(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 March 2020 (26.03.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/061355 A2

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 25/28* (2006.01)

(21) International Application Number:
PCT/US2019/051992

(22) International Filing Date:
19 September 2019 (19.09.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/733,934	20 September 2018 (20.09.2018)	US
62/743,313	09 October 2018 (09.10.2018)	US
62/843,851	06 May 2019 (06.05.2019)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

(54) Title: SERUM NEUROFILAMENT PROTEIN FOR GUIDING THERAPEUTIC INTERVENTION IN MULTIPLE SCLEROSIS

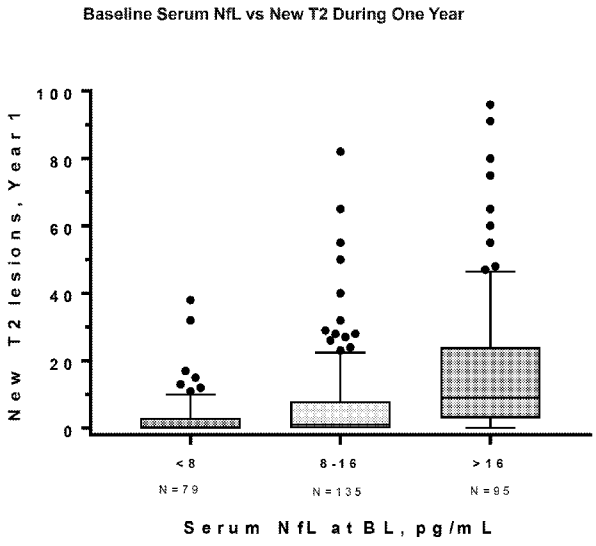


Figure 1A

(57) Abstract: In one aspect, the invention provides a method for alleviating symptoms in a patient with multiple sclerosis, comprising administering an efficacious therapeutic that is efficacious to the patient, the efficacious therapeutic, when administered to the patient, resulting in a level of a neurofilament protein in serum of the patient that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the therapeutic. In some embodiments, the patient is currently being administered with or has formerly been administered with either a non-efficacious therapeutic or a therapeutic that is different than the efficacious therapeutic.

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KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

SERUM NEUROFILAMENT PROTEIN FOR GUIDING THERAPEUTIC INTERVENTION IN MULTIPLE SCLEROSIS

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 62/733,934, filed September 20, 2018, U.S. Provisional Application No. 62/743,313, filed October 9, 2018, and U.S. Provisional Application No. 62/843,851, filed May 6, 2019. The content of each of the foregoing applications are incorporated by reference herein in their entirety.

Background of the Invention

[0002] The invention relates to the field of biology and medicine, and particularly to the field of multiple sclerosis.

[0003] Multiple sclerosis (MS) is a chronic, disabling disease of the central nervous system. In MS, the myelin sheath covering nerve fibers is damaged, resulting in disruption and breakdown in the patient's nervous system. Symptoms of MS include vision problems, numbness, pain, slurred speech, fatigue, muscle weakness, dizziness, and problems with bowel and bladder function.

[0004] There are different types of MS. Most patients have relapsing-remitting MS, in which they experience periods of new symptoms, or relapses, that can last days or weeks, which relapsing periods are interspersed with quiet periods of disease remission during which the symptoms partially or completely improve. In relapsing-remitting MS, the quiet periods may last years.

[0005] Another type of MS is secondary progressive MS. In secondary progressive MS, there is a progressive worsening of neurological function over time, thus resulting in an accumulation of disability. Although there may be occasional relapses, unlike relapsing-remitting MS, patients with secondary progressive MS who do have non-relapsing periods do not experience improvement in symptoms at that time. Rather, following a relapse (which is rare in secondary progressive MS), the start of the non-relapsing period has the lowest severity of symptoms, and the symptoms will either stay the same or progressively worsen with time during the non-relapsing period. Thus, it can be difficult to distinguish a non-relapsing period from a relapsing period in secondary progressive MS patients. Often, a patient starts out with relapsing-remitting MS and progresses over time to secondary progressive MS.

[0006] Yet another type of MS that rarely occurs is primary progressive MS. In primary progressive MS patients, no relapses occur. Rather, the patient's neurological function steadily worsens over time.

[0007] There are several therapeutics for alleviating the symptoms of MS, including but not limited to beta-interferons, dimethyl fumarate, natalizumab, alemtuzumab, fingolimod, ocrelizumab, and teriflunomide. However, not every therapeutic alleviates symptoms in all patients, and there is currently no known cure for multiple sclerosis.

[0008] Thus, there remains a need for identifying new therapeutics that will alleviate the symptoms of MS in patients.

Brief Summary of the Invention

[0009] In some embodiments, the invention provides methods for identifying whether a therapeutic is efficacious for that patient and will thus alleviate the symptoms of MS and slow down disability progression in that patient. In some embodiments, the invention provides methods of identifying a severe MS disease early so an appropriate therapeutic is initiated as soon as possible. In some embodiments, the invention provides clinically-relevant cutpoints for specific context of use. In some embodiments, the invention provides methods to determine if an MS patient is responding to an administered therapeutic (i.e., if the therapeutic is efficacious in that patient), and methods to treat an MS patient with a therapeutic that will alleviate that patient's symptoms.

[00010] Accordingly, in a first aspect, the invention provides a method for alleviating symptoms in a patient with multiple sclerosis, comprising administering an efficacious therapeutic that is efficacious to the patient, the efficacious therapeutic, when administered to the patient, resulting in a level of a neurofilament protein in serum of the patient that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum (or equal to an amount equivalent to 16 pg serum neurofilament light chain per ml serum) or lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the efficacious therapeutic. In some embodiments, the patient is currently being administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the efficacious therapeutic.

[00011] In another aspect, the invention provides a method for determining if a therapeutic administered to a patient with multiple sclerosis is efficacious to the patient, comprising obtaining or having obtained a blood or serum sample from the patient administered with the therapeutic; and measuring or having measured a level of a

neurofilament protein in blood or serum of the patient to obtain a post-treatment level, wherein a post-treatment level that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum (or equal to an amount equivalent to 16 pg serum neurofilament light chain per ml serum) or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the therapeutic identifies the therapeutic as being efficacious to the patient. In some embodiments, the patient is currently being administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the efficacious therapeutic.

[00012] In another aspect, the invention provides method for treating a patient with multiple sclerosis with an efficacious therapeutic, comprising: (i) determining whether symptoms of multiple sclerosis in the patient will be alleviated by a candidate therapeutic by: (a) obtaining or having obtained a blood or serum sample from the patient following administration of the candidate therapeutic; and (b) measuring or having measured a level of a neurofilament protein in blood or serum of the patient to obtain a post-treatment level; and if the post-treatment level is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum (or equal to an amount equivalent to 16 pg serum neurofilament light chain per ml serum) or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the candidate therapeutic, then the candidate therapeutic is an efficacious therapeutic and (ii) administering the efficacious therapeutic to the patient, wherein the efficacious therapeutic will alleviate the symptoms of multiple sclerosis in the patient with multiple sclerosis. In some embodiments, the patient is currently being administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the candidate therapeutic.

[00013] In yet another aspect, the invention provides a method for identifying a efficacious therapeutic that will alleviate the symptoms of multiple sclerosis in a patient with multiple sclerosis, comprising administering a candidate therapeutic to the patient and measuring a level of a neurofilament protein in serum of the treated patient after the patient has been administered for X weeks with the candidate therapeutic to obtain a post-treatment level, wherein a post-treatment level that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum (or equal to an amount equivalent to 16 pg serum neurofilament light chain per ml serum) or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the candidate therapeutic, identifies the candidate therapeutic as an efficacious therapeutic that will alleviate the symptoms of multiple sclerosis in the patient. In some embodiments, the patient is currently being

administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the candidate therapeutic.

[00014] In some embodiments, X is 48. In some embodiments, X is 12, 24, or 36, or 48, or 72, or 96. In some embodiments, X is 10-20, 20-40, 40-80, or 80-100.

[00015] In another aspect, the invention provides a method for alleviating symptoms in a patient with multiple sclerosis by administration of an efficacious therapeutic, comprising measuring a level of a neurofilament protein in blood or serum of the patient, the patient currently being administered with a therapeutic that is (a) a non-efficacious therapeutic or (b) different than the efficacious therapeutic and, if the level is greater than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or is greater than a level of neurofilament protein in a patient with multiple sclerosis not administered the efficacious therapeutic, then stopping administration of the therapeutic to the patient and starting administration of the efficacious therapeutic to the patient.

[00016] In accordance with any method described herein, in some embodiments, the method comprises obtaining or having obtained a blood or serum sample from the patient administered with the therapeutic 2-8 months (e.g., 2-3 months, 3-4 months, 4-5 months, 5-6 months, 6-7 months, or 7-8 months) after initiating treatment with the therapeutic. In accordance with any method described herein, in some embodiments, the method comprises measuring or having measured a post-treatment level of neurofilament protein 2-8 months (e.g., 2-3 months, 3-4 months, 4-5 months, 5-6 months, 6-7 months, or 7-8 months) after initiating treatment with the therapeutic.

[00017] In some embodiments, the patient has relapsing remitting multiple sclerosis (RRMS). In some embodiments, the patient has secondary progressive multiple sclerosis (SPMS). In some embodiments, the patient has clinically isolated syndrome (CIS). In some embodiments, the patient has had his/her first demyelinating event. In some embodiments, the patient has had less than 3 (e.g., less than 3, 2, or 1) demyelinating events, e.g., at the time of initiating treatment with a therapeutic, e.g., a therapeutic described herein. In various embodiments, the patient is human.

[00018] In various embodiments of the various aspects of the invention, the patient is currently being administered with a non-efficacious therapeutic. In various embodiments of the various aspects of the invention, the patient is currently being administered with a therapeutic that is different than the candidate therapeutic. In various embodiments of the various aspects of the invention, the patient is currently being administered with a therapeutic that is different than the efficacious therapeutic.

[00019] In various embodiments of the various aspects of the invention, the patient has been formerly been administered with a non-efficacious therapeutic. In various embodiments of the various aspects of the invention, the patient has been formerly administered with a therapeutic that is different than the candidate therapeutic. In various embodiments of the various aspects of the invention, the patient is currently being administered with a therapeutic that is different than the efficacious therapeutic.

