to the “extension period” for an additional 12 months of treatment. A total of 297 subjects were enrolled, 103 subjects were randomized to placebo group, 95 subjects were randomized to the 30 mg/d group and 99 subjects were randomized to the 60 mg/d group. Two-year completion of the 60 mg/d group was 86%.

Core period (12 m) 1st year	# of enrolled subject	Extension period (12 mo) 2nd year	# of enrolled subjects

	(N)		(N)
Placebo	103	P-30mg	49
		P-60mg	48

During the 12-month double-blind core-period,the adverse events most frequently reported (> 5%) occurred in the SOCs of Infections and Infestations (36%), Nervous System Disorders (19%), Gastrointestinal Disorders (15%), Psychiatric Disorders (13%), General Disorders and Administration Site Conditions (9%), and Musculoskeletal and Connective Tissue Disorders (7%). No significant differences in TEAEs by SOC were observed between treatment groups. There was a slight dose related increase in the percent of subjects with gastrointestinal AEs: 8%, 12%, and 15% for placebo, 30 mg/d, and 60 mg/d treatment groups, respectively.

During the core-period, more treatment-related TEAEs were experienced by subjects in the 60 mg/d group (26 TEAEs) compared to the 30 mg/d (18 TEAEs) or placebo group (12 TEAEs). The most common treatment-related AEs were headache (n =3) and nausea (n =2) in the 30 mg/d; nausea (n =4), headache (n =3), vomiting (n=2) and dizziness (n =2) in the 60 mg/d group; and upper respiratory infection (n =5) and headache (n =2) in the placebo group.

During the 12-month open-label extension period, five additional treatment-related TEAEs occurred. These TEAEs were night cramps (1 subject 60 mg/d), hepatotoxity (1 subject 60 mg/d), depression (1 subject 60 mg/d), and pruritus (2 subjects in placebo to 60 mg/d).

Over the entire 24-month study, the most frequently occurring AEs (≥ 2% of subjects) were nasopharyngitis (20%), headache (14%), urinary tract infection (9%), pharyngitis (6%), nausea (5%), anxiety (4%), insomnia (4%), asthenia (4%), pyrexia (4%), diarrhoea (3%),vomiting (3%) iron deficiency anemia (3%), hypertension (3%), depressed mood (3%), depression (3%), upper respiratory tract infection (3%), hypercholesterolaemia (2%), back pain (2%), rhinitis (2%), and sinusitis (2%). Gastrointestinal AEs occurred in a percentage of subjects: 10%, 12%, 17%, and 18% for placebo to 30 mg/d, placebo to 60 mg/d, 30 mg/d, and 60 mg/d treatment groups, respectively. There was a slight increase in gastrointestinal AEs in the percent of subjects who received MN-166 treatment for 24 months.

Over the entire 24-month study, a total of 21 SAEs were reported by 20 subjects. SAEs were reported by 12 subjects in the core period and nine SAEs were reported by 7 subjects during the extension period. More subjects in the 60 mg/d dose group (10/99 [10.1%]) experienced an SAE compared to other active group. Four subjects in placebo group (4/103 [3.8%]) experienced SAEs during core period while on placebo, 1 subject in placebo-60 mg subject (1/48 [2.1%]) experienced an SAE during the extension-period while on 60 mg/d dosage and 4 subjects in the 30mg/d dose group (4/95 [4.2%]) experienced an SAE. The SAEs were as follows: acetabulum fracture, duodenal ulcer perforation, calculus ureteric, and menorrhagia in the placebo group; inguinal hernia, epilepsy, peptic ulcer perforation, fibula fracture and menorrhagia in the 30 mg/d group; and bladder diverticulum, epilepsy, intervertebral disc protrusion, pneumonia haemophilus, uterine cancer, gastrointestinal malignant neoplasms, retinal detachment, hip surgery, depression, acute pancreatitis, ankle fracture and congenital anomaly resulting in death (partner pregnancy) in the 60 mg/d group . All of these SAEs were considered by the

Investigator to be unlikely related or not related to study drug. Eight of the SAEs were considered by the Investigator to be severe or life threatening. Subject SE-001-009 who was diagnosed with gastrointestinal malignant neoplasms died due to this SAE approximately 1 year after he withdrew from the study due to this SAE.

A total of nine subjects discontinued from the study due to a TEAE. Two of these subjects experienced a TEAE that were considered to be related to study drug; hepatic steatosis (severe) and hepatotoxicity (moderate) treated with 30 mg/d and 60 mg/d, respectively. None of the other TEAEs (duodenal ulcer perforation, osteoarthritis, tachycardia, epilepsy, uterine cancer, gastrointestinal neoplasm, acute pancreatitis) that resulted in study discontinuation were considered to be related to study medication by investigator.

4.6.4. Abnormal Laboratory Test Values in the clinical trials

Ibudilast-related changes in clinical chemistry or hematology endpoints have been infrequent and not clearly dose-related. Two treatment-related adverse events of ALT and AST increases have been observed in Phase 1 studies AV411-016 and AV411-026. In a single dose study in healthy volunteers (AV411-016), one protocol-defined dose limiting toxicity was encountered in one subject in the 50mg cohort. Subject 0203 experienced adverse events of pruritis and uticaria on Day 1 and increased ALT and AST on Day 2. All of these AEs were considered by the Investigator to be possibly related to study drug. On Day 3, the subject's ALT was 82 U/L, slightly over twice the ULN; AST value is unknown. Liver function tests returned to normal on Day 20. One diabetic subject (subject 2004) in study AV411-026 experienced increases in ALT to 83 IU/L on Day 15 (one day post last dose of AV411 50 mg bid), 74 IU/L on Day 16 and 117 IU/L on Day 24 (10 days post last dose of study drug) which were considered by the Investigator to be possibly related to study medication; on Day 36, ALT levels returned to normal limits. In the MN-166-CL-001 study in MS patients, 3 subjects had clinically significant ALT/AST elevations. One subject (SE-004-069) on MN-166 30mg/d experienced the adverse event of hepatic steatosis at 8 month. This subject's ALT was 323 U/L, AST 153 U/L. This event was considered possibly related to study drug by the Investigator and the subject was withdrawn from the study. His ALT and AST level returned to normal as recorded at the 9 month early termination visit. One patient (subject SE-004-068), after receiving 10 months of MN-166 60 mg/d, he had an acute pancreatitis event with ALT increased to 691 U/L and the AST to164 U/L. Amylase was 855 U/L at the time of admission into the hospital. The patient was withdrawn from the study and the event was considered by the Investigator to be not related to study drug. The lab values 1 week after admission were: ALT 141 U/L, AST 105 U/L and GGT 251 U/L. His early termination follow up lab value at 11 months was ALT 85 U/L, GGT 117 U/L; his other labs returned to normal. Another patient (SE-003-057) after receiving 22 months of N-166 60 mg/d had an adverse event of hepatotoxicity (ALT 98 U/L, AST 87 U/L and GGT 259 U/L) which was considered by the Investigator to be moderate in severity and related to study drug. This patient was withdrawn from the study and ALT was 76 U/L and other values returned to normal at 23 months early termination visit . According to the Investigator, this subject may have overdosed. The subject was non-compliant and was taking "24 capsules more."

Two isolated GGT elevation have also been observed in the MN-166 and placebo treatment groups: one healthy volunteer in the AV411-009 trial had an adverse event of a mild elevation

in GGT (from 60 U/L to 78 U/L) on Day 16 (after completing 14 days 60 mg/d dose regimen) which was considered by the Investigator to be related to study drug treatment In an ongoing study IBU-002, one subject in the placebo group exhibited a 6 fold increase in GGT after approximately two-weeks of dosing without notable changes in ALT, AST, BUN and creatinine: these changes were thought to be related to study drug.

4.6.5. Safety Overview for Higher Dosage

In general, clinical trials involving ibudilast dosing with 8 days (up to 100 mg/d) or 2 weeks (to 80 mg/d) or 2 years (to 60 mg/d), safety findings that warrant concern have not appeared to increase with increased duration of treatment. The results to date suggest acceptable safety and tolerability at doses of 60 to 100 mg/d, without an apparent accumulation of AEs or toxicity over time. Any potential study drug-related adverse events can be identified by regular clinical monitoring and scheduled lab tests and exams as proposed. Additionally, in the recently completed trial in medication overuse headache (MOH) subjects at 80 mg/d for 2 months, the most common AE reported was nausea, which occurred more frequently in the MN-166-treated group but was generally mild and was managed by over-the-counter anti-emetics and/or temporarily ceasing the medication and reinstating at a lower dose and titrating upward to the original dose. In the UCLA methamphetamine addiction study, subjects received 100 mg/d MN-166 for 7 d following 1 week at 40 mg/d before or after of 1 week of placebo. No significant difference or trend was observed in the frequency of study drug-related AEs. In the ongoing opioid dependency study and alcohol abuse studies, subjects receive 100 mg/d MN-166 for 3 weeks or 7 d and are progressing without apparent safety/tolerability concerns.

Focusing on the clinical trials involving single and multiple dosing regimens of 80-100 mg/d, which is most relevant to the proposed protocol, AEs have been generally GI-related as summarized in the table below. Tolerability issues with ibudilast dosing tend to be gastrointestinal (GI) in nature and are somewhat, but not consistently, dose-related. Nausea tends to be more commonly observed followed by diarrhea and emesis in a small subset of patients. In the RRMS trial and in other repeated dosing trials, when GI distress was observed, there appeared to be some accommodation to these side effects within 1-2 weeks of dosing.

A summary of frequently reported treatment related AEs in the 80-100 mg /d dosage group from completed and ongoing studies are summarized below.

	MN-166 (ibudilast) related adverse events of interest (GI symptom, headache, Lab change) in 80-100 mg/d dosing regimen: *Completed studies*											
	AV411-016				**AV411-026**				**AV411-010**		**AV411-OWA***	
	80 mg single dose N =9	**80 mg fasting single dose N =8**	**100 mg single dose N =9**	**Placebo N=15**	**80 mg (2d) -100 mg (8d) total 10 days**		**Placebo**		**60 mg (2d) 80 mg (12d) total 14 days**	**Placebo**	**80 mg x 14 days**	**Placebo**
					HV N =9	**DM N =9**	**HV N =3**	**DM N =3**	**N =16**	**N =10**	**N =10**	**N =10**
Nausea	-	1/8	3/9	-	-	1/9	-	-	5/16	1/10	1/10	2/10
Dyspepsia	-	-	-	-	-	4/9	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	3/9	-	1/3	1/16	-	-	-
Vomiting	-	-	-	-	-	1/9	-	-	-	-	1/10	2/10
GI upset	-	-	-	-	-	-	-	-	-	-	2/10	4/10
Headache	1//9	1/8	1/9	3/15	2/9	3/9	1/3	-	4/16	3/10	2/10	-
ALT/AST elevation	-	-	-	-	-	1/9	-	-	-	-	-	-

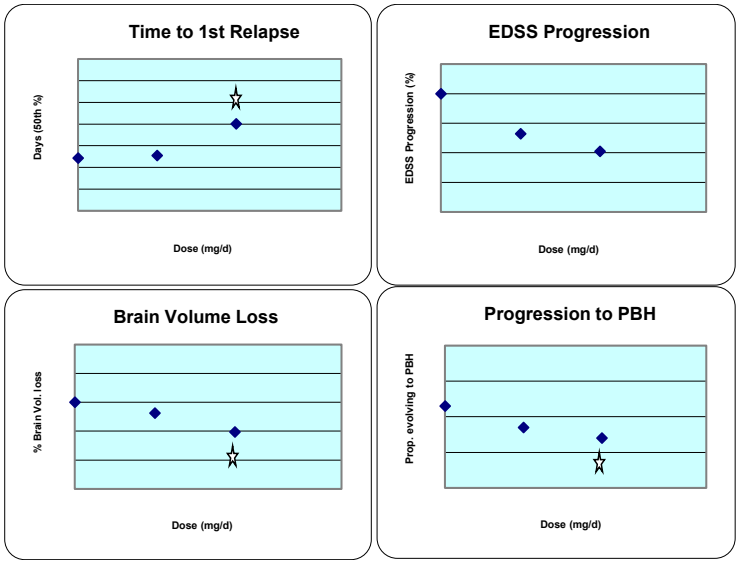
*AV411-OWA study: TRAEs during double-blind phase (Weeks 2 and 3)

	MN-166 (ibudilast) related frequently observed AEs (GI symptom, Headache, Lab changes) in 80-100 mg/d regimen: completed & ongoing study						
	IBU-002-MOH		**UCLA-Meth Ph1 CO***		**AV411-SA CO***	**UCLA Alcohol abuse CO***	**UCLA Meth Ph2**
	80 mg/d N=15	**Placebo N=19**	**100 mg/d phase N=11**	**Placebo phase N=11**	**100 mg/d and Placebo**	**100 mg/d and Placebo**	**100 mg/d or Placebo**
Nausea/ GI upset	**10/15**	**2/19**	**4/11**	**4/11**	**Ongoing No SAE**	**Ongoing No SAE**	**Ongoing No SAE**
Diarrhea	**2/15**	**-**	**-**	**-**			
Vomiting	**-**	**-**	**-**	**-**			
Headache	**4/15**	**2/19**	**5/11**	**3/11**			
ALT/AST elevation	**-**	**-**					
GGT elevation	**-**	**6-fold UNL @ 2weeks**					
Withdrawn from study	**2/15** **1:Unable to attend remaining study visit** **1:worsening pre-exist headache**	**2/19** **1: worsening headache** **1: withheld study for GGT elevation** **No longer meet IC when resolved**					

*study is cross-over design

4.7. Dose rationale

The efficacy result from the previous Phase 2 MS trial indicated that patients receiving 60 mg/d exhibited significantly reduced brain volume loss, reduced progression to persistent black hole formation (from gadolinium-enhancing lesions), extended time to first relapse and reduced EDSS progression vs. Placebo (Barkhof et al 2010) (see figure below). A dose-response was evident between the 30 and 60 mg/d regimens, with 60 mg/d statistically significant, for all but EDSS. Inflammation MRI endpoints were not significantly affected, implicating ibudilast as perhaps one of the first therapies with potential neuroprotective action independent of overt anti-inflammatory or immunosuppressive action.



☆ p < *0.05 at 60 mg/d for Time to 1st relapse, Brain Volume Loss, and Progression to PBH*

Brain atrophy, as measured by percent brain volume change (PBVC), was -0.95% and -1.2% in the placebo group at 8 and 12 months, respectively. Ibudilast treatment attenuated atrophy by ~13% at the low dose and ~35% ($p<0.05$) at 60 mg/d.

The Phase 2 RRMS trial results suggest that MN-166 is best positioned for primary neuroprotection in MS patients (Fox 2010) and the dose-response data here and in other translational neuropharmacology suggest that doses ≥60 mg/d may be suitable to demonstrate efficacy in patient population.

Pain is reported in ~50% of MS patients, is considered to be neuropathic in the majority of those patients, and is more prevalent in progressive forms of MS (Grau-Lopez et al 2011; Österberg et al 2005). There is a recognized need for effective pharmacotherapies for pain management in

MS (Truini et al 2011). Given ibudilast's preclinical central and peripheral neuropathic pain efficacy validation and preliminary indicators of efficacy in neuropathic pain patients, a secondary endpoint assessment of pain modulation might confer important insights on potential clinical utility therein.

A summary of clinical trial outcomes related to dose rationale is as follows:

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, Dependence	McGill pain survey, Subjective Opioid Withdrawal Scale	40 80	Not available +

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

4.8. Potential Drug-Drug Interactions

Ibudilast is not expected to exhibit pharmacokinetic drug-drug interactions with immunomodulating therapies in patients in this trial. Patients in the proposed progressive MS trial may remain on their glatiramer acetate or interferon-beta therapy. While the metabolism and pharmacokinetics of ibudilast are described in the Investigator's Brochure, specific notation of potential interactions with glatiramer acetate or interferon-beta is not noted herein.

Pharmacokinetic interactions are not anticipated with ibudilast co-administration in either case: glatiramer acetate is a small polypeptide mixture and not reported to involve cytochrome P450 metabolism nor induction; interferon-beta-1 does not appear to be a major substrate for cytochrome P450 metabolism although it may have some inhibitory action on CYP1A2 (medicine.iupui.edu/clinpharm). As ibudilast is neither a potent inducer nor an inhibitor of 1A2 and is not notably metabolized by 1A2 in human isozyme metabolic studies, pharmacokinetic interactions are not anticipated. Moreover, pharmacodynamic interactions of note have not been reported, to our knowledge. One remote option considered relates to bupropion. It is a substrate of CYP2B6 and *if* ibudilast were to induce CYP2B6 then there may be some increased metabolism, reduced effectiveness, of this anti-depressant. There has been some concomitant medication history with Wellbutrin® in our trials with no apparent pharmacodynamics adverse outcomes. Moreover, as has been published separately, and as we have observed in our own clinical trials, there have been no declines in steady-state plasma ibudilast levels with multi-day/week dosing which provides some additional support that neither 2B6 nor other enzymes participating in ibudilast metabolism are not noticeably induced. Finally, ibudilast in conjunction with other drugs has not yielded DDI (drug-drug interaction), at least in terms of plasma ibudilast

levels or pharmacodynamic outcomes, to date in MediciNova/Avigen trials involving concomitant administrations.

5. TRIAL OBJECTIVES AND PURPOSE

This is a multicenter, double-blind, placebo-controlled study designed to evaluate the safety, tolerability and activity of ibudilast (MN-166) in subjects with primary and secondary progressive multiple sclerosis.

5.1. Primary Objective

The primary objectives of the study are:

- to evaluate the activity of ibudilast (MN-166) (100 mg/d) versus placebo at 96 weeks as measured by quantitative magnetic resonance imaging (MRI) analysis for whole brain atrophy using brain parenchymal fraction (BPF).
- to evaluate the safety and tolerability of ibudilast (MN-166) (100 mg/d) versus placebo administered orally in subjects with primary and secondary progressive multiple sclerosis.

5.2. Secondary Objectives

The major secondary objectives are to evaluate the activity of ibudilast (MN-166) at 96 weeks versus placebo as measured by:

- Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
- Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
- Retinal nerve fiber layer as measured by Optical coherence tomography (OCT)
- Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm (CLADA)

The additional secondary outcomes are to measure the activity of ibudilast (MN-166) at 96 weeks versus placebo on:

- Inflammatory disease activity, as measured by T1 lesion volume, T2 lesion volume, and annualized relapse rate
- Disability, as measured by Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC)
- Cognitive impairment as measured by the Symbol Digit Modalities Test and the Selective Reminding Test
- Quality of Life, as measured by Multiple Sclerosis Impact Scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36)
- Neuropathic pain, as measured by Brief Pain Inventory (BPI)

5.3. Tertiary Objectives

The first set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at one year versus placebo as measured by the primary and secondary imaging outcome measures: whole brain atrophy using brain parenchymal fraction (BPF), diffusion tensor imaging (DTI) in descending pyramidal white matter tracts, magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue, retinal nerve fiber layer (RNFL) as measured by Optical

coherence tomography (OCT), and cortical atrophy as measured by cortical longitudinal atrophy detection algorithm (CLADA).

The second set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at 96 weeks versus placebo as measured by, whole-brain gray matter fraction, magnetization transfer ratio (MTR) in gray matter, new T1 lesions since baseline, and new T2 lesions since baseline.

5.4. Exploratory Objectives

The exploratory objectives include evaluation of the pharmacokinetics (PK) of ibudilast (MN-166) using a population PK approach, correlations of cerebrospinal fluid (CSF) and serum biomarkers with imaging and clinical measures of progressive disability, identification of unique phase 2 endpoints, and composite MRI scales (combining BPF, MTR, and DTI).

Blood samples for analysis of ibudilast and its metabolite, 6,7-dihydrodiol (DHD) will be collected during scheduled visits on Weeks 8, 48, and 96. The exact sampling time and time relative to the previous dose will be documented in the case report forms. Population PK modeling using the NONMEM program (Icon Development Solution) will be used to characterize the pharmacokinetic properties of ibudilast in healthy subjects and subjects with MS. The population analysis will evaluate selected covariates to determine if they contribute to differences in PK parameter estimates among individuals. The covariates will likely include demographic variables (age, gender, body weight, and race), creatinine clearance (as a marker of renal function), liver enzyme levels (as a marker of hepatic function), blood chemistry variables, and relevant disease covariates at baseline, among others. Further, the effect of concomitant medications on the pharmacokinetics of ibudilast will also be assessed.

6. OVERALL STUDY DESIGN AND PLAN: DESCRIPTION

This is a multicenter, randomized, double-blind, placebo-controlled, parallel-group study designed to evaluate the safety, tolerability and activity of ibudilast (MN-166) administered twice daily over a 96 week period in subjects with primary or secondary progressive multiple sclerosis who are currently untreated with long-term MS disease modifying therapy (DMT) or who are receiving either glatiramer acetate (GA) or interferon beta (IFNβ-1a [Avonex, Rebif] or IFNβ-1b [Betaseron, Extavia]) treatment. Study drug will be administered as an adjunct to glatiramer or beta interferon treatment.

A total of 250 male and female subjects from 21 to 65 years old, inclusive, are planned to be enrolled into one of two treatment arms (ibudilast 100 mg/d or matching-placebo). Randomization of subjects will be stratified by disease status (PPMS or SPMS), and by immunomodulating therapy status: "untreated", GA, or IFN. Subjects will return to the clinic for follow-up visits on a regular basis at Week 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 (see Table 1 Schedule of Assessments).

After 30 patients have been enrolled for at least 30 days and then again after 60 patients have been enrolled for at least 60 days, the Medical Safety Monitor (MSM) will review safety data provided by the Data Coordinating Center. Safety data will be segregated by treatment group (Group A, Group B), but the treatment assignment for each group (ibudilast, placebo) will not be identified. The MSM will prepare a report of study safety based upon the 30-day and 60-day safety data. A National Institute of Neurological Disorders and Stroke (NINDS) Data and Safety Monitoring Board (DSMB) will meet to review the 30-day and 60-day safety data report from the MSM. The DSMB will then meet every six months during the study (and more often, if deemed necessary), and will review segregated (but treatment group blinded) safety data including adverse events and serious adverse events (SAE). One member of the DSMB will know the specific treatment group assignment. The membership of the DSMB and its mandates are described in the NeuroNEXT DSMB Guideline.

The NeuroNEXT Clinical Coordinating Center (CCC) located at the Massachusetts General Hospital will be responsible for site and project management of the study in conjunction with the Lead Principal Investigator and study Sponsor. The NeuroNEXT Data Coordinating Center (DCC) located at the University of Iowa will coordinate all data and statistical services for the study, as well as clinical monitoring for all sites.

The study phases are described below and displayed in Figure 1. The schedule of assessments, is displayed in Table 1. Table 2 displays the Schedule of Unplanned Procedures and Assessments.

6.1. Screening Phase (up to 45 days)

A total of up to 45 days will be allowed to complete the screening assessments.

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

6.1.1. Diagnosis at Screening

Subjects must have a confirmed diagnosis of SPMS or PPMS according to 2010 International Panel Criteria, typical MS lesions on brain MRI according to Swanton's MRI Criteria, and

clinical evidence of disability progression in the preceding 2 years, as measured by any of the following:

- worsening overall EDSS of at least 0.5 points (may be assessed retrospectively, but cannot be during a clinical relapse) or
- 20% worsening in 25-foot walk (25-FW) or
- 20% worsening in 9-hole peg test (9-HPT) in either hand.

The diagnosis must be noted in the source documents.

6.1.2. Treatment Phase (96 weeks)

The double-blind Treatment Phase consists of a Baseline visit followed by 10 scheduled clinic visits. The Baseline Visit must occur within 45 days following the Screening Visit. At the Baseline Visit (Day 1), subjects who have completed all of the screening assessments and continue to meet eligibility criteria will be randomized to one of two treatment groups (MN-166 100 mg/d or placebo) in a 1:1 ratio.

Subjects will take their first dose of study medication (30 mg MN-166 or placebo) 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 through Day 14. Subjects randomized to MN-166 will start at 60 mg/d (30 mg BID) on Day 2 and will remain on 60 mg/d through Day 14. Beginning on Day 15, all subjects will increase dosing to 5 capsules BID regimen; those randomized to the MN-166 treatment arm will be taking 100 mg/d.

After Day 15, subjects with intolerable side-effects (e.g., nausea, diarrhea, vertigo) may reduce their dose to either 4 capsules twice a day (80 mg/d for those taking ibudilast) or 3 capsules twice a day (60 mg/d for those taking ibudilast). Subject with intolerable side-effects (e.g., nausea, diarrhea, vertigo) at the end of Day 14 may continue to taking 3 capsules twice a day at the Investigator's discretion. At the investigator's discretion, the daily dose of ibudilast can be changed between 3 capsules twice a day, 4 capsules twice a day, and 5 capsules twice a day over the first 8 weeks of treatment. At the investigator's discretion, 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). The dosing interval may be twice a day or 3 times per day, at the investigator's discretion. The dosing interval may be changed between twice and three times per day over the course of the entire study, at the investigator's discretion.

Subjects will return to the clinic for follow-up visits on a regular basis at Week 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 (see Table 1: Schedule of Assessments).

Subjects who experience symptoms suggestive of a possible relapse will return to the clinic within three days of notifying the Investigator and will undergo assessments described in Table 2 Schedule of Unplanned Procedures and Assessments).

Subjects who prematurely discontinue study medication will be asked to continue to be followed off study medication on a semi-annual basis (Table 2: Schedule of Unplanned Procedures and

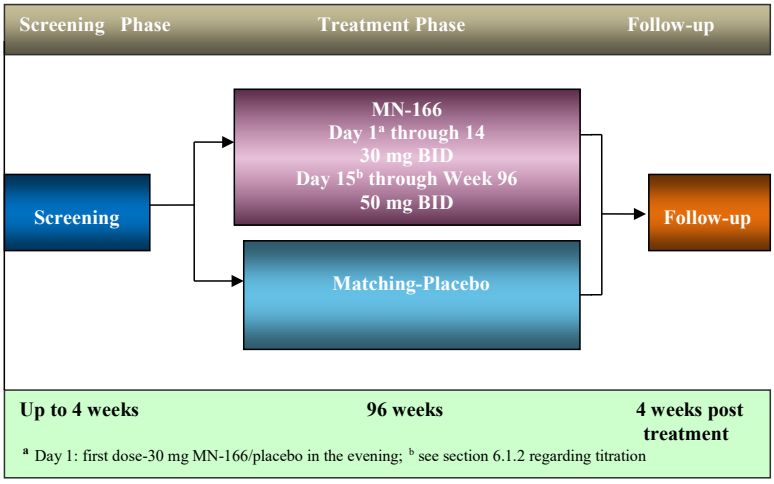
Assessments) until the end of the study (Week 96). See section 7.3.1 for addition details regarding drug discontinuation procedures.

Randomization will be performed at the double-blind baseline visit using an Interactive Voice/Web Response System (IXRS).

6.1.3. Follow-up Phase

All subjects who complete the study will return for a safety visit 4 weeks after their last study visit to assess the subject's general health status and assess adverse events, if applicable.

Figure 1: Study Design



7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1. Clinical Trial Population

The population for this trial will include male and female subjects ≥ 21 and ≤ 65 years of age with a diagnosis of primary or secondary progressive multiple sclerosis who are currently untreated with immunomodulating medications or who are receiving either glatiramer acetate (GA) or interferon beta (IFNβ-1a or IFNβ-1b) treatment.

It is estimated that in order to complete 250 subjects, approximately 280 subjects will need to be screened.

