Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Additional Methodology

Patients

Inclusion criteria

Patients eligible for inclusion in the study had to fulfill all the following 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 secondary progressive multiple sclerosis or primary progressive multiple sclerosis according to 2010 International Panel Criteria
- Typical multiple sclerosis 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)
- Expanded Disability Status Scale 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 Expanded Disability Status Scale 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-foot walk) or
    - 20% worsening in 9-hole peg test (9-hole peg test) 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

Patients fulfilling any of the following criteria were not eligible for inclusion in this study. No additional exclusions could be applied by the investigator, to ensure that the study population was representative of all eligible patients.

- 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 (i.e., monthly or bimonthly intravenous methylprednisolone)
- Use of oral immunosuppressants (e.g., azathioprine, methotrexate, cyclosporine, teriflunomide) 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 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 due to potential drug-drug interactions or QT prolongation.
- 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

- 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, e.g. 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 the MRI scans required for this study.
- Subject is unable to lie sufficiently still in an MRI to obtain a high quality MRI image.

Re-enrollment of patients (reattempt of enrolment into the protocol at some future time-point after the subject was determined to be initially ineligible) was allowed in certain cases where the patient's condition changed such that would meet entry criteria. All screening assessments were repeated when a patient was re-screened.

Randomization

Randomization was stratified according to disease status (primary or secondary progressive MS) and by use of immunomodulating therapy (yes or no). A list of random treatment assignments was generated for each strata using randomized block methods. Block sizes of four and six were randomly chosen and within each block, treatment assignments were randomly generated in a 1:1 ratio.

EDSS Assessments

EDSS assessments were performed by examiners that were trained and certified by Neurostatus, University Hospital Basel, Basel, Switzerland.

Magnetic Resonance Imaging

MRI procedures

All MRIs were conducted using contemporary Siemens (Trio/Prisma or Skyra) or GE (version 12X or higher) 3T systems. The image acquisition includes 3D spoiled gradient-recalled echo; proton density weighted and T2 weighted 2D turbo/fast spin-echo; 2D T2-weighted FLAIR; 3D spoiled gradient-recalled echo with selective excitation, with and without magnetization transfer pulse; 64-direction high angular resolution diffusion imaging (twice refocused spin echo, single-shot EPI readout for Siemens Trio; Monopolar Plus for Siemens Skyra; Stejskal-Tanner single-shot EPI readout for GE).

Scans were transmitted from each clinical site to the primary imaging coordinating center (NeuroRx, Montreal, Canada), and subsequently to two additional imaging centers: Department of Biomedical Engineering, Cleveland Clinic, and Imaging Institute, Cleveland Clinic. All three imaging center performed quality control assessments for overall MRI sequence and acquisition

accuracy, as well as magnetization transfer ratio (NeuroRx), whole brain atrophy and cortical atrophy (Department of Biomedical Engineering, Cleveland Clinic) and diffusion tensor imaging (Imaging Institute, Cleveland Clinic) within 3 days of receipt of images. Common reasons to reject a scan include incorrect MRI acquisition parameters, incorrect imaging hardware, incorrect head angle, and patient motion. When repeat imaging was needed, clinical sites were asked to repeat MRI acquisition as soon as possible.

MRI quality control

Central imaging physicists visited each imaging site prior to subject enrollment to review the study scanning protocol and phantom scan acquisition with local site MRI technologists. One healthy volunteer at each imaging site underwent two scanning sessions on the same scanner to ensure accurate scanning procedures and acceptable scanner stability. In addition, scanner performance was monitored with monthly scans of the Biomedical Informatics Research Network (BIRN) phantom using an abbreviated imaging protocol. Quantitative procedures to detect low-level spiking, evaluate eddy current and Nyquist ghosting artifacts were used along with alarm thresholds for determining minimal acceptable signal-to-noise ratio. Excessive change in any of these metrics triggered a request to the site to have a field service engineer investigate and implement necessary repairs. Details of these procedures and their results are reported in Zhou et al.[1]