[00020] In various embodiments, the neurofilament protein is a neurofilament light chain. In various embodiments, the neurofilament protein is a neurofilament heavy chain. In various embodiments, the neurofilament protein is a neurofilament medium chain. In various embodiments, the neurofilament protein is internexin. In various embodiments, the neurofilament protein is peripherin.

[00021] In various embodiments, the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic is at least 10% lower (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more, lower) than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

[00022] In various embodiments, the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least three months is at least 10% lower (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more, lower) than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

[00023] In various embodiments, the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least six months is at least 10% lower (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more, lower) than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

[00024] In various embodiments, the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least twelve months is at least 10% lower (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more, lower) than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

[00025] In various embodiments, the efficacious therapeutic comprises a very late antigen (VLA-4)-binding agent (e.g., natalizumab), interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide,

fingolimod, dalfampridine, dimethyl fumarate, pegylated interferon beta-1a, or any combination thereof.

[00026] In various embodiments, the efficacious therapeutic is a very late antigen 4 (VLA-4)-binding agent. In various embodiments, the VLA-4-binding agent is natalizumab.

[00027] In various embodiments, the efficacious therapeutic is a fumarate, e.g., dimethyl fumarate, diroximel fumarate, or monomethyl fumarate.

[00028] In various embodiments, the efficacious therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine and dimethyl fumarate. In various embodiments, the efficacious therapeutic is interferon beta-1a. In various embodiments, the interferon beta-1a is pegylated. In various embodiments, the efficacious therapeutic comprises a combination therapy comprising one or more efficacious therapeutics described herein.

[00029] In various embodiments, the administration of the efficacious therapeutic slows the progression of the MS disease in the patient.

[00030] In another aspect, the invention provides a method of treating multiple sclerosis in a human subject in need thereof, comprising: administering one or more doses of a therapeutic to the human subject; optionally ceasing the administration of the therapeutic for a predetermined time period (e.g., at least 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, or more); measuring a serum neurofilament light chain level in a sample obtained from the human subject; and if the measured serum neurofilament light chain level is greater than an amount equivalent to 16 pg per ml, then administering one or more further doses of the therapeutic to the human subject, and if the measured serum neurofilament light chain level is equal to or lower than an amount equivalent to 16 pg per ml, then further measuring serum neurofilament light chain levels in the human subject one or more times prior to further administering the therapeutic.

[00031] In some embodiments, the therapeutic is not administered to the human subject for at least 1 week (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks) prior to measuring a serum neurofilament light chain level in a sample obtained from the human subject.

[00032] In some embodiments, the therapeutic is a very late antigen 4 (VLA-4)-binding agent (e.g., natalizumab).

[00033] In some embodiments, the therapeutic is a fumarate, e.g., dimethyl fumarate, diroximel fumarate, or monomethyl fumarate.

[00034] In some embodiments, the therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine, and dimethyl fumarate.

[00035] In another aspect, the invention provides a method of treating multiple sclerosis in a human subject in need thereof, comprising: administering initial doses of a therapeutic to the human subject, wherein each of the initial doses is in the same amount and is administered at the same dosing interval between doses; measuring a serum neurofilament light chain level that is equal to or lower than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and administering further doses of the therapeutic to the human subject, wherein each of the further doses is in the same or lesser amount and at the same or lengthened dosing interval as compared to the initial doses.

[00036] In some embodiments, the therapeutic is a very late antigen 4 (VLA-4)-binding agent (e.g., natalizumab).

[00037] In some embodiments, the therapeutic is a fumarate, e.g., dimethyl fumarate, diroximel fumarate, or monomethyl fumarate.

[00038] In some embodiments, the therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine, and dimethyl fumarate.

[00039] In another aspect, the invention provides a method of treating multiple sclerosis in a human subject in need thereof, comprising: administering initial doses of a therapeutic to the human subject, wherein each of the initial doses is in the same amount and is administered at the same dosing interval between doses; measuring a serum neurofilament light chain level that is greater than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and administering further doses of the therapeutic to the human subject, wherein each of the further doses is in an increased amount and/or at a shortened dosing interval as compared to the initial doses.

[00040] In some embodiments, the therapeutic is a very late antigen 4 (VLA-4)-binding agent (e.g., natalizumab).

[00041] In some embodiments, the therapeutic is a fumarate, e.g., dimethyl fumarate, diroximel fumarate, or monomethyl fumarate.

[00042] In some embodiments, the therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine, and dimethyl fumarate.

[00043] In another aspect, the invention provides a method of treating multiple sclerosis in a human subject in need thereof, comprising: (i) administering one or more doses of a therapeutic to the human subject; (ii) measuring a serum neurofilament light chain level that is equal to or lower than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and (iii) further measuring the serum neurofilament light chain level in the human subject, wherein the therapeutic is not further administered to the human subject in the interval between the measuring in step (ii) and the further measuring in step (iii).

[00044] In some embodiments, the therapeutic is a very late antigen 4 (VLA-4)-binding agent (e.g., natalizumab).

[00045] In some embodiments, the therapeutic is a fumarate, e.g., dimethyl fumarate, diroximel fumarate, or monomethyl fumarate.

[00046] In some embodiments, the therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine, and dimethyl fumarate.

Brief Description of the Drawings

[00047] Figure 1A is a graph showing the number of new T2 lesions in comparison to the levels of neurofilament light chain in the serum of MS patients with relapsing-remitting MS a year earlier.

[00048] Figure 1B is a line graph showing that if serum neurofilament light chain level is greater than 16pg/ml, the patient has a greater than 90% of having a new T2 lesion in the year following the serum neurofilament light level measurement date.

[00049] Figure 2A is a bar graph showing the percentage of patients with increased disability (as measured by EDSS) 5 years after their neurofilament light chain levels in serum were measured.

[00050] Figure 2B describes that patients with high serum NfL levels have several fold (5-10 fold) greater odds ratio of progressing to EDSS 6.0 or greater at 8 and 15 years later.

[00051] Figures 2C and 2D are graphs showing the volume of T2 lesions in MS patients with less than 8 pg/ml serum Neurofilament light chain, between 8 and 16 pg/ml neurofilament light chain, and greater than 16 pg/ml neurofilament light chain 5 years after their neurofilament light chain levels in serum were measured (Fig. 2C) and 10 years after their neurofilament light chain levels in serum were measured (Fig. 2D).

[00052] Figure 2E is a graph showing the percentage change of brain parenchymal fraction (BFP or brain atrophy) in MS patients with less than 8 pg/ml serum Neurofilament light chain, between 8 and 16 pg/ml neurofilament light chain, and greater than 16 pg/ml neurofilament light chain 5 years after their neurofilament light chain levels in serum were measured.

[00053] Figures 3A-3E are line graphs showing the level of neurofilament light chain in blood serum levels in patients after 48 weeks of treatment with placebo (Fig. 3A), 48 weeks of treatment with pegylated interferon beta-1a (Fig. 3B), 96 weeks of treatment with natalizumab + IFN-beta-1a (Fig. 3C), 48 weeks of treatment with natalizumab (FIG. 3D), and 12 weeks of treatment with dimethyl fumarate (FIG. 3E).

[00054] Figures 4A-4C are graphs showing the brain volume changes after 96 weeks following measurement of neurofilament light chain in patient serum. As can be seen in Fig. 4A, those patients with serum neurofilaments between 13.4-154 picograms/ml (lowest tertile) at baseline (i.e., 96 weeks prior to brain measurement) showed the highest amount of brain volume loss 96 weeks late. Figure 4B and 4B show the brain loss in patients with Gd+ (i.e., active) lesions 96 weeks after baseline and brain loss in patients with Gd- (i.e., non-active) lesions 96 weeks after baseline, respectively.

[00055] Figure 5 is a line graph showing the measurements of serum neurofilament light chain in patients that had no increase in EDSS score (i.e., no progression, circles) and increase in EDSS score (i.e., progression, squares).

[00056] Figure 6 is a line graph showing the measurements of serum neurofilament light chain in patients that had no increase T25FW score (i.e., no progression, circles) and increase in T25FW score (i.e., progression, squares).

[00057] Figure 7 is a line graph showing the measurements of serum neurofilament light chain in patients that had no increase 9HPT-D (dominant hand) score

(i.e., no progression, circles) and increase in 9HPT-D (dominant hand) score (i.e., progression, squares).

[00058] Figure 8 is a line graph showing the measurements of serum neurofilament light chain in patients that had no increase 9HPT-ND (non-dominant hand) score (i.e., no progression, circles) and increase in 9HPT-ND (non-dominant hand) score (i.e., progression, squares).

[00059] Figure 9 is a line graph showing the serum neurofilament levels in patients administered with natalizumab (circles) and patients administered with placebo (squares)

[00060] Figures 10A and 10B are line graphs showing the serum neurofilament light chain levels in patients administered with natalizumab (circles, solid line) and patients administered with placebo (circles, dotted line), where the patients had Gd+ lesions at week 0 (Fig. 1-A) and where the patients did not have Gd+ lesions at week 0 (Fig. 10B).

[00061] Figures 11A and 11B are line graphs showing the serum neurofilament levels in MS patients administered with natalizumab (circles, solid line) and patients administered with placebo (circles, dotted line), where the patients had one or more relapses within 2 years prior to week 0 (Fig. 11A) and where the patients did not have any relapses within two years prior to week 0 (Fig. 11B).

[00062] Figures 12A and 12B are line graphs showing the serum neurofilament levels in patients administered with natalizumab (circles, solid line) and patients administered with placebo (squares, dotted line), where the patients had inflammatory activities during the 96 weeks of treatment (Fig. 12A) and where the patients did not have any inflammatory activities during the 96 weeks of treatment (Fig. 12B).

[00063] Figures 13A and 13B are two line graphs showing that patients with no evident disease activity (NEDA; Fig. 13A) have consistently low and stable serum NfL levels, and patients with evident disease activity (EDA; Fig. 13B) have higher and more variable serum NfL levels. The highest variability was seen in EDA patients with more than 1% brain volume loss per year (see Fig. 13B).