7.2. Inclusion/Exclusion Criteria

7.2.1. Inclusion Criteria

1. Subject provides written informed consent and is willing and able to comply with the protocol in the opinion of the Investigator.
2. Male or female subjects who are ≥ 21 and ≤ 65 years of age, on the day of signing the informed consent.
3. Confirmed diagnosis of SPMS or PPMS according to 2010 International Panel Criteria
4. Typical MS lesions on MRI according to Swanton's MRI Criteria (at least one lesion in two or more of the following regions: periventricular, juxtacortical, infratentorial [brainstem/cerebellum], spinal cord)
5. EDSS 3.0-6.5, inclusive
6. Clinical evidence of disability progression in the preceding 2 years, as measured by any of the following (excluding progression during clinical relapses):
   - worsening overall EDSS of at least 0.5 points (may be assessed retrospectively, but cannot be during a clinical relapse)
     or
   - 20% worsening in 25-foot walk (25FW)
     or
   - 20% worsening in 9-hole peg test (9HPT) in either hand
7. Existing multiple sclerosis pharmacotherapy status may include interferon-beta or glatiramer acetate or none (i.e., untreated).
8. Females of child-bearing potential must have a negative serum ß-hCG at screening and must be willing to use appropriate contraception (as defined by the investigator) for the duration of study treatment and 30 days after the last dose of study treatment.
9. Male subjects should practice contraception as follows: condom use and contraception by female partner.
10. Subject is in good physical health on the basis of medical history, physical examination, and laboratory screening, as defined by the investigator.

11. Subject is willing and able to comply with the protocol assessments and visits, in the opinion of the study nurse/coordinator and the Investigator.

7.2.2. Exclusion Criteria

Note: If any laboratory exclusion criteria are outside the normal range, the site may have the subject retested. If upon retesting the value remains outside the normal range, the significance of this value must be discussed with the Medical Safety Monitor for enrollment consideration.

1. Progressive neurological disorder other than SPMS or PPMS
2. Relapse and/or systemic corticosteroid treatment within 3 months of screening. Inhaled or topical steroids are allowed.
3. Current use of intermittent systemic corticosteroids (i.e., monthly or bimonthly intravenous methylprednisolone).
4. Use of oral immunosuppressants (e.g., azathioprine, methotrexate, cyclosporine, teriflunomide [Aubagio®]) within 6 months of screening
5. Use of mitoxantrone, natalizumab, or IVIg within 6 months of screening, or use of alemtuzumab within the prior 10 years
6. Use of fingolimod or dimethyl fumarate [Tecfidera®] within 3 months of screening
7. Use of rituximab or other B-cell therapy within 12 months of screening
8. Current use of other MS disease-modifying treatments (DMTs) besides glatiramer acetate, IFNβ-1 (any formulation) and the above listed medications.
9. Current use of cimetidine, cyclosporine, dronedarone, lopinavir, probenecid, quinidine (including Neudexta), ranolazine, rifampin, ritonavir, or tipranavir.
10. Subject has clinically significant cardiovascular disease, including myocardial infarct within last 6 months, unstable ischemic heart disease, congestive heart failure or angina.
11. Subject has a resting pulse < 50 bpm, SA or AV block (Type II or greater), uncontrolled hypertension, or QTcF > 450 ms
12. Clinically significant pulmonary conditions, including severe COPD, fibrosis, or tuberculosis
13. Subject demonstrates evidence of acute hepatitis, clinically significant chronic hepatitis, or evidence of clinically significant impaired hepatic function through clinical and laboratory evaluation including ALP > 1.5x ULN; ALT or AST > 2x ULN; GGT > 3x ULN
14. Immune system disease (other than multiple sclerosis and autoimmune thyroid disease)
15. Subject has a history of stomach or intestinal surgery or any other condition that could interfere with or is judged by the Investigator to interfere with absorption, distribution, metabolism, or excretion of study drug.
16. Subject has any abnormal laboratory parameter at screening that indicates a clinically significant medical condition as determined by the Investigator or has any of the following abnormalities at screening:
    - Creatinine: females > 0.95 mg/dL; males > 1.17 mg/dL
    - WBCs < 3,000 mm^3
    - Lymphocytes < 800 mm^3
    - Platelets < 90,000 mm^3

17. Subject has a history of malignancy < 5 years prior to signing the informed consent, except for adequately treated basal cell or squamous cell skin cancer or *in situ* cervical cancer
18. History of HIV, clinically significant chronic hepatitis, or other active infection
19. Subject currently has a clinically significant medical condition (other than MS) including the following: neurological, metabolic, hepatic, renal, hematological, pulmonary, cardiovascular (including uncontrolled hypertension), gastrointestinal, urological disorder, or central nervous system (CNS) infection that would pose a risk to the subject if they were to participate in the study or that might confound the results of the study.

    Note: Active medical conditions that are minor or well-controlled are not exclusionary if, in the judgment of the Investigator, they do not affect risk to the subject or the study results. In cases in which the impact of the condition upon risk to the subject or study results is unclear, the Medical Safety Monitor should be consulted. Any subject with a known cardiovascular disease or condition (even if controlled) must be discussed with the Medical Safety Monitor before being screened.

20. Subjects with moderate to severe depression as determined by the Beck Depression Inventory-Fast Screen (BDI-FS).
21. Subject has a history of alcohol or substance abuse (DSM-IV-TR criteria) within 3 months prior to screening or alcohol or substance dependence (DSM-IV-TR criteria) within 12 months prior to screening. The only exceptions include caffeine or nicotine abuse/dependence.
22. Subject has poor peripheral venous access that will limit the ability to draw blood as judged by the Investigator.
23. Subject is currently participating, or has participated in, a study with an investigational or marketed compound or device within 3 months prior to signing the informed consent.
24. Subject is unable to cooperate with any study procedures, unlikely to adhere to the study procedures and keep appointments, in the opinion of the Investigator, or was planning to relocate during the study.
25. Subject is unable to undergo MRI imaging because of having an artificial heart valve, metal plate, pin, or other metallic objects (including gun shots or shrapnel) in their body or is unable to complete all the five MRI scans required for this study.
26. Subject is unable to lie sufficiently still in an MRI to obtain a high quality MRI image.

Additional Exclusion Criteria for Lumbar Puncture Sub-Study
The following additional exclusion criteria apply to subjects electing to enroll in the lumbar puncture sub-study:

27. A clinical history of a bleeding disorder.
28. Current treatment with blood thinner medications such as warfarin (Coumadin), or clopidogrel (Plavix), dipyridamole (Persantine), ticlopidine (Ticlid), warfarin, heparin, although aspirin is allowed.

7.3. 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 this study. The following instances may 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 11.8 for reporting requirements and follow-up of the pregnancy;
- Any Grade 3 or higher CTCAE;
- Abnormal laboratory values as described in section 8.6.2.

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, every effort should be made to ensure all Early Withdrawal (EW) procedures and evaluations are performed.

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

Study drug re-administration may be considered as noted below.

7.3.1. Follow-up Procedures Upon Discontinuation/Withdrawal

If a subject discontinues study medication for any reason, the subject will be encouraged to continue to be followed within the study, but on a reduced study visit schedule. Depending upon when the subject discontinued study drug, the reduced study visit schedule will include study visits at Week 24, 48, 72, and 96. The timing of all study visits will remain relative to the original baseline date. For subjects who discontinue study drug between regularly scheduled study visits, the subject should complete the next regularly scheduled study visit after study drug discontinuation, and then shift to the reduced study visit schedule. For subjects who discontinue study drug at a regularly scheduled study visit, the subject should shift immediately to the reduced study visit schedule. For subjects discontinuing study drug but remaining in the study for follow-up, study visits will occur on an approximate semi-annual basis until Week 96. Table 2 outlines the procedures and assessments to be conducted in subjects remaining in the study for follow-up but are off study medication. After study drug discontinuation, new-onset AEs will not be collected.

If a subject discontinues/withdraws prior to study completion and does not wish to continue follow-up within the study (i.e. return for semi-annual visits), then all assessments scheduled for the Early Withdrawal visit (Table 2) should be performed at the time of study discontinuation.

A 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.

For all subjects who complete the study on study medication, a follow-up visit (Week 100) will be conducted 4 weeks after the last visit to assess general health and adverse event status.

8. TREATMENT OF SUBJECTS

8.1. Description of Study Drug

MN-166 and matching-placebo will be provided in bottles containing MN-166 10 mg capsules or matching-placebo capsules, and will be stored at room temperature.

8.2. Concomitant Medications

Subjects may continue the use of IFN-β or glatiramer acetate while participating in this study. During the study, subjects may be allowed to change medications from one injectable to the other. Pegylated interferon beta-1 will be allowed (if approved by the FDA).

Inhaled and topical steroids are allowed.

If a relapse episode occurs, a single course of systemic corticosteroids is permitted, as prescribed by the treating neurologist.

8.3. Prohibited Medications

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

- Systemic corticosteroid treatment within 3 months prior to screening (inhaled or topical steroids are allowed).
    - A single course of systemic corticosteroid treatment will be allowed for treatment of a clinical relapse
- Current use of intermittent systemic corticosteroids (i.e., monthly or bimonthly intravenous methylprednisolone)
- Oral immunosuppressants (e.g., azathioprine, methotrexate, cyclosporine, teriflunomide [Aubagio®]) within 6 months of screening
- Mitoxantrone or natalizumab within 6 months of screening
- Fingolimod or dimethyl fumarate [Tecfidera®] within 3 months of screening
- Rituximab or other B-cell therapy within12 months of screening
- Current use of other MS disease-modifying therapies (DMTs) besides glatiramer acetate and IFNβ-1 (any formulation).

The following medications are prohibited during study participation:

- cimetidine, cyclosporine, dronedarone, lopinavir, probenecid, quinidine (including Neudexta), ranolazine, rifampin, ritonavir, or tipranavir

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 for drug accountability. Compliance will be assessed at weeks 24, 72, and 96 by counting capsules and dividing the actual number of doses taken (per capsule count) by the number of days the subject took study drug. 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 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

The study will consist of a screening phase (up to 45 days), followed by a double-blind treatment phase (96 weeks) and a follow-up visit (4 weeks after the 96 week visit). Following the screening phase, subjects who continue to meet entry criteria will be randomly assigned to one of two treatment groups: MN-166 100 mg/d or matching placebo. A total of 250 subjects, aged 21 to 65 years, will be enrolled and randomized into one of two treatment groups (MN-166 vs. placebo) in a 1:1 ratio. Randomization of subjects will be stratified by immunomodulating therapy status (IFN/GA vs. no DMT), and disease status (PPMS vs. SPMS). The NeuroNEXT DCC will generate a randomization table for each of the strata using a permuted block design with random block sizes.

8.6. Dosing Guidelines

8.6.1. Treatment Phase

Capsules containing either MN-166 (10 mg capsules) or matching-placebo 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 Dosing Interruption for Abnormal Laboratory Values

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
- Creatinine > 1.2 x ULN
- White blood cell 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 central laboratory) until resolution or stabilization of the laboratory value.

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

8.6.3 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 Section 8.6.2 must permanently discontinue study treatment.

8.7. Written Informed Consent

Each subject is required to provide written informed consent prior to undergoing any study procedures. 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.

During the consent process, subjects will be informed that there is an FDA-approved treatment option available (i.e., mitoxantrone) for secondary progressive multiple sclerosis.

8.8. Assessments

Table 1: Schedule of Assessments

Table 2: Schedule of Unplanned Procedures and Assessments

Figure 12: Study Design

Visits Week 4, 8, 24, 48, 72, and 96 will have a window of ± 5 days. Safety visits conducted at Week 12, 36, 60, 84 and 100 will have a window of ± 14 days. (See Table 1 Schedule of Assessments)

Clinical laboratory evaluations will be performed by a central laboratory. 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. Assessments by Visit

The following is a summary of assessments by study visit:

8.8.1.1. Screening

Study Visit 1

- Informed consent

- Inclusion/Exclusion
- Medical history, MS history
- Physical examination
- Height/body weight
- Vital signs
- Prior/concomitant medication review
- Brief Pain Inventory (BPI)
- Cognitive tests [Symbol Digit Modalities Test (SMDT) and the Selective Reminding Test (SRT)]
- Short Form-36 Health Survey (SF-36)
- Multiple Sclerosis Impact Scale-29 (MSIS-29)
- EuroQoL 5 Dimensions (EQ-5D)
- Beck Depression Inventory-Fast Screen (BDI-FS)
- Hematology, chemistry, lipid profile, and urinalysis labs
- Serum biomarker samples
- Serum β-hCG (Serum pregnancy test) (in females)
- Electrocardiogram (12-lead ECG)
- Multiple Sclerosis Functional Composite (MSFC)
- Expanded Disability Status Scale (EDSS) by blinded neurologist
- Brain MRI*
- Optical Coherence Tomography (OCT)
- Lumbar puncture (optional)**

*MRI must be approved by the MRI Reading Center prior to randomization.

**Screening LP can be performed anytime between Screening Visit (inclusive) and Baseline Visit (inclusive), but prior to taking study drug. If MRI is done after screening LP, it must be at least 3 days after screening LP.

8.8.1.2. Treatment Phase

Study Visit 2 (Baseline-Day 1)

- Inclusion/Exclusion review
- Randomize
- Vital signs
- Interval history

- SBQ-R
- Concomitant medication review
- Brief Pain Inventory (BPI)
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Urine pregnancy test (urine β-hCG) (in females)
- Cognitive Tests (SDMT and SRT)
- MS Functional Composite (MSFC)
- EDSS by blinded neurologist
- Adverse event monitoring
- Study drug dispensing

Study Visit 3 (Week 4)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review
- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- Study drug accountability

Study Visit 4 (Week 8)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review
- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum biomarker samples
- Serum pregnancy test (in females)
- Plasma sample for PK
- ECG (12-lead)
- Study drug accountability

Study Visit 5 (Week 12)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review
- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- Study drug accountability

Study Visit 6 (Week 24)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- Relapse assessment
- Cognitive tests (SDMT and the SRT)
- BPI
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- MSFC
- EDSS by blinded neurologist
- Brain MRI
- OCT
- Return of used/unused study drug bottles, study drug accountability and compliance check
- Dispense study drug

Study Visit 7 (Week 36)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review

- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- Study drug accountability

Study Visit 8 (Week 48)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- Relapse assessment
- Cognitive tests (SDMT and SRT)
- BPI
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- Hematology, chemistry, lipid profile, and urinalysis labs
- Serum biomarker samples
- Serum pregnancy test (in females)
- Plasma sample for PK
- ECG (12-lead)
- MSFC
- EDSS by blinded neurologist
- Brain MRI
- OCT

- Lumbar puncture (optional)
- Return of used/unused study drug bottles, study drug accountability and compliance check
- Dispense study drug

Study Visit 9 (Week 60)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review
- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- Study drug accountability

Study Visit 10 (Week 72)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- Relapse assessment
- Cognitive tests (SDMT and the SRT)
- BPI
- SF-36
- MSIS-29

- EQ-5D
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- MSFC
- EDSS by blinded neurologist
- Brain MRI
- OCT
- Return of used/unused study drug bottles, study drug accountability and compliance check
- Dispense study drug

Study Visit 11 (Week 84)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event review
- Concomitant medication review
- Relapse assessment
- BPI
- SBQ-R
- Hematology, chemistry, and urinalysis labs
- Serum pregnancy test (in females)
- ECG (12-lead)
- Study drug accountability

Study Visit 12 (Week 96)

- Physical examination
- Body weight
- Vital signs

- Interval history
- Adverse event monitoring
- Concomitant medication review
- Relapse assessment
- Cognitive tests (SDMT and SRT)
- BPI
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- Hematology, chemistry, lipid profile, and urinalysis labs
- Serum biomarker samples
- Serum pregnancy test (in females)
- Plasma sample for PK
- ECG (12-lead)
- MSFC
- EDSS by blinded neurologist
- Brain MRI
- OCT
- Lumbar puncture (optional)
- Return of used/unused study drug bottles, study drug accountability and compliance check

Follow-up Visit 13 (Week 100)

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- Relapse assessment
- SBQ-R

Unscheduled Visits

Relapse Evaluation

Between scheduled study visits, subjects with new or significant worsening MS symptoms that last 24 hours in duration and occur in the absence of fever or infection should notify the Investigator immediately and be seen for an Unscheduled Relapse Evaluation Visit within 3 days of notifying the Investigator. The following assessment should be performed at the Relapse Evaluation Visit:

- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- SF-36
- MSIS-29
- SBQ-R
- BPI
- EDSS by blinded neurologist

Subjects continuing follow-up off study medication do not need to return for Unscheduled Relapse Evaluation Visits. If during a regularly scheduled study visit, a relapse episode is noted, complete the relapse evaluation assessments above along with the scheduled assessments for that study visit.

Semi-Annual Visits (for subjects who discontinue study medication)

Subjects who discontinue study medication and remain in the study for follow-up will be followed on a semi-annual basis (Week 24, 48, 72, and 96, relative to Baseline). The following assessments will be performed at each semi-annual visit:

- Relapse assessment
- Cognitive tests (SDMT and SRT)
- BPI
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- MSFC

- EDSS
- Brain MRI
- OCT

Early Withdrawal Visit

Subjects who discontinue study medication and choose not to be followed on a semi-annual basis will undergo the following assessments:

- Physical examination
- Body weight
- Vital signs
- Interval history
- Adverse event monitoring
- Concomitant medication review
- Cognitive tests (SDMT and SRT)
- BPI
- SF-36
- MSIS-29
- EQ-5D
- SBQ-R
- Hematology, chemistry, lipid profile, and urinalysis lab
- Serum biomarker samples
- ECG (12-lead)
- MSFC
- EDSS
- Brain MRI
- OCT
- Lumbar puncture (optional)
- Return of used/unused study drug bottles, study drug accountability and compliance check

Laboratory Re-test visit

Subjects who have a clinically significant abnormal lab finding may return for a laboratory re-test visit and will have the following assessments performed at the discretion of the CSSPI:

- Interval history
- Hematology, chemistry, or urinalysis lab, as indicated

8.8.2. Procedures/Assessment Details

8.8.2.1. Informed Consent

The Clinical Study Site Principal Investigator (CSSPI) 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 including history of multiple sclerosis will be obtained by the CSSPI or qualified designee as listed on the Form FDA 1572. If the subject's historical MS care was provided at another institution or location, efforts must be made to obtain these outside records to verify that the subject meets all inclusion and none of the exclusion criteria. This must be accomplished during the screening period and added to the subject's medical record.

8.8.2.3. Prior/Concomitant Medication Review

Site study staff will record all medications used to treat multiple sclerosis taken 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. Relapse Assessment

Relapse Definition

A protocol-defined clinical relapse is defined as new or recurrent neurological symptoms, not associated with fever or infection, lasting for at least 24 hours, which is following a period of at least 30 days of stability or improvement. In addition, a protocol-defined clinical relapse requires an increase in the EDSS Functional System corresponding to symptom(s) of the relapse, or an increase in the overall EDSS secondary to a functional change related to symptom(s) of the relapse. Because relapse is an expected event in patients with MS, a relapse episode will not be considered an adverse event.

Relapse Treatment

Clinical relapses that meet the above definition may be treated with a course of corticosteroids at the investigator's direction. MRI studies must be postponed until 1 month after completion of any systemic (excludes topical, inhaled) corticosteroid treatment.

8.8.2.5. Physical Examination

The physical exams must be performed by the CSSPI 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 examination.

8.8.2.6. Vital Signs, Height, and Weight

Blood pressure (BP), heart rate (HR) measurements will be taken. Respiratory rate and temperature will also be measured and all measurements will be recorded in the CRF. Clinically significant changes from the signing of the ICF should be captured as AEs in the CRF.

Weight will be measured in pounds or kilograms. Height will be recorded only at Visit 1 (screening).

8.8.2.7. Electrocardiogram (12-Lead ECG)

All subjects will have standard resting 12-lead ECGs performed and interpreted. A central facility will be used in this study for interpretation and analysis of ECGs. 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-hour clock).

The CSSPI 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. If a CSSPI has reviewed, initialed and dated the original ECG tracing and the results on the tracing match those on the report, the report will be considered as reviewed and signed by the CSSPI. If the results vary, the CSSPI must also review, sign and date the report. Results will be captured in the subject's study chart, not in the CRF. 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.

8.8.2.8. Interval History

During each visit, adverse events and concomitant medications will be documented. For subjects who discontinue study medication and are followed on a semi-annual basis, adverse events that occur post-study drug discontinuation will not be collected. Only those adverse events that

occurred while on study drug will be followed until the event resolves or stabilizes or until all study related visits have been completed.

8.8.2.9. Brain Magnetic Resonance Imaging (MRI)

Brain MRI will be performed in a standard fashion, as outlined in the MRI procedure manual. Each subject's baseline MRI must be approved by the MRI Reading Center prior to randomization.

Brain MRIs will be read clinically for non-MS pathology by a board-certified neuroradiologist, with a report entered into the study database within ten days and reported back to the treating neurologist through standard clinical results reporting methods. Urgent findings identified on brain MRI will be reported to the treating neurologist immediately at the discretion of the reading neuroradiologist.

At any time during the study, subjects who received unscheduled corticosteroid treatment must postpone their MRI until 1 month after completion of the corticosteroid treatment.

All sites will perform a test (or "dummy") MRI scan prior to study initiation. This test MRI will ensure adequate performance of the study MRI.

It is required that the same MRI scanner be used to acquire all MRI scans over the course of the study. If during the study period a study site needs to change the MRI scanner or the current MRI scanner undergoes significant upgrades (hardware or software), the investigator should notify the Clinical Coordinating Center. For all changes in scanner and significant scanner upgrades, the investigator will be requested to obtain MRI scans of 4 volunteers acquired prior to changing/upgrading the MRI scanner and the same 4 volunteers again after changing scanners (a total of 8 scans). The site will be allowed to acquire ibudilast trial subject scans on the new scanner only after obtaining approval of the new or upgraded scanner.

8.8.2.10. Optical Coherence Tomography (OCT)

OCT is a non-invasive imaging technique used to obtain high resolution cross-sectional images of the retina. OCT measures the thickness of the ten layers in the retina.

8.8.2.11. Lumbar Puncture (LP)

An optional lumbar puncture for the collection of cerebrospinal fluid will be performed in subjects who consent for LP. If LP is performed, it must be performed after the MRI is done and cannot be performed prior to MRI.

8.8.2.12. Adverse Event (AE) Monitoring

The CSSPI or a qualified designee listed on Form FDA 1572 must assess the severity and relationship to study medication for all AEs (see Section 11.3).

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.

Each CSSPI and research team are responsible for identifying adverse events and reporting them through the DCC Online Adverse Event Reporting System.

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 11.3) 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).

The DCC will prepare aggregate reports of all adverse events (serious/not serious, expected/unexpected and relationship to study drug) for the Independent Medical Monitor (IMM) on a quarterly basis and the DSMB on a semi-annual basis, or as requested by either the Independent Medical Safety Monitor or the DSMB. In addition, all adverse events will be coded using the Medical Dictionary for Regulatory Affairs (MedDRA) system. A separate report detailing protocol compliance will also be available from the DCC for DSMB as requested.

8.8.2.13. Laboratory Evaluations

Laboratory evaluations will include the tests listed in Appendix 1.

8.8.2.14. Instruments and Rating Scales

For subjects who do not speak English with sufficient fluency to complete English versions of the Instruments and Rating Scales, a validated foreign language version will be utilized. Where an appropriate foreign language version is not available, the instrument or rating scale will be omitted.

8.8.2.14.1. Brief Pain Inventory (BPI)-short form

The BPI-short form is designed for use in clinical trials to provide information on the intensity of pain as well as the degree to which pain interferes with function. The BPI also asks questions about pain relief, pain quality, and the subject's perception of the cause of pain. It takes approximately 5-10 minutes to complete.

8.8.2.14.2. Short Form-36 Health Survey (SF-36)

The SF-36 is a multipurpose short-form (SF) generic measure of health status. The SF-36 measures eight concepts commonly represented in widely used surveys: physical functioning, role limitations due to physical health problems, bodily pain, general health, vitality (energy/fatigue), social functioning, role limitations due to emotional problems and mental health (psychological distress and psychological well-being). The standard (4-week) recall version will be utilized.

8.8.2.14.3. Multiple Sclerosis Impact Scale-29 (MSIS-29)

The MSIS-29 is an instrument measuring the physical (20 questions) and psychological (9 questions) impact of multiple sclerosis.

8.8.2.14.4. Multiple Sclerosis Functional Composite (MSFC)

The MSFC was originally defined as a three-part, standardized, quantitative assessment instrument for use in clinical trials. The three original components measure leg function/ambulation through 25-foot-walk test (25 FW), arm/hand function through 9-hole peg test (9-HPT), and cognitive function through Paced Auditory Serial Addition Test. Contemporary revisions to the MSFC have added a visual component (a low-contrast visual acuity test) and replaced the Paced Auditory Serial Addition Test with the Symbol Digit Modality Test (SDMT). This trial's implementation of the MSFC will utilize this four-component version: 25 FW, 9-HPT, SDMT, and Low Contrast Sensitivity (2.5% chart). The MSFC measures will be administered by a trained and certified examiner and takes approximately 20 to 30 minutes to complete.

8.8.2.14.5. Expanded Disability Status Scale (EDSS)

The Kurtzke EDSS is a rating system used to quantify disability in subjects with multiple sclerosis and monitor changes in the level of disability over time. The EDSS scale ranges from 0 to 10 in 0.5 unit increments, where a higher score represents higher levels of disability. Scoring is based on an examination by a neurologist. EDSS in this study will be performed by a blinded neurologist, who is unaware of subject symptoms and adverse events.

8.8.2.14.6. EuroQol 5-Dimension (EQ-5D)

The EQ-5D is a standardized instrument for use as a measure of health outcome (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). It is designed for self-completion by respondents and takes a few minutes to complete.

8.8.2.14.7. Beck Depression Inventory-Fast Screen (BDI-FS)

The BDI-FS is a brief 7-item self-report instrument which assesses dysphoria, anhedonia, suicidal ideation, and cognition-related symptoms on a three-point scale. The test is usually completed in less than 5 minutes.

8.8.2.14.8. Suicide Behaviors Questionnaire-Revised (SBQ-R)

The 4-item SBQ-R is a measure of past suicidal thoughts and attempts which have proved to be significant predictors of future suicidality. The items ask if the respondent has ever thought about or attempted suicide, how frequent was suicidal thoughts in the past year, have they told someone about such thoughts, and what is the likelihood of attempting suicide in the future.

8.8.2.14.9. Symbol Digit Modalities Test (SDMT)

The Symbol-Digit Modalities Test (SDMT) is a five minute test that quickly assesses the participant for cerebral dysfunction using a simple substitution task. It is easy to administer and remarkably accurate when it comes to detecting the presence of brain damage and other changes in a patient's cognitive functioning. It is effective because those with cerebral dysfunction will

always perform poorly due to deficiencies in attention span, scanning abilities and motor skills. For this trial, SDMT will be both a component of the MSFC and a separate outcome.

8.8.2.14.10. Selective Reminding Test (SRT)

The Selective Reminding Test (SRT) measures memory performance. The test measures the following:

- Total Recall (TR)
- Long Term Retrieval (LTR)
- Long Term Storage (LTS)
- Short Term Retrieval (STR)
- Consistent Long Term Retrieval (CLTR)
- Number of correct recognized Multiple Choice items (MCR)
- Delayed Recall (DR)

9. STUDY DRUG MATERIALS AND MANAGEMENT

The investigational product is MN-166 50 mg taken twice daily in the morning and evening for a total daily dose of 100 mg/d.

9.1. Study Drug

Investigational Drug:	ibudilast (MN-166); previously known as AV411
Active Ingredient:	ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine)
Formulation:	10 mg delayed-release capsules (specially-manufactured Pinatos®)
Frequency:	5 capsules administered twice daily (in the AM and PM) except during titration where 3 capsules will be administered twice daily (or wherein a final 4 capsule BID (80 mg/d) regimen is maintained). Subjects will be instructed to take study medication with food or within an hour of eating.
Storage Conditions:	Store at room temperature (preferably 18-23^{0}C, but 15-25^{0}C acceptable)
Packaging Description:	Polyethylene bottles

Placebo is identical to ibudilast (MN-166) in color, shape, and packaging.

9.2. Study Drug Packaging and Labeling

Medicinova will manufacture the study drug and matching placebo and provide primary packaging services. The Clinical Materials Services Unit (CMSU), at the University of Rochester will provide primary labeling and secondary packaging, labeling and distributions services.