Magnetization Transfer Ratio

The scan sequences prior to site activation and subject enrollment at each imaging site also included magnetization transfer ratio sequences. To account for between-scanner differences, magnetization transfer ratio in the normal appearing brain tissue was calibrated across scanners using data from a healthy volunteer at each site.[2]

Ocular Coherence Tomography

Ocular Coherence Tomography Training

Optical coherence tomography site training, acquisition, central reading, quality control, and data management were coordinated through an established regulatory-compliant reading center, Digital Optical Coherence Tomography Reading Center at the Digital Angiography Reading Center, which is affiliated with Cleveland Clinic. All technicians and research coordinators involved in optical coherence tomography acquisition underwent training for the SPRINT-MS study via live webinar. Each site was supplied with a study procedures handbook and quick reference guide specific to the SPRINT-MS study and the specific instrument used at each site. Optical coherence tomography technicians at each site were individually certified by Digital Optical Coherence Tomography Reading Center via submission of sample scans, which were required to pass quality criteria prior to acquisition of on-study optical coherence tomography scans at their site.

Ocular Coherence Tomography Scan Acquisition

Heidelberg Spectralis utilized Glaucoma Retinal Nerve Fiber Layer 768x496 scan with an ART of 100, with scan quality required to be >25/40. Zeiss Cirrus utilized Optic Disc Cube 200 x 200 with scan quality required to be ≥ 6/10. Neither the OSCAR-IB criteria for optical coherence tomography quality in clinical trials[3] nor the NIH Common Data Elements for Optical Coherence Tomography in Multiple Sclerosis[4] were available when the SPRINT-MS study protocol was developed. However, scan requirements and training in SPRINT-MS emphasized similar elements of scan quality to these now-published standards. General requirements for images

were that the fundus image was in focus and well-illuminated (and iris in focus if Cirrus images), with scan appropriately centered and no breaks of blood vessels (saccades). Technicians were encouraged to submit a minimum of two scans of each type from each eye, with no maximum number of scans that could be transmitted for each study visit.

Ocular Coherence Tomography Quality Control and Analysis

All submitted scans for each subject and visit were received at the central reading center and evaluated by two independent certified optical coherence tomography graders blinded to treatment assignment. These certified graders selected the highest quality scans from each study visit and entered retinal nerve fiber layer measurements into the study database. Certified graders evaluated images with attention to the scan being correctly centered, possible algorithm failure, and possible alternative retinal pathology. If a grader's retinal nerve fiber layer measurement was more than 7.5% from the mean value of the two measurements, then a third certified grader would independently analyze the image. Masked image evaluation data was submitted to the Medical Director for final review. The average retinal nerve fiber layer thickness measured by each certified grader was utilized for analysis in the NN102/SPRINT-MS study.

Sample Size

Sample size estimates were computed from published and unpublished data from clinical trials and preliminary studies using analytic formulas for linear mixed model analysis and yielded a sample size of N=125 patients per treatment arm to provide 82.5% power to measure a treatment effect on the rate of brain atrophy of 33% or larger.

Statistical Analysis

All models included patient specific random intercepts and slopes that were assumed to be distributed according to a multivariate normal distribution with an unstructured covariance. We assumed the random errors to be normally and identically distributed and independent of the random effects. We further assumed an overall common fixed effect intercept to constrain the baseline means to be equal in order to control for any initial imbalance that might occur due to chance.[5] For each imaging endpoint, Akaike's Information Criteria[6] was used to select between models that assumed a linear trend over time and models that included time as a categorical variable to allow for non-linear trends. For all reported outcomes, the linear model provided the best fit. Models were adjusted for disease type (primary or secondary progressive MS) and concurrent immunomodulating therapy use (yes or no).

Sensitivity analyses were conducted to assess the impact of the Missing at Random assumption by fitting a number of pattern-mixture models incorporating a variety of assumptions for non-missing at random data, including an extreme assumption that the non-completers on ibudilast were a group that would receive no benefit from treatment (i.e., were similar to controls). Additional prespecified sensitivity analyses included the effects of covariates imbalanced at baseline and a per-protocol analysis, which included patients with no major protocol deviations, 75% - 125% trial medication compliance, and only utilized data collected prior to any early discontinuation of trial medication.