[00064] Figures 14A-14C are graphs showing that a stronger increase of serum NfL from BL to Year 1 in PEG-IFN treated patients is associated with higher accumulation of new T2 lesions over 1 year (Fig. 14A), 2 years (Fig. 14B) and 4 years (Fig. 14C).

[00065] Figures 15A and 15B show that patients with higher average NfL levels have higher brain atrophy over 2 years in patients on PEG-IFN treatment (Figure 15A,

NfL average from baseline, month 3 and month 6; Figure 15B, NfL average of baseline, month 3, month 6, month 9 and month 12).

[00066] Figures 16A-16B are graphs showing the ROC curves for average NfL measurement in PEG-IFN treated MS patients having no evident disease activity over 6 months (Fig. 16A) and 1 year (Fig. 16B).

[00067] Figures 17A-17C are line graphs showing that serum neurofilament light chain levels were significantly lower in patients treated with pegylated interferon as compared to placebo as early as 3 months after the start of treatment. Fig. 17A shows patients with baseline neurofilament levels of less than 8 pg/ml serum, Fig. 17B shows patients with baseline neurofilament levels of 8-16 pg/ml serum; and Fig. 17C shows patients with baseline neurofilament levels of greater than 16 pg/ml serum.

[00068] Figure 18 is a graph showing the association between baseline sNfL concentrations and % normalized brain volume change from baseline to year 2.

[00069] Figure 19 is a panel of graphs showing the sNfL levels in natalizumab-treated versus placebo-treated subjects, binned by the subject's baseline sNfL levels. NfL data is shown as mean ± 95% CI.

[00070] Figure 20A is a graph showing the association between baseline (BL) Gd+ lesion count and BL sNfL concentration. Figure 20B is a graph showing the association between BL sNfL and new T2 lesions between BL to year 1.

[00071] Figure 21A is a graph showing the association between baseline sNfL levels and time to clinically definite multiple sclerosis (CDMS) over 5 years. Figure 21B is a graph showing the association between baseline sNfL and percent of patients reaching EDSS score of >= 3.5 at 5 years.

[00072] Figure 22 is a graph showing the distribution of sNfL levels among healthy controls at different ages.

[00073] Figure 23A is a graph showing the association between sNfL level and change in peri-papillary retinal nerve fiber layer (pRNFL). Figure 23B is a graph showing the association between sNfL level and change in ganglion cell layer and inner plexiform layer (GCIPL).

[00074] Figure 24 is a panel of graphs showing sNfL levels at 6 months post treatment initiation associated with longer term MRI and clinical outcomes.

[00075] Figure 25 is a graph and table showing the sNfL profile in a single patient.

Detailed Description

[00076] In some embodiments, the invention provides methods to determine if an MS patient will respond to a therapeutic, and methods to treat an MS patient with a therapeutic that will alleviate that patient's symptoms. In some embodiments, the patient is a human.

[00077] The publications (including patent publications), web sites, company names, and scientific literature referred to herein establish the knowledge that is available to those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter.

[00078] All amino acid sequences shown herein are in the amino (N) to carboxy (C) direction, unless indicated otherwise. All nucleotide sequences shown herein are in the 5' to 3' direction, unless indicated otherwise. When comprising or comprises is used in reference to an indicated amino acid sequence, it is meant that one or more amino acid residues can appear at the N' end of the indicated amino acid sequence, at the C' end of the indicated amino acid sequence, or at both end; but that one or more amino acid residues do not appear between stated amino acid residues in the indicated amino acid sequence. When comprising or comprises is used in reference to an indicated nucleotide sequence, it is meant that one or more nucleotide residues can appear at the 5' end of the indicated nucleotide sequence, at the 3' end of the indicated nucleotide sequence, or at both ends; but that one or more nucleotide residues do not appear between stated nucleotide residues in the indicated nucleotide sequence.

[00079] Terms defined or used in the description and the claims shall have the meanings indicated, unless context otherwise requires. Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter. As used herein, the following terms have the meanings indicated. As used in this specification, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise. The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the

numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

[00080] Multiple Sclerosis (MS) is a chronic disease in which the myelin sheath covering nerve fibers are damaged, resulting in disruption of communication between the brain and the rest of the body. MS takes several forms, with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms). Between relapses, symptoms (e.g., muscle weakness, double vision, coordination loss) may disappear completely; however, permanent neurological problems often remain, especially as the disease advances.

[00081] Most people with MS start with a relapsing-remitting disease course (Relapsing remitting multiple sclerosis or RRMS). RRMS patients experience periods of new symptoms or relapses that develop over days or weeks and usually improve partially or completely in the quiet periods in between the relapses. The quiet periods of disease remission that can last months or even years.

[00082] However, about 60 to 70 percent of people with relapsing-remitting MS eventually develop a steady progression of symptoms, with or without periods of remission, known as secondary-progressive MS. The worsening of symptoms usually includes problems with mobility and gait. The rate of disease progression varies greatly among people with secondary-progressive MS.

[00083] It has been found that more damage can be incurred in the first years of the disease than in later stages of MS (see Kuhlmann et al. *Brain.* 125:2202-2212, 2002). Thus, without wishing to be bound by any particular theory, it may be that if a MS patient, such as a patient with RRMS, is treated early in the disease progression (e.g., shortly after diagnosis) with a disease modifying therapy (DMT) that is best suited to alleviate the symptoms of that particular patient, the patient's disease may progress more slowly and thus that RRMS patient's progression to secondary progressive MS may be slowed. Similarly, if an MS patient with secondary-progressive MS is treated early (e.g., shortly after diagnosis), the rate of his or her disease progression may be slowed.

[00084] Currently, disease progression in MS is measured by functional tests and by the appearance of lesions, or plaques, in the brain and/or spinal cord as observed using magnetic resonance imaging (MRI). Such functional tests, including the Expanded Disability Status Scale (EDSS) test and the Timed 25-foot walk (T25FW) test, are well known to practicing health care providers and scientists, and are described in brief below. Physical

tests, including the observation of gadolinium (Gd) enhancing lesions, T1 lesions and/or T2 lesions on MRI are also well known and described in brief below.

[00085] There is no cure for multiple sclerosis, and although there are therapeutics that are approved for administration to MS patients, not every MS patient will respond to every therapeutic. Thus, the treatment of MS is challenging because not all MS patients respond the same to the same therapeutic, and choosing the correct therapeutic for a particular patient can be difficult. Currently, it is up to a health care provider to select a particular therapeutic for use on a particular patient. The health care provider (e.g., physician) currently selects a particular therapeutic for treatment of a particular patient based on the alleviation of MS symptoms in that particular patient, but there is no certainty that the particular therapeutic selected will, in fact, alleviate the symptoms of MS in that particular patient.

[00086] To determine if a therapeutic alleviates the MS symptoms in a particular patient, a therapeutic is judged by its ability to slow the progression of the MS disease and/or reduce symptoms of MS in a patient administered with the therapeutic as compared to a patient not administered with the therapeutic (e.g., an untreated patient or a patient prior to treatment). Various tests have been developed to assess a patient's disability, including the Expanded Disability Status Scale (EDSS) score, the Multiple Sclerosis Severity Score, the Timed 25-Foot Walk (T25FW) assessment, the 9-Hole Peg Test (9HPT) assessment, the 12-item Multiple Sclerosis Walking Scale (MSWS-12), change in patient-reported manual ability based on the ABILHAND questionnaire, patient-reported quality of life with the Multiple Sclerosis Impact Scale-29 (MSIS-29) physical score, number and/or frequency of Gadolinium enhancing lesions as measured by magnetic resonance imaging (MRI), number of T2 lesions in the brain as measured by MRI, and number of T1 lesions in the brain as measured by MRI.

[00087] The EDSS score is a test used to describe the severity of disability in an MS patient (see, e.g., Zackowski et al., Mult. Scler. Rel. Dis. 4(1): 67-74, 2015). EDSS is a method of quantifying disability in multiple sclerosis and monitoring changes in the level of disability over time. It is widely used in clinical trials and in the assessment of people with MS. The EDSS scale ranges from 0 to 10 in 0.5 unit increments that represent higher levels of disability. Scoring is based on an examination by a neurologist.

[00088] EDSS scores 1.0 to 4.5 refer to people with MS who are able to walk without any aid and is based on measures of impairment in eight functional systems (FS): pyramidal – weakness or difficulty moving limbs; cerebellar – ataxia, loss of coordination

or tremor; brainstem – problems with speech, swallowing and nystagmus; sensory – numbness or loss of sensations; bowel and bladder function; visual function; and cerebral (or mental) functions. Each functional system is scored on a scale of 0 (no disability) to 5 or 6 (more severe disability).

[00089] EDSS scores 5.0 to 9.5 are defined by the impairment to walking. In general, the EDSS scores are as follows:

EDSS Score	Description
1.0	No disability, minimal signs in one functional system (FS)
1.5	No disability, minimal signs in more than one functional system (FS)
2.0	Minimal disability in one FS
2.5	Mild disability in one FS or minimal disability in two FS
3.0	Moderate disability in one FS or mild disability in three or four FS. No impairment to walking.
3.5	Moderate disability in one FS and more than minimal disability in several other FS. No impairment to walking.
4.0	Significant disability but self-sufficient and up and about for about 12 hours a day. Able to walk without aid or rest for 500 meters
4.5	Significant disability but up and about much of the day. Able to walk without aid or rest for 300 meters
5.0	Disability severe enough to impair full daily activities and ability to work a full day without special provisions. Able to walk without aid or rest for 200 meters
5.5	Disability severe enough to preclude full daily activities. Able to walk without aid or rest for 100 meters
6.0	Requires a walking aid – cane, crutch, etc. – to walk about 100m with or without resting
6.5	Requires two walking aids – pair of canes, crutches, etc. – to walk about 20m without resting
7.0	Unable to walk beyond approximately 5m even with aid. Essentially restricted to wheelchair; though wheels self in standard wheelchair and transfers alone. Up and about in wheelchair some 12 hours a day
7.5	Unable to take more than a few steps. Restricted to wheelchair and may need aid in transferring. Can wheel self but cannot carry on in standard wheelchair for a full day and may require a motorized wheelchair
8.0	Essentially restricted to bed or chair or pushed in wheelchair. May be out of bed itself much of the day. Retains many self-care functions. Generally has effective use of arms
8.5	Essentially restricted to bed much of the day. Has some effective use of arms. Retains some self-care functions.