Study drug will be packaged as follows:

- Each bottle will contain 500* capsules 10 mg ibudilast or matching placebo. Each bottle will be sufficient for 50 days of dosing at the 100 mg/d dosage (5 capsules BID).
- Four (4) 500 capsule count bottles will be placed in a kit box which will be sufficient for 6 months of dosing. Each subject is expected to receive a new kit box every 6 months for a total of 4 kit boxes or 40 bottles of study drug.

*Subsequent shipments throughout the study may be packaged with less than 500 capsules per bottle.

At a minimum the following information will be included on each six (6) month kit box and each bottle within the kit box:

- Name of Sponsor
- Name and address of distribution center
- Study number/Acronym/IND number
- Unique drug kit number/kit box
- Additional information on each bottle within the kit box: unique bottle number (01-04), plus each bottle will contain the unique kit box number
- Drug treatment (Generically listed as either 10 mg ibudilast *or* placebo capsule)
- Pharmaceutical dosage form
- 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 clinical study drug MN-166 and the placebo capsules should be stored at room temperature (preferably 18-23°C, but 15-25°C acceptable).

9.4. Study Drug Dispensation and Handling

CMSU will provide each site with a predetermined number of six (6) month active and placebo kit boxes, each kit box with a unique kit box number. Par levels will be determined in advanced based on anticipated enrollment. Study centers will use Interactive Voice/Web Response System (IXRS) for assignment of a uniquely identified kit box (active or placebo) based on the randomization assignment.

The subject will be instructed to return all unused study drug to the clinical trial site at each visit.

9.5. 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 at home following the directions given to them in the clinic.

9.6. Study Drug Accountability

Investigational clinical supplies must be received by the CSSPI 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 CSSPI 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 the CRF: visit medication dispensed, dosing start/stop dates, dosage level achieved (60 – 100 mg/d) number of capsules dispensed and number of capsules returned.

10. ASSESSMENT OF ACTIVITY

10.1. Primary and Secondary Endpoints

The primary endpoint is:

- Covariate-adjusted mean rate of change in brain atrophy over 96 weeks as measured by brain parenchymal fraction (BPF).

The following major secondary endpoints will be evaluated at 96 weeks:

- Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
- Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
- Retinal nerve fiber layer as measured by Optical coherence tomography (OCT)
- Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm [CLADA]

The additional secondary outcomes are to measure the activity of ibudilast (MN-166) at 96 weeks versus placebo on:

- Inflammatory disease activity, as measured by T1 lesion volume, T2 lesion volume, and annualized relapse rate
- Disability, as measured by Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC)
- Quality of Life, as measured by Multiple Sclerosis Impact Scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36)
- Cognitive impairment, as measured by Symbol Digit Modalities Test and the Selective Reminding Test
- Neuropathic pain, as measured by Brief Pain Inventory (BPI).

10.2. Tertiary Endpoints

1. The first set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at 48 weeks versus placebo as measured by the primary and secondary imaging outcome measures: whole brain atrophy (BPF), diffusion tensor imaging (DTI) in descending pyramidal white matter tracts, magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue, and retinal nerve fiber layer as measured by Optical coherence tomography (OCT), and cortical atrophy as measured by CLADA.

2. The second set of tertiary objectives are to evaluate the activity of ibudilast (MN-166) at 96 weeks versus placebo as measured by whole-brain gray matter fraction, new T1 lesions since baseline, and new T2 lesions since baseline.

10.3. Exploratory Endpoints

The exploratory endpoints at selected sites include analysis of the pharmacokinetics (PK) of ibudilast (MN-166) using a population PK approach, potential pharmacokinetic-pharmacodynamic analyses, and the correlation of cerebrospinal fluid (CSF) and serum biomarkers – e.g., neurofilament light chain measurement and correlation analysis with imaging metrics.

Blood samples for analysis of ibudilast and its metabolite, 6,7-dihydrodiol (DHD) will be collected during scheduled visits on Weeks 8, 48, and 96. The exact sampling time and time relative to the previous dose will be documented in the case report forms. Population PK modeling using the NONMEM program (Icon Development Solution) will be used to characterize the pharmacokinetic properties of ibudilast in healthy subjects and subjects with MS. The population analysis will evaluate selected covariates to determine if they contribute to differences in PK parameter estimates among individuals. The covariates will likely include demographic variables (age, gender, body weight, and race), creatinine clearance (as a marker of renal function), liver enzyme levels (as a marker of hepatic function), blood chemistry variables, and relevant disease covariates at baseline, among others. Further, the effect of concomitant medications on the pharmacokinetics of ibudilast will also be assessed.

11. ASSESSMENT OF SAFETY

11.1. Primary Safety Parameters

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

- treatment emergent adverse events (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.

During the study, if ≥ 5 new or enlarging T2 lesions should apply to any scans at any time points, the MSM should be notified and an evaluation of the subject should be made as to whether the subject may continue on study drug.

11.2. Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study subject who was 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 or under placebo.

11.3. Assessment of Adverse Events

The CSSPI 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 11.6). These assessments will be made in accordance with the standard ratings detailed in the following sections.

Each CSSPI and research team is responsible for identifying adverse events and reporting them through the DCC Online Adverse Event Reporting System (AERS).

11.3.1. Severity Assessment

The severity of AEs will be determined as described below.

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 and urgent medical/therapy intervention required hospitalization probable.
Death Grade 5	Death related to AE.

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 grade of 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.

11.3.2. Relationship to Study Drug

One of the following categories in Table 6 should be selected based on medical judgment, considering the definitions below and all contributing factors.

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 (e.g., 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.

As this is a double-blind study, the causality assessment should be made under the assumption that the subject is receiving active study medication. If considering unblinding, this assessment should be made prior to unblinding to avoid bias.

11.4. 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, (i.e., the subject has discontinued or completed the study) through the follow-up visit with the exception of those subjects who discontinue study medication during the study and are followed on a semi-annual basis. Subjects who discontinue study medication but continue follow-up within the study will not have AEs collected after study drug discontinuation. 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 CSSPI 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 CSSPIs 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.

11.5. 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.

11.6. 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.

11.6.1. SAE Reporting Requirements

The CSSPI or an authorized delegate is responsible for submitting the requested information via the DCC Online Adverse Event Reporting System 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 CSSPI or an authorized delegate should report SAEs "spontaneously" if considered at least possibly related to study medication. Upon entry of a serious adverse event by a CSSPI, the DCC Online Adverse Event Reporting System will immediately notify the Medical Safety Monitor (MSM).

- Within **24 hours** (of learning of the event), investigators must report any Serious Adverse Events (SAEs). Investigators must report all other AEs within **5 working days/7 calendar days** (of learning of the event).

Serious adverse events: The CSSPI determines causality (related, probably related, possibly related, unlikely to be related, and unrelated) of the adverse event. The IMM will review the SAE report. The IMM may request further information if necessary. The Online Adverse Event Reporting System maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study. The IMM may determine that the Serious Adverse Event requires expedited reporting to the Sponsor and FDA (Food and Drug Administration). The DCC will prepare a Medwatch safety report for submission to the FDA. If warranted, the IMM will notify the DSMB chair. The DSMB may suggest changes to the protocol or consent form to the Study Chair as a consequence of adverse events.

As a minimum requirement, the initial notification will provide the following information:

- Subject ID number
- Date of SAE onset
- An event term
- Details of SAE (if sufficient information is available)
- Criterion/criteria for classification as "serious"
- 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 CSSPI or an authorized delegate through review of these documents will be submitted on a follow-up SAE CRF page via the DCC Online Adverse Event Reporting System. The Independent Medical Monitor may also request additional information on the SAE.

Non-serious adverse events: Non-serious adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DCC in a timely fashion (within 5 working days). The events will be presented in tabular form and given to the IMM on a quarterly basis or as requested. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DCC will prepare aggregate reports of all adverse events (serious/not serious, expected/unexpected and relationship to study drug) for the IMM on a quarterly basis and the DSMB on a semi-annual basis, or as requested by either the IMM or DSMB. In addition, all adverse events will be coded using the MedDRA system. A separate report detailing protocol compliance will also be available from the DCC for DSMB review monthly or as requested.

Any AE fulfilling the criteria for expedited reporting will be reported by MediciNova and NeuroNEXT to regulatory authorities and Investigators, respectively, in accordance with the NeuroNEXT Network and company's standard operating procedures (SOPs) and local regulatory requirements.

Clinical Study Site Principal Investigators are responsible for complying with NeuroNEXT Central Institutional Review Board's (CIRB) reporting requirements for all safety reports.

Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file. **Emergency Procedures**

If the CSSPI or an authorized delegate needs urgent advice regarding the management of an SAE or any other safety issue, an "on call" Independent Medical Safety Monitor(s) will be available 24 hours a day by telephone/pager. A neurologist experienced with MS trials and pharmacotherapy trial medical monitoring, independent of MediciNova or NINDS/NeuroNEXT or CCF, will be the primary medical safety monitor.

11.6.2. Emergency Treatment Code-Break

In the case of a medical emergency, where knowledge of study treatment by the CSSPI or an authorized delegate is essential for immediate medical management, the CSSPI or an authorized delegate should contact the study IMM to discuss the code break prior to revealing the treatment identity.

A 24-hour code-break service will be available via the DCC.

All treatment code breaks must be fully documented and signed with the time, date, reason, and name of person responsible for breaking the blind and tracked on the unblinding log. The breaking of the blind must result in the withdrawal of the subject, and the subject should return for a final study assessment.

11.7. Guidance for Overdose

There is no clinical experience with MN-166/AV411overdose in humans and there is no available specific antidote to the effects of MN-166/AV411. Standard symptomatic support measures should be used in the case of excessive pharmacological effects or overdose.

11.8. 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 CSSPI or an authorized delegate should submit a Pregnancy Report Form to the DCC database within 24 hours of the CSSPI 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 via the DCC Online Adverse Event Reporting System within 24 hours of the CSSPI 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 CSSPI 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 via the DCC electronic data capture system within 24 hours of the CSSPI 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 CSSPI 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's representative will provide instructions on how to collect pregnancy information in accordance with local requirements.

11.9. 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.)

11.10. Data and Safety Monitoring Board (DSMB)

The monitoring of subject safety and data quality will follow the NINDS Guidelines for Data and Safety Monitoring in Clinical Trials. A DSMB appointed by the NIH /NINDS will meet semi-annually to review partially unblinded study data provided by the study statistician. This committee will monitor rates of adverse events and endpoints in the trial and will monitor the performance of the trial. The format of DSMB meetings, reports, and guidelines for interim analysis will be agreed prior to study subject enrollment.

12. STATISTICS

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

12.1. Data Analysis

12.1.1. Analysis Populations

The modified Intent-to-Treat (mITT) Population: The primary population for analysis is the mITT, which is defined as all subjects who are randomized and receive at least one dose of study medication and have at least one efficacy assessment in the double-blind phase. Subjects will be analyzed based on the treatment they are randomized.

The Per Protocol (PP) Population: The per-protocol population includes all mITT subjects who satisfy the following conditions:

- Have 75% -125% compliance, both limit values inclusive in the double-blind phase,
- Have no major protocol deviations, determined by a blinded data review.

Commented [CCS1]: OPTIONAL – We removed this from the SAP, since this is repetitive with the second criteria in that you can't have 75%-125% compliance without taking the study medication as randomized. If others feel we don't need to change this, I'm fine with that.

12.1.2. Statistical Analysis Plan

All imaging endpoints will be statistically evaluated using linear mixed models (LMMs: Laird and Ware, 1982). LMMs are advantageous for longitudinal clinical trials because they can account for the dependency due to repeated measures with relatively few parameters, which potentially enhances statistical efficiency. Furthermore, LMMs can accommodate incomplete cases (i.e., missing data), which is expected in this study due to dropout. LMMs are typically estimated using maximum likelihood methods (Verbeke and Molenberghs, 2000) that yield valid inferences with incomplete cases under the widely applicable assumption that the missing data are ignorable (Little and Rubin 2002). For all analyses, the residuals of the fitted statistical models will be examined for evidence of departure from assumptions, such as normality. If assumptions appear to be grossly violated, then transformations of response variables might be considered. Alternatively, generalized LMMs might be used because of their ability to accommodate a wider range of distributional forms (e.g., beta distribution). The primary analysis will be conducted using a modified intent-to-treat analysis, which includes all subjects who are randomized, receive at least one dose of study medication in the double-blind phase, and meet the conditions specified in 12.1.1. Since this is a phase II proof of concept study, statistical significance will be determined by any test that exceeds the 0.10 significance level. To account for baseline imbalance due to randomization vagaries, the baseline group means (intercepts) in the statistical analysis will be constrained to be equal.

Primary Objective #1: ***To evaluate the activity of Ibudilast (MN-166 100 mg/d) versus placebo at two years, as measured by quantitative magnetic resonance imaging (MRI) analysis for whole brain atrophy using brain parenchymal function (BPF).***

The first primary objective of the proposed study is to evaluate the activity of ibudilast (treatment) versus placebo. For the first primary objective, the endpoint is the rate of change in brain parenchymal fraction (BPF) of the treatment group versus the placebo group. The evaluation of activity is defined as the test of the null hypothesis that the BPF rates of change in

the treatment and placebo groups are equal. The null hypothesis can be evaluated based on parameter estimates in the LMM, the details of which are now presented.

Suppose Y_{ij} is the BPF value for the ith patient ($i = 1, \ldots, N$) at the jth month ($j = 1, \ldots, n_i$), t_{ij} denotes time in weeks, P stands for placebo, and T stands for treatment. Assuming linear change over time, the LMM can be written as the following,

$$Y_{ij} = \alpha_P + \beta_P t_{ij} + (\gamma + \delta t_{ij}) g_i + (a_i + b_i + \epsilon_{ij}) \qquad (1)$$

where g_i is a dummy variable for group,

$$g_i = \begin{cases} 0 & if\ P, \\ 1 & if\ T. \end{cases}$$

In Equation (1), α_P, β_P, γ, and δ are fixed effects; α_P is the intercept for the placebo group, β_P is the slope for the placebo group, $\gamma = \alpha_T - \alpha_P = 0$ is the difference of the intercepts, and $\delta = \beta_T - \beta_P$ is the difference of the slopes. Setting $\gamma = 0$ constrains the baseline means to be equal, which controls for initial imbalance that occurs in empirical randomization (Senn, 2013). The terms a_i and b_i are random effects (random intercepts and slopes, respectively), and ϵ_{ij} is random error. We make the typical assumptions

$$[a_i \quad b_i]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{G}),\ \epsilon_{ij} \sim \mathcal{N}(0, \sigma^2 \boldsymbol{I}_i), \text{ and } [a_i \quad b_i]^T \perp \epsilon_{ij}.$$

The prime object of inference is δ, as this is the index of longitudinal activity of T versus P, namely, the difference in rates of change. The null hypothesis of no longitudinal ibudilast effect (equality of T and P slopes) is H_0: $\delta = 0$, and can be evaluated with the likelihood ratio test, or a Z-test, provided a sufficiently large sample size that is provided in the proposed study ($N = 250$, see Sample Size Justification below). The Z statistic is $Z = \hat{\delta}/SE(\hat{\delta})$ and leads to the rejection of H_0 when $|Z| > Z_{1-\alpha}$, with the latter value being the $100 \cdot (1 - \alpha)$th quartile of the standard normal distribution (single-tailed test).

Longitudinal measurements will span up to five time points per participant (Baseline and 24, 48, 72, 96 weeks), and there is a possibility that BFP trajectories will not be linear. In order to assess the sensitivity of the results to the assumption of linearity, non-linear trends will be modeled with polynomials and compared to the fit of the linear trend model of Equation (1). If polynomials of order greater than one are required, then the evaluation of T versus P involves parameters in addition to δ from Equation (1), for example, the difference in quadratic terms. In this case, an appropriate Wald test will be performed, which is the multiparameter extension of the Z-test. Interpretation of the results will be in terms of T versus P differences in instantaneous rate of change (ie, the first derivative) at different time points.

Accounting for multiple sites. The proposed study involves multiple sites, which is a potential source of additional variation. Patients within a site tend to be correlated due to similarity of environment, eg, because of testing by the same set of clinicians (Localio et al 2001). It is potentially important to account for the nesting of patients within sites in order to produce proper precision estimates (ie, standard errors) for statistical testing. To account for site variation, the Equation (1) model may be augmented with additional random effects and associated variance components.

Suppose that Y_{hij} is BPF for the jth score of the ith patient in the hth site ($h = 1, \ldots, S$). Then the LMM has the form in the left side of the table below. The right hand side of the table identifies the types of effects in each piece of the equation.

Linear Mixed Model with Site Effects

Equation	Types of Effects
$Y_{hij} = \alpha_P + \beta_P t_{hij} + (\gamma + \delta t_{hij}) g_{hi}$	[fixed effects]
$+ (a_{hi} + b_{hi} t_{hij})$	[patient random effects]
$+ (a_h + b_h t_{hij} + c_h g_{hi} + d_h g_{hi} t_{hij})$	[site random effects]
$+ \epsilon_{hij}$	[random error]

The fixed effects and patient random effects are similar to Equation (1), and we again set $\gamma = 0$ to account for baseline imbalance. There are four site random effects, with d_h potentially being the most important, as it represents site variation in the longitudinal treatment effect, ie, a site by treatment interaction. It is assumed that

$[a_{ih} \quad b_{ih}]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{G})$, $[a_h \quad b_h \quad c_h \quad d_h]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{H})$, $\epsilon_{ij} \sim \mathcal{N}(0, \sigma^2 \boldsymbol{I}_i)$, and $[a_i \quad b_i]^T \perp [a_h \quad b_h \quad c_h \quad d_h]^T \perp \epsilon_{ij}$.

If individual site variance components in ***H*** are negligible, then the associated random effects will be omitted to simplify the model. In the case that all ***H*** components are negligible, we will revert to Equation (1) for the analysis.

Primary Objective #2: ***To evaluate the safety and tolerability of ibudilast versus placebo.***

The main assessment of safety will involve a comparison of treatment-related SAEs across the two treatment groups. This will be assessed in two ways. First, the percentage of subjects who experience any treatment-related SAE in each group will be compared using a chi-square test. Then, the rates of treatment-related SAEs in each group will be compared using a Poisson regression model. We will also assess the values of laboratory assessments over time across the two groups using a LMM. To assess tolerability, the percentage of subjects who complete the entire treatment period will be compared across the two groups using a chi-square test. Appropriate constraints for baseline imbalance will be used in all statistical models.

Major Secondary Objectives: ***To evaluate the activity of ibudilast at two years versus placebo, as measured by:***

(1) Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts

(2) Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue

(3) Retinal nerve fiber thickness as measured by optical coherence tomography (OCT)

(4) Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm (CLADA)

The secondary objectives will be analyzed using a model similar to that described above for the assessment of BPF. An exception is the OCT analysis will require additional random effects for eyes nested within patients. Additional details are provided below. The baseline intercepts will again be constrained to be equal to account for baseline imbalance.

Other Objectives

Commented [CCS2]: Per email from R. Fox on 09/09/2016, removed this section and moved the text into the exploratory objectives section below.

The proposed study will provide a wealth of additional information. Correspondingly, a number of additional exploratory hypotheses will be conducted. For example, there is a great need to identify imaging metrics for measuring potential neuroprotective therapies in neurodegenerative diseases. Using data collected simultaneously from the same patients over two years, we will directly compare different atrophy measures, MTR, DTI, and OCT for variability, sensitivity to change over time, cost, ease of implementation, and correlation with clinical disability measures, patient self-report, and CSF parameters. In addition, we will correlate imaging = changes in the initial 6-12 months with clinical changes over 2 years. A multivariate version of the LMM will be used (Fieuws & Verbeke, 2004) to simultaneously model two response variables. This will allow the statistical comparison of the slopes, for example, of brain atrophy and a self-report measure to see if they are changing similarly over time. Through these analyses, we will attempt to make recommendations regarding the most robust and practical outcome for implementation in future Phase 2 trial of progressive MS, acknowledging that different neuroprotective therapies may affect advanced imaging metrics differently. The baseline intercepts in all the models will again be constrained to be equal to account for baseline imbalance.

Additional exploratory analyses will examine one year changes in the variables involved in the primary and secondary objectives above,as well as one or two year changes in a number of additional variables: T1 lesion volume; T2 lesion volume; annualized relapse rate; disability as measured by the Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC); cognitive impairment as measured by the Symbol Digit Modality Test (SDMT) and Selective Reminding Test; quality of life as measured by Multiple Sclerosis impact scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36); and neuropathic pain, as measured by the Brief Pain Inventory (BPI) in a subset of subjects with central neuropathic pain. All will be assessed using either a linear mixed model (for continuous outcomes) or a generalized linear mixed model (for binary our count data). However, it is important to note that these are not the main focus of this study. Hence, we did not power for any of these comparisons, they are not involved directly in the “go”/”no go” decision, and should be considered as exploratory hypothesis generating analyses. We will also assess the activity of ibudilast at 96 weeks versus placebo as measured by whole-brain gray matter fraction, MTR in gray matter, new T1 lesions since baseline and new T2 lesions since baseline. Finally, additional exploratory analyses will seek pharmacokinetics (PK) of ibudilast using a population PK

approach, and to correlate the cerebrospinal fluid (CSF) and serum biomarkers with imaging and clinical measures of progressive disability.

12.1.3. Sample Size Justification

Primary Objective: Estimated required sample size for the primary objective was computed based on two pilot data sets and relevant literature. The first pilot data set (DS1) consisted of N = 30 PP subjects (n=15 female; mean age 58.6 years; mean disease duration 7.3 years; mean EDSS 4.97) who participated in the placebo arm of a clinical trial for the chemotherapy drug mitoxantrone (Kita et al 2004). A maximum of three repeated measures were available, collected over 24 months.

The second pilot data set (DS2) consisted of N = 42 RR and SP participants who had BPF data from 3T scans. Similar to DS1, a maximum of three repeated measures were available, collected over 24 months, and the DS2 pilot participants were in the control group.

The effect size for the power analysis was defined as the percentage difference in linear slope relative to a hypothetical treatment group. That is, the percentage reduction in linear deterioration of the treated group in a hypothetical intervention. The pilot data and the survey of (Altmann et al 2009) suggested a reasonable range of percentage difference was 30% to 50%. Also of note, an earlier study was performed assessing the activity of ibudilast in relapsing MS, and this study included measurement of brain atrophy. While the earlier study used a different atrophy measure (SIENA) than in this proposal (BPF), and had only two time points, it did show effects sizes of approximately 33%-36%, (Barkhof et al 2010) which could be considered an appropriate target for the power analysis.

Power calculations were based on a LMM appropriate for clinical trials (Yi and Panzarella, 2002; Heo and Leon, 2009). Because there was only one site for the pilot data, the LMM of Equation (1) was used as the basis for the power analysis. Presentation of the required sample size equations is facilitated by using general notation. Consider the following equalities for the parameters of Equation (1),

$$[\alpha_P \quad \beta_P \quad \gamma \quad \delta]^T = [\beta_0 \quad \beta_1 \quad \beta_2 \quad \beta_3]^T = \boldsymbol{\beta}^T,$$
$$[a_i \quad b_i]^T = [b_{0i} \quad b_{1i}]^T = \boldsymbol{b}_i^T. \qquad (1)$$

(Recall that γ= 0 to constrain the groups to be equal at baseline.) Then the LMM of Equation (1) can be written in matrix notation as

$$\boldsymbol{Y}_i = \boldsymbol{X}_i\boldsymbol{\beta} + \boldsymbol{Z}_i\boldsymbol{b}_i + \boldsymbol{\epsilon}_i,$$

where each matrix has row dimension n_i; $\boldsymbol{X}_i$ is the design matrix of the fixed effects, and $\boldsymbol{Z}_i$ is the design matrix for the random effects. $\boldsymbol{X}_i$ has four columns, the first being a vector of 1s, the second being t_{ij}, the third being g_i, and the fourth being $t_{ij} \cdot g_i$. $\boldsymbol{Z}_i$ consists of the first two columns of $\boldsymbol{X}_i$.

The required sample size depends on the variance of the responses,

$$\boldsymbol{V}_i = Var(\boldsymbol{Y}_i) = \boldsymbol{Z}_i\boldsymbol{G}\boldsymbol{Z}_i^T + \sigma^2\boldsymbol{I}_i$$

and the required sample size for a single arm (half the total sample size) is computed with the following formula

$$\frac{N}{2} = \frac{\left(Z_{1-\alpha/2}+Z_{1-\beta}\right)^2 \cdot \left(\boldsymbol{X}_P^T\boldsymbol{V}^{-1}\boldsymbol{X}_P + \boldsymbol{X}_T^T\boldsymbol{V}^{-1}\boldsymbol{X}_T\right)^{-1}_{4,4}}{\delta^2} \qquad (2)$$

where $\boldsymbol{X}_P$ is the design matrix for the placebo group, $\boldsymbol{X}_T$ is the design matrix for the treatment group, and the subscript (4,4) indicates the element in the 4th row and 4th column of the matrix. The value of δ is varied to represent effect sizes of 30%, 35%, 40%, 45%, and 50%, which is the hypothetical slope difference between the treatment and placebo groups.

The analysis used the proposed time points of 0, 24, 48, 72, and 96 weeks. Power curves were computed for DS1, DS2, and for their average (Avg). Avg was based on averaging the LMM parameter estimates across the data sets (eg, average slope = [DS1 slope + DS2 slope] / 2). Avg was an attempt to pool information from the two data sets without the raw data (the raw data for DS2 was unavailable). A 10% dropout rate was assumed and all estimated sample sizes were inflated by this percentage (ie, final sample size = initial sample size × 1.10). Given the resources required for obtaining and analyzing 3T scans, the type I error rate was set at $\alpha = 0.10$. Figure 2 shows power curves for the three smallest effect sizes (30%, 35%, 40%). Focusing on Avg for the middle effect size (35%), the figure indicates that a single-arm $N / 2 = 125$ provides close to 80% (power = 0.80 is the horizontal dashed line). The middle effect size is important as it nearly corresponds to the upper effect size of the aforementioned ibudilast study (effect size = 36%). The Avg data source is preferred because it approximates a pooling of the information from both samples. The feasibility of $N / 2 = 125$ as the single-arm sample size was examined by calculating power for effect sizes with $\alpha = 0.10$ for all three data sources (DS1, DS2, Avg). The power by effect size curve and data source is shown in Figure 3. The figure clearly shows that the study has adequate power across the range of possible values suggested by these two data sources. For the "best" data (DS1), our study has slightly less than 90% power to detect effects of approximately 40% or larger, and more than 90% power to detect effects on the upper end of the range of interest (50%). Most importantly, the figure shows that the power based on the Avg data source is very high for effects in the 33% - 36% range of greatest interest (approximately 80% - 85%). Therefore, based on these assumptions, we are confident that a per-arm sample size of $N / 2 = 125$ ($N = 250$ total participants) is sufficient to help ensure statistically reliable results for the proposed study.

Figure 2: Power as a Function of Single-arm sample size, effect size, and data set

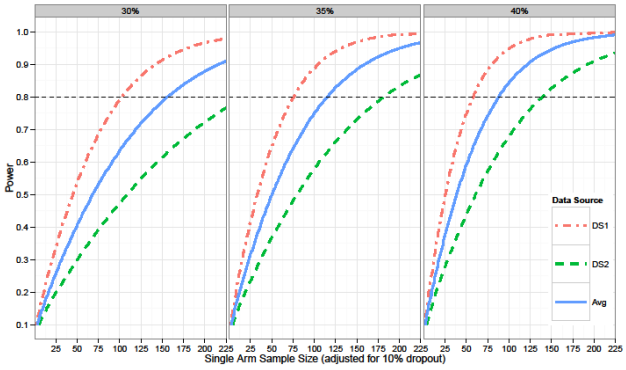
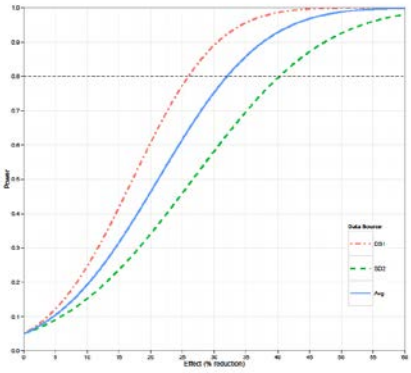


Figure 3: Power as a Function of Effect Size and data source for sample size of 125 and alpha = 0.10



Major Secondary Objectives: The required sample size for the primary endpoint was estimated to be 125 per arm. Since the primary analysis fixes the size of the sample, it is of interest to estimate the power level for the analysis of the secondary endpoints. Estimated power was computed for the secondary endpoints of DTI, OCT, and MTR.