An interim analysis was conducted after approximately half of patients completed the trial and included futility and overwhelming efficacy stopping criteria. The interim analysis used the Lan-DeMets alpha spending function approach with the O'Brien-Fleming stopping boundaries.

Placing BPF Into Context

The primary outcome of SPRINT-MS found that treatment of ibudilast was associated with a slowing in progression of brain atrophy of 0.0009 brain parenchymal fraction (BPF) units per year.

Using the baseline group mean brain parenchymal fraction (BPF) of 0.80 and each group's observed annualized BPF progression rate:

Placebo: 0.80 – (0.0019 BPF units/year x 96 weeks) = 0.7965 BPF units at 96 weeks

Ibudilast: 0.80 – (0.0010 BPF units/year x 96 weeks) = 0.7982 BPF units at 96 weeks

Difference = 0.0017 BPF units over 96 weeks

BPF is derived from dividing the brain tissue volume (BV) by the outer contour volume (OCV) of the brain: BPF = BV / OCV.[7] Using the mean OCV of 1472.63 ml from the 255 randomized subjects at baseline, 0.0017 BPF units translates to sparing on average of about 2.50 ml brain tissue with ibudilast compared to placebo in a patient with progressive MS.

Figure S1. Consort Diagram

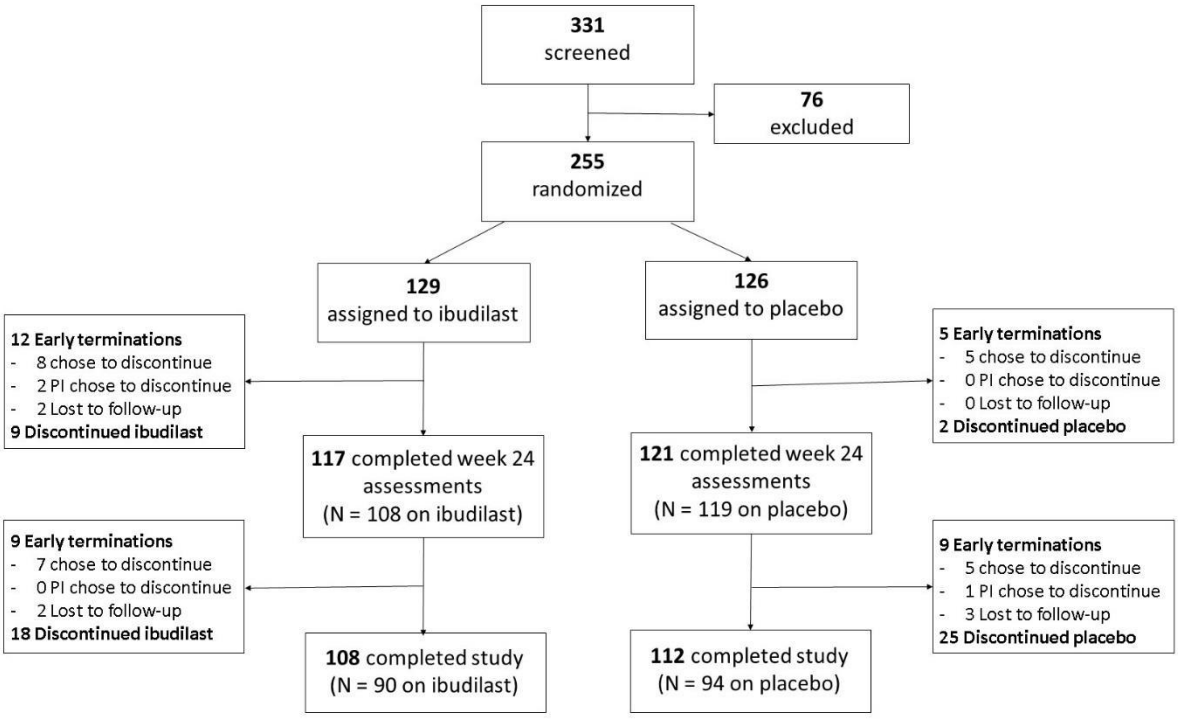

Figure S1. Consort Diagram. A schematic of patient disposition in the NN102/SPRINT-MS trial.