9.0	Confined to bed. Can still communicate and eat
9.5	Confined to bed and totally dependent. Unable to communicate effectively or eat/swallow.
10.0	Death due to MS

[00090] In some embodiments, a therapeutic is said to alleviate the symptoms of MS in a particular patient (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least three months, the EDSS score of the patient stays the same, decreases, or increases by 0.5 or less as compared to the patient's EDSS score prior to start of administration of the therapeutic. For example, a therapeutic is said to alleviate the symptoms of MS if, prior to administration, the patient had an initial EDSS score of 4.0 and after three months of treatment with the therapeutic, the patient's EDSS score remains 4.0 or decreases (e.g. to 3.5 or 3.0) or increases to 4.5. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six months, the EDSS score of the patient stays the same, same, decreases, or increases by 0.5 or less as compared to the patient's EDSS score prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the EDSS score of the patient stays the same, same, decreases, or increases by 0.5 or less as compared to the patient's EDSS score prior to start of administration of the therapeutic.

[00091] The Timed 25-foot walk (T25-FW) test is a quantitative mobility and leg function performance test based on a timed 25-foot walk (see, e.g., Zackowski et al., Mult. Scler. Rel. Dis. 4(1): 67074, 2015). In this test, the patient is directed by a trained examiner (e.g., a nurse or physician or other trained professional) to one end of a clearly marked 25-foot course and is instructed to walk 25 feet as quickly as possible, but safely. The time is calculated from the initiation of the instruction to start and ends when the patient has reached the 25-foot mark. The task is immediately administered again by having the patient walk back the same distance. Patients may use assistive devices when doing this task. The score for the T25-FW is the average of the two completed trials.

[00092] The average walking speeds of healthy individuals (e.g., individuals not diagnosed with multiple sclerosis) are known (see, e.g., Bohannon et al., Age Ageing. 26(1):15–9, 1997). As an example, men aged 40-49 have a 3.10 second T25FW score on average and women aged 40-49 have a 3.95 second T25FW score on average.

[00093] In some embodiments, a therapeutic is said to alleviate the symptoms of MS (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least

three months, the T25-FW score of the patient stays the same, decreases, or increases by less than 5% as compared to the patient's T25-FW score prior to start of administration of the therapeutic. For example, a therapeutic is said to alleviate the symptoms of MS if, prior to administration, the patient had an initial T25-FW score of 6.3 seconds and after three months of treatment with the therapeutic, the patient's T25-FW score remains T25-FW or decreases (e.g. to 6.25 seconds) or increases to 6.615 seconds or less. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six months, the T25-FW score of the patient stays the same, decreases, or increases by less than 5% as compared to the patient's T25-FW score prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the T25-FW score of the patient stays the same, decreases, or increases by less than 5% as compared to the patient's T25-FW score prior to start of administration of the therapeutic.

[00094] The 9-HPT is a brief, standardized, quantitative test of upper extremity function (see, e.g., Grice et al., Amer. J. Occupational Therapy 57: 570-573, 2003). Both the dominant and the non-dominant hand are measured, giving scores of 9HPT-D and 9HPT-ND, respectively. The test is administered by a trained examiner (e.g., a nurse or physician or other trained professional). To do the test, the patient is seated at a table with a small, shallow container holding nine pegs and a wood or plastic block containing nine empty holes. On a start command when a stopwatch is started, the patient picks up the nine pegs one at a time as quickly as possible, puts them in the nine holes, and, once they are in the holes, removes them again as quickly as possible one at a time, replacing them into the shallow container. The total time to complete the task is recorded. Two consecutive trials with the dominant hand are immediately followed by two consecutive trials with the non-dominant hand. The score for the 9-HPT is an average of the four trials. The two trials for each hand are averaged, converted to the reciprocals of the mean times for each hand and then the two reciprocals are averaged. In healthy adults

[00095] In some embodiments, a therapeutic is said to alleviate the symptoms of MS (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least three months, the 9-HPT score (on either hand or both hands) of the patient stays the same, decreases, or increases by less than 5% as compared to the patient's the 9-HPT score (on either hand or both hands) prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six months, the 9-HPT score (on either hand or both hands) of the

patient stays the same, decreases, or increases by less than 5% as compared to the patient's the 9-HPT score (on either hand or both hands) prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the 9-HPT score (on either hand or both hands) of the patient stays the same, decreases, or increases by less than 5% as compared to the patient's the 9-HPT score (on either hand or both hands)prior to start of administration of the therapeutic.

[00096] Although the functional tests such as the EDSS test, the T25-FW test, and the 9-HPT test are non-invasive, they are time consuming to administer, often show changes only after months and sometimes years. Moreover, once function declines, it may be difficult to regain it. Thus, disease progression of MS can also be detected by keeping track of physical changes, preferably before evidence of functional decline. For example, physical changes can be detected using magnetic resonance imaging (MRI) scanning.

[00097] One common method for is the use of Gadaolinium (Gd) contrast with MRI scanning. Gadaolinium (Gd) is a large compound that is injected into a patient's vein during an MRI scan. Gadolinium normally cannot pass from the bloodstream into the brain or spinal cord due to the blood brain barrier. But during active inflammation within the brain or spinal cord, as during an MS relapse, the blood-brain barrier is disrupted, allowing gadolinium to pass through. As a result, the gadolinium can enter the brain or spinal cord and thus leak into an MS lesion, lighting it up and causing a highlighted spot on the MRI scan. If a lesion on the MRI lights up, it means that active inflammation has occurred within the two to three months prior to the scan. Active inflammation means that myelin sheath covering the nerve fiber is being damaged and/or destroyed by a person's immune cells.

[00098] If a lesion on an MRI does not light up after gadolinium is injected, then it's likely an older lesion—one that occurred more than 2 to 3 months prior to the scan. Thus, use of contrast helps a neurologist and/or other health care provider determine the age of a lesion.

[00099] In some embodiments, a therapeutic is said to alleviate the symptoms of MS in a patient administered with the therapeutic (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least three months, the number of gadolinium enhanced lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of gadolinium enhanced lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six

months, the number of gadolinium enhanced lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of gadolinium enhanced lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the number of gadolinium enhanced lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of gadolinium enhanced lesions in an MRI scan of the patient prior to start of administration of the therapeutic.

[000100] T2 lesions, found during a T2 weighted MRI scan, are the white spots observed on MRI using the typical imaging sequences acquired to diagnose and monitor Multiple Sclerosis. MS lesions in the brain will show up as white or bright spots on T2 weighted images, and these lesions are often referred to as MS or T2 plaques. If the T2 lesion is recently formed (i.e., within 2-3 months prior to the scan), it will light up if gadolinium was injected into the patient prior to the scan. Sometimes T2 plaques can heal and repair themselves, and the lesion will disappear over time. However, if a T2 plaque continues to become re-inflamed, it may eventually turn into a T1 lesion.

[000101] In some embodiments, a therapeutic is said to alleviate the symptoms of MS (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least three months, the number of T2 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T2 lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six months, the number of T2 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T2 lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the number of T2 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T2 lesions in an MRI scan of the patient prior to start of administration of the therapeutic.

[000102] T1 lesions, found during a T1 weighted MRI scan, are black spots or "holes" observed within the white matter of the brain. The T1 lesions or "black holes" within the brain can be caused by temporary process like acute inflammation or permanent scaring and tissue damage. The way to determine if a "black hole" is a permanent scar is to determine if it persists over a period usually greater than 3 months. Usually, the blacker the lesion, the

more likely it is that the tissue in the region has been destroyed. Eventually, the tissue is replaced entirely by water and appears totally black on a T1 weighted scan.

[000103] In some embodiments, a therapeutic is said to alleviate the symptoms of MS (i.e., be efficacious in that patient) if, after administration of the therapeutic for at least three months, the number of T1 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T1 lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least six months, the number of T1 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T1 lesions in an MRI scan of the patient prior to start of administration of the therapeutic. In some embodiments, a therapeutic is said to alleviate the symptoms of MS if, after administration of the therapeutic for at least twelve months, the number of T1 lesions in an MRI scan of the patients is the same or decreased in number as compared to the number of T1 lesions in an MRI scan of the patient prior to start of administration of the therapeutic.

[000104] However, the current tests to detect physical changes require expensive and time-consuming scanning such as magnetic resonance imaging (MRI) scanning. Because MRI scans use a strong magnet, they cannot be performed on patients with, for example, implanted pacemakers. Additionally, some patients experience side effects from the contrast agents, such as gadolinium contrast agents. Thus, tests to detect physical changes cannot be administered too frequently, and often scans are separated by six months or longer. During this length of time, physical changes can occur without detection.

[000105] In some embodiments, a therapeutic is said to alleviate the symptoms of MS in a particular patient (i.e., be efficacious in that patient) if, after administration of the therapeutic, e.g., for at least three months, the patient has less thinning (e.g., exhibits a slower rate of change in thickness) of a ganglion cell layer and inner plexiform layer (GCIPL) or peri-papillary retinal nerve fiber layer (pRNFL) (e.g., in one or two eyes), e.g., compared to a patient not administered the therapeutic.

[000106] Thus, the current tests for determining if a particular therapeutic will be efficacious (i.e., alleviate the symptoms) in a particular patient with MS rely on either the loss of function or the appearance of physical damage (e.g., appearance lesions in the brain and/or spinal cord) after such loss or function or appearance of physical damage occurs. While quiet time (i.e., no relapse) may allow the patient to recover that loss of function

and/or heal the physical lesion, a full recovery may not be achieved. Over time, disease progression worsens.

[000107] Thus, there is a need to find a way to identify a therapeutic will be efficacious in a particular patient that is not based on loss of function and/or appearance of physical damage (e.g., appearance of lesions).