DTI: The power analysis was based on longitudinal DTI pilot data with the outcome measure being longitudinal diffusivity (LD) within corticospinal tracts among RRMS and SPMS starting therapy with natalizumab (Fox 2010). Eighteen MS patients were measured five times over 24 months. Equation (2) was the basis for computing required sample size based on the LMM. In order to express power ($1 - \beta$) as a function of the fixed sample size (N / 2 = 125) and the other quantities, the following equation can be used

$$1 - \beta = \Phi\left[\left(\sqrt{\frac{125 \cdot \delta^2}{\left(\sum_k^2 \sum_l^L \boldsymbol{X}_{kl}^T \boldsymbol{V}_{kl}^{-1} \boldsymbol{X}_{kl} \boldsymbol{P}_{kl}\right)^{-1}_{4,4}}}\right) - Z_{1-\alpha/2}\right] \qquad (3)$$

where $\Phi[\cdot]$ is the cumulative distribution function of the standard normal distribution. Estimated power was calculated using Equation (3) for the δ effect sizes discussed previously. The results show that the estimated power was greater than 0.90 for the smallest effect size (30% difference in slopes). This result implies that $N = 250$ patients is sufficient to provide high power for testing the equality of LD slopes in the treatment and control groups for the range of plausible effect sizes that is anticipated in the proposed study.

OCT: Estimated power for OCT was based on pilot data supplied by Dr. Laura Balcer (University of Pennsylvania) from an unpublished multi-center natural history observational study of RRMS and SPMS patients. A sample of $N = 373$ MS patients was measured an average of 2.7 times over an average of 1.4 years. A complexity of OCT is that it is measured in both eyes. Therefore, the LMM must be augmented to include random effects for the additional level of nesting. Ignoring site, we define Y_{ijk} to be the OCT value for the kth week ($k = 1, \ldots, n_{ij}$) of the jth eye ($j = 1, 2$) of the ith patient ($i = 1, \ldots, N$). Then the LMM is

$$Y_{ijk} = \alpha + \beta t_{ijk} + (\gamma + \delta t_{ijk}) g_i + a_i + b_i t_{ijk} + a_{ij} + b_{ij} t_{ijk} + \epsilon_{ijk} \quad (4)$$

where $\gamma = 0$ is set to constrain the groups to be equal at baseline, a_i and b_i are patient-specific random effects and a_{ij} and b_{ij} are random effects for eyes nested within patients. It is assumed that

$$[a_i \quad b_i]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{G}), [a_{ij} \quad b_{ij}]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{H}), \epsilon_{ij} \sim \mathcal{N}(0, \sigma^2 \boldsymbol{I}_i), \text{ and}$$
$$[a_i \quad b_i]^T \perp [a_{ij} \quad b_{ij}]^T \perp \epsilon_{ij}.$$

The three levels of nesting represented by Equation (4) (month nested within eye nested within patient) presents a challenge for deriving power equations, and analytic results are usually based on simplifying assumptions (eg, fewer random effects than in Equation (4)) (Heo and Leon, 2009). In order to base the power estimate on the LMM of Equation (4), a simulation approach was used. For the simulation, the LMM of Equation (4) was fit to the pilot data and then the resulting estimates were treated as parameters in the following replications. For each replication, a random number generator was used to compute random effects and random error for $N = 250$ hypothetical treatment and control patients based on the appropriate covariance structures (eg, $\boldsymbol{G}$). Then simulated OCT response values were computed based on the fixed and random effects and random error of Equation (4). For each replication, the null hypothesis of H_0: $\delta = 0$ was evaluated with the Z-test based on a LMM fit to the simulated data. The process was repeated 1000 times for each effect size value of δ and the average number of rejections of H_0 was taken to be the estimated power. Results of the simulation are shown in the table below.

Power as a Function of Effect Size based on the OCT Simulation Study

Effect Size	Power
30%	0.515
35%	0.603
40%	0.665
45%	0.782
50%	0.855

As the table shows, the power surpassed the conventional cutoff of 0.80 only for the largest effect size, but the next lower size (45%) was close to the cutoff. The results indicate that $N = 250$ might provide adequate power for the treatment and placebo slope comparison of OCT if the effect size is large, but power might be lower than the conventional cutoff for medium and smaller effect sizes.

MTR: Pilot data was not available for the MTR power estimates. Descriptive statistics were provided by Dr. Douglas Arnold (Director of NeuroRx) for a study of the efficacy of BG-12 (BG00012, dimethyl fumarate), which is a novel therapy in the development for relapsing MS. Ibudilast is purported to have neuroprotective effects, and so these data were used as a model to project the power of ibudilast in this proposed trial. A total of N =448 MS patients had whole-brain MTR measured at baseline, 24 weeks, 1 year, and 2 years as part of a multi-center phase III clinical trial using 1.5T MRIs and the stock manufacturer pulse sequence. Only descriptive

statistics were available for change between baseline and two years. There was a placebo group and two treatment groups, one receiving BG-12 240 mg twice a day (b.i.d.) and the second receiving BG-12 240 mg three times per day day (t.i.d.). The statistics available for the study were means of change scores and their standard deviations. Thus, power was estimated based on an independent *t*-test of the mean baseline to 2 year difference in MTR scores for the treatment (BG-12) and placebo groups. Power was computed using the conventional formula

$$1 - \beta = \Phi\left(\frac{|\delta|\sqrt{125}}{\sigma} - Z_{1-\frac{\alpha}{2}}\right)$$

where δ is the difference of the mean change scores and σ^2 is the pooled variance of the difference. A subset of patients had no new or enlarging T2 legions, and the analysis was performed twice: once for all the patients and another time excluding the patients with no lesions. The results of the power analysis are shown in the table below. As seen in the table, the lowest estimated power (t.i.d., all patients) was greater than the conventional cut-off of 0.80. When only subjects with no new lesions were considered (which more closely approximates what we expect in the proposed trial), estimated power was even greater than the conventional level. The table suggests that N =250 might provide sufficient power to detect T and P differences in change over time for the proposed study. The improved image acquisition from 3T MRIs and tailored pulse sequences are expected to increase the study power.

Power as a Function of Treatment and Type of Patient for MTR

Treatment	Patients	Power
b.i.d.	All	0.856
t.i.d	All	0.803
b.i.d.	No Lesions	0.925
t.i.d.	No Lesions	0.816

CLADA and NFL from CSF: No pilot data or other effect size estimates were available for CLADA or NFL measured from CSF. However, we expect both CLADA and NFL effects to be consistent with those of the other secondary endpoints. Therefore, we are confident that statistical power for CLADA will be sufficient to detect effect sizes similar to those discussed for the other secondary endpoints. The power for NFL will depend upon the number of subjects who agree to have lumbar punctures.

Safety Objective: With an overall significance level of 0.10, the study will have 80% or greater power to detect differences in safety or tolerability of 15% or greater across the two groups. Thus, minor safety concerns may not be detected in this study – and would need to be assessed in a larger, future phase 3 trial. Nevertheless, the study is adequately powered to detect major safety concerns.

12.1.4. Interim Monitoring Plan

The monitoring of subject safety and data quality will follow the NINDS Guidelines for Data and Safety Monitoring in Clinical Trials. An NIH-appointed Data and Safety Monitoring Board (DSMB) will meet at approximately 6-month intervals to review study progress (eg, enrollment,

site performance, subject retention, etc.) as well as blinded study data (safety and efficacy) provided by the DCC. The frequency and format of DSMB meetings, reports, and guidelines for interim analysis will be agreed upon prior to study initiation.

We propose to have one interim efficacy analysis at a time when half of the subjects have completed their Week 96 follow-up visit. For this interim analysis, we propose to use the Lan-DeMets spending function approach with O'Brien-Fleming stopping boundaries. Table 10 illustrates the proposed stopping boundaries under the assumption of 2 equally spaced analyses (one interim analysis and the planned final analysis). However, the Lan-DeMets method allows for analyses at unequal intervals and without having to pre-specify the time of any interim analyses. Hence, the proposed method has the flexibility to adapt should the DSMB request an interim analysis at an alternative time point or if the DSMB doesn't meet after exactly half of the patients complete their Week 96 assessment. We also propose to conduct a formal futility assessment at the time of the interim efficacy analysis. The futility assessment will be based on a determination of the predictive power. If this predictive power is below 20% at the time of the interim analysis, then we propose that the trial should stop for futility. In the event of early study termination because of overwhelming efficacy, all participants would be offered open-label active treatment, if feasible. The protocol and informed consent would be revised accordingly. Similar to all previous analysis, the statistical models will constrain the groups to be equal at baseline to account for vagaries in randomization that result in initial imbalance (Senn, 2013).

O'Brien-Fleming Stopping Boundaries for a Single Interim Analysis

Efficacy Analysis	Number of Subjects Completing 96 Week Follow-Up	Nominal P-Value to Conclude Efficacy
1	125	0.0104
2	250	0.0816

Commented [CCS3]: At the time we were finalizing the SAP, we realized that this was not modified to account for a significance level of 0.10 instead of 0.05.

12.2. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

By signing this protocol, the CSSPI 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 CSSPI also agrees to allow monitoring, audits, NeuroNEXT CIRB review, and regulatory agency inspection of study-related documents and procedures and provide for direct access to all study-related source data and documents.

The CSSPI agrees not to seek reimbursement from subjects, their insurance providers, or from government programs for procedures included as part of the study reimbursed to the CSSPI by the NeuroNEXT CCC.

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

The CSSPI 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 CSSPI 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.

In the event the Sponsor prematurely terminates a particular study site, the CSSPI will promptly notify the NeuroNEXT CIRB.

12.3. Study Monitoring

This study will be monitored at all stages of its development by the DCC at The University of Iowa, and clinical research personnel employed by the Sponsor or its representative. Monitoring will include on-site, and centralized monitoring to assure that the investigation is conducted according to protocol, to protect subject rights and safety, and to confirm data integrity and quality.

On-site monitoring will assess critical study procedures, including study data endpoints, subject safety, protocol compliance, and Regulatory compliance, and will involve the following:

a. Audit data listings to source documentation.
b. Ensure subject eligibility
c. Verify reporting of adverse events.
d. Assess investigational product accountability
e. Assess compliance with protocol.
f. Audit regulatory files
g. Ensure adequate site personnel training

Investigators are required to store all source documents.

Centralized monitoring processes at the DCC occur at regular intervals, and will include:

- Data quality checks resulting in reports by:
    - monitoring of the data entry system

  - use of data entry quality controls
  - use of an online query system
  - use of an eCRF (electronic case report form) management module
- Site performance checks resulting in reports by:
  - Production of data trend analyses to recognize patterns and trends
  - Use of an eCRF management module to monitor data quality
- Safety reporting monitoring, resulting in scheduled reports
- Regulatory document tracking, using an online system to upload and store regulatory documents
  - Results in reports listing missing documents and pending renewals

12.4. Audits and Inspections

The CSSPI and appropriate personnel may be periodically requested to attend meetings/workshops 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 CSSPI 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 CSSPI has to inform the Sponsor if he/she is approached for a regulatory audit.

12.5. Institutional Review Board (IRB)

Before initiation of the study, the CSSPI must obtain approval or favorable opinion of the research protocol, informed consent form (ICF), and any advertisement for subject recruitment from the NeuroNEXT CIRB complying with the provisions specified in the Code of Federal Regulations (CFR) 21 Part 56 and applicable government regulations. The Investigator must assure CIRB compliance with the applicable regulations.

A copy of written CIRB approval of the protocol, ICF, and advertising (if applicable) must be provided to the Sponsor or their designee prior to initiation of the study.

12.6. 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 NeuroNEXT CCC 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 everyone listed on the FDA 1572.

13. QUALITY CONTROL AND QUALITY ASSURANCE

By signing this protocol, the Sponsor and Clinical Study Sites 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 NeuroNEXT network to ensure that studies are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

14. ETHICS

14.1. Ethics Review

Documented approval from the NeuroNEXT CIRB 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 the NeuroNEXT CCC, DCC, MediciNova Inc. and/or Regulatory Authority representatives at any time. The CSSPI must agree to the inspection of clinical trial-related records by the NeuroNEXT CCC/ DCC/Regulatory Authority/ 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 CIRB 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 CSSPI or a sub-Investigator who is listed on the Form FDA 1572 if the CSSPI 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 CSSPI 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 CSSPI 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 CSSPI when the destruction of documents is permitted.

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 by the Protocol Steering Committee, NINDS, DSMB, and the CIRB. The CSSPI will not make any changes to the conduct of the study or the protocol without first obtaining written approval from the Sponsor and the CIRB, except where necessary to eliminate an apparent immediate hazard to a study subject.

16.2. Discontinuation of the Study

The NeuroNext Network, in collaboration with the company and DSMB, if appropriate, reserve the right to discontinue the study at site(s) for safety or administrative reasons at any time. For example, a site that does not recruit at an acceptable rate may be discontinued. 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 CSSPI 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 CSSPI 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 CSSPI also consents to the transmission of this information to MediciNova Inc. for these purposes.

17. REFERENCES

1. Fujimoto, et al. Ibudilast, a phosphodiesterase inhibitor, ameliorates experimental autoimmune encephalomyelitis in Dark August rats. Journal of Neuroimmunology 1999: 35-42.
2. Mizuno T et al. Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. Neuropharmacology 2004;46:404-11
3. Sakoda et al. Ibudilast a nonselective phosphodiesterase inhibitor, for treatment of multiple sclerosis-a pilot study. Unpublished Manuscript draft
4. Feng J et al. Ibudilast, a nonselective phosphodiesterase inhibitor, regulates Th1/Th2 balance and NKT cell subset in multiple sclerosis. Mult Scler. 2004; 10:494-8.
5. Barkhof, F. et al, Matsuda, K, Landin R. Ibudilast in relapsing-remitting multiple sclerosis. A neuroprotectant? *Neurology* 2010; 74:1033-1040.
6. Fox R. Primary neuroprotection: the Holy Grail of multiple sclerosis therapy. Neurology, 2010; 74:1018-1019
7. Laird, N. M., and Ware, J. H. (1982). Random-Effects Models for Longitudinal Data. Biometrics, 38(4), 963-974.
8. Verbeke, G. and Molenbergs, G. (2000). Linear mixed models for longitudinal data. New York: Springer.
9. Little, RJA and Rubin, DB (2002). Statistical Analysis with Missing Data, Second Edition. New York: Wiley.
10. Altmann D et al. Sample sizes for brain atrophy outcomes in trials for secondary progressive multiple sclerosis. Neurology 2009;72:595-601.
11. Kawasaki A et al., A novel antiasthmatic agent on airway hypersensitivity in bronchial asthma. J Asthma 1992; 29:245-52.
12. Fukuyama, H et al. Pharmacological effects of ibudilast on cerebral circulation: a PET study. Neurol Res 1993: 15:169-173
13. Ledeboer, A. et al., and Johnson, K.W. The Glial Modulatory Drug AV411 Attenuates Mechanical Allodynia in Rat Models of Neuropathic Pain. Neuron Glia Biology (Pain special issue). 2007: 2:279-291.
14. Ledeboer, A., et al. and Johnson, K. AV411 (ibudilast): a new class therapeutic candidate for neuropathic pain and opioid withdrawal syndromes. Expert Opinion on Investigational Drugs. 2007; 16:935-950.
15. Rolan P, Hutchinson M, Johnson K. Ibudilast: a review of its pharmacology, efficacy and safety in respiratory and neurological disease. Expert Opin Pharmacother 2009;10:2897-904.
16. Gibson L et al. The inhibitory profile of ibudilast against the human phosphodiesterase enzyme family. Eur J Pharmacol 2006; 538:39-42.

17. Cho, Y., et al., Johnson, K., and Lolis, E. AV411 (Ibudilast) and AV1013 are allosteric inhibitors of macrophage migration inhibitory factor: Enzymatic, functional, and structural studies. PNAS – USA, 2010; 107:11313-8.
18. Powell, N. D., et al. Cutting edge: macrophage migration inhibitory factor is necessary for progression of experimental autoimmune encephalomyelitis. J. Immunol. 2005: 175:5611–5614.
19. Kithcart A et al., A small-molecule inhibitor of macrophage migration inhibitory factor for the treatment of inflammatory disease. Faseb J. 2010; 11:4459-4466.
20. Nishio Y et al., Deletion of MIF attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. Acta Neuropathol. 2009; 117:321-328.
21. Inacio, A et al. Macrophage migration inhibitory factor promotes cell death and aggravates neurologic deficits after experimental stroke. Journal Cerebral Bl Flow & Metab. 2011; 31:1093–1106.
22. Chen R et al. Broad spectrum neuroprotection profile of phosphodiesterase inhibitors as related to modulation of cell-cycle elements and caspase-3 activation. Neurosci Lett 2007;418:165-9.
23. Nakamizo T et al. Phosphodiesterase inhibitors are neuroprotective to cultured spinal motor neurons. J Neurosci Res 2003; 71:485-95.
24. Sloane E, et al., Anti-inflammatory cytokine gene therapy decreases sensory and motor dysfunction in experimental Multiple Sclerosis: MOG-EAE behavioral and anatomical symptom treatment with cytokine gene therapy. Brain Behav. Immun. 2009; 23:92-100.
25. Watkins L and Maier S: Glia: a novel drug discovery target for clinical pain. Nat. Rev. Drug Discov. 2003; 2: 973-985.
26. DeLeo J and Yezierski R. The role of neuroinflammation and neuroimmune activation in persistent pain. Pain 2001; 90:1-6.
27. Kagitani-Shimono K et al. Anti-inflammatory therapy by ibudilast, a phosphodiesterase inhibitor, in demyelination of twitcher, a genetic demyelination model. J Neuroinflammation 2005; 2:10-22.
28. Sanftner, et al. Cross-species comparisons of the pharmacokinetics of ibudilast. Xenobiotica. 2009; (12): 964-977.
29. Grau-López L et al., Specific T-cell proliferation to myelin peptides in relapsing-remitting multiple sclerosis. Eur J Neurol. 2011; 18:1101-4.
30. Österberg A et al., Central pain in multiple sclerosis--prevalence and clinical characteristics. Eur J Pain. 2005 Oct; 9:531-42.
31. Truini A et al. Treating pain in multiple sclerosis. Expert Opin Pharmacother. 2011; 12:2355-68.
32. Senn S. Seven myths of randomization in clinical trials. Statistics in Medicine. 2013; 32:1439-1450.

33. Localio AR, Berlin JA, Ten Have TR, et al. Adjustments for center in multicenter studies: an overview. Ann Intern Med, 2001;135:112–23.

34. Fieuws, S. and Verbeke, G. (2004). Joint modeling of multivariate longitudinal profiles: pitfalls of the random effects approach. Statistics in Medicine, 23:3093–3104.

35. Kita, M. Cohen JA, Fox RJ, Evangelista A, Pelletier D, Stone J, Goodkin DE, Zamvil SS. A Phase II Trial of Mitoxantrone in Patients with Primary Progressive Multiple Sclerosis, Neurology 2004; 62:A99.

36. Yi, Q. and Panzarella, T. (2002). Estimating sample size for tests on trends across repeated measurements with missing data based on the interaction term in a mixed model. Controlled Clinical Trials, 23:481–496.

37. Heo, M. and Leon, A. C. (2009). Sample size requirements to detect an intervention by time interaction in longitudinal cluster randomized clinical trials. Statistics in Medicine, 28:1017–1027.

18. APPENDICES

Appendix 1: Laboratory Safety Tests for Ibudilast/MN-166

Appendix 2: Suicide Behaviors Questionnaire-Revised (SBQ-R)

Appendix 3: Beck Depression Inventory-Fast Screen (BDI-FS)

Appendix 4: Modeled Informed Consent

Appendix 5: DSMB Guidelines

Appendix 1: Laboratory Safety Tests for Ibudilast (MN-166)

Blood Chemistry Tests
aspartate aminotransferase (AST)
alanine aminotransferase (ALT)
albumin
alkaline phosphatase
bicarbonate
blood urea nitrogen
calcium
chloride
creatinine
gamma-glutamyl transferase (GGT)
phosphorous
potassium
sodium
total bilirubin[a]
total protein
lactate dehydrogenase

Blood Chemistry Tests
triglyceride
Lipid Profile
fasting (non-random) serum cholesterol
fasting (non-random) serum high-density lipoprotein cholesterol
fasting (non-random) serum low-density lipoprotein cholesterol
fasting glucose
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
Urinalysis Tests
color
appearance
total ketones
urobilinogen
bilirubin
red blood cells
leukocyte esterase
nitrite
pH
protein
specific gravity
glucose
microscopic evaluation[b]

[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.

[b] Microscopic evaluation will be performed if dipstick analysis indicates the presence of any significant abnormality.

Appendix 2: Suicide Behaviors Questionnaire-Revised (SBQ-R)

Patient Name_____Date of Visit_____

Instructions: Please check the number beside the statement or phrase that best applies to you.

1. Have you ever thought about or attempted to kill yourself? (check one only)

1. Never
2. It was just a brief passing thought

3a. I have had a plan at least once to kill myself but did not try to do it
3b. I have had a plan at least once to kill myself and really wanted to die
4a. I have attempted to kill myself, but did not want to die
4b. I have attempted to kill myself, and really hoped to die

2. How often have you thought about killing yourself in the past year? (check one only)

1. Never
2. Rarely (1 time)
3. Sometimes (2 times)
4. Often (3-4 times)
5. Very Often (5 or more times)

3. Have you ever told someone that you were going to commit suicide, or that you might do it?

1. No
2a. Yes, at one time, but did not really want to die
2b. Yes, at one time, and really wanted to die
3a. Yes, more than once, but did not want to do it
3b. Yes, more than once, and really wanted to do it

4. How likely is it that you will attempt suicide someday? (check one only)

0. Never
1. No chance at all
2. Rather unlikely
3. Unlikely
4. Likely
5. Rather likely
6. Very unlikely

Appendix 3: Beck Depression Inventory-Fast Screen (BDI-FS)

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

BDI–FastScreen

This questionnaire consists of groups of statements. Please read each group of statements carefully, then pick out the one statement in each group which best describes the way you have been feeling during the past 2 weeks, including today! Circle the number beside the statement you picked. If several statements in the group seem to apply equally well, circle the statement which has the largest number.

1.
- **0** I do not feel sad.
- **1** I feel sad much of the time.
- **2** I am sad all the time.
- **3** I am so sad or unhappy that I can't stand it.

2.
- **0** I am not discouraged about my future.
- **1** I feel more discouraged about my future than I used to be.
- **2** I do not expect things to work out for me.
- **3** I feel my future is hopeless and will only get worse.

3.
- **0** I do not feel like a failure.
- **1** I have failed more than I should have.
- **2** As I look back, I see a lot of failures.
- **3** I feel I am a total failure as a person.

4.
- **0** I get as much pleasure as I ever did from the things I enjoy.
- **1** I don't enjoy things as much as I used to.
- **2** I get very little pleasure from the things I used to enjoy.
- **3** I can't get any pleasure from the things I used to enjoy.

5.
- **0** I feel the same about myself as ever.
- **1** I have lost confidence in myself.
- **2** I am disappointed in myself.
- **3** I dislike myself.

6.
- **0** I don't criticize or blame myself more than usual.
- **1** I am more critical of myself than I used to be.
- **2** I criticize myself for all of my faults.
- **3** I blame myself for everything bad that happens.

7.
- **0** I don't have any thoughts of killing myself.
- **1** I have thoughts of killing myself, but I would not carry them out.
- **2** I would like to kill myself.
- **3** I would kill myself if I had the chance.

NOTICE: This form is printed with both green and black ink. If your copy does not appear this way, it has been photocopied in violation of copyright laws.

_____ Total

281382-2 987654

PEARSON

22 23 24 A B C D E

Pearson Executive Office 5601 Green Valley Drive Bloomington, MN 55437
800.627.7271 www.PsychCorp.com

PsychCorp

Product Number 0158019431

Appendix 4: Modeled Informed Consent

NeuroNEXT Consent Form Template
Version Number: v11.0; Version Date: February 17, 2015

Protocol Title:A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability and Activity of Ibudilast (MN-166) in Subjects with Progressive Multiple Sclerosis #NN102

Protocol Principal Investigator: Robert J. Fox, MD

Site Principal Investigator:

NeuroNEXT Clinical Study Site:

Description of Subject Population: Adults with Progressive Multiple Sclerosis

About this consent form

Please read this form carefully. It tells you important information about a research study. A member of our research team will also talk to you about taking part in this research study. People who agree to take part in research studies are called "subjects." This term will be used throughout this consent form.

{Name of site} is **{description of site}**. We are doing this research as part of the Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT), which is supported by the National Institute of Neurological Disorders and Stroke (NINDS).

If you have any questions about the research or about this form, please ask us. Taking part in this research study is up to you. If you decide to take part in this research study, you must sign this form to show that you want to take part. We will give you a signed copy of this form to keep.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. That website will not include information that can identify you. At most, the website will eventually include a summary of the results of the study. You can search this website at anytime.

Why is this research study being done?

The study will be conducted by the NeuroNEXT Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT) in about 28 sites around the United States.

{Place any Conflict of Interest disclosures mandated by your institution or the Central IRB here.}

We are doing this research study to find out if the drug ibudilast can help people with progressive multiple sclerosis (MS). We will also find out if it is safe to take without causing too many side effects.

Ibudilast is not approved for use by the US Food and Drug Administration (FDA). This means that ibudilast can only be used in research studies. This drug has been given to approximately 450 people who were healthy volunteers, had diabetes, had relapsing remitting MS or were in trials for neuropathic pain.

During this study, we plan to compare ibudilast to placebo. The 'placebo' looks like the study drug, ibudilast capsules, but contains no active study drug. We will assign you by chance (like a coin toss) to the ibudilast group or the placebo group. You and the Study Doctor cannot choose your study group. You will have a 1 out of 2 chance of being assigned to ibudilast. You will have a 1 out of 2 chance of being assigned to placebo.

You are being asked to take part in this study because you have been diagnosed with a progressive form of multiple sclerosis. Currently, mitoxantrone is the only FDA-approved therapy for secondary progressive MS, but is not commonly used because of its risks of heart injury and blood cancers (i.e., leukemia).

About 250 people will take part in this study. We plan to enroll about 10-15 people at **{Insert Site Name}**.

This research study is supported by a federal grant awarded by the National Institute of Health/National Institute of Neurological Disorders and Stroke (NINDS) through the Network for Excellence in Neurological Clinical Trials (NeuroNEXT). The National Multiple Sclerosis Society and MediciNova, Inc. are also supporting this research study. The study drug (ibudilast and placebo) used for this research study is supplied by MediciNova, Inc. a biopharmaceutical company developing this drug.

How long will I take part in this research study?

It will take you about 26 months (a little over 2 years) to complete this study. You can expect a total of 13 study visits during this time, although more visits may be required if your Study Doctor decides they are needed for medical reasons.

What will happen in this research study?

You will go to the **{Insert Site Name}** for all of your study visits. **{Insert Site Investigator Name}**, your main Treating Doctor (called the "Study Doctor" in this Consent Form), will explain to you in more detail about the method of gathering information that is important in conducting clinical research.
If you choose to take part in this study, we will ask you to sign this consent form before we do any research procedures.

Screening Visit
The Screening Visit will take about 4-5 hours. At this visit, we will do some tests and procedures to see if you qualify to take part in this research study. We will ask that you fast for 12 hours prior to this visit. The Study Doctor will review the results of these tests and procedures. If you don't qualify, the Study Doctor will tell you why.