Figure S2. Major Secondary Outcomes

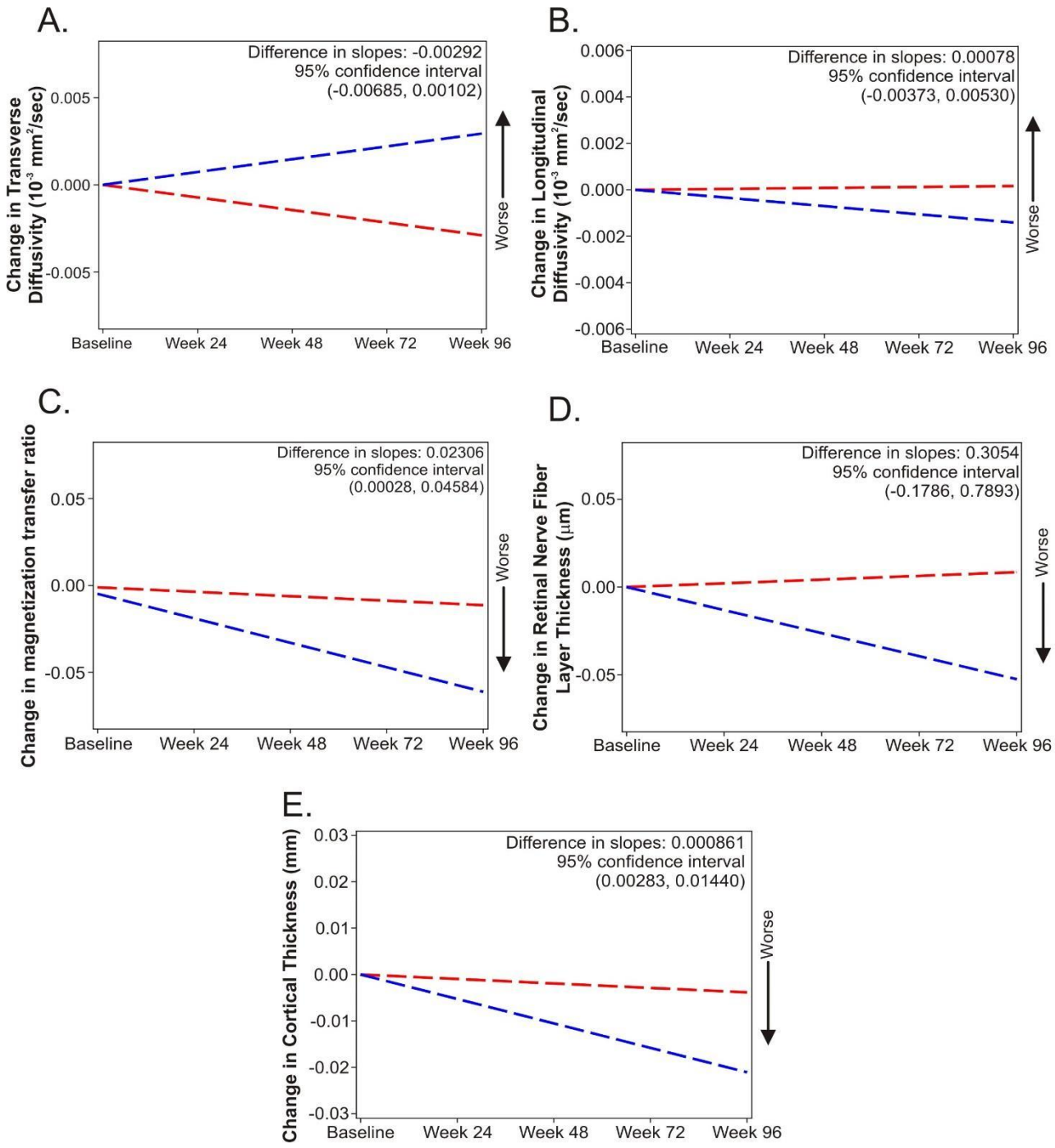

Figure S2. Major secondary outcomes. 96-week change in the major secondary outcomes for ibudilast group (red line) and placebo group (blue line) as measured by (A) mean transverse diffusivity in the corticospinal tracts; (B) mean longitudinal diffusivity in the corticospinal tracts; (C) magnetization transfer ratio in normal appearing brain tissue; (D) thickness of retinal nerve fiber layer; and (E) mean cortical thickness.