[000108] Neurofilaments (NF) are proteins found in the cytoplasm of neurons. They are protein polymers measuring approximately 10 nm in diameter and many micrometers in length. Together with microtubules and microfilaments, they form the neuronal cytoskeleton. Neurofilaments are composed of different proteins including neurofilament protein L (low molecular weight, NFL, also called neurofilament light chain), neurofilament protein M (medium molecular weight; NFM, also called neurofilament medium chain) and neurofilament protein H (high molecular weight; NFH, also called neurofilament heavy chain). Neurofilaments in the mammalian nervous system also contain the protein internexin and that neurofilaments in the peripheral nervous system can also contain the protein peripherin.

[000109] Thus, as used herein, "a neurofilament protein" is meant neurofilament protein L (low molecular weight, NFL, also called neurofilament light chain), neurofilament protein M (medium molecular weight; NFM, also called neurofilament medium chain), neurofilament protein H (high molecular weight; NFH, also called neurofilament heavy chain), internexin and peripherin. The amino acid sequence of human neurofilament light chain is provided in SEQ ID NO:1 and in Julien et al., Biochimica et Biophysica Acta 909: 10-20, 1987 (see also NCBI Reference Sequence: NP_006149.2 and NCBI Reference Sequence: NG_008492.1). The amino acid sequences of human neurofilament heavy chain are provided in SEQ ID NO:2 and SEQ ID NO:3, and in Lees et al., EMBO J. 7(7): 1947-1955, 1988 (see also NCBI Reference Sequence: NG_008404.1 and NCBI Reference Sequence: NP_066554.2). The amino acid sequences of human neurofilament medium chain are provided in SEQ ID NO:4 and SEQ ID NO:5 and in Myers et al., EMBO J. 6(6): 1617-1626, 1987. SEQ ID NO:6 provides the sequence of human internexin protein. SEQ ID NO:7 provides the sequence of human peripherin protein.

[000110] Because of their specific structural role in neurons, neurofilaments can be used to assess neuron integrity. When a neuron is damaged, neurofilament escapes the damaged neuron and can be found in cerebral spinal fluid (CSF) or even in blood (e.g., in the serum component of the blood). Neurofilament light chain has been reported to be increased

in patients with Multiple Sclerosis (MS) (See Kuhle et al., Multiple Sclerosis Journal 22(12): 1550-1559, 2016).

[000111] Thus, in some embodiments, the neurofilament protein is a neurofilament light chain. In some embodiments, the neurofilament light chain is phosphorylated. Assays for measuring neurofilament light chain in serum has been described (see, e.g., Gaiottino et al., PLoS ONE 8: e75091, 2013; Kuhle et al., J. Neurol. Neurosurg. Psychiatry 86(3): 273-279, 2014. For example, blood serum from the patient centrifuged at 1000g for 10 minutes at room temperature and stored at −80°C within 2 hours of collection. Serum neurofilament light chain (NfL) concentrations can be measured (e.g., in duplicate) using ready-to-use enzyme linked immunosorbent assay (ELISA) diluent; Mabtech AB, Nacka Strand, Sweden) or an electrochemiluminescence (ECL) immunoassay described in Gaiottino et al., PLoS ONE 8: e75091, 2013, or a single molecule array (SIMOA) method described in Disanto et al., Ann. Neurol. 81(6): 857-870, 2017. The three assay methods have been compared in Kuhl et al., Clinical Chemistry and Laboratory Medicine 54 (10): 1655-1661, 2016. The SIMOA assay (particularly called the Simoa NF-light Advantage kit) is commercially available from Quanterix Corp. (Lexington, MA, USA).

[000112] In accordance with the methods described herein, a particular serum neurofilament light chain per ml serum level/concentration refers to (a) such a concentration/level as measured using a SIMOA assay described herein (e.g., the Simoa NF-light Advantage Kit assay described herein) or (b) an equivalent level/concentration of serum neurofilament light chain as measured by a different assay. For example, a 16 pg serum neurofilament light chain per ml serum level refers to (a) 16 pg serum neurofilament light chain per ml serum as measured using a SIMOA assay described herein (e.g., the Simoa NF-light Advantage Kit assay described herein) or (b) an equivalent level or concentration of serum neurofilament light chain as measured by a different assay.

[000113] The medium chain and/or heavy chain of neurofilament protein can also be measured in according with some embodiments of the invention. For example, the SimplePlex platform can be used to measure the levels of phosphorylated Nf heavy chain (pNf-H). SimplePlex is commercially available from Protein Simple (San Jose, CA, USA) (See Dysinger M, *et al. J Immunol Methods*. 451:1-10, 2017). In some embodiments, the neurofilament heavy chain is phosphorylated.

[000114] Although elevated neurofilament light chain levels in the CSF and serum have been described as correlating with physical damage as observed with MRI, as described herein, the inventors have discovered that elevated (or increased) levels of

neurofilament protein in the serum and/or CSF can predict future physical damage or functional loss in an MS patient. Thus, by keeping track of the level of a neurofilament protein in the serum (or CSF) of a patient on a particular therapeutic, a health care provider can predict whether the patient is responding to the therapeutic (i.e., whether the therapeutic alleviates the symptoms in that patient) *before* the patient shows functional loss and/or physical damage.

[000115] In one aspect, the invention provides a method for alleviating multiple sclerosis symptoms in a patient with multiple sclerosis, comprising administering a therapeutic that maintains or reduces the level of a neurofilament protein in serum of the patient as compared to the level of the neurofilament protein in serum of (a) a patient not administered with the therapeutic or (b) the patient prior to administration of the therapeutic.

[000116] In another aspect, the invention provides a method to determine if a candidate therapeutic will be efficacious in a particular patient and thus useful for alleviating the symptoms of MS disease in that patient by determining if the candidate therapeutic is able to reduce the level of serum neurofilament protein in the patient, as compared to the level in the patient prior to treatment with that candidate therapeutic. If the candidate therapeutic is able to reduce the level of serum neurofilament in the patient as compared to the level in the patient prior to treatment with the candidate therapeutic, that candidate therapeutic is identified as being efficacious in that patient.

[000117] In another aspect, the invention provides a method for alleviating symptoms in a patient with multiple sclerosis, comprising administering an efficacious therapeutic that is efficacious to the patient, the efficacious therapeutic, when administered to the patient, resulting in a level of a neurofilament protein in serum of the patient that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the therapeutic. In some embodiments, the patient is currently being administered with or has formerly been administered with either a non-efficacious therapeutic or a therapeutic that is different than the efficacious therapeutic.

[000118] In some embodiments, the neurofilament protein is a neurofilament light chain. In some embodiments, the neurofilament protein is a neurofilament medium chain. In some embodiments, the neurofilament protein is a neurofilament heavy chain. In some embodiments, the neurofilament protein is a human protein. In some embodiments, the level (i.e., amount or quantity) of neurofilament protein in blood or serum or cerebral spinal fluid is measured.

[000119] It will be understood that when a level of a neurofilament protein (e.g., neurofilament light chain, or NfL) is described as being increased (i.e., raised), the same as (or equal to), or decreased (i.e., reduced), that level is being compared to a level obtained with the same assay method from the same type of fluid. For example, an after-treatment level of serum neurofilament light chain (sNfL) obtained from a patient's serum using a particular assay (e.g., the Simoa assay, e.g., as described herein) will be compared with a baseline (i.e., before start of treatment) level of sNfL from the patient using the particular assay (e.g., the Simoa assay, e.g., as described herein) or will be compared to the level of sNfL as measured by the particular assay (e.g., the Simoa assay, e.g., as described herein) in serum of a patient that is not being treated. It will also be understood that the "level" of a neurofilament protein can be described in any manner, for example, in picograms of a neurofilament protein (e.g., neurofilament protein described herein, e.g., neurofilament light chain) per mL of blood or serum.

[000120] By "therapeutic" is simply meant a drug or agent that may be used to treat multiple sclerosis. The therapeutic need not actually cure the disease, and there is currently no known cure for multiple sclerosis. Thus, a patient being "treated" with a therapeutic simply means that he/she is being administered a therapeutic that might be efficacious for his or her disease. The therapeutic may not be efficacious in all multiple sclerosis patients. Rather, therapeutics for multiple sclerosis are sometimes referred to as disease-modifying therapies or disease-modifying drugs, (DMTs or DMDs), because their administration reduces the symptoms of MS in some patients, and thus allow those patients with MS who respond to the therapeutic to have an improved quality of life and/or improved neuromuscular function. Current therapeutics, if efficacious, slow the progression of the MS disease and/or alleviate symptoms in the responding patients.

[000121] Thus, by "efficacious" is meant that a therapeutic will reduce the symptoms of a MS patient.

[000122] Several therapeutics have received regulatory approval in the US, Europe, and elsewhere for use in treating multiple sclerosis. These include, without limitation, fingolimod (e.g., FTY720) (sold under the trade name Gilenya), teriflunomide (sold under the trade name Aubagio), dimethyl fumarate (sold under the trade name Tecfidera), glatiramer acetate (sold under the trade name Copaxone), dalfampridine (sold under the trade name Ampyra), alemtuzumab (sold under the trade name Lemtrada), interferon beta-1a (sold under the trade names Avonex, Plegridy, and Rebif), interferon beta-1b (sold under the trade names Betaseron, Betaferon, and Extavia), an anthracenedione

molecule such as mitoxantrone (sold under the trade name Novantrone), an antibody or fragment thereof against alpha-4 integrin such as natalizumab (sold under the trade name Tysabri), an antibody or fragment thereof against the alpha subunit of the IL-2 receptor of T cells (CD25) such as Daclizumab (sold under the trade name Zinbryta) and ocrelizumab (sold under the trade name Ocrevus). Additional new types of therapeutics for treating MS and/or alleviating MS symptoms that are still in development include, without limitation, ozanimod (Cellgene) and opicinumab (Biogen).

[000123] By "administration" or "administering" is meant delivery of a therapeutic to a patient. The route of administration can be by any means including, without limitation, oral, rectal, intravenous, intrathecal, intraperitoneal, intramuscular, subcutaneous, nasal, topical, parenteral, and sublingual. The dosage amount and dosing schedule for a therapeutic can be according to the label of the regulatory agency (e.g., the U.S. Food and Drug Administration or the European Medicines Agency). A patient who is administered a therapeutic may be referred to as being treated with that therapeutic, regardless of if the patient actually responds to the administered therapeutic. If a patient does not respond to an administered therapeutic, that therapeutic is non-efficacious in that patient.