At this visit, we will:

- Ask you questions about your medical history, including your MS.
- Ask you about medications you are taking or have taken. Bring bottles (both empty and full) of medications you currently use.
- Perform a physical exam and measure your vital signs, including heart rate and blood pressure.
- Measure your weight.
- Ask you to complete the Expanded Disability Status Scale (EDSS).This test helps to measure your multiple sclerosis disease status. For this test, we will give you a complete neurological exam and ask you to walk 500 meters.
- Ask you to complete the Multiple Sclerosis Functional Composite (MSFC). This test measures your walking ability, how well you can move your hands, and how well you can see.
- Ask you to complete some cognitive tests. These tests will tell us about your cognition (thinking).
- Perform Ocular Coherence Tomography (OCT). This is a painless scan of the retina in the back of your eyes, one of the areas affected by MS.
- Draw blood samples. We will draw a total of about 15 tablespoons of blood over the course of this research study.
- Ask you for a urine sample.
- Test your blood for pregnancy, if you are a woman who is able to become pregnant. Pregnant women cannot take part in this research study.

- Give you an MRI (magnetic resonance imaging) scan. An MRI scan is a large medical imaging device that uses powerful magnets to create a picture of your brain (scan) that can determine the areas affected by multiple sclerosis. This test is painless and takes about 60 minutes.
- Perform an optional spinal tap.For this test, we collect some spinal fluid from the bottom of your spine with a needle. There may be some discomfort with this test.
- Perform an electrocardiogram (ECG), a test to measure your heart function. For this test we will place several small, sticky pads on your chest, arms and legs. Each pad has a wire attached. The wires connect to a machine that makes a recording of your heart rhythm. This painless test takes about 15 minutes.
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.

Optional Spinal Tap
If you are willing, we will ask you to undergo a spinal tap at the Screening Visit, Week 48 (visit 8) and Week 96 (visit 12). The Screening Visit Spinal Tap may be completed at any time prior to your first dose of study drug. This is being done to find out if spinal fluid, scans and physical exams all give us different information about disease progression. You do not need to have the spinal tap in order to take part in the rest of this study. For this test, we collect some spinal fluid from the bottom of your spine with a needle. This test takes about 20-30 minutes.

A local anesthetic (numbing medicine) will be injected into the area on your back. You might feel a burning sensation until the medicine begins to work. When the area is numb, a hollow needle is inserted in the lower back between the two lumbar vertebrae (bones in your lower spine). This sometimes causes a pressure sensation. The spinal canal is penetrated, and fluid is collected. The spinal cord is not touched by the needle during the test. You might feel some discomfort.

Baseline Visit (Visit 2)
This visit will take about 2 hours. At this visit, we will:
- Ask you about your health and medications.
- Ask you about any side effects you may have experienced and how you have been feeling.
- Measure your vital signs.
- Draw a blood sample.
- Ask for a urine sample.
- Test your urine for pregnancy, if you are a woman who is able to become pregnant.
- Perform an MRI.
- Ask you to complete the EDSS and MSFC tests.
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.

If you still qualify for this study, we will assign you by chance (like a coin toss) to the ibudilast group or the placebo group. You and the Study Doctor cannot choose which group you are in.
You will have a 1 in 2 chance of receiving either ibudilast or placebo.

You and the Study Doctor will not know which group you are in, but the Study Doctor can find out if medically necessary.

Taking the Study Drug
You will begin taking 3 capsules of your assigned study drug (ibudilast or placebo) by mouth twice daily (dosage 60 mg/day) for 14 days. Beginning on Day 15, we will increase your dose to 5 capsules taken by mouth twice daily (dosage 100 mg/day). *Study drug should always be taken with food.* However, in the event that you are having difficulty tolerating the study drug (nausea, diarrhea) by the end of Day 14, you may continue taking 3 capsules twice a day until Day 21. If, after 21 days, you are still having difficulty tolerating the study drug, you may stop taking the study drug. If tolerability is acceptable after 21 days, your dosage will be increased to 5 capsules twice a day starting from Day 22. An optional smart phone application (or "app") will be available for you to download to receive reminders to take your study drug. This is a free app, therefore there will be no charge to you. Your phone number will not be shared with anyone outside of **{Insert Site Name}**. If interested, please let your study team know so that they may set this up for you.

If you are unable to tolerate 5 capsules twice a day, your Study Doctor may choose to reduce the dosage. Do not make any changes to your dosing schedule without discussing with your Study Doctor first.

Study Drug Follow-Up Visits (Visits 3 through 12)

These visits will take place at 4, 8, 12, 24, 36, 48, 60, 72 84 and 96 weeks after you start taking the study drug. Visit 3 (week 4), 4 (week 8), 5 (week 12), 7 (week 36), 9 (week 60), 11 (week 84) will take about 3 hours. Visits 6 (week 24), 8 (week 48), 10 (week 72) 12 (week 96) will take about 4 hours. We will ask that you fast prior for 12 hours to the week 48 and 96 visits. Bring bottles (both empty and full) all medications you are currently taking, including the study drug bottles, to all of these study visits. At these visits, we will:

- Ask you about your health and medications.
- Ask you about taking the study drug.
- Give you a physical exam and measure your vital signs.
- Measure your weight.
- Ask you about any side effects you may have experienced and how you have been feeling.
- Draw a blood sample.
- Ask for a urine sample
- Perform an MRI (Visit 6 ,8, 10 and 12 only)
- Perform an optional spinal tap (Visit 8 and 12 only).
- Perform an ECG.
- Ask you to complete the EDSS and MSFC tests (Visit 6, 8, 10 and 12 only).
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.
- Ask you to complete OCT (Visit 6, 8, 10 and 12 only).
- Ask you to complete some cognitive Tests (Visit 6, 8, 10 and 12 only).

Unscheduled Visit: Relapse Evaluation

If you experience new or worsening neurologic symptoms that suggest you may be having a relapse during the course of this study, you should contact your Study Doctor immediately. Your Study Doctor may ask you to visit the **{Insert Site Name}** for evaluation. However, do not stop taking the study drug or make changes to your dosing schedule. Your Study Doctor will determine whether any changes need to be made.

At the relapse visit, we will:

- Ask you about your health and medications including taking the study drug
- Perform a physical exam and measure your vital signs.
- Ask you about any side effects you may have experienced and how you have been feeling.
- Ask for a urine sample.
- Ask you to complete the EDSS.
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.

Unscheduled Visit: Semi-Annual Visits

If you discontinue study medication and remain in the study, you will be followed on a semi-annual basis. At these visits, we will:

- Perform an MRI
- Ask you to complete the EDSS and MSFC tests
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.
- Ask you to complete OCT
- Ask you to complete some cognitive Tests
- Perform an optional spinal tap

Unscheduled Visit: Early Withdrawal Visit

If you discontinue study medication and choose not to be followed on a semi-annual basis, we will:

- Ask you about your health and medications.
- Give you a physical exam and measure your vital signs.

- Measure your weight.
- Ask you about any side effects you may have experienced and how you have been feeling.
- Draw a blood sample.
- Perform an MRI
- Perform an optional spinal tap
- Perform an ECG.
- Ask you to complete the EDSS and MSFC tests
- Ask you to complete some questionnaires about your health, daily life, how you are feeling, and your MS.
- Ask you to complete OCT
- Ask you to complete some cognitive Tests

Abnormal Lab follow up
For abnormal lab follow up, we will ask you to come into the clinic in 2 week intervals to draw additional blood samples. We will also ask you about any side effects you may have experienced and how you have been feeling.

Final Follow-Up Visit (Visit 13)
This visit will take place about 4 weeks after you stop taking study medication. We anticipate this will be at the Week 96 visit, but may be scheduled earlier if you have to discontinue study medication for any reason. Visit 13 will take about 1 hour. At this visit we will:

- Perform a physical exam
- Measure your vital signs.
- Measure your weight.
- Ask you about your health and medications.
- Ask you about any side effects you may have experienced and how you have been feeling.

Stopping the Study Early
If you choose to stop taking part from the study, you will be asked to make one last study visit to the **{Insert Site Name}**, return all study drugs, and undergo safety tests. This visit will be just like visit 8 except that we will also draw blood samples, ask for a urine sample, and ask you to complete some questionnaires.

If you just want to or need to stop taking the study drug, you will be asked to continue follow-up according to the original schedule if you are willing, even though you are no longer taking study drug.

Use and Storage of Study Information and Samples
We will use your samples and information for the research described in this form and for other future research. We will label your samples and health information with a code instead of your name. The key to the code connects your name to your samples and health information. The Study Doctor will keep the key to the code in a password protected computer/locked file. All study-related clinical lab samples (including blood, urine and spinal fluid) collected from this site and from other participating sites will be sent to centralized laboratory facilities supported by NeuroNEXT for storage and testing. Similarly MRI, ECG, OCT and all other collected data will be sent to specialized facilities for storage and analysis.

The sponsor and researchers may use health information that identifies you to do the research described in this form, and to do related research. This means research related to ibudilast drug being studied alone or in combination with other drugs or devices or other neurological disorders. The sponsor and researchers may also use health information that no longer identifies you to do any type of research. The use of blood and CSF samples will be limited to research for progressive MS and other neurological diseases.

Do you agree to share you samples for future uses in progressive MS and other neurological diseases?

☐ YES ☐ NO Initials_____

You can change your mind about this later. If you change your mind, tell the Study Doctor.

What are the risks and possible discomforts from being in this research study?

Risks of Taking Ibudilast

1. Ibudilast has been used for the treatment of asthma and post-stroke disorders in Japan for more than 20 years. About 3 out of every 100 people who took ibudilast in Japan reported side effects. The most common reported side effects were:

- appetite loss
- nausea
- diarrhea
- elevated liver enzymes which is an indication of liver damage**-(uncommon, between 1 and 10 out of a 1000)**
- headache

2. Approximately 450 people**,** including nearly 300 with MS, have been treated with ibudilast in multiple research studies. Generally good safety and tolerability have been seen. The side effects that were seen in a 2 year study were GI (gastrointestinal, meaning the stomach and intestines; include nausea and diarrhea; increase in liver enzymes) related.

3. Ibudilast has been given to nearly 300 patients with relapsing remitting MS. There were 20 serious adverse events (side effects) reported. Most of the serious adverse events were gastrointestinal in nature or having to do with bone fractures. All of these serious adverse events were considered by the Investigator to be unlikely or not related to ibudilast. Six of the serious adverse events were considered severe and two were considered life-threatening. Nine subjects discontinued from the study due to a side effect. Two side effects (liver disorders) were possibly related to taking ibudilast.

As in the earlier trials, the most common side effects were:

- Nausea
- Diarrhea
- Headache
- Increase in liver enzyme

As noted from the above mentioned studies, GI distress, which includes nausea and diarrhea, appear to be the most common side effects. The GI symptoms associated with ibudilast tend to occur early (within 1-2 weeks) and usually last for only a few days or weeks, and then improve. These symptoms may be relieved with anti-diarrheal or anti-nausea drugs, if necessary. Usually a majority of those receiving ibudilast recover from these symptoms within several days (with or without anti-diarrheal drugs). Talk to your Study Doctor if you experience these symptoms, particularly if they last for more than 2-3 weeks.

Other rare side effects associated with ibudilast are cold symptoms, itching sensation, rash, dizziness, tremors, insomnia, drowsiness, sudden blushing, anorexia (decreased appetite), abdominal pain, abdominal bloating, palpitations, flushed appearance, anemia (low red blood cell counts), low white blood cells, tiredness, facial edema (swelling), abnormal sound sensitivity (changes in your hearing) and metallic taste in mouth.

Risk of Allergic Reaction

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or serious, and can even result in death in some cases. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing. If you think you are having an allergic reaction, call the Study Doctor right away. If you are having trouble breathing, call 911 immediately.

Unknown Risks
There may be risks or side effects of taking ibudilast that we don't know about at this time. New information about ibudilast may become available during this study. If this happens, your Study Doctor will tell you about any new information that could affect your willingness to take part in the study. We may ask you to sign a new Consent Form that includes the new information.

Pregnancy and Fertility Risks
The effect of ibudilast on an embryo or fetus (developing baby still in the womb), or on a breastfeeding infant, is unknown and may be harmful. Because of these unknown risks**, if you are capable of giving birth to or fathering a child, you and your sexual partner should use adequate birth control measures while you are in this study.** If you are a female who is sexually active and able to become pregnant, or a man with a sexual partner who is able to become pregnant, you must agree to use one of the birth control methods listed below. You must use birth control for the entire study and at least 30 days after your last dose of study drug. Acceptable birth control methods for this study are:

- Abstinence (no sex)
- Oral contraceptives (birth control pills)
- IUD (a small T-shaped device containing either copper or a hormone inserted into the womb for birth control)
- Diaphragm with spermicide (a foam, cream or gel that kills sperm)
- Norplant (birth control capsules that are inserted in the skin of the upper arm of a female)
- Approved hormone injections
- Condoms with spermicide

Women cannot take part in this study if they are:

- Pregnant
- Trying to become pregnant
- Breastfeeding

If during the study you think you are pregnant, you must tell your Study Doctor and stop taking the study drug immediately. Your Study Doctor will ask you about the outcome of your pregnancy.

Men cannot take part in this study if they are actively trying to get their sexual partner pregnant. During this study if you think your partner is pregnant you must tell the Study Doctor immediately. Your Study Doctor will ask you about the outcome of your partner's pregnancy.

Risks of MRI
MRI examination releases radio waves, which are very noisy. We will give you earplugs. You may experience brief claustrophobia (fear as a result of being in a small, enclosed space) during the MRI procedure. Some patients feel discomfort associated with lying still within the magnet. If you need it, the doctor can give you some medication to make you less anxious and help you relax.

Because MRI uses strong magnets, people with cardiac pacemakers, certain artificial heart valves, metal plates, pins, or other metallic objects in your body (including gun shot debris or shrapnel) or other metallic/electronic material in their body cannot undergo MRI and will not be eligible for this study.

Risks of Unexpected Findings
You will have several MRIs during this study. MRIs are generally not performed in patients with progressive MS. The MRIs done for this study will not affect the care you receive for your disease. The study MRIs are being done to learn more about the effects of the study drug on the brain.

It is possible these MRIs could show us something that looks like an abnormality or problem. If we do see something that looks like a medical problem, we will ask a radiologist (a doctor who specializes in x-rays/scans/test

results of this sort) to review the results. If the radiologist thinks there might be a problem, we will tell you and help you get follow-up care.

If the radiologist thinks that you might have a medical problem, but it turns out that you don't, we may have caused you to worry needlessly about your health.

Risks of Blood Draw
You may have a bruise (a black and blue mark) or pain where we take the blood samples. There is also a small risk of feeling lightheaded, fainting, or infection.

Risks of Optional Spinal Tap
Spinal tap is a standard procedure used in medical practice. When spinal fluid is removed during a spinal tap, the risks include headache, bleeding and pain at the site where the needle was put in, and infection. Pain during the spinal tap procedure will be prevented or minimized by using local anesthesia (lidocaine). Infection after a spinal tap is very rare, but serious, and would be treated with antibiotics.

About 1 out of 3 people who have a spinal tap develop a post-spinal tap headache. Headache can occur if the lining around the spinal fluid (dura) is torn and some of the fluid leaks out. Post-spinal tap headaches are more common in females and in people less than 30 years old. This headache can be mild to severe. You may also have nausea, dizziness and ringing in the ears.

If you develop a headache, you will need to lie down to reduce the headache pain and symptoms. Post-spinal tap headaches get worse when you are sitting or standing. Occasionally, the headache may be severe enough to interfere with your normal daily activities, such as going to work or school. If this happens, there are no plans to pay you for time missed from work or school or for other costs, such as paying for a babysitter.

If you get a headache, you should contact [ENTER PI NAME], who is in charge of this study. Pain medication will be given to you, if needed. If the headache lasts more than three days, a procedure called a blood patch may be performed. This procedure involves taking blood from your arm and injecting it in the same place where the spinal needle was put in during the spinal tap. The clotting of the blood in this space should stop further fluid leaking and stop the headache.

Bleeding may occur in 1-2% of patients that undergo spinal tap. If you are currently taking blood thinner medications you may be at a higher risk of bleeding and will not be eligible to undergo spinal tap.

Do you agree to let us do a spinal tap at the Screening Visit and the Week 48 and Week 96 Visits?

☐ YES ☐ NO Initials_____

You can change your mind about this later. If you change your mind, tell the Study Doctor.

What are the possible benefits from being in this research study?

You may not experience any medical benefit from taking part in this research study. While you are in this study, your MS may improve, but it is also possible that your MS may remain the same, or get worse.

What we learn from this study may help to identify a new therapy for progressive MS patients and/or identify new tools to monitor MS disease progression in progressive MS patients. Others with progressive MS may benefit in the future from what we learn in this study.

What other treatments or procedures are available for my condition?

You do not have to take part in this study to be treated for your progressive MS. Currently, mitoxantrone is the only FDA-approved therapy for secondary progressive MS, but is not commonly used because of its risks of heart injury and blood cancers (i.e., leukemia). Some symptoms of progressive MS may be helped by exercise, stretching, and physical and occupational therapy. There are also medications which may be used to treat some symptoms of progressive MS such as bladder and bowel urgency (an urgent need to go to the bathroom), erectile problems, spasticity, and pain, if such treatments are needed. You can talk to the Study Doctor and your own doctor about other treatments and procedures that are available for progressive MS, including other research studies.

Can I still get medical care within {Insert Site Name} if I don't take part in this research study, or if I stop taking part?

Yes. Your decision won't change the medical care you get from us now or in the future. There will be no penalty, and you won't lose any benefits you receive now or have a right to receive.

Taking part in this research study is up to you. You can decide not to take part. If you decide to take part now, you can change your mind and drop out later. We will tell you if we learn new information that could make you change your mind about taking part in this research study.

What should I do if I want to stop taking part in the study?

If you take part in this research study, and want to drop out, you should tell us. We will also talk to you about follow-up care, if needed. We will make sure that you stop the study safely. If you decide to stop taking the study drug, you will be asked to come in for visit 13, which should occur approximately 4 weeks after you stop taking study drug. You will be asked to continue following within study, even though you have discontinued study drug.

It is possible that we will have to ask you to drop out before you finish the study. If this happens, we will tell you why. We will also help arrange other care for you, if needed.

Will I be paid to take part in this research study?

Yes. We will pay you $50 for each in-person study visit, including any relapse visits, $25 for each lab re-check visit, and $100 for each spinal tap. We will pay you approximately $700 over the two-year study period if you complete all the in-person visits for this study but do not complete the 3 optional spinal taps, or $1,000 if you complete all the in-person visits for this study and do complete the 3 optional spinal taps.

We may also use your samples and information to develop a new product or medical test to be sold. The sponsor, hospital, and researchers may benefit if this happens. You will not be given any additional payments if this happens.

What will I have to pay for if I take part in this research study?

Study funds will pay for study related tests, procedures, and visits. One of the study sponsors (MediciNova Inc.) will provide the study drug at no cost to you. The cost of routine tests and services that would normally be performed even if you don't take part in the study will be billed to you or your insurance provider. You will be responsible for payment of any deductibles and co-payments required by your insurer for this routine care or other billed care. If you have any questions about costs to you that may result from taking part in the research, please speak with the Study Doctors and study staff. If necessary, they will arrange for you to speak with someone in the **{Insert Site Name}** Patient Financial Services about these costs.

What happens if I am injured as a result of taking part in this research study?

We will offer you the care needed to treat any injury that directly results from taking part in this research study. We reserve the right to bill your insurance company or other third parties, if appropriate, for the care you get for the injury. We will try to have these costs paid for, but you may be responsible for some of them. For example, if the care is billed to your insurer, you will be responsible for payment of any deductibles or co-payments required by your insurer.

Injuries sometimes happen in research even when no one is at fault. There are no plans to pay you or give you other compensation for an injury, should one occur. {**Insert any site-specific injury statement or compensation here.}** However, you are not giving up any of your legal rights by signing this form.

If you think you have been injured or have experienced a medical problem as a result of taking part in this research study, tell the person in charge of this study as soon as possible. The researcher's name and phone number are listed in the next section of this consent form.

If I have questions, concerns or complaints about this research study, whom can I call?

You can call us with your questions, concerns or complaints. Our telephone numbers are listed below. Ask questions as often as you want.

{Insert name and academic degrees} is the person in charge of this research study. You can call him/her at **{Insert phone number} {insert when person is available M-F 9-5 or 24/7}**. You can also call **{Insert name(s) of local IRB or institutional contact}** at **{Insert phone number(s)} {insert when each person is available M-F 9-5 or 24/7}** with questions, concerns or complaints about this research study.

If you have questions about the scheduling of appointments or study visits, call **{Insert name(s)}** at **{Insert phone number(s)}**.If you want to speak with someone **not** directly involved in this research study, please contact the NeuroNEXT Central IRB (the Partners HealthCare System Human Research office) in Boston Massachusetts. The Central IRB is the ethics board that oversees the research conducted by NeuroNEXT. You can call them at 617-424-4100.

You can talk to them about:

- Your rights as a research subject
- Your concerns about the research
- A complaint about the research

Also, if you feel pressured to take part in this research study, or to continue with it, they want to know and can help.

If I take part in this research study, how will you protect my privacy?

During this research, identifiable information about your health will be collected. In the rest of this section, we refer to this information simply as "health information." In general, under federal law, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use, and share your health information for research and why they may need to do so.

In this study, we may collect health information about you from:

- Past, present, and future medical records
- Research procedures, including research office visits, tests, interviews, and questionnaires

Who may see, use, and share your identifiable health information and why they may need to do so:

- Research staff involved in this study
- Non-research staff within the institution who need this information to do their jobs (such as for treatment, payment (billing), or health care operations
- The sponsor(s) of this study, and the people or groups it hires to help perform this research
- MediciNova, Inc., one of the sponsors of this study
- Other researchers and medical centers that are part of this NeuroNEXT clinical study (NeuroNEXT Clinical Study Sites) and their ethics boards
- Partners HealthCare System, Inc. ("Partners"), Brigham and Women's Hospital and Massachusetts General Hospital ("MGH") and their ethics boards (the NeuroNEXT Central IRB)
- MGH (the NeuroNEXT Clinical Coordinating Center)
- University of Iowa (the NeuroNEXT Data Coordinating Center)
- The University of Rochester (a university center that will provide pharmacy and laboratory services for the study)
- The Cleveland Clinic (the site of the Principal Investigator of the study)
- A group that oversees the data (study information) and safety of this research
- People from organizations that provide independent accreditation and oversight of hospitals and research
- People or groups that we hire to do work for us, such as data storage companies, insurers, and lawyers
- Federal and state agencies (such as the Food and Drug Administration, the Department of Health and Human Services, the National Institutes of Health, and other US or foreign government bodies that oversee or review research)
- Public health and safety authorities (for example, if we learn information that could mean harm to you or others, we may need to report this, as required by law)
- Other: The organizations mentioned below will be provided some of the data and specimens collected in this study in a de-identified form (meaning that any information that can identify you will be removed and the sample will only contain a code).
    - NeuroRx, a centralized organization that has been selected to read MRI scans.
    - Cardiocore, an organization that has been selected to read ECG scans.
    - Angiography Reading Center/Digital OCT Reading Center, an organization that has been selected to read OCT scans.
    - Blizard Institute of London, England, an organization that has been selected to do cerebrospinal fluid analysis.

Some people or groups who get your health information might not have to follow the same privacy rules that we follow. We share your health information only when we must, and we ask anyone who receives it from us to protect your privacy. However, once your information is shared outside our institution, we cannot promise that it will remain private.

Because research is an ongoing process, we cannot give you an exact date when we will either destroy or stop using or sharing your health information.

The results of this research study may be published in a medical book or journal, or used to teach others. However, your name or other identifying information **will not** be used for these purposes without your specific permission.

Your Privacy Rights

You have the right **not** to sign this form that allows us to use and share your health information for research; however, if you don't sign it, you can't take part in this research study.

You have the right to withdraw your permission for us to use or share your health information for this research study. If you want to withdraw your permission, you must notify the person in charge of this research study in writing. Once permission is withdrawn, you cannot continue to take part in the study.

If you withdraw your permission, we will not be able to take back information that has already been used or shared with others.

You have the right to see and get a copy of your health information that is used or shared for treatment or for payment. To ask for this information, please contact the person in charge of this research study. You may only get such information after the research is finished.

Informed Consent and Authorization

Statement of Study Doctor or Person Obtaining Consent

- I have explained the research to the study subject.
- I have answered all questions about this research study to the best of my ability.

_____ _____

Study Doctor or Person Obtaining Consent Date/Time

Statement of Person Giving Informed Consent and Authorization

- I have read this consent form.
- This research study has been explained to me, including risks and possible benefits (if any), other possible treatments or procedures, and other important things about the study.
- I have had the opportunity to ask questions.
- I understand the information given to me.

Signature of Subject:

I give my consent to take part in this research study and agree to allow my health information to be used and shared as described above.

_____ _____

Subject Date/Time

Consent of Non-English Speaking Subjects Using the "Short Form" in the Subject's Spoken Language

Statement of Hospital Medical Interpreter

As someone who understands both English and the language spoken by the subject, I interpreted, in the subject's language, the researcher's presentation of the English consent form. The subject was given the opportunity to ask questions.

_____ _____

Hospital Medical Interpreter Date/Time

OR
Statement of Other Individual (Non-Interpreter)

As someone who understands both English and the language spoken by the subject, I represent that the English version of the consent form was presented orally to the subject in the subject's own language, and that the subject was given the opportunity to ask questions.

_____ _____

Name Date/Time

Appendix 5: DSMB Guidelines

Data and Safety Monitoring Board for the Network of Excellence in Neuroscience Clinical Trials (NeuroNEXT)

Data and Safety Monitoring Board Guidelines

1. Introduction

The Data and Safety Monitoring Board will act in an advisory capacity to the National Institute of Neurological Disorders and Stroke (NINDS) to monitor participant safety, data quality, and to evaluate the progress and overall conduct of NeuroNEXT-supported trials.

DSMB monitoring is required for trials which may modify the current standards of treatment or public health policy, result in the licensing of a therapeutic agent or device, or extend approved indications to new groups of patients. This includes Phase III clinical trials and possibly some earlier phase trials (eg, this could include trials that involve multiple sites, pose significant risk to participants, are conducted in vulnerable populations, or use certain controversial interventions).

2. Responsibilities

The DSMB is responsible for assuring the NINDS that study participants are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to high scientific and ethical standards. Specifically, the DSMB will:

- Review the research protocol, informed consent documents and plans for safety monitoring and advise the NINDS on readiness to begin enrollment
- Assess the performance of the trial with respect to participant recruitment, retention and follow-up, protocol adherence, and data quality and completeness, in order to ensure the likelihood of successful and timely trial completion.
- Review the statistical analysis plan, including the interim analysis plan, stopping rules and randomization scheme.
- One DSMB member, when possible a clinician, may be unblinded to treatment assignment. Monitor interim data regarding the safety and efficacy of the study regimens, so that the trial will be concluded as soon as there is convincing evidence of the treatment effects. Review abstract and publications of main findings prior to submission to ensure the study is being reported appropriately.
- Review and consider any protocol modifications or ancillary studies proposed by the study investigators after the main trial begins to ensure that these do not negatively impact on the main trial. For example, addition of an ancillary study could burden the study participants so much that they are likely to discontinue participation in the trial. Protocol modifications will be considered in the context of their potential impact on scientific integrity and participant safety. The DSMB will be responsible for the data and safety monitoring of the ancillary study.
- Monitor recruitment progress.
- Monitor lost to follow-up.
- Review data completeness and quality.
- Monitor missing data.

- Recommend planned adaptations based on pre-specified plans and decision rules.
- Advise the NINDS and the study investigators as to whether a protocol should continue as scheduled or undergo a modification due to findings that emerged as a result of the monitoring process.
- Consider factors external to the study when relevant information becomes available, such a scientific or therapeutic developments that may have an impact on the safety of participants or the ethics of the trial.
- Review abstract and publication of main findings prior to submission to ensure the study is being reported appropriately.