Figure S3. Change in Brain Atrophy

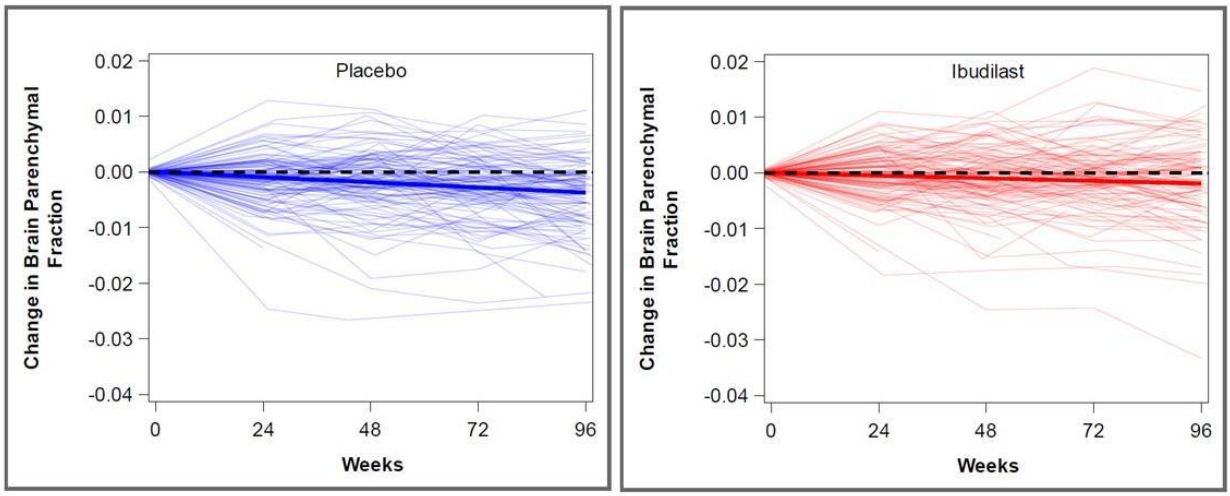

Figure S3. Change in whole brain atrophy. Individual patient values and linear mixed model of the slope of change model for each treatment group.

Supplemental Table S1

Serious Adverse Events in Ibudilast and Placebo groups

	Placebo (N = 126)	Ibudilast (N = 129)	P value
Serious Adverse Events (number, percentage)	24 (19%)	20 (16%)	0.46
	Atrial Fibrillation Back pain Bladder Prolapse Bladder Transitional Cell Carcinoma Breast Cancer Cerebrovascular Accident Cervical Spinal Stenosis Choleothiasis Colonic Obstruction Convulsion Cystitis Endometrial Cancer Forearm Fracture Gastroenteritis Hyponatremia Injury Intestinal Obstruction Kidney Infection Muscular Weakness Parotidectomy Pneumonia Aspiration Pulmonary Emoblism Pyrexia Sepsis (2) Skin Infection (2) Thrombocytopenia Tooth Infection Urinary Tract Infection (2)	Asthenia Ataxia Back pain Cerebral Hemorrhage Cervical Carcinoma Stage 0 Clostridium Difficile Colitis Dehydration (2) Febrile Neutropenia Fracture Hypercalcaemia Hypokalemia Metastatic Malignant Melanoma Multiple Fractures Myocardial Infarction Nephrolithiasis (2) Pain Pain in extremity Rotator Cuff Syndrome Sepsis (2) Sinus Tachycardia Spondylitic myopathy Transient Acantholytic Dermotosis Urinary Tract Infection (2)	

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