[000124] By "symptoms of MS" is meant any of the symptoms that a patient with multiple sclerosis may experience. Symptoms of MS include, without limitation, pain in specific areas (e.g., in the back or in the eye), pain that may be associated with movement, tremor, muscular pains or mobility problems (e.g., cramping, difficulty walking, inability to rapidly change motions, involuntary movements, muscle paralysis, muscle rigidity, muscle weakness, coordination problems, stiff muscles, clumsiness, muscle spasms, over-reactive reflexes, fatigue, dizziness, heat intolerance, poor balance, vertigo, sensory issues (e.g., abnormality of taste, reduced sensation of touch, tingling, feeling pins and needles), urinary issues (e.g., excessive urination at night, leaking of urine, persistent urge to urinate, urinary retention), visual problems (e.g., blurred vision, double vision, or vision loss), sexual dysfunction, anxiety, mood swings, slurred speech, impaired voice, and other symptoms that individuals with multiple sclerosis experience. For the avoidance of doubt, neurofilament protein (e.g., neurofilament light chain or neurofilament heavy chain) in the serum or blood or cerebral spinal fluid is not a symptom of MS. Therefore, a therapeutic that simply reduces a neurofilament protein in the blood or serum or CSF without reducing any symptoms of MS in a patient is non-efficacious in that patient.

[000125] As used herein, by "alleviate the symptoms of MS" is meant that a therapeutic will reduce MS disease symptoms in a patient and/or slow the progression of the

MS disease. A patient whose MS symptoms are alleviated by being administered a therapeutic is said to respond to, or to be responsive to, the indicated therapeutic. If a therapeutic is able to alleviate the symptoms of MS upon administration of the therapeutic to that patient, the therapeutic is referred to as being efficacious in that patient.

[000126] It should be noted that reference to a patient "not being administered the therapeutic" is simply a patient (e.g., an MS patient) who has not been treated with the indicated therapeutic. Thus, this patient may be the same patient that is, at some future point in time, administered the indicated therapeutic, or this might be a completely different patient who is not being administered the indicated therapeutic. Note that the patient not being administered the therapeutic might be currently being treated with another therapeutic, or may have been treated with the indicated therapeutic in the past, but is not currently being treated with the indicated therapeutic.

[000127] In some embodiments, the maintenance or reduction of the level of a neurofilament protein (e.g., neurofilament light chain) in the blood or serum of a patient that will have his or her MS symptoms alleviated by the therapeutic (as compared to the level of the untreated patient) is detectable at least three months, or at least six months, or at least one year prior to observation of alleviation of MS symptoms (as determined by one of the assessments described herein).

[000128] In some embodiments, alleviation of MS symptoms in a patient is assessed by the Expanded Disability Status Scale (EDSS) assessment, the Multiple Sclerosis Severity Score assessment, the Timed 25-Foot Walk (T25FW) assessment, the 9-Hole Peg Test (9HPT) assessment (on either or both hands), the confirmed composite progression test, the 12-item Multiple Sclerosis Walking Scale (MSWS-12) assessment, change in patient-reported manual ability based on the ABILHAND questionnaire, patient-reported quality of life with the Multiple Sclerosis Impact Scale-29 (MSIS-29), or any combination of two or more of the foregoing assessments.

Sequence Disclosure

SEQ ID NO:1 (human neurofilament light chain amino acid sequence)

MSSFSYEPYYSTSYKRRYVETPRVHISSVRSGYSTARSAYSSYSAPVSSSLSVRRSYSSSSGSLMPSLEN
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QEIRDLRLAAEDATNEKQALQGEREGLEETLRNLQARYEEEVLSREDAEGRLMEARKGADEAALARAELE
KRIDSLMDEISFLKKVHEEEIAELQAQIQYAQISVEMDVTKPDLSAALKDIRAQYEKLAAKNMQNAEEWF
KSRFTVLTESAAKNTDAVRAAKDEVSESRRLLKAKTLEIEACRGMNEALEKQLQELEDKQNADISAMQDT
INKLENELRTTKSEMARYLKEYQDLLNVKMALDIEIAAYRKLLEGEETRLSFTSVGSITSGYSQSSQVFG
RSAYGGLQTSSYLMSTRSFPSYYTSHVQEEQIEVEETIEAAKAEEAKDEPPSEGEAEEEEKDKEEAEEEE
AAEEEEAAKEESEEAKEEEEGGEGEEGEETKEAEEEEKKVEGAGEEQAAKKKD

SEQ ID NO:2 (human neurofilament heavy chain amino acid sequence)

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SPSRFRGAGAASSTDSLDTLSNGPEGCMVAVATSRSEKEQLQALNDRFAGYIDKVRQLEA
HNRSLEGEAAALRQQQAGRSAMGELYEREVREMRGAVLRLGAARGQLRLEQEHLLEDIAH
VRQRLDDEARQREEAEAAARALARFAQEAEAARVDLQKKAQALQEECGYLRRHHQEEVGE
LLGQIQGSGAAQAQMQAETRDALKCDVTSALREIRAQLEGHAVQSTLQSEEWFRVRLDRL
SEAAKVNTDAMRSAQEEITEYRRQLQARTTELEALKSTKDSLERQRSELEDRHQADIASY
QEAIQQLDAELRNTKWEMAAQLREYQDLLNVKMALDIEIAAYRKLLEGEECRIGFGPIPF
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EEGKEEEGGEEEEAEGGEEETKSPPAEEAASPEKEAKSPVKEEAKSPAEAKSPEKEEAKS
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PEKEEAKSPEKAKSPVKAEAKSPEKAKSPVKAEAKSPEKAKSPVKEEAKSPEKAKSPVKE
EAKSPEKAKSPVKEEAKTPEKAKSPVKEEAKSPEKAKSPEKAKTLDVKSPEAKTPAKEEA
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KAAKGK

SEQ ID NO:3

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 121 hnrslegeaa alrqqqagrs amgelyerev remrgavlrl gaargqlrle qehllediah
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 241 llgqiqgsga aqaqmqaetr dalkcdvtsa lreiraqleg havqstlqse ewfrvrldrl
 301 seaakvntda mrsaqeeite yrrqlqartt elealkstkd slerqrsele drhqadiasy
 361 qeaiqqldae lrntkwemaa qlreyqdlln vkmaldieia ayrkllegee crigfgpipf
 421 slpeglpkip svsthikvks eekikvveks eketviveeq teetqvteev teeeekeake
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 841 kaeeekapat pkteekkdsk keeapkkeap kpkveekkep avekpkeskv eakkeeaedk
 901 kkvptpekea pakvevkeda kpkektevak kepddakake pskpaekkea apekkdtkee
 961 kakkpeekpk teakakeddk tlskepskpk aekaekssst dqkdskppek atedkaakgk
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SEQ ID NO:4 (human neurofilament medium chain amino acid sequence)

MSYTLDSLGNPSAYRRVTETRSSFSRVSGSPSSGFRSQSWSRGSPSTVSSSYKRSMLAPR
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EAAEQNKEAIRSAKEEIAEYRRQLQSKSIELESVRGTKESLERQLSDIEERHNHDLSSYQ
DTIQQLENELRGTKWEMARHLREYQDLLNVKMALDIEIAAYRKLLEGEETRFSTFAGSIT
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AVSMKEEKKEAAEEKEEEPEAEEEEVAAKKSPVKATAPEVKEEEGEKEEEEGQEEEEEED
EGAKSDQAEEGGSEKEGSSEKEEGEQEEGETEAEAEGEEAEAKEEKKVEEKSEEVATKEE
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VEEKGKSPVSKSPVEEKAKSPVPKSPVEEAKSKAEVGKGEQKEEEEKEVKEAPKEEKVEK
KEEKPKDVPEKKKAESPVKEEAVAEVVTITKSVKVHLEKETKEEGKPLQQEKEKEKAGGE
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EKVTSHAIVKEVTQSD

SEQ ID NO:5

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SEQ ID NO:6

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ALEAELAALRQRHAEPSRVGELFQRELRDLRAQLEEASSARSQALLERDGLAEEVQRLRA
RCEEESRGREGAERALKAQQRDVDGATLARLDLEKKVESLLDELAFVRQVHDEEVAELLA
TLQASSQAAAEVDVTVAKPDLTSALREIRAQYESLAAKNLQSAEEWYKSKFANLNEQAAR
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SEQ ID NO:7

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LEEELRQLKEEMARHLREYQELLNVKMALDIEIATYRKLLEGEESRISVPVHSFASLNIK
TTVPEVEPPQDSHSRKTVLIKTIETRNGEVVTESQKEQRSELDKSSAHSY

[000129] The following examples are provided which are meant to illustrate but not limit the invention described herein.

Example 1: Elevation of Serum Neurofilament Light Chain in MS Patients precedes MS symptoms

[000130] A clinical trial was performed to look at serum neurofilament levels in human patients.

[000131] Serum neurofilament levels were measured in patients and compared with observations of new T2 lesions (as observed on brain MRI scans) a year after the serum neurofilament level was taken.

[000132] Serum neurofilament light chain levels were measured in 309 MS patients. One year later, MRI scans were taken of the patients.

[000133] As shown in Figure 1, serum neurofilament light chain levels could predict which patients would have new T2 lesions one year later. As shown, when patients had a serum neurofilament light chain level of less than 8 picograms per ml serum at baseline ("BL", one year prior to MRI) (79 patients), very few had any T2 lesions one year later. When patients had a serum neurofilament light chain level of between 8-16 pg/ml serum at baseline (135 patients), the occurrence of new T2 lesions increased. However, in the patients who had greater than 16 pg/ml serum neurofilament light chain at baseline, multiple patients had multiple T2 lesions a year later. In other words, if a patient's serum NfL levels are greater than 16 pg/mL, it is highly likely that the patient will have new T2 lesions over the next year.