Enrollment in a study cannot begin until the DSMB's recommendation for approval to begin has been accepted by NINDS and IRB approval has been obtained at participating sites.

3. Membership

The DSMB is appointed by and advisory to the NINDS. The voting members may include physicians, laboratory scientists, statisticians, ethicists and patient advocates. Collectively, they will have appropriate expertise in the relevant scientific and safety monitoring areas. The precise number and areas of expertise of DSMB members will be dictated by the complexity of the study. Study investigators may suggest to the NINDS appropriate individuals to serve on the DSMB. NINDS representatives will participate on the DSMB as non-voting members.

The number of DSMB members for each NeuroNEXT trial may vary depending on the size, disease, and complexity of the trial and could range from three to five or more members. It is expected that DSMB members attend every meeting and every call. However since that might not always be possible, a quorum is considered to be half of the standing members plus one. The Board may wish to decide if particular expertise is needed within the quorum for the meeting to be valid.

To avoid any appearance of conflict of interest, it is critical that DSMB members not be involved in the studies have no vested interest in their outcome, have no ties to the study investigators (eg, not from the same institution and no history of extensive collaboration), and have no financial ties to any commercial concerns likely to be affected by the studys' outcome. If at any time a DSMB member perceives that he/she or another member of the Board (including an NINDS representative) has a potential conflict of interest, he/she is obligated to bring the issue to the attention of the full DSMB for open discussion and resolution. The NINDS requires DSMB members to complete a conflict of interest disclosure form and a statement of confidentiality on an annual basis.

4. Initial Meeting

Before any study is opened to subject accrual, the DSMB will meet in conjunction with the study prinicipal investigator (PI) and study statistician to review the study protocol, particularly the specific outcome definitions, the analysis plan, the procedures for recording and reporting SAEs, the monitoring proposal, pre-specified interim analysis plan and decision rules, and pre-specified plans for adaptation and decision rules. The informed consent document/process also will be inspected to ensure that all required elements have been included in language understandable to a typical study participant to be enrolled in the trial. It is possible that the DSMB will recommend modification or clarification of the protocol, especially relating to the monitoring plan. A carefully considered, final monitoring plan is important to establish at the outset, because any subsequent deviation from the pre-specified plan may diminish the scientific integrity and credibility of the study.

During the initial DSMB discussion for each trial, the Board will formulate its operating procedures. These procedures will include such issues as: the types and formats of reports it will receive from the PI and study statistician; the policy on whether and how the members may be unblinded; what interim data (if any) may be released to the study investigators (eg, overall event rate); and how SAEs will be submitted for DSMB review.

The DSMB decides in their first meeting if DSMB members will be unblinded. If the DSMB decides to remain blinded, they should consider assigning one DSMB member, when possible a clinician, to be unblinded to treatment assignment. The unblinded DSMB member may decide to unblind other DSMB members as indicated, and for example based on concerns over SAE imbalances between study groups

5. Meeting Frequency and Format

The DSMB will meet regularly to monitor the cumulative safety data during the period when participants are receiving study intervention and during the participant follow-up period. Meetings are generally held four times a year to review and discuss new and ongoing studies and will be generally scheduled in February, May, August and November each year. Additional meetings or conference calls will be scheduled as needed should participant safety questions or other unanticipated problems arise. Up to two meetings annually will be held in-person in the Washington, DC area. Typically, the DSMB will review each ongoing trial at least twice a year; in no instance should more than 12 months elapse between DSMB reviews of cumulative safety data after the first subject has enrolled. The DSMB responsibilities for monitoring and oversight conclude when the study is done and data has been verified and ready for publication.

The NINDS DSMB meeting format consists of three sessions: Closed Executive Sessions, Open Sessions, and Optional DSMB Sessions with Program Official. The meeting format, including the number of open and closed sessions, and the participation in these sessions is at the discretion of the DSMB. The DSMB Chair, in conjunction with the NINDS DSMB Liaison, is responsible for the DSMB operations and will set the meeting agenda. Before each meeting, the DSMB Liaison will ask all DSMB members to state whether they have developed any new conflicts of interest since the last formal report to the NINDS.

Closed Executive Sessions: Only DSMB members and NINDS DSMB Liaison participate in closed executive sessions, to ensure complete objectivity as they discuss outcome results by treatment arm as needed, make decisions, and formulate recommendations regarding the study. The DSMB Chair may request additional participants during this session, eg, MSM, unblinded study statistician.
Open Sessions: DSMB members, NINDS Staff, study PIs and study statistician(s) attend this session, at which data concerning study conduct and aggregate safety data are discussed.
Optional DSMB Sessions with Program Official: DSMB members, NINDS DSMB Liaison, and NINDS Administrative PD attend this session. During this session no unblinded data (closed report) will be discussed, but other trial issues, such as recruitment, can be discussed in the absence of the investigators.

6. Interim Data Reports

For each trial, the format and reporting requirement of unblinded data should be discussed and agreed upon at the first DSMB meeting. In general, the study statistician will prepare study data reports and send these to the NINDS DSMB Liaison at least 14 days prior to the meeting. These materials will be reviewed for completeness and forwarded to the DSMB members. These reports will contain the most up-to-date data permitted by the timeframe necessary for the statistician to prepare and review the analyses.

Interim data reports will usually consist of two parts, corresponding to the Open and Closed Sessions of the DSMB meeting. Only the DSMB members will receive copies of the Closed Session report, and at the conclusion of the meeting the statistician or the NINDS representative will collect all copies of the report.

The Open Session report will focus on study participant accrual and demographics, data completeness, and other study performance measures, any new information (on the intervention or disease/disorder) that may affect the outcome of the trial, and a list of publications or presentations. All data in the Open Session report will be presented in the aggregate, ie, not by treatment assignment. The Closed Session report will divide study participants according to cumulative data or coded treatment assignment (eg, Treatments A vs. B), comparing participant demographics and baseline characteristics, rates of and reasons for treatment discontinuation and loss to follow-up, rates of SAEs and, if an interim efficacy analysis is scheduled, rates of efficacy outcomes (depending on the trial).

Typically, the PI will have prepared a report addressing specific concerns he or she anticipates the DSMB will have regarding the conduct of the study. This report should be sent to the NINDS Liaison for distribution to the DSMB along with the Open Session report as noted above. Likewise, the study statistician's report for the Closed Session will usually contain his or her assessment of the progress of the trial, including recommendations on whether it should be terminated or modified. Interim data reports will generally include the following types of information, although only the Closed Session data reports will include comparisons by treatment group. If the randomization is stratified (eg, by age), these tables and figures are presented by strata:

- A summary of monthly accrual and cumulative accrual, overall and by clinical center, compared to targets.
- A summary of baseline characteristics, overall and by treatment group.
- A summary of the completeness and quality of data collection forms.
- A summary of the status of enrolled participants, overall and by treatment group. (Study status includes whether the subject is on study or off study. For participants who are on study, there should be an indication as to whether they are on study treatment or off treatment. For participants who are off study, the reason should be indicated (ie, completed study, died, refused further participation, lost-to-followup, or other).
- Summaries of participants off treatment, including a listing by subject ID number of those who have permanently discontinued study treatment and summaries (overall and by treatment group) of the reasons for going off treatment, the proportion of participants off treatment prior to reaching the study outcome, and the proportion of participants going off treatment each study month.
- Assessments of whether the clinical centers have followed eligibility criteria and other protocol requirements.
- An assessment (eg, based on pill counts or diaries) of subject adherence to the treatment regimen, overall and by treatment group.
- A summary of outcome rates by treatment group, if an interim efficacy analysis is scheduled.
- A listing of individual SAEs by subject ID number and a table of event-specific cumulative rates, overall and by treatment group.
- A listing of AEs by treatment group and body system.
- A listing of protocol violations, if any.

An outline for the DSMB Report is available on the NINDS website:
http://www.ninds.nih.gov/research/clinical_research/policies/dsmb_outline.htm

SAE Reporting: see protocol section 11.6.1 for SAE reporting.

Planned Interim analyses: see section 12.1.4 for interim analyses plan.

7. Communication of DSMB Recommendations

At the conclusion of each DSMB meeting, the DSMB will provide a verbal report to the study PI indicating areas of concern regarding performance and safety. The DSMB must not communicate any information that could lead to the unblinding of investigators or suggest interim treatment-specific results.

The DSMB Chair will provide meeting minutes, including the DSMB's recommendations to the Director of the NINDS Office of Clinical Research, through the NINDS DSMB Liaison. DSMB recommendations need not be generated by consensus of all DSMB members, and should include the views of all members if there is disagreement about a particular issue. The NINDS OCR Director will determine if the NINDS concurs with the DSMB's recommendations. The NINDS OCR Director or designee will communicate concurrence or not with the study PI. The NINDS DSMB Liaison will provide the DSMB minutes and recommendations to the study PI, along with a memorandum documenting: (a) the date of the review; (b) that all relevant interim safety and efficacy data were reviewed; (c) recommendations concerning the study execution or modifications to the study protocol; and (d) the anticipated date of the next review. The Principal Investigator will promptly forward a copy of this memorandum to each participating study investigator for submission to their local IRBs, pursuant to the NIH's Guidance on Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multicenter Clinical Trials (release date: June 11, 1999, http://grants.nih.gov/grants/guide/notice-files/not99-107.html).

If the DSMB recommends an amendment to the protocol, it must be approved by the NINDS representative, the IRBs and, for IND or IDE studies, the FDA. NINDS concurrence is required because some decisions may have significant programmatic implications. For example, a decision to extend the duration of a trial or increase the sample size has implications for the NINDS budget.

If as a result of interim data monitoring the DSMB determines that a trial: (a) has answered the primary study question; (b) is futile or will not be able to reach a firm conclusion; (c) cannot recruit participants within a reasonable timeframe (as determined by the NINDS); (d) is not being conducted according to high scientific or ethical standards; or (e) poses an unreasonable or unnecessary risk to study participants, the DSMB will recommend to the NINDS representative that the study protocol be amended, temporarily suspended, or terminated, as appropriate. If the NINDS OCR Director concurs, this recommendation will be conveyed to and discussed with the PI before any action is taken. It will be important to ensure that the PI understands the DSMB's rationale. In addition, prior to a public announcement of a trial's early termination, a plan should be developed and implemented for notifying the study investigators, the IRBs and the study participants. When a study is conducted under an IND or IDE, the FDA and the involved biopharmaceutical companies or device manufacturers must also be notified. A decision to modify or terminate a trial may influence the conduct of another, similar clinical trial and the NINDS may arrange to debrief that trial's study investigators or its DSMB in advance of the public announcement.

8. Communications from Study Investigators

Communication with DSMB members will be primarily through the NINDS DSMB Liaison. Neither the investigators nor the DSMB members should directly communicate on any study-related issues outside of the DSMB meetings (including protocols, procedure manuals, reports, recommendations). The study PI will typically perform ongoing monitoring of study implementation parameters (eg, recruitment, follow-up, compliance) in order to manage day-to-day study aspects and ensure quality control. These data reviews do not involve treatment group comparisons. If in the course of such monitoring, the investigator uncovers issues that may threaten the integrity of the study or subject safety (eg, excessive dropout rate or unexpectedly high rate of adverse events), he or she should alert the NINDS DSMB Liaison who will consult with the DSMB Chair as to whether a special meeting or conference call of the Board should be held. Except as explicitly authorized by the DSMB, it is critical that study investigators will remain blinded to the interim data because knowledge of emerging trends between treatment arms may influence participant enrollment, management and evaluation, thus compromising the study by introducing bias.

In addition, for clinical trials funded in whole or in part by the NINDS and involving an IND or an IDE (regardless of who the official sponsor is), a participating study investigator is obligated to inform the NINDS of any significant communication from the FDA concerning the trial, including warning letters, investigator disqualification notices, clinical holds, etc., within 72 hours of first learning of this FDA communication. Formal notification should be made in writing, but initial notification may be done by telephone if a written notice would delay the notification. It should include a statement of the action taken or contemplated and the assistance needed to resolve the situation. This policy is detailed in the Notice to NIH Grantees/Contractors Regarding Letters or Notices from the Food and Drug Administration (FDA) (release date: September 22, 2000, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-053.html). The NINDS will bring the matter to the attention of the DSMB.

9. Medical Safety Monitor and Safety Monitoring by the DSMB

The NINDS requires that each DSMB-monitored trial have an assigned **Medical Safety Monitor (MSM)**, independent of the study investigators, who is responsible for review of individual serious adverse events (SAEs) as they occur, and regular reporting of SAEs to the DSMB and others, as appropriate. Some SMC's may also identify a MSM to review SAEs.

Each multi-center clinical trial supported by the NINDS will have an independent Medical Safety Monitor (MSM), nominated by the principal study investigator before subject enrollment begins and subsequently approved by the NINDS Program director (PD). The Medical Safety Monitor is a physician who is not involved in the study and who has no conflict of interest. The Medical Safety Monitor is responsible for ongoing monitoring of reports of SAEs submitted by the clinical centers in real time to ensure good clinical practice and to quickly identify safety concerns. The Medical Safety Monitor may suggest protocol modifications to prevent the occurrence of particular adverse events, eg, modifying the protocol to require frequent measurement of laboratory values predictive of the event or to improve expeditious identification of SAEs. To minimize bias, the Medical Safety Monitor will usually evaluate SAEs blinded to treatment assignment, unless the DSMB/SMC approves partial or complete unblinding. Specific procedures for Medical Safety Monitor activities will necessarily vary from trial to trial in order to protect the safety of participants. For selected trials, the Medical Safety Monitor may serve as a resource to the clinical investigators for advice about management of SAEs but may not be involved in other aspects of the trial.

The Medical Safety Monitor will prepare regular reports concerning SAEs (not segregated by treatment group) for submission to the principal study investigator, the DSMB and, as appropriate, the FDA and collaborating biopharmaceutical companies or device manufacturers. Typically, such

reports will be submitted on a regular basis, to be determined by the DSMB, for example real time, monthly or quarterly.

In the event of unexpected SAEs or an unduly high rate of SAEs, the Medical Safety Monitor will promptly contact the principal study investigator and the NINDS Program Director (PD) and if applicable, NINDS DSMB representative, who will notify the DSMB Chair. The DSMB may convene a meeting or teleconference of the Board to consider the concerns and plan appropriate action.

The DSMB could consider recommending that the trial go on hold if a clear imbalance in the rates of a serious adverse event emerged between the treatment groups. The DSMB would discuss whether to recommend that the study should 1) proceed with additional monitoring, 2) proceed with protocol modifications, 3) be placed on hold, or 4) should be terminated.

In the event that the MSM is unavailable for an extended period of time (ie, extended vacation, sabbatical, illness, etc.) a back-up MSM will be nominated by the study PI and approved by the study PD. The responsibilities of the MSM are worked out between the Steering Committee and DSMB in advance of starting the trial.

10. DSMB Review of Study Publications

The DSMB will have the opportunity to review and comment on all study manuscripts and abstracts prior to submission.

NN102

Statistical Analysis Plan

A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Activity of Ibudilast (MN-166) in Subjects with Progressive Multiple Sclerosis

Robert J. Fox, MD FAAN
Mellen Center for Multiple Sclerosis
The Cleveland Clinic
Protocol Principal Investigator

Christopher S. Coffey, PhD
University of Iowa Data Coordinating Center
Principal Investigator

Merit E. Cudkowicz, MD
Massachusetts General Hospital Clinical Coordinating Center
Principal Investigator

VERSION 1.1
September 05, 2017

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

NN102

[SPRINT-MS: A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Activity of Ibudilast (MN-166) in Subjects with Progressive Multiple Sclerosis]

Principal Investigator Approval

Signature: _____ **Date:** _____

Name: Robert J. Fox, MD, FAAN

NeuroNEXT Clinical Coordinating Center Approval

Signature: _____ **Date:** _____

Name: Merit Cudkowicz, MD

NeuroNEXT Data Coordinating Center Approval

Signature: _____ **Date:** _____

Name: Christopher S. Coffey, PhD

SUMMARY OF CHANGES

SEPTEMBER 2016 - VERSION 1.0

- Initial version finalized prior to presenting first interim efficacy and futility analysis for the primary endpoint to the DSMB (first unblinded data presented to the DSMB).

SEPTEMBER 2017 - VERSION 1.1

- Modified language in sections 2.2 and 7.2.1 in order to better clarify distinction between treatment-emergent and treatment-related AEs/SAEs
- Corrected language in XXX to clarify that analyses will be based on actual strata in situations where a subject was mis-stratified due to incorrect information at the time of randomization

PREFACE

This Statistical Analysis Plan (SAP) describes the planned analyses for the NeuroNEXT NN102 (SPRINT-MS) study [National Institute of Neurological Disorders and Stroke (NINDS) grant # U01NS082329]. The planned analyses identified in this SAP are intended to support the completion of the Final Study Report (FSR) and will be included in regulatory submissions and/or future manuscripts. All interim analyses will involve only the primary study endpoint, and will be performed once the specified number of randomized subjects have completed the full study period. All final, planned analyses identified in this SAP will be performed only after the last randomized subject has completed the full study period. Once all data have been cleaned and verified, a "locked" version of the data will be used for reporting the final study results. Key statistics and study results will be made available to the Protocol Principal Investigator (PPI) following database lock and prior to completion of the final FSR.

1. STUDY DESIGN

This is a multicenter, phase II, randomized, double-blind, placebo controlled, parallel group study designed to generate proof-of-concept evidence to evaluate the activity of Ibudilast (MN-166) on imaging measures of brain atrophy and tissue integrity, to evaluate the safety and tolerability of Ibudilast over two years, and to identify imaging markers for measuring biologic activities of potential therapies in progressive MS. A total of 250 male and female subjects from 21 to 65 years old, inclusive, will be enrolled into two treatment groups across sites within the NeuroNEXT network. Subjects will be randomized in a 1:1 fashion to receive either Ibudilast (100 mg/day; n = 125) or matching placebo (n = 125). Randomization of subjects will be stratified by disease status [primary progressive MS (PPMS) or secondary progressive MS (SPMS)] and immunomodulation therapy [untreated with long-term MS disease modifying therapy or receiving either glatiramer acetate (GA) or interferon beta (IFNβ-1a: Avonex, Rebix or IFNβ-1b: Betaseron, Etavia) treatment]. Study drug will be administered either two times per day (i.e., MN-166 50 mg or placebo taken in the morning and evening) or three times per day, depending on subject's tolerance to study drug. The study will consist of a screening phase (up to 45 days), followed by a treatment phase (96 weeks), and a follow-up visit (1 month post week 96 visit). Thus, subjects will be involved for approximately 100 weeks in the various phases of the study.

During the screening phase, subjects will be assessed for study eligibility. The baseline visit must occur within 45 days following the screening visit. At the baseline visit (day 1), subjects who have completed all of the screening assessments and continue to meet eligibility criteria will be randomized to one of two treatment groups and will take 3 capsules of study medication on the evening of day 1. On the morning of day 2, all subjects will begin a three capsule BID (twice daily) dosing regimen through day 14. Subjects randomized to MN-166 will start at 60 mg/day (30 mg BID) and will remain on 60 mg/day through day 14. Beginning on day 15, all subjects will begin a 5 capsule BID regimen; those randomized to MN-166 will therefore be taking 100 mg/day (50 mg BID). After day 15, subjects with intolerable side effects (e.g., nausea, diarrhea, vertigo) may reduce their dose to either 4 capsules twice a day (80 mg/day for those taking MN-166) or 3 capsules twice a day (60 mg/day for those taking MN-166). Subjects with intolerable side effects at the end of day 14 may continue taking 3 capsules twice a day at the Investigator's discretion. At the Investigator's discretion, the daily dose of Ibudilast can be changed between 3 capsules twice a day, 4 capsules twice a day, and 5 capsules twice a day over the first 8 weeks of treatment. At the end of the first 8 weeks of treatment, the subject must maintain their then-current daily dose of study medication (3 capsules twice per day, 4 capsules twice per day, or 5 capsules twice per day) for the duration of the trial. Additionally, at the

Investigator's discretion, the daily dose of Ibudilast may be divided and taken three times per day to improve tolerability. Subjects will return to the clinic for follow-up visits on a regular basis at weeks 4, 8, 12, 24, 36, 48, 60, 72, and 96. All subjects who complete the study on drug will return for a follow-up safety visit at week 100 (4 weeks after their last study visit) to assess general health and adverse event status.

Subjects who experience a relapse will return to the clinic within three days of notifying the Investigator, and will undergo assessments as described in Table 2 (Schedule of Unplanned Procedures and Assessments) of the study protocol.

Subjects who prematurely discontinue study medication will continue to be followed on a semi-annual basis until the end of the study. For such subjects, adverse events will not be collected post study drug discontinuation. Existing AEs will be followed until the AE resolves or stabilizes.

1.1 Primary Objectives

Primary Objective #1: *Evaluate the activity of Ibudilast (MN-166: 100 mg/day) versus placebo at 96 weeks as measured by quantitative MRI analysis for whole brain atrophy using brain parenchymal fraction (BPF).*

Primary Objective #2: *Evaluate the safety and tolerability of Ibudilast (MN-166) versus placebo*

1.2 Major Secondary Objectives

Major Secondary Objective: *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by:*
(1) *Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts*
(2) *Magnetization transfer ration (MTR) imaging in normal-appearing brain tissue*
(3) *Retinal nerve fiber layer thickness as measured by Optical coherence tomography (OCT)*
(4) *Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm (CLADA)*

Brain MRI will be performed in a standard fashion, as outlined in the MRI procedure manual. Each subject's baseline MRI must be approved by the MRI Reading Center prior to randomization. All sites will perform a test (or "dummy") MRI scan prior to study initiation. This test MRI will ensure adequate performance of the study MRI. It is required that the same MRI scanner be used to acquire all MRI scans over the course of the study. If during the study period a study site needs to change the MRI scanner or the current MRI scanner undergoes significant upgrades (hardware or software), the investigator should notify the Clinical Coordinating Center (CCC). For all changes in scanner and significant scanner upgrades, the investigator will be requested to obtain MRI scans of 3 volunteers acquired prior to changing/upgrading the MRI scanner and the same 3 volunteers again after changing scanners (a total of 6 scans). The site will be allowed to acquire trial subject scans on the new scanner only after obtaining approval of the new or upgraded scanner. Data will be captured in the study database flagging that a major scanner change/upgrade has occurred. However, all primary and secondary analyses will not attempt to adjust for the scanner change.

2. PRIMARY ENDPOINTS

2.1. Brain Parenchymal Fraction (BPF)

Using computerized image analysis software, brain volume loss is detectable at all stages of disease (Miller et al, 2002; Bermel & Bakshi, 2006). Atrophy is an indirect marker of destructive pathologic processes in MS, including the net effects of focal tissue damage in white matter and grey matter, but also diffuse pathologic processes in normal-appearing brain tissue. More than 20 studies show

significant cross-sectional correlations between whole brain atrophy and overall clinical disability (Fisher, 2011a; Fisher, 2011b). Whole brain atrophy (WBA) also correlates with cognitive impairment (Edwards et al, 2001; Rao et al, 1985; Hohol et al, 1997; Benedict et al, 2004; Lazeron et al, 2005), depression (Feinstein et al, 2004), fatigue (Marrie et al, 2005; Tedeschi et al, 2007), and quality of life (Janardhan & Bakshi, 2000; Rudick et al, 2001). Multivariate analysis suggests that disability correlates better with WBA than with lesion measures (Benedict et al, 2004). WBA progresses over the course of MS, with a mean rate in untreated MS patients generally ranging from 0.5-1.5% per year (Fisher, 2011b). Although one small (n=21) early study found atrophy to slow in later disease (Fox et al, 2000), contemporary studies with larger sample sizes (n=69 and n=963) found similar or faster rates of atrophy in PMS compared with RRMS (Fisher et al, 2008; De Stefano et al, 2010). Atrophy progression in PPMS is similar to SPMS (Kalkers et al, 2001). Longitudinal studies found patients with greater rates of atrophy are more likely to worsen clinically (Dastidar et al, 1999; Molyneux et al, 2000; Fisher et al, 2000; Fisher et al, 2002; Dalton et al, 2004; Minneboo et al, 2008; Di Fillippo et al, 2010). For example, a longitudinal study found WBA progression is predictive of cognitive deterioration (Amato et al, 2007). These clinical correlations appear to be stronger later in the disease (i.e., SPMS compared to RRMS – Fisher et al, 2000; Ge et al, 2000). Altogether although additional treatment trial data is needed, face validity, concurrent validity, and predictive validity of WBA in PMS are significant enough to use it as the primary outcome metric in PMS trials. Based on accumulated evidence, an international consensus panel recommended WBA as the primary outcome of phase II PMS trials (Barkhof et al, 2009). Indeed, several recent PMS studies used WBA as the primary outcome, including trials evaluating lamotrigine (Huang et al, 2002) and simvastatin (Chataway et al, 2012). For currently available RRMS therapies, the treatment effects on atrophy range from 30%-50% compared to placebo, including a 34% slowing of atrophy in a phase II trial of Ibudilast in RRMS (Barkhof et al, 2010) and a 49% slowing over 2 years with simvastatin in SPMS (Chataway et al, 2012).

The primary outcome of this trial is WBA, estimated as the change in brain parenchymal fraction (BPF). BPF is a normalized measure of whole brain volume calculated as the volume of brain parenchymal tissue divided by the total volume within a smoothed outer surface of the brain (Rudick et al, 1999). The fully-automated algorithm takes partial volume effects into account, can utilize either FLAIR or dual echo PD-/T2-weighted images, and has a scan-rescan coefficient of variability < 0.2%. Sensitivity analyses for BPF include SIENA and an advanced SIENA technique that utilizes a single longitudinally co-registered image within each patient, which may further reduce variability.

For the purposes of this trial, BPF will be computed based on MRI scans performed at screening, and at weeks 24, 48, 72, and 96. The images will be processed, and reviewed for quality and completeness at the Cleveland Clinic MS MRI Analysis Center. Images will be checked for adherence to protocol, consistency over time, image quality, and completeness of the electronic transfer. This step determines if the MRI data set can be approved for BPF analysis or if it should be rejected, reacquired, or resent from NeuroRx. Once approved, the images will be set up to be analyzed. The image analysis pipeline automatically records numerical results in an internal database. Database records for which the atrophy results have been visually verified will be transmitted to the NeuroNEXT Data Coordinating Center (DCC) in accordance with the procedures outlined in the corresponding data transfer agreement.

2.2. Safety

The primary assessment of safety will compare the percentage of subjects in each group with:

- Treatment-emergent adverse events (TEAEs)
- Treatment-emergent serious adverse events (TESAEs)

For the purposes of this study, a treatment-emergent adverse event (AE) is any untoward medical occurrence in a study subject who was 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 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 or under placebo.

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

- 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 (de-challenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory re-challenge 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). Re-challenge 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 re-challenge information. Typically explained by extraneous factors (e.g., concomitant disease, environmental factors, or other medications or chemicals).

For the purposes of this study, primary interest involves an examination of treatmed-related AEs - defined as any AE deemed to be at least possibly related to study treatment. As this is a double-blind study, the causality assessment should be made under the assumption that the subject is receiving

active study medication. If considering unblinding, this assessment should be made prior to unblinding to avoid bias.

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

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.

Dr. Steven Krieger will serve as the Medical Safety Monitor (MSM) for this trial. Dr. Krieger will work closely with the DCC, and will use the online AE reporting system to review all reported SAEs in near real time and evaluate them to identify the need for timely intervention. For any reported SAEs, an automatic email will be sent to Dr. Krieger to prompt a review of the event for determination of whether the event is unanticipated and/or whether it is related to study drug. With the assistance of the coordinators at the DCC, Dr. Krieger has the option of requesting additional information about any SAE. He will complete a form for each review, and this information will be entered into the online data entry system.

Thus, in summary, the determination of whether an AE or SAE is treatment-related (at least possibly related to treatment) differs. Because the MSM only reviews SAEs in real-time, the determination of whether or not a non-serious AE is considered treatment-related will be made at the site level. However, for SAEs, the MSM determination of whether or not an SAE is treatment-related will take precedent over the classification at the site level.