[000134] When data from several multiple sclerosis clinical studies were combined (from the ADVANCE, CHAMPS, and SENTINEL MS clinical studies), it was also found that sNfL levels were associated with Gd+ lesion count and new T2 lesions during the following year. See Figures 20A-20B. The data showed that sNfL levels >16 pg/mL were associated with a high probability (PPV = 91%) of new T2 lesion development in the following year. (PPV = positive predictive value).

[000135] The use of serum neurofilament light chain was a predictor even earlier than one year. Indeed, serum neurofilament light chain levels can predict which patients, five years after measuring the serum neurofilament light chain levels, will progress in MS symptoms such that their EDSS is greater than 3.5 units. As shown in Figure 2A, when patients had a level of neurofilament light chain in their sera of between 1.14 to 10.26

picograms/ml serum at baseline (i.e., 5 years prior to the EDSS assessment), only 3.8% of the patients (2 out of 52) had a EDSS score of 3.5 or higher five years later. But, when patients who had a level of neurofilament light chain in their sera of between 10.37 to 18.3 picograms/ml serum at baseline (i.e., 5 years prior to the EDSS assessment), 8.3% (5 out of 60) had an EDSS score of 3.5 or higher five years later. Finally, the patients who had serum neurofilament light chain levels of 18.47 picograms/ml serum or higher at baseline had a 13.3% chance (6 patients out of 45) of having an EDSS score of 3.5 or higher five years later.

[000136] The data (e.g., from the CHAMPS clinical study) showed that baseline sNfL levels were associated with longer-term clinical outcomes in patients with CIS and MS. In patients with greater than 16 picograms neurofilament light chain per ml serum, 12.5% reached an EDSS score of greater than or equal to 3.5 within 5 years after their serum neurofilament light chain level was measured. However, in those patients that had a measurement of between 8-16 pg neurofilament light chain per ml serum, 7.0% reached an EDSS score of greater than or equal to 3.5 within 5 years after their serum neurofilament light chain level was measured. In those patients that had a measurement of less than 8 pg neurofilament light chain per ml serum, only 3.3% reached an EDSS score of greater than or equal to 3.5 within 5 years after their serum neurofilament light chain level was measured. FIG. 21A shows the association between sNfL and time to clinically definite multiple sclerosis (CDMS) over 5 years. Baseline sNfL levels were significantly associated with time to CDMS and the proportion of patients reaching EDSS score >= 3.5 at 5 years. See FIGs. 21A-21B.

[000137] Thus, serum neurofilament light chain levels were able to predict overall MS progression five years later. Indeed, serum neurofilament light chain levels were able to predict MS symptom progression as early as eleven years before the EDSS assessment. Figure 2B shows the odds for MS progression to an EDSS greater than or equal to 6.0 over 5 years, and over 12 years based on neurofilament levels. As can be seen in Fig. 2B, patients with high serum NfL levels have several fold (5-10 fold) greater odds ratio of progressing to EDSS 6.0 or greater at 8 and 15 years later.

[000138] As shown in Figures 2C and 2D, the volume of T2 lesions in MS patients with less than 8 pg/ml serum Neurofilament light chain at baseline is very low 5 years and 10 years later (see left-most columns in Figs. 2C and 2D, respectively). When the serum neurofilament light chain levels are 8 and 16 pg/ml at baseline, the volume of T2 lesions 5 years and 10 years later increases (see middle columns in Figs. 2C and 2D, respectively) When serum neurofilament light chain levels are greater than 16 pg/ml, this

group had the largest volume of T2 lesion 5 years and 10 years after baseline (see right columns in Figs. 2C and 2D, respectively).

[000139] Thus, serum neurofilament light chain levels were able to predict MS progression in terms of volume of T2 lesions 10 years later.

[000140] Moreover, serum neurofilament light chains were also able to predict brain atrophy 5 years later. As shown in Figure 2E, patients that had the largest decline in BPF (i.e., greatest increase in brain atrophy) had greater than 16 pg/ml serum neurofilament light chain five years earlier.

[000141] Thus, serum neurofilament light chain levels were found to be a good predictor of progression of multiple sclerosis symptoms. Based on these results, a relapsing-remitting MS patient with high levels of serum neurofilament light chain (e.g., over 16 picograms per ml serum) may have an increased likelihood that he/she will have a relapse within one year of the high serum neurofilament light chain measurement. Of course, more frequent relapses may indicate the progression of a RRMS patient toward secondary progressive MS.

Example 2. Treatment of MS patients will reduce the level of neurofilament light chain levels in serum.

[000142] Although there is no cure for multiple sclerosis, current therapeutics that are used to treat MS also result in a decrease in the level of serum neurofilament light chain levels. As shown in Figures 3A-3C, as compared to placebo (Fig 3A), treatment of patients with either Pegylated interferon-1beta-1a (Pegridy; Fig. 3B), with natalizumab combined with interferon-1beta-1a (Fig. 3C), with natalizumab alone (Fig. 3D), or with dimethyl fumarate (Fig. 3E) resulted in decreased serum neurofilament levels over time. In Fig. 3A, in patients treated with placebo, 35% of the 126 patients started out with a serum NfL level of greater than 16 pg/ml serum; after 48 weeks, that number was 30%. In Figure 3B, in patients treated with Pegridy, 27% of the 182 patients started out with a serum NfL level of greater than 16 pg/ml serum; after 48 weeks, that number fell to 13%. In Figure 3C, in patients treated with natalizumab combined with interferon-1beta-1a, 30% of the 81 patients started out with a serum NfL level of greater than 16 pg/ml serum; after 48 weeks, that number fell to 4%. In FIG. 3D, in patients treated with natalizumab alone, 41% of the patients started out with a serum NfL level of greater than 16 pg/mL; after 48 weeks, that number fell to 3%. In FIG. 3E, in patients treated with dimethyl fumarate, 29% of patients started out with a serum NfL level of >16 pg/mL; after 12 weeks, that number fell to 5%.

sNfL levels were significantly lower in the experimental treatment arms.

Example 3: Serum neurofilament light chain levels predict treatment outcome in MS

[000143] Multiple sclerosis is a debilitating disease, and most MS patients have relapsing remitting MS (RRMS), in which relapses (or flare-ups) are following by periods of recovery during which the patient can recover the majority of functional loss.

[000144] However, over time, RRMS patients lose function as assessed by standard methods including the assessment methods described above. Indeed, more damage can be incurred in the first years of the disease than in later stages of MS (see Kuhlmann et al. *Brain.* 125:2202-2212, 2002).

[000145] Thus, it is important to treat an MS patient with a therapeutic that he/she will respond to as soon as possible after the initial diagnosis, to prevent progression of the disease.

[000146] Unfortunately, not all patients respond to therapeutics in the same way. In other words, a given therapeutic will not alleviable the symptoms of all MS patients. Rather, it is up to the physician of a particular patient to try to prescribe administration of a particular therapeutic to each individual patient. Since in the early stages of MS, relapses occur infrequently, it may take some time to determine if the therapeutic chosen by the patient's physician is a therapeutic that that patient will respond to. As discussed above, symptoms of MS are assessed by functional tests such as EDSS, the T25FW test, and the 9HPT-D test. Unfortunately, once the patient starts to show a loss of function (e.g., an increased time in the T25FW test, that loss of function is difficult to regain. By the time a new therapeutic is selected by the patient's physician to try to treat the patient's symptoms, the patient's condition may have deteriorated still further.

[000147] In an attempt to more rapidly detect whether a particular patient will respond to a particular therapeutic, a clinical study was performed using natalizumab as a therapeutic to see if serum neurofilament light chain levels could be used to predict whether natalizumab would alleviate MS symptoms.

[000148] In this study, 748 patients (aged 18-58 years of age), who had not been treated with natalizumab, were enrolled. These 748 patients were diagnosed as having secondary progressive multiple sclerosis for 2 years or more. The patients in the study had disability progression unrelated to relapses in the previous year, and had Expanded Disability Status Scale (EDSS) scores of 3.0 to 6.5.

[000149] For this study, serum neurofilament light chain levels were measured using the single molecule array (SIMOA) assay available from Quanterix (Lexington, MA, USA). Serum neurofilament light chain levels were measured at baseline (i.e., before treatment started), at 48 weeks after start of treatment, and at 96 weeks after start of treatment. Of the 748 patients, 379 patients received natalizumab (administered according to the FDA label for Tysabri), and 365 patients received placebo (administered via the same administration route (i.v.) as natalizumab).

[000150] As shown in Figure 4A, baseline ("BL") serum neurofilament light chain levels were associated with brain volume change. Patients with serum neurofilament light chain levels from 3.09 to 9.09 pig/ml at baseline had almost no brain volume change 96 weeks later. Patients with serum neurofilament light chain levels from 9.09 to 13.4 pig/ml at baseline had slightly more brain volume change 96 weeks later. Patients with serum neurofilament light chain levels from 13.4 pig/ml or higher at baseline had the highest amount of brain volume change 96 weeks later.

[000151] Also, as shown in FIG. 18, baseline sNfL concentrations were associated with brain atrophy over two years, with subjects having higher baseline sNfL concentrations (e.g., 8-16 pg/mL or >16 pg/mL) associating with a larger % of brain volume loss over two years.

[000152] Interestingly, as shown in Figs. 4B and 4C, these changes occurred in both patients with active lesions (i.e., Gd+ lesions) and patients without inflammatory activity (i.e., Gd-lesions).

[000153] Figure 5 shows that serum neurofilament levels at week 48 of treatment predicted whether a patient would have EDSS progression at week 96 of treatment. Those patients who did not have EDSS progression by week 96 of treatment (circles) had a decrease in serum neurofilament light chain (as compared to prior to treatment at week 0) at week 48, and this level remained decreased from week 0 by week 96. In contrast, the patients who did have EDSS progression by week 96 of treatment showed no decrease in serum neurofilament light chain levels at week 48 as compared to week 0.

[000154] The results in Figure 6 show that serum neurofilament light chain levels at baseline (i.e., week 0) are a relatively good predictor of loss of lower extremities function (as measured by the T25FW test) 96 weeks later.