2.3. Tolerability

The primary assessment of tolerability will compare the percentage of subjects in each group who:

- Discontinue treatment early (early study termination and/or early study drug withdrawal) due to treatment-emergent AEs or SAEs
- Discontinue treatment early (early study termination and/or early study drug withdrawal) for any reason

3. MAJOR SECONDARY ENDPOINTS

3.1. Diffusion Tensor Imaging (DTI)

Diffusion tensor imaging (DTI) quantifies the magnitude and direction of water diffusion. Areas of tissue injury have altered diffusion, thus offering a quantitative, dynamic measure of tissue integrity. The four common DTI metrics that describe water diffusion are axial diffusivity ($\lambda_{\parallel}$, diffusion along the length of a fiber tract), radial diffusivity ($\lambda_{\perp}$, diffusion across the breadth), mean diffusivity (MD, overall measure of diffusion), and fractional anisotropy (FA, degree of anisotropy of diffusion – Molyneux et

al, 2000; Fisher et al, 2000). Several animal and human studies suggest that $\lambda_{\parallel}$ roughly reflects axonal integrity and $\lambda_{\perp}$ roughly reflects myelin integrity (Song et al, 2002; Song et al, 2003; Song et al, 2005). DTI measures correlate with disability (Dalton et al, 2004; Amato et al, 2007). A longitudinal study of MS patients showed an increase in FA within acute MS lesions during recovery, with a concomitant decrease in $\lambda_{\perp}$ suggestive of possible remyelination (Fox et al, 2011). Furthermore, the magnitude of $\lambda_{\perp}$ as baseline predicted the evolution of lesions into T1 black holes (areas with more significant tissue injury) at 1 year (Fox et al, 2011). DTI is performed differently by different MRI manufacturers, which can yield different DTI values. However, with careful attention to pulse sequence implementation, we have demonstrated the ability to standardize image acquisition to yield DTI images with very similar values (Fox et al, 2012; Magnotta et al, 2012).

The DTI images will be processed at the Cleveland Clinic Mellen Center Research MRI Laboratory (Director: Mark Lowe). The images will be reviewed for quality and completeness as they are received by the lab. Images will be checked for adherence to protocol, image quality, and completeness of the electronic transfer. This step determines if the MRI dataset can be approved for DTI analysis or if it should be rejected, reacquired, or resent from NeuroRx. Once approved, the images will be analyzed. The image analysis pipeline results in numerical results recorded in a comma separated value (csv) text file. Cumulative results will be transmitted to the NeuroNEXT DCC in accordance with the procedures outlined in the corresponding data transfer agreement.

Based on this information, there will be two DTI outcomes under consideration, both measured separately on the left and right side:

- Axial Diffusivity (LD)
- Radial Diffusivity (TD)

3.2. Magnetization Transfer Ratio (MTR)

Magnetization transfer (MT) imaging is an indirect study of semi-solid tissue components such as cell membranes, whose T2 relaxation times are too short to be imaged directly (Hohol et al, 1997; Benedict et al, 2004). The MT effect is usually quantified by calculating voxel-by-voxel maps of the percent decrease in signal between images with and without MT pulse, thus producing a MTR image (Fillippi & Grossman, 2002). Decreased MTR correlates strongly with loss of myelin and axons within lesions are more pronounced in NAWM in SPMS (Feinstein et al, 2004; Marrie et al, 2005), and correlate with disability (Tedeschi et al, 2007). MTR is also abnormal in normal-appearing grey matter (NAGM) in MS (Ge et al, 2002; Fisniku et al, 2009; Fillippi et al, 2011), and recent MR/pathology correlation studies show that MTR can detect cortical demyelination (Chen et al, 2013). These findings suggest that whole-brain normal-appearing brain tissue (NABT) and gray matter MTR may be useful biomarkers of tissue damage in trials of neuroprotective therapies in MS.

A number of small, single-center studies of MS therapies have incorporated MTR. But, relatively few multi-center studies have done so, presumably because of the added complexity of standardizing acquisition protocols and performing a robust centralized analysis. Two randomized, placebo-controlled multi-center studies in SPMS (interferon beta-1b and IVIG) demonstrated annual changes in whole-brain measures of MTR of about 2.5%-3.5% in their respective placebo groups (Inglese et al, 2003; Fillippi et al, 2004). While neither study showed a benefit of treatment on MTR, the magnitude of change over time suggests that detecting a treatment effect on MTR for a neuroprotective agent in SPMS is feasible. The recent DEFINE BG-12 phase III trial in RRMS (analyzed by NeuroRX) included MTR imaging in 64 sites, with n=126-135 per treatment group. Using a similar sample size to our proposed study, a significant benefit on both whole-brain and

NABT MTR was seen in patients treated with BG-12 relative to placebo (Arnold et al, 2014). These results, combined with the ability to implement MTR imaging on most modern scanners with whole-brain coverage, high resolution, and high signal-to-noise ratio (SNR), suggest that MTR is both feasible and useful to deploy in a multi-center trial in progressive MS (Dastidar et al, 1999; Kalkers et al, 2001; De Stefano et al, 2010).

The MTR data will be transmitted to the NeuroNEXT DCC directly from NeuroRX in accordance with the procedures outlined in the corresponding data transfer agreement.

Based on this information, there will be two MTR outcomes under consideration:

- Median MTR in normal appearing brain tissue (NABT)
- Median MTR in normal appearing grey matter (NAGM)

3.3. Optical Coherence Tomography

Optical Coherence Tomography (OCT) non-invasively uses near-infrared light to measure the thickness of the retinal nerve fiber layer (RNFL) and macular ganglion cell layer (GCL), which comprise first-order sensory neurons for the visual pathway. Both RNFL and GCL decline proportional to disease progression in MS (even in the absence of overt visual systems, signs, or symptoms – Rudick et al, 1999) and brain atrophy (Gordon-Lipkin et al, 2007). OCT metrics correlate strongly with visual function (Walter et al, 2012) and patient-reported quality of life in MS (Mowry et al, 2009). Being able to selectively quantify the contributions of unmyelinated axonal (peripherally RNFL) vs. neuronal (macular GCL) pathology has substantial relevance for understanding the pathological processes in MS (Waxman & Black, 2007). Analogous to MRI, data from different OCT platforms are not equivalent; however, the change in RNFL thickness from the two main study OCT platforms (Zeiss Cirrus and Heidelberg Spectralis) can be combined in a clinical trial (Waxman et al, 2011). In summary, OCT may represent a simple, quick, non-invasive, and relatively inexpensive method to assess neurodegeneration and the potential benefit of putative neuroprotective therapies.

The OCT data will be transmitted to the NeuroNEXT DCC from the Digital Angiography Reading Center (DARC) lab in accordance with the procedures outlined in the corresponding data transfer agreement. For each subject, there is one record per eye.

For the purposes of this trial, the analysis will focus on a comparison of the mean retinal nerve fiber layer (RNFL) thickness. All subjects are assessed by either a Spectralis or Cirrus machine. For each machine, three separate measurements are obtained – unless the first two readings differ by more than 7.5%, in which case an additional reading is taken. The mean RNFL thickness (within each eye) is defined as the mean of the two variables that are closest to one another.

3.4. Cortical Longitudinal Atrophy Detection Algorithm (CLADA)

Additional atrophy measures include GM atrophy, which will be measured using two different approaches – GM faction (GMF) and cortical thickness (CTh). Cortical thickness is measured at each time point using CLADA, a longitudinal algorithm based on deformation of a patient-specific cortical model (Nakamura et al, 2011), and will be considered as an additional major secondary endpoint for this study. The cortical model is generated from longitudinal T1-weighted MPRAGE images. The point correspondence that define the distance between the inner and outer cortical surfaces are maintained throughout, leading to greater reproducibility. Change in CTh is calculated as the slope in CTh over time, with a scan-rescan variability of 0.45%.

The CTh data will be processed at the Cleveland Clinic MS MRI Analysis Center, and will part of the

same electronic data transfer containing the BPF data. In accordance with the procedures described in the corresponding data transfer agreement, the BPF analysis and verification will be completed first. After the MRI data for all study visits for a given subject have been received, the other atrophy measurements, GMF and CTh, will be measured and verified.

4. ENROLLMENT & RANDOMIZATION

Following the screening phase, subjects who continue to meet entry criteria will be enrolled and randomly assigned in a 1:1 manner to one of two treatment groups: MN-166 100 mg/d or matching placebo. A total of approximately 250 subjects will be randomized into the study. Randomization of subjects will be stratified by immunomodulation therapy status [untreated with long-term MS disease modifying therapy (DMT) vs. those receiving either glatiramer acetate (GA) or interferon beta (IFNβ-1a – Avonex, Rebif, or IFNβ-1b – Betaseron, Etavia)], and disease status [primary progressive MS (PPMS) vs. secondary progressive MS (SPMS)]. The NeuroNEXT DCC will generate a randomization table for each of the strata using a permuted block design with random block sizes.

5. PRELIMINARY TABULTIONS

All subjects who provide informed consent will be accounted for in this study. Regularly generated enrollment reports will describe:

- Number of subjects consented, eligible, and randomized by site
- Ongoing study status of all randomized subjects
- Reasons for ineligibility
- Protocol deviations
- Early drug withdrawals
- Early study terminations

Subject data will also be summarized by treatment group (MN-166 vs. placebo) with respect to important demographic characteristics. Distribution of categorical variables will be tabulated by treatment group. Continuous variables will be summarized as mean, median, standard deviation, minimum, and maximum by treatment group and overall. Variables to be collected will include:

- Gender
- Race
- Ethnicity
- Age
- Participation in lumber puncture study
- T2 lesion volume (CCs) at screening

6. ANALYSIS POPULATIONS

Due to the exploratory nature of this study, all analyses to address the primary and major secondary objectives will be conducted at the 0.10 significance level. The analysis population of interest will differ depending on the objectives of any particular analysis.

6.1. Primary and Major Secondary Efficacy Analyses

6.1.1. Modified Intent-to-Treat (mITT) Population

The primary analysis for all primary and major secondary efficacy objectives will be implemented using a modified intent-to-treat (mITT) approach, which is defined as all subjects who are:

- Randomized
- Received at least one dose of study medication
- Have at least one efficacy assessment (i.e., week 24, 48, 72, or 96 visit) during the double-blind phase of the study

All subjects meeting this criteria will be analyzed based on the treatment to which they were randomized, whether or not they remained compliant with respect to taking study medication.

6.1.2. Per Protocol (PP) Population:

To assess the sensitivity of the results, and to obtain knowledge regarding the potential effects when the protocol was strictly adhered to, we will also replicate all primary and major secondary efficacy objectives using a per protocol population. The per-protocol population includes the subset of all mITT subjects who satisfy both of the following conditions:

- Have 75% -125% compliance (both limit values inclusive) with assigned study medication as randomized in the double-blind phase
- Have no major protocol deviations (defined as any alteration/modification to the protocol that has the potential to negatively impact subject safety, integrity of the study, ability to draw conclusions from the study data, or affect the subject's willingness to participate in the study)

6.2. Safety/Tolerability Population

All safety/tolerability analyses will be implemented using an intent-to-treat approach, which is defined as all subjects who are randomized and receive at least one dose of study medication.

7. PRIMARY ANALYSES

7.1. ***Primary Objective #1:*** *Evaluate the activity of Ibudilast (MN-166: 100 mg/day) versus placebo at 96 weeks as measured by quantitative MRI analysis for whole brain atrophy using brain parenchymal fraction (BPF).*

The first primary objective of the proposed study is to evaluate the activity of Ibudilast (MN-166) versus placebo by assessing the difference in rates of change in brain parenchymal fraction (BPF) between the Ibudilast and placebo treatment groups as measured at baseline, weeks 24, 48, and 96 post randomization. The evaluation of activity is defined as the test of the null hypothesis that the BPF rates of change in the treatment and placebo groups are equal.

The raw data will be summarized graphically, and in the following table:

Table 7.1: Descriptive statistics of BPF over time

Visit	Ibudilast	Placebo
Baseline		
Mean (SD)	x.xx (xx)	x.xx (xx)
Min. – max.	x.xx-x.xx	x.xx-x.xx
missing	xx	xx
Week 24		
Mean (SD)	x.xx (xx)	x.xx (xx)
Min. – max.	x.xx-x.xx	x.xx-x.xx
missing	xx	xx
Week 48		
Mean (SD)	x.xx (xx)	x.xx (xx)
Min. – max.	x.xx-x.xx	x.xx-x.xx
missing	xx	xx
Week 72		
Mean (SD)	x.xx (xx)	x.xx (xx)
Min. – max.	x.xx-x.xx	x.xx-x.xx
missing	xx	xx
Week 96		
Mean (SD)	x.xx (xx)	x.xx (xx)
Min. – max.	x.xx-x.xx	x.xx-x.xx
missing	xx	xx

The null hypothesis can be evaluated based on an assessment of parameter estimates from a linear mixed model (LMM: Laird and Ware, 1982). LMMs are advantageous for longitudinal clinical trials because they can account for dependency due to repeated measures with relatively few parameters, which potentially enhances statistical efficiency. Furthermore, LMMs can accommodate incomplete cases (i.e., missing data), which is expected in this study due to dropout. LMMs are typically estimated using maximum likelihood methods (Verbeke and Molenberghs, 2000) that yield valid inferences with incomplete cases under the widely applicable assumption that the missing data are ignorable (Little and Rubin, 2002).

For this analysis, assuming linear change over time, the LMM can be written in the following manner:

$$Y_{ij} = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 t_{ij} x_{3i} + \beta_4 t_{ij} x_{4i} + a_i + b_i + \epsilon_{ij}$$

where:

- Y_{ij} is the BPF value for the i^{th} subject at the j^{th} time point
- α is the common intercept parameter
- t_{ij} denotes time (in weeks)
- x_{1i} = 1 if the subject was thought to be receiving immunomodulation therapy at randomization (IFN/GA), and 0 if the subject was not thought to be receiving immunomodulation therapy
- x_{2i} = 1 if the subject was thought to have primary progressive MS at randomization, and 0 if the subject was thought to have secondary progressive MS
- x_{3i} = 1 if the subject is in the treatment group
- x_{4i} = 1 if the subject is in the placebo group
- a_i and b_i are random effects (random intercepts and slopes)
- ε_{ij} is a random error term

After randomization, it is expected that a small number of enrolled subjects may be discovered to fall into a different stratum than that which was thought to be true at the actual time of randomization. For any such subjects, the true strata value discovered post-randomization (not the value thought to be true at the time of randomization) will be used for the models.

Assuming a common intercept constrains the baseline means to be equal, which controls for any initial imbalance that might occur due to empirical randomization (Senn, 2013). We make the typical assumptions:

$$\begin{bmatrix} a_i \\ b_i \end{bmatrix} \sim N(\mathbf{0}, \boldsymbol{G}), \qquad \varepsilon_{ij} \sim N(0, \sigma^2 \boldsymbol{I}_i), \qquad and \begin{bmatrix} a_i \\ b_i \end{bmatrix} \perp \varepsilon_{ij}$$

In this model, the parameters β_1 and β_2 are included to adjust the model for the two stratification variables (immunomodulation therapy status at randomization & disease status at randomization). The main parameters of interest are β_3 and β_4, which correspond to the estimated slopes in the treatment and placebo groups, respectively. The desired test of interest can be obtained by testing the null hypothesis of the following contrast:

$$H_0: \theta_1 = \beta_4 - \beta_3 = 0$$

against the alternative

$$H_1: \theta_1 \neq 0.$$

This null hypothesis can be evaluated with the likelihood ratio test, or a Z-test, provided a sufficiently large sample size which is provided in the proposed study. The Z statistic of interest is:

$$Z = \hat{\theta}_1 / SE(\hat{\theta}_1) ,$$

and leads to the rejection of H_0 when

$$|Z| < Z_{1-\alpha/2}$$

with the latter being the 100(1-α/2)th quartile of the standard normal distribution.

If we fail to reject H_0, then we will conclude that there is no significant difference in the change of BPF between the treatment group and the placebo group over 2 years. If H_0 is rejected in favor of the alternative, and θ_1 is greater than zero, then we will conclude that the study provides evidence that the rate of BPF change is slower in the Ibudilast treatment group relative to the placebo group. However, if H_0 is rejected in favor of the alternative, and θ_1 is less than zero, then we will conclude that the study provides evidence that the rate of BPF change is faster in the Ibudilast treatment group relative to the placebo group. It is important to note that failure to reject the null hypothesis does not imply acceptance of the null hypothesis of no difference in slopes over time for the two groups.

The data from the fitted model will be summarized graphically, and in the following table:

Table 7.2: Estimated rate of BPF change by treatment group

Treatment group	Estimated rate of BPF change 90% CI	p-value for difference in rate of change
Ibudilast	xx (xx, xx)	0.xx
Placebo	xx (xx, xx)	

Since the longitudinal measurements will span up to five time points per participant (baseline, 24, 48,

72, and 96 weeks), there is a possibility that the BPF trajectories will not be linear. In order to assess the sensitivity of the results to the assumption of linearity, non-linear trends will be modeled by inserting time as a categorical variable. For this modelling approach, visits that were performed outside of an expected scheduled visit will be categorized into the next expected scheduled visit. We will compare model fit between the non-linear and linear models using the AIC, and will report results from the non-linear model if the AIC suggests that the nonlinear model provides a better fit. In this case, the primary comparison will involve a comparison of the estimated means of the two groups at each time point, and will be displayed in a table similar to the following:

Table 7.3: Model based estimates of BPF over time

Model Based			
Visit	**Ibudilast**	**Placebo**	**p-value for difference**
Baseline Estimate (SE) 90% CI	 xx (xx) (xx, xx)	 xx (xx) (xx, xx)	0.xx
Week 24 Estimate (SE) 90% CI	 xx (xx) (xx, xx)	 xx (xx) (xx, xx)	0.xx
Week 48 Estimate (SE) 90% CI	 xx (xx) (xx, xx)	 xx (xx) (xx, xx)	0.xx
Week 72 Estimate (SE) 90% CI	 xx (xx) (xx, xx)	 xx (xx) (xx, xx)	0.xx
Week 96 Estimate (SE) 90% CI	 xx (xx) (xx, xx)	 xx (xx) (xx, xx)	0.xx

The residuals of the fitted statistical models will be examined for evidence of departure from assumptions, such as normality. If assumptions appear to be grossly violated, then transformations of response variables might be considered. It is also possible that some baseline characteristics will be imbalanced between the two treatment groups. In this case, the model may be expanded to include adjustments for the imbalanced characteristics. As specified in section 6, the primary analysis will be conducted using a modified intent-to-treat analysis, which includes all subjects who are randomized, receive at least one dose of study medication, and have at least one efficacy assessment in the double-blind phase.

Finally, the proposed study involves multiple sites, which is a potential source of additional variation. Patients within a site tend to be correlated due to similarity of environment, e.g. because of testing by the same set of clinicians (Localio et al, 2001). Sensitivity analyses may also be conducted to account for site variation by augmenting the models described above with additional random effects and associated variance components.

7.2. ***Primary Objective #2:*** *Evaluate the safety and tolerability of Ibudilast (MN-166) versus placebo*

7.2.1 Safety

The primary assessment of safety will compare the percentage of subjects in each group with:

- Treatment-emergent adverse events (TEAEs)
- Treatment-emergent serious adverse events (TESAEs)

The main assessment of safety and tolerability will involve a comparison of treatment-related AEs and SAEs across the two treatment groups. First, the percentage of subjects who experience any treatment-related AE in each group will be compared using the following logistic regression model:

$$\text{logit}(Y_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \epsilon_i$$

where

- Y_i represents an indicator of whether or not i^{th} subject had at least one treatment related AE.
- x_{1i} = 1 if i^{th} subject was thought to be receiving immunomodulation therapy at randomization (IFN/GA), and 0 if the subject was not thought to be receiving immunomodulation therapy
- x_{2i} = 1 if i^{th} subject was thought to have primary progressive MS at randomization, and 0 if the subject was thought to have secondary progressive MS
- x_{3i} = 1 if i^{th} subject was randomized to the Ibudilast group, and 0 if the subject was randomized to placebo group
- ϵ_i is random error for the i^{th} subject

To determine if the percentage of subjects having any treatment related AEs differ across treatment group we will test the following hypothesis:

$$H_o: \beta_3 = 0 \text{ vs. } H_A: \beta_3 \neq 0$$

If the null hypothesis is rejected, and $\beta_3 > 0$, we will conclude that Ibudilast was responsible for a significantly greater frequency of treatment-related AEs. Similarly, if $\beta_3 < 0$, we will conclude that Ibudilast was responsible for a significantly lower frequency of treatment-related AEs. In addition to an overall comparison, this hypothesis will be repeated for assessing the percentage of subjects having at least one treatment-related AE within each MedDRA system organ class (SOC). If there are significant differences between groups within any specific SOC, then additional tests will compare differences across groups for specific MedDRA preferred terms in order to further explore the cause of the observed differences.

In addition to the comparison of percentages in the manner described above, the rates of treatment-related AEs in each group will be compared using the following Poisson regression model:

$$\log\left(\frac{Y_i}{T_i}\right) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \epsilon_i$$

where

- Y_i represents the number of treatment related SAEs experienced by the i^{th} subject.
- T_i represents the number of months between the date of randomization and the date of last follow-up for the i^{th} subject.
- x_{1i} = 1 if i^{th} subject was thought to be receiving immunomodulation therapy at randomization (IFN/GA), and 0 if the subject was not thought to be receiving immunomodulation therapy
- x_{2i} = 1 if i^{th} subject was thought to have primary progressive MS at randomization, and 0 if the subject was thought to have secondary progressive MS
- x_{3i} = 1 if i^{th} subject was randomized to the Ibudilast group, and 0 if the subject was randomized to placebo group
- ϵ_i is random error for the i^{th} subject

To determine if the rate of treatment related SAEs differ across treatment group we will test the following hypothesis:

$$H_o: \beta_3 = 0 \text{ vs. } H_A: \beta_3 \neq 0$$

If the null hypothesis is rejected, the direction of β_3 will indicate the direction of the observed effect. Values of $\beta_3 > 0$ indicate an increased rate of treatment-related AEs associated with the Ibudilast group, while values of $\beta_3 < 0$ indicate a decreased rate of treatment-related AEs associated with the Ibudilast group.

Treatment-related SAEs will be analyzed in the same manner described above. Additional safety analyses will also assess all treatment-emergent AEs, treatment-emergent SAEs, unanticipated SAEs, and treatment-related & unanticipated SAEs in a similar manner.

7.2.2 Tolerability

The assessment of tolerability will involve assessing the percentage of subjects in each group who:

- Discontinue treatment early (early study termination and/or early study drug withdrawal) due to treatment-emergent AEs or SAEs
- Discontinue treatment early (early study termination and/or early study drug withdrawal) for any reason

Both will be assessed using a model similar to that described in section 7.2.1 for the assessment of treatment-related AEs and SAEs.

8. SECONDARY ANALYSES

The major secondary objective of the SPRINT-MS study is to measure activity of Ibudilast at 96 weeks versus placebo using a variety of additional imaging metrics. Four separate imaging metrics will be considered.

- Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
- Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
- Retinal nerve fiber layer as measured by optical coherence tomography (OCT)
- Cortical atrophy as measured by cortical longitudinal atrophy detection algorithm

8.1. Major Secondary Objective (a): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by diffusion tensor imaging (DTI) in pyramidal white matter tracts.*

For both DTI outcomes of interest [Axial Diffusivity (LD), Radial Diffusivity (TD)], the comparison of outcomes for subjects on MN-166 versus placebo will be analyzed using a model similar to the described in section 7.1 for the primary objective, with the exception that the outcome variable will be modified to represent the corresponding DTI outcome of interest and the model will include an additional random effect to account for measurements taken on both sides within the same subject.

8.2. Major Secondary Objective (b): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by magnetization transfer ratio (MTR) imaging in normal appearing brain tissue.*

Because both MTR outcome measures of interest [Median MTR in normal appearing brain tissue (NABT) and Median MTR in normal appearing grey matter (NAGM)] record the values at each of the time points as the change from baseline, the LMM used to address this objective will be slightly modified from that described in section 7.1. Assuming linear change over time, the LMM can be

written in the following manner:

$$Y_{ij} = \alpha + \beta_0 x_{0i} + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 t_{ij} x_{3i} + \beta_4 t_{ij} x_{4i} + a_i + b_i + \epsilon_{ij}$$

where:

- Y_{ij} is the change in the MTR value from baseline for the i^{th} subject at the j^{th} time point
- x_{1i} = the baseline MTR value for the i^{th} subject

and all other variables are defined in the same manner as described for the model in section 7.1. The major secondary hypothesis of interest will involve a comparison of the β_3 and β_4 parameters, and will proceed in the same manner as described for the model in section 7.1.

8.3. Major Secondary Objective (c): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by retinal nerve fiber layer, as measured by optical coherence tomography (OCT).*

For the analysis of mean RNFL thickness from OCT, the comparison of outcomes for subjects on MN-166 versus placebo will be analyzed using a model similar to the described in section 7.1 for the primary objective, with the exception that the outcome variable will be modified to represent the mean RNFL thickness values and the model will include an additional random effect to account for measurements taken on both eyes within the same subject.

8.4. Major Secondary Objective (d): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by cortical atrophy, as measured by cortical longitudinal atrophy detection algorithm.*

For the analysis of cortical thickness (CTh) as measured by CLADA, the comparison of outcomes for subjects on MN-166 versus placebo will be analyzed using a model similar to that described in section 7.1 for the primary objective, with the exception that the outcome variable will be modified to represent the cortical thickness values.

8.5. Additional Secondary, Tertiary, and Exploratory Analyses

A number of additional secondary, tertiary, and exploratory analyses are also planned, but will not be included as part of the FSR. These additional analyses include, but are not limited to:

- Inflammatory disease activity, as measured by T1 lesion volume, T2 lesion volume, and annualized relapse rate
- Disability, as measured by the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC)
- Cognitive impairment, as measured by the Symbol Digit Modalities Test and the Selective Reminding Test
- Quality of Life as measured by the Multiple Sclerosis Impact Scale (MSIS-29), EuroQol 5 Dimensions (EQ-5D), and Short Form-36 Health Survey (SF-36)
- Neuropathic pain, as measured by the Brief Pain Inventory
- Evaluating the activity of Ibudilast (MN-166) at 48 weeks versus placebo as measured by the primary and major secondary imaging outcome measures:
    - Whole brain atrophy using brain parenchymal fraction (BPF)
    - Diffusion tensor imaging (DTI) in descending pyramidal white matter tracts
    - Magnetization transfer ratio (MTR) imaging in normal-appearing brain tissue
    - Retinal nerve fiber layer as measured by optical coherence tomography (OCT)

  - Cortical atrophy, as measured by cortical longitudinal atrophy detection algorithm (CLADA)
- Evaluating the activity of Ibudilast (MN-166) at 96 weeks versus placebo as measured by:
  - Whole-brain gray matter fraction
  - Magnetization transfer ratio (MTR) in gray matter
  - New T1 lesions since baseline
  - New T2 lesions since baseline
- Evaluating the pharmacokinetics (PK) of Ibudilast (MN-166) using a population PK approach
- Correlation of cerebrospinal fluid (CSF) and serum biomarkers with imaging and clinical measures of progressive disability
- Identification of unique phase 2 endpoints, and composite MRI scales (combining BPF, MTR, and DTI)

9. SAMPLE SIZE JUSTIFICATION

9.1. ***Primary Objective #1:*** *Evaluate the activity of Ibudilast (MN-166: 100 mg/day) versus placebo at 96 weeks as measured by quantitative MRI analysis for whole brain atrophy using brain parenchymal fraction (BPF).*

Estimated required sample size for the primary objective was computed based on two pilot data sets and relevant literature. The first pilot data set (DS1) consisted of *N* = 30 PP subjects (n=15 female; mean age 58.6 years; mean disease duration 7.3 years; mean EDSS 4.97) who participated in the placebo arm of a clinical trial for the chemotherapy drug mitoxantrone (Kita et al 2004). A maximum of three repeated measures were available, collected over 24 months.

The second pilot data set (DS2) consisted of *N* = 42 RR and SP participants who had BPF data from 3T scans. Similar to DS1, a maximum of three repeated measures were available, collected over 24 months, and the DS2 pilot participants were in the control group.

The effect size for the power analysis was defined as the percentage difference in linear slope relative to a hypothetical treatment group. That is, the percentage reduction in linear deterioration of the treated group in a hypothetical intervention. The pilot data and the survey of (Altmann et al 2009) suggested a reasonable range of percentage difference was 30% to 50%. Also of note, an earlier study was performed assessing the activity of Ibudilast in relapsing MS, and this study included measurement of brain atrophy. While the earlier study used a different atrophy measure (SIENA) than in this proposal (BPF), and had only two time points, it did show effects sizes of approximately 33%-36%, (Barkhof et al 2010) which could be considered an appropriate target for the power analysis.

Power calculations were based on a LMM appropriate for clinical trials (Yi and Panzarella, 2002; Heo and Leon, 2009). Because there was only one site for the pilot data, the LMM of Equation (1) was used as the basis for the power analysis. Presentation of the required sample size equations is facilitated by using general notation. Consider the following equalities for the parameters of the primary model described in section 7.1:

$$[\alpha_P \quad \beta_P \quad \gamma \quad \delta]^T = [\beta_0 \quad \beta_1 \quad \beta_2 \quad \beta_3]^T = \boldsymbol{\beta}^T,$$
$$[a_i \quad b_i]^T = [b_{0i} \quad b_{1i}]^T = \boldsymbol{b}_i^T.$$

(Recall that $\gamma = 0$ to constrain the groups to be equal at baseline.) Then the LMM of Equation (1) can be written in matrix notation as

$$\boldsymbol{Y}_i = \boldsymbol{X}_i\boldsymbol{\beta} + \boldsymbol{Z}_i\boldsymbol{b}_i + \boldsymbol{\epsilon}_i,$$

where each matrix has row dimension n_i; $\boldsymbol{X}_i$ is the design matrix of the fixed effects, and $\boldsymbol{Z}_i$ is the design matrix for the random effects. $\boldsymbol{X}_i$ has four columns, the first being a vector of 1s, the second being t_{ij}, the third being g_i, and the fourth being $t_{ij} \cdot g_i$. $\boldsymbol{Z}_i$ consists of the first two columns of $\boldsymbol{X}_i$.

The required sample size depends on the variance of the responses,

$$\boldsymbol{V}_i = Var(\boldsymbol{Y}_i) = \boldsymbol{Z}_i\boldsymbol{G}\boldsymbol{Z}_i^T + \sigma^2\boldsymbol{I}_i$$

and the required sample size for a single arm (half the total sample size) is computed with the following formula

$$\frac{N}{2} = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \cdot (\boldsymbol{X}_P^T\boldsymbol{V}^{-1}\boldsymbol{X}_P + \boldsymbol{X}_T^T\boldsymbol{V}^{-1}\boldsymbol{X}_T)_{4,4}^{-1}}{\delta^2}$$

where $\boldsymbol{X}_P$ is the design matrix for the placebo group, $\boldsymbol{X}_T$ is the design matrix for the treatment group, and the subscript (4,4) indicates the element in the 4th row and 4th column of the matrix. The value of δ is varied to represent effect sizes of 30%, 35%, 40%, 45%, and 50%, which is the hypothetical slope difference between the treatment and placebo groups.

The analysis used the proposed time points of 0, 24, 48, 72, and 96 weeks. Power curves were computed for DS1, DS2, and for their average (Avg). Avg was based on averaging the LMM parameter estimates across the data sets (e.g., average slope = [DS1 slope + DS2 slope] / 2). Avg was an attempt to pool information from the two data sets without the raw data (the raw data for DS2 was unavailable). A 10% dropout rate was assumed and all estimated sample sizes were inflated by this percentage (i.e., final sample size = initial sample size $\times$ 1.10). Given the resources required for obtaining and analyzing 3T scans, the type I error rate was set at $\alpha = 0.10$. Figure 7.1 shows power curves for the three smallest effect sizes (30%, 35%, and 40%). Focusing on Avg for the middle effect size (35%), the figure indicates that a single-arm $N / 2 = 125$ provides close to 80% (power = 0.80 is the horizontal dashed line). The middle effect size is important as it nearly corresponds to the upper effect size of the aforementioned Ibudilast study (effect size = 36%). The Avg data source is preferred because it approximates a pooling of the information from both samples. The feasibility of $N / 2 = 125$ as the single-arm sample size was examined by calculating power for effect sizes with $\alpha = 0.10$ for all three data sources (DS1, DS2, Avg). The power by effect size curve and data source is shown in Figure 7.2. The figure clearly shows that the study has adequate power across the range of possible values suggested by these two data sources. For the “best” data (DS1), our study has slightly less than 90% power to detect effects of approximately 40% or larger, and more than 90% power to detect effects on the upper end of the range of interest (50%). Most importantly, the figure shows that the power based on the Avg data source is very high for effects in the 33% - 36% range of greatest interest (approximately 80% - 85%). Therefore, based on these assumptions, we are confident that a per-arm sample size of $N / 2 = 125$ ($N = 250$ total participants) is sufficient to help ensure statistically reliable results for the proposed study.

Figure 9.1: Power as a Function of Single-arm sample size, effect size, and data set

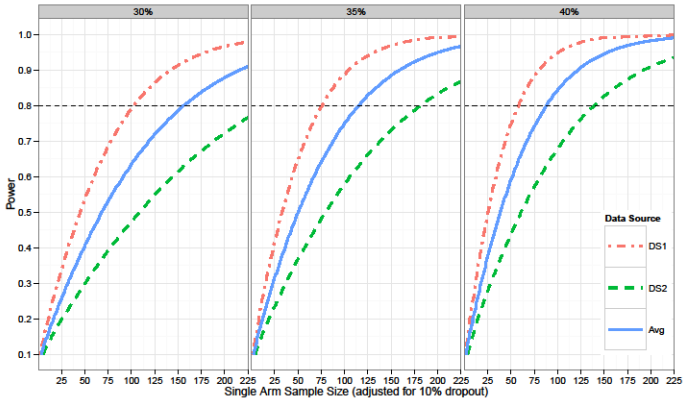
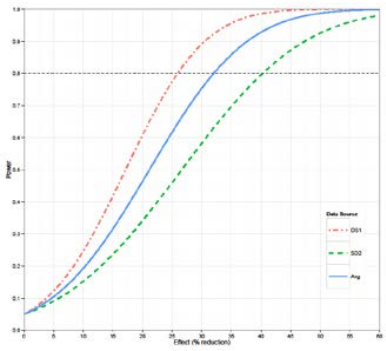


Figure 9.2: Power as a Function of Effect Size and data source for sample size of 125 and alpha = 0.10



9.2. *Primary Objective #2:* *Evaluate the safety and tolerability of Ibudilast (MN-166) versus placebo*

With an overall significance level of 0.10, the study will have 80% or greater power to detect differences in safety or tolerability of 15% or greater across the two groups. Thus, minor safety concerns may not be detected in this study – and would need to be assessed in a larger, future phase 3 trial. Nevertheless, the study is adequately powered to detect major safety concerns.

9.3. Major Secondary Objective (a): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by diffusion tensor imaging (DTI) in pyramidal white matter tracts.*

The power analysis was based on longitudinal DTI pilot data with the outcome measure being longitudinal diffusivity (LD) within corticospinal tracts among RRMS and SPMS starting therapy with natalizumab (Fox 2010). Eighteen MS patients were measured five times over 24 months. Equation (2) was the basis for computing required sample size based on the LMM. In order to express power $(1-\beta)$ as a function of the fixed sample size (N / 2 = 125) and the other quantities, the following equation can be used

$$1-\beta = \Phi\left[\left(\sqrt{\frac{125\cdot\delta^2}{\left(\sum_k^2\sum_l^L \boldsymbol{X}_{kl}^T \boldsymbol{V_{kl}}^{-1} \boldsymbol{X}_{kl}\, \boldsymbol{P}_{kl}\right)_{4,4}^{-1}}}\right) - Z_{1-\alpha/2}\right]$$

where $\Phi[\cdot]$ is the cumulative distribution function of the standard normal distribution. Estimated power was calculated using Equation (3) for the δ effect sizes discussed previously. The results show that the estimated power was greater than 0.90 for the smallest effect size (30% difference in slopes). This result implies that *N* = 250 patients is sufficient to provide high power for testing the equality of LD slopes in the treatment and control groups for the range of plausible effect sizes that is anticipated in the proposed study.

9.4. Major Secondary Objective (b): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by magnetization transfer ratio (MTR) imaging in normal appearing brain tissue.*

Pilot data was not available for the MTR power estimates. Descriptive statistics were provided by Dr. Douglas Arnold (Director of NeuroRx) for a study of the efficacy of BG-12 (BG00012, dimethyl fumarate), which is a novel therapy in the development for relapsing MS. Ibudilast is purported to have neuroprotective effects, and so these data were used as a model to project the power of Ibudilast in this proposed trial. A total of *N* =448 MS patients had whole-brain MTR measured at baseline, 24 weeks, 1 year, and 2 years as part of a multi-center phase III clinical trial using 1.5T MRIs and the stock manufacturer pulse sequence. Only descriptive statistics were available for change between baseline and two years. There was a placebo group and two treatment groups, one receiving BG-12 240 mg twice a day (b.i.d.) and the second receiving BG-12 240 mg three times per day (t.i.d.). The statistics available for the study were means of change scores and their standard deviations. Thus, power was estimated based on an independent *t*-test of the mean baseline to 2 year difference in MTR scores for the treatment (BG-12) and placebo groups. Power was computed using the conventional formula

$$1-\beta=\Phi\left(\frac{|\delta|\sqrt{125}}{\sigma}-Z_{1-\frac{\alpha}{2}}\right)$$

where δ is the difference of the mean change scores and σ^2 is the pooled variance of the difference. A subset of patients had no new or enlarging T2 legions, and the analysis was performed twice: once for all the patients and another time excluding the patients with no lesions. The results of the power analysis are shown in the table below. As seen in the table, the lowest estimated power (t.i.d., all patients) was greater than the conventional cut-off of 0.80. When only subjects with no new lesions were considered (which more closely approximates what we expect in the proposed trial), estimated power was even greater than the conventional level. The table suggests that *N* =250 might provide sufficient power to detect *T* and *P* differences in change over time for the proposed study. The improved image acquisition from 3T MRIs and tailored pulse sequences are expected to increase the study power.

Table 9.1. Power as a Function of Treatment and Type of Patient for MTR

Treatment	Patients	Power
b.i.d.	All	0.856
t.i.d	All	0.803
b.i.d.	No Lesions	0.925
t.i.d.	No Lesions	0.816

9.5. Major Secondary Objective (c): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by retinal nerve fiber layer, as measured by optical coherence tomography (OCT).*

Estimated power for OCT was based on pilot data supplied by Dr. Laura Balcer (University of Pennsylvania) from an unpublished multi-center natural history observational study of RRMS and SPMS patients. A sample of N = 373 MS patients was measured an average of 2.7 times over an average of 1.4 years. A complexity of OCT is that it is measured in both eyes. Therefore, the LMM must be augmented to include random effects for the additional level of nesting. Ignoring site, we define Y_{ijk} to be the OCT value for the kth week (k = 1, …, n_{ij}) of the jth eye (j = 1, 2) of the ith patient (i = 1, …, N). Then the LMM is

$$Y_{ijk} = \alpha + \beta t_{ijk} + (\gamma + \delta t_{ijk}) g_i + a_i + b_i t_{ijk} + a_{ij} + b_{ij} t_{ijk} + \epsilon_{ijk}$$

where $\gamma = 0$ is set to constrain the groups to be equal at baseline, a_i and b_i are patient-specific random effects and a_{ij} and b_{ij} are random effects for eyes nested within patients. It is assumed that

$$[a_i \quad b_i]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{G}),\ [a_{ij} \quad b_{ij}]^T \sim \mathcal{N}(\mathbf{0}, \boldsymbol{H}),\ \epsilon_{ij} \sim \mathcal{N}(0, \sigma^2 \boldsymbol{I}_i), \text{ and } [a_i \quad b_i]^T \perp [a_{ij} \quad b_{ij}]^T \perp \epsilon_{ij}.$$

The three levels of nesting represented by Equation (4) (month nested within eye nested within patient) presents a challenge for deriving power equations, and analytic results are usually based on simplifying assumptions (e.g., fewer random effects than in Equation (4)) (Heo and Leon, 2009). In order to base the power estimate on the LMM of Equation (4), a simulation approach was used. For the simulation, the LMM of Equation (4) was fit to the pilot data and then the resulting estimates were treated as parameters in the following replications. For each replication, a random number generator was used to compute random effects and random error for N = 250 hypothetical treatment and control patients based on the appropriate covariance structures (e.g., $\boldsymbol{G}$). Then simulated OCT response values were computed based on the fixed and random effects and random error of Equation (4). For each replication, the null hypothesis of H_0: $\delta = 0$ was evaluated with the Z-test based on a LMM fit to the simulated data. The process was repeated 1000 times for each effect size value of δ and the average number of rejections of H_0 was taken to be the estimated power. Results of the simulation are shown in the table below.

Table 9.2. Power as a Function of Effect Size based on the OCT Simulation Study

Effect Size	Power
30%	0.515
35%	0.603
40%	0.665
45%	0.782
50%	0.855

As the table shows, the power surpassed the conventional cutoff of 0.80 only for the largest effect size, but the next lower size (45%) was close to the cutoff. The results indicate that N = 250 might provide adequate power for the treatment and placebo slope comparison of OCT if the effect size is large, but power might be lower than the conventional cutoff for medium and smaller effect sizes.

9.6. Major Secondary Objective (d): *Evaluate the activity of Ibudilast (MN-166) versus placebo as measured by cortical atrophy, as measured by cortical longitudinal atrophy detection algorithm.*

No pilot data or other effect size estimates were available for CLADA. However, we expect CLADA effects to be consistent with those of the other secondary endpoints. Therefore, we are confident that statistical power for CLADA will be sufficient to detect effect sizes similar to those discussed for the other secondary endpoints.

10. SAFETY MONITORING

10.1. Assessment & Recording of Adverse Events

The clinical study site PI (CSSPI) or an authorized physician will assess all AEs for severity, relationship with study medication, and whether it meets the criteria for classification as an SAE, requiring immediate notification to the Sponsor or designee. These assessments will be made in accordance with the standard ratings detailed in the following sections. Each CSSPI and research team is responsible for identifying adverse events and reporting them through the DCC Online Adverse Event Reporting System (AERS).

Adverse events should be collected and recorded for each subject from the date the informed consent form was signed until the end of their participation in the study (i.e., the subject has discontinued or completed the study) through the follow-up visit with the exception of those who discontinue study medication during the study and are followed on a semi-annual basis. Subjects who discontinue study medication but continue follow-up within the study will not have AEs collected after study drug discontinuation. Only those AEs that occurred while on study drug that have not been resolved will be followed until resolution or stabilization.

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 CSSPIs 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.

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.

The CSSPI or an authorized delegate is responsible for submitting the requested information via the DCC Online Adverse Event Reporting System 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 CSSPI or an authorized delegate should report SAEs “spontaneously” if considered at least possibly related to study medication. Upon entry of a serious adverse event by a CSSPI, the DCC Online Adverse Event

Reporting System will immediately notify the Medical Safety Monitor (MSM).

10.2. Severity Assessment

The severity of AEs will be determined as described below:

- 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 and urgent medical/therapy intervention required. Hospitalization probable.
- Death (Grade 5): Death related to AE.

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 grade of 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.

10.3. Medical Safety Monitor

As previously indicated, Dr. Steven Krieger will serve as the MSM for this trial. In addition to performing real-time reviews of all SAEs (as described in section 2.2), Dr. Krieger will also receive quarterly tabulations, by blinded treatment group, of all AEs/SAEs for the purpose of determining if any safety trends exist that may raise concerns. Safety will be assessed in two ways – both the percentage of subjects who experience any AE and the rate of AEs will be compared by body system across the two groups. The additional questions related to whether the AE/SAE is related to treatment and/or whether the AE/SAE is unanticipated will be used to subset these into a series of additional tables. This quarterly review will identify any disconcerting discrepancy in the frequency of any AE/SAE between the two groups.

11. INTERIM ANALYSES

11.1. Interim Safety Monitoring

After 30 patients have been enrolled for at least 30 days and 60 patients have been enrolled for at least 60 days, the Medical Safety Monitor will review pooled (i.e., blinded to treatment) safety data provided by the DCC. Beginning after approximately month 3 of enrollment start, a National Institute of Neurological Disorders and Stroke (NINDS) Data and Safety Monitoring Board (DSMB) will meet every six months during the study (and more often, if deemed necessary), and will review blinded safety data including adverse events and serious adverse events (SAE). The DSMB will be empowered to recommend stopping the study at any time due to safety concerns.

11.2. Interim Efficacy and Futility Assessment

One interim efficacy assessment will occur when 125, or approximately half, of the 250 total planned subjects have completed their week 96 follow-up visit. For these analyses, the Lan-DeMets alpha

spending function approach with the O'Brien-Fleming stopping boundaries will be used. Table 11.1 below shows the stopping boundaries under the assumption of two equally spaced analyses (one interim and one final analysis).

Table 11.1 Stopping Boundaries for Interim and Final Analyses

Efficacy Analysis	Number of Subjects Completing Week 96 Visit	Nominal p-value to Conclude Efficacy
1	125	0.0104
2	250	0.0816

A formal futility assessment, based on the determination of predictive power, will also be conducted at the time of the interim analysis. If the predictive power is below 20% at the time of the interim analysis, then we would recommend that the study be stopped early for futility.

REFERENCES

Altmann DR, Jasperse B, Barkhof F et al (2009). "Sample sizes for brain atrophy outcomes in trials for secondary progressive multiple sclerosis," Neurology, 72:595-601.

Amato MP, Portaccio E, Goretti B et al (2007). "Association of neocortical volume changes with cognitive deterioration in relapsing-remitting multiple sclerosis," Arch Neurol, 64:1157-1161.

Arnold DL, Gold R, Kappos L et al (2014). "Magnetization transfer ratio in the delayed-release dimethyl fumerate DEFINE study," Journal of Neurology, 261:2429-2437.

Barkhof F, Calabresi PA, Miller DH et al (2009). "Imaging outcomes for neuroprotection and repair in multiple sclerosis trials," Nat Rev Neurol, 5:256-266.

Barkhof F, Hulst HE, Drulovic et al (2010). "Ibudilast in relapsing-remitting multiple sclerosis: A neuroprotectant?," Neurology, 74:1033-1040.

Benedict RH, Carone DA, and Bakshi R (2004). "Correlating brain atrophy with cognitive dysfunction, mood disturbances, and personality disorder in multiple sclerosis," J Neuroimaging, 14:36S-45S.

Benedict RH, Weinstock-Guttman B, Fishman I et al (2004). "Prediction of neuropsychological impairment in multiple sclerosis: comparison of conventional magnetic resonance imaging measures of atrophy and lesion burden," Arch Neurol, 61:226-230.

Bermel RA and Bakshi R (2006). "The measurement and clinical relevance of brain atrophy in multiple sclerosis," Lancet Neurol, 5:158-70.

Chataway J, Alsanousi A, Chan D et al (2012). "The MS-STAT trial: high dose simvastatin demonstrates neuroprotection without immune-modulation in secondary progressive multiple sclerosis (SPMS) - a phase II trial," In: ECTRIMS; Lyon, France.

Chen JT, Easle K, Schneider C, et al (2013). "Clinically feasible MTR is sensitive to cortical demyelination in MS," Neurology, 80:246-252.

Dalton CM, Chard DT, Davies GR et al (2004). "Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes," Brain, 127:1101-1107.

Dastidar P, Heinonen T, Lehtimaki T et al (1999). "Volumes of brain atrophy and plaques correlated with neurological disability in secondary progressive multiple sclerosis," J Neurol Sci, 165:36-42.

De Stefano N, Giorgio A, Battaglini M et al (2010). “Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes,” Neurology, 74:1868-1876.

Di Filippo M, Anderson VM, Altmann DR et al (2010). “Brain atrophy and lesion load measures over 1 year relate to clinical status after 6 years in patients with clinically isolated syndromes,” J Neurol Neurosurg Psychiatry, 81:204-208.

Edwards SG, Liu C, and Blumhardt LD (2001). “Cognitive correlates of supratentorial atrophy on MRI in multiple sclerosis,” Acta Neurol Scand, 104:214-223.

Feinstein A, Roy P, Lobaugh N et al (2004). “Structural brain abnormalities in multiple sclerosis patients with major depression,” Neurology, 62:586-590.

Filippi M and Grossman RI (2002). “MRI techniques to monitor MS evolution: the present and the future,” Neurology, 58(8):1147-1153.

Filippi M, Rocca MA, De Stefano N et al (2011). “Magnetic resonance techniques in multiple sclerosis: the present and the future,” Arch Neurol, 68:1514-1520.

Filippi M, Rocca MA, Pagani E et al (2004). “European study on intravenous immunoglobulin in multiple sclerosis: Results of magnetization transfer magnetic resonance imaging analysis,” Arch Neurol, 61:1409-1412.

Fisher E (2011). “Measurement of CNS Atrophy,” In: Cohen JA, Rudick RA, eds. Multiple Sclerosis Therapeutics, 4th Edition. New York: Cambridge University Press.

Fisher E, Lee JC, Nakamura K et al (2008). “Gray matter atrophy in multiple sclerosis: a longitudinal study,” Ann Neurol, 64:255-265.

Fisher E, Rudick RA, Cutter G et al (2000). “Relationship between brain atrophy and disability: an 8-year follow-up study of multiple sclerosis patients,” Mult Scler, 6(6):373-377.

Fisher E, Rudick RA, Simon JH et al (2002). “Eight-year follow-up study of brain atrophy in patients with MS,” Neurology, 59:1412-1420.

Fisniku LK, Altmann DR, Cercignani M et al (2009). “Magnetization transfer ratio abnormalities reflect clinically relevant grey matter damage in multiple sclerosis,” Mult Scler, 15:668-677.

Fox NC, Jenkins R, Leary SM et al (2000). “Progressive cerebral atrophy in MS. A serial study using registered volumetric MRI,” Neurology, 54:807-812.

Fox RJ, Cronin T, Lin J et al (2011). “Measuring myelin repair and axonal loss with diffusion tensor imaging,” Am J Neuroradiol, 32:85-91.

Fox RJ, Sakaie K, Lee JC et al (2012). “A validation study of multicenter diffusion tensor imaging: Reliability of fractional anisotropy and diffusivity values,” AJNR Am J Neuroradiol, 33:695-700.

Ge Y, Grossman RI, Udupa JK et al (2000). “Brain atrophy in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis: Longitudinal quantitative analysis,” Radiology, 214:665-670.

Ge Y, Grossman RI, Udupa JK et al (2002). “Magnetization transfer ratio histogram analysis of normal-appearing gray matter and normal-appearing white matter in multiple sclerosis,” J Comput Assist Tomogr, 26:62-68.

Gordon-Lipkin E, Chodkowski B, Reich DS et al (2007). “Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis,” Neurology, 69:1603-1609.

Heo, M. and Leon, A.C. (2009). “Sample size requirements to detect an intervention by time interaction in longitudinal cluster randomized clinical trials,” Statistics in Medicine, 28:1017-1027.

Hohol MJ, Guttmann CR, Orav J et al (1997). “Serial neuropsychological assessment and magnetic resonance imaging analysis in multiple sclerosis,” Arch Neurol, 54:1018-1025.

Huang D, Tani M, Wang J et al (2002). “Pertussis toxin-induced reversible encephalopathy dependent on monocyte chemoattraction protein-1 overexpression in mice,” J Neurosci, 22:10633-10642.

Inglese M, van Waesberghe JH, Rovaris M et al (2003). “The effect of interferon beta-1b on quantities derived from MT MRI in secondary progressive MS,” Neurology, 60:853-860.

Janardhan V and Bakshi R (2000). “Quality of life and its relationship to brain lesions and atrophy on magnetic resonance images in 60 patients with multiple sclerosis,” Arch Neurol, 57:1485-1491.

Kalkers NF, Bergers E, Castelijns JA et al (2001). “Optimizing the association between disability and biological markers in MS,” Neurology, 57:1253-1258.

Kita M., Cohen JA, Fox RJ et al (2004). “A phase II trial of Mitoxantrone in patients with primary progressive multiple sclerosis,” Neurology, 62:A99.

Lazeron RH, Boringa JB, Schouten M et al (2005). “Brain atrophy and lesion load as explaining parameters for cognitive impairment in multiple sclerosis,” Mult Scler, 11:524-531.

Magnotta VA, Matsui JT, Liu D et al (2012). “Multi-center reliability of diffusion tensor imaging,” Brain Connect, 2:345-355.

Marrie RA, Fisher E, Miller DM et al (2005). “Association of fatigue and brain atrophy in multiple Sclerosis,” J Neurol Sci, 228:161-166.

Miller DH, Barkhof F, Frank JA et al (2002). “Measurement of atrophy in multiple sclerosis: Pathological basis, methodological aspects and clinical relevance,” Brain, 125:1676-1695.

Minneboo A, Jasperse B, Barkhof F et al (2008). “Predicting short-term disability progression in early multiple sclerosis: Added value of MRI parameters,” J Neurol Neurosurg Psychiatry, 79:917-923.

Molyneux PD, Kappos L, Polman C et al (2000). “The effect of interferon beta-1b treatment on MRI measures of cerebral atrophy in secondary progressive multiple sclerosis. European Study Group on Interferon beta-1b in secondary progressive multiple sclerosis,” Brain, 123:2256-2263.

Mowry EM, Loguidice MJ, Daniels AB et al (2009). “Vision related quality of life in multiple sclerosis: correlation with new measures of low and high contrast letter acuity,” J Neurol Neurosurg Psychiatry, 80:767-772.

Nakamura K, Fox R, and Fisher E (2011). “CLADA: Cortical longitudinal atrophy detection algorithm,” Neuroimage, 54:278-289.

Rao SM, Glatt S, Hammeke TA, et al (1985). “Chronic progressive multiple sclerosis. Relationship between cerebral ventricular size and neuropsychological impairment,” Arch Neurol, 42:678-682.

Rudick RA, Cutter G, Baier M et al (2001). “Use of the Multiple Sclerosis Functional Composite to predict disability in relapsing MS,” Neurology, 56:1324-1330.

Rudick RA, Fisher E, Lee JC et al on behalf of the Multiple Sclerosis Collaborative Research Group (1999). “Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS,” Neurology, 53:1698-1704.

Song SK, Sun SW, Ju WK et al (2003). “Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia,” Neuroimage, 20:1714-1722.

Song SK, Sun SW, Ramsbottom MJ et al (2002). “Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water,” Neuroimage, 17:1429-1436.

Song SK, Yoshino J, Le TQ et al (2005). “Demyelination increases radial diffusivity in corpus callosum of mouse brain,” Neuroimage, 26:132-140.

Tedeschi G, Dinacci D, Lavorgna L et al (2007). “Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability,” J Neurol Sci, 263:15-19.

Walter SD, Ishikawa H, Galetta KM et al (2012). “Ganglion cell loss in relation to visual disability in multiple sclerosis,” Ophthalmology, 119:1250-11257.

Warner CV, Syc SB, Stankiewicz AM et al (2011). “The impact of utilizing different optical coherence tomography devices for clinical purposes and in multiple sclerosis trials,” PLoS One, 6:e22947.

Waxman SG and Black JA (2007). “Retinal involvement in multiple sclerosis,” Neurology, 69:1562-1563.

Yi Q and Panzarella T (2002). “Estimating sample size for tests on trends across repeated measurements with missing data based on the interaction term in a mixed model,” Controlled Clinical Trials, 23:481-496.