[000155] The results in Figures 7 and 8 are striking. Although both groups of patients start out with similar serum neurofilament levels at baseline, by 48 weeks, those that will eventually show progression by week 96 in 9HPT-D (dominant hand) score (Fig. 7,

squares) and 9HPT-ND (non-dominant hand) score (Fig. 8, squares) have a slight but consistent increase in their serum neurofilament light chain levels at 48 weeks (i.e., 48 weeks before their hands show symptoms). In contrast, those patients that do not show progression by week 96 in 9HPT-D (dominant hand) score (Fig. 7, circles) and 9HPT-ND (non-dominant hand) score (Fig. 8, circles) have levels of serum neurofilament light chains that are either maintained (i.e., stay the same as) or are reduced as compared to baseline both 48 and 96 weeks later. These results show that increase in serum neurofilament light chain levels are a good predictor of future MS progression as measured by upper extremities function.

[000156] Based on these results, it can be concluded that if a patient's serum neurofilament light chain levels are increased at week 48 as compared to week 0, that patient will likely have loss of function by week 96. In some embodiments, the loss of function measured by upper extremities function e.g., using the 9HPT-D (dominant hand) score and 9HPT-ND (non-dominant hand) score. In some embodiments, if serum neurofilament light chain level increases by at least 1% at week 48 compared to week 0, that patient will likely have loss of function by week 96. In some embodiments, if serum neurofilament light chain level increases by at least 3% at week 48 compared to week 0, that patient will likely have loss of function by week 96. In some embodiments, if serum neurofilament light chain level increases by at least 5% at week 48 compared to week 0, that patient will likely have loss of function by week 96. In some embodiments, if serum neurofilament light chain level increases by at least 10% at week 48 compared to week 0, that patient will likely have loss of function by week 96.

Example 4: Serum Neurofilament Light Chain in Patients treated with an Efficacious Therapeutic

[000157] An analysis was done on the serum neurofilament levels in patients treated with natalizumab, a non-limiting therapeutic.

[000158] In this study, 744 patients (aged 18-58 years of age), who had not been treated with natalizumab, were enrolled. These 748 patients were diagnosed as having secondary progressive multiple sclerosis for 2 years or more. The patients in the study had disability progression unrelated to relapses in the previous year, and had Expanded Disability Status Scale (EDSS) scores of 3.0 to 6.5.

[000159] For this study, serum neurofilament light chain levels were measured using the single molecule array (SIMOA) assay available from Quanterix (Lexington, MA, USA). Serum neurofilament light chain levels were measured at baseline (i.e., before

treatment started), at 48 weeks after start of treatment, and at 96 weeks after start of treatment. Of the 748 patients, 379 patients received natalizumab (administered according to the FDA label for Tysabri), and 365 patients received placebo (administered via the same administration route (i.v.) as natalizumab).

[000160] As Figure 9 shows, although all patients (those treated with placebo, squares, dotted line) and those treated with natalizumab (circles, solid line) started out with approximately the same level of serum neurofilament light chain at week 0 (baseline), after 48 weeks of treatment, the patients receiving natalizumab shows a significant reduction in their serum neurofilament light chain as compared to baseline. This reduction continued to week 96 of treatment. In contrast, the placebo treated group showed a steadily increasing level of serum neurofilament light chain. The reduction in serum neurofilament levels for those patients on natalizumab after 48 and 96 weeks was greater in patients with active lesions at week 0 (Gd+ lesions, Fig. 10A) than those patients with inactive lesions at week 0 (Gd- lesions, Fig. 10B).

[000161] In other words, if serum neurofilament levels are lower than 16 pg per mL serum, there is a high probability that the patient will have no GD+ lesions. For example, if baseline serum neurofilament levels are lower than 16 pg/ml serum (or lower than 8 pg/ml serum), no Gd+ are likely.

[000162] As discussed above, if serum neurofilament levels are higher than 16 pg per mL serum, there is a high probability that the patient will develop new T2 lesions in the following year. In other words, if a patient has a serum neurofilament level that is higher than 16 pg per ml serum, within a year, that patient is likely to develop a new T2 lesion. If that patient is being treated with a therapeutic, that therapeutic is likely not efficacious to that patient, and that patient should be switched to another therapeutic that is efficacious to that patient.

[000163] Regardless of whether a patient had had relapses 2 years prior to week 0, natalizumab treatment was effective in lowering serum neurofilament light chain levels. Compare Fig. 11A (with relapses in the 2 years prior to week 0) to Fig. 11B (with no relapses in the 2 years prior to week 0).

[000164] Serum neurofilament levels in natalizumab treated patients were reduced at weeks 48 and 96 as compared to week 0 regardless of whether the patient showed inflammatory activity during the treatment period. Compare Figs. 12A, with inflammatory activities, to Figure 12B, without inflammatory activities, where inflammatory activities is

defined as having Gd+ lesions, new T2 lesions, newly enlarging T2 lesions, or relapse at any time during the 96 week treatment.

[000165] Thus, these studies found that after 96 weeks of treatment with natulizumab, serum neurofilament light chain levels were significantly higher with disease progression as compared to those without progression during the 96 weeks. Progression of MS disease was identified using EDSS, T25FW, or 9HTP at both weeks 48 and 96.

[000166] As shown in these studies, administration of natalizumab reduced the levels of neurofilament light chain in the patient serum as compared to the level of neurofilament light chain in serum of patients administered the placebo. This was true both in patients with and also without acute inflammatory activity.

[000167] In addition, these studies showed that the change in sNfL levels over 96 weeks differed by baseline sNfL levels. As shown in FIG. 19, subjects with a higher baseline sNfL level (e.g., 8-16 pg/mL or >16 pg/mL) had a larger change in sNfL level over 96 weeks of natalizumab treatment (e.g., compared to placebo or baseline sNfL level).

[000168] In sum, these studies show that baseline serum neurofilament light chain (sNfL) levels were significantly associated with baseline age ($p<0.05$), number of Gd+ lesions ($p<0.0001$), T2 lesion volume ($p<0.0001$), Timed 25-Foot Walk time(T25FW, $p<0.0001$), and 9-Hole Peg Test time (9HPT, $p<0.0001$). Baseline sNfL levels were also associated with brain atrophy over 96 weeks ($p<0.0001$). At week 96, sNfL levels were significantly higher in patients with progression compared to those without progression during the study, as defined using EDSS ($p<0.01$), T25FW ($p<0.05$), or 9HPT ($p<0.01$ for both week 48 and week 96). Finally, sNfL levels at week 48 and week 96 were significantly lower in natalizumab-treated patients compared to those on placebo [ratio 0.84, 95% CI (0.79, 0.89), $p<0.001$ and ratio 0.80, 95% CI (0.7, 0.85), $p<0.001$, respectively]. Statistically significant differences in sNfL levels between natalizumab and placebo groups were observed in patients with and without enhancing lesions at baseline, relapses in the two years prior to the study enrollment, and inflammatory activity during the study.

[000169] Thus, baseline sNfL levels of secondary progressive MS patients were associated with baseline disease activity measures and future brain atrophy rates. Natalizumab reduced sNfL levels compared to placebo in SPMS patients with and without acute inflammatory activity. These findings suggest that serum neurofilament light chain levels might not only reflect inflammation driven neuro-axonal damage but also non-inflammatory neurodegeneration in MS patients.

[000170] Figure 13A shows that patients with no evident disease activity (NEDA) have consistently low and stable serum NfL levels. In contrast, patients with evident disease activity (EDA) have higher and more variable serum NfL levels (Fig. 13B). The highest variability was seen in EDA patients with more than 1% brain volume loss per year (see Fig. 13B). NEDA is defined as no Gd+ lesions, no new T2 lesions, and no relapses. In sum, NEDA is no confirmed progression.

[000171] The results presented here are not limited to treatment with natalizumab. Figures 14A-14C show that patients being treated with pegylated interferon had similar results. Patients who had elevated serum neurofilament light chain before start of treatment, and had a greater than 50% decrease of serum neurofilament light chain at year 1 of treatment, had fewer lesions at year 1 as compared with patients that had a less than 50% decrease in serum neurofilament light chain (Figure 14A), Patients who had elevated serum neurofilament light chain before start of treatment, and had a greater than 50% decrease of serum neurofilament light chain at year 1 of treatment, had fewer lesions at year 2 as compared with patients that had less than 50% decrease in serum neurofilament light chain (Fig. 14B). Patients who had elevated serum neurofilament light chain before start of treatment, and had a greater than 50% decrease of serum neurofilament light chain at year 1 on treatment, had fewer lesions at year 4 as compared with patients that had less than 50% decrease in serum neurofilament light chain (Fig. 14C).

[000172] In other words, even if the patient had elevated serum neurofilament light chain at baseline, an increase of serum neurofilament light chain level within the first year of treatment with pegylated interferon was predictive of new T2 lesions even after 4 years of treatment.

[000173] Figures 15A and 15B show that patients with higher average NfL levels have higher brain atrophy over 2 years in patients on PEG-IFN treatment (Figure 15A, NfL average of baseline and of month 6; Figure 15B, NfL average of baseline and of month 12). Note that in Figs. 15A-15B, the left column (0, 8) are the patients with less than 8 pg neurofilament light chain per ml serum at baseline; the middle column (8,16) are the patients with between 8-16 pg neurofilament light chain per ml serum at baseline; and the right column (16,100) are the patients with greater than 16 pg neurofilament light chain per ml serum at baseline

[000174] The ability of a therapeutic, such as pegylated interferon, to hold a patient at an average serum neurofilament level is predictive of that therapeutic's ability to alleviate the MS symptoms of that patient. As shown in Figures 16A and16B, MS patients

being treated with pegylated interferon who had having no evident disease activity over 6 months ("BL-M6" in Fig. 16A) and 1 year ("BL-Y1" in Fig. 16B) had average levels of serum neurofilament levels.

[000175] Indeed, serum neurofilament levels were found to be significantly lower in patients on PEG-IFNβ-1a treatment (i.e., 125 mg every 2 weeks) as early as 3 months after the start of treatment, and certainly significantly lower after 6 months of treatment. Fig. 17A shows patients who had a baseline of less than 8 pg neurofilament light chain per ml serum; Fig. 17B shows patients who had a baseline of 8-16 pg neurofilament light chain per ml serum; and Fig. 17C shows patients who had a baseline of greater than 8 pg neurofilament light chain per ml serum: at 3 months later (NFLM3 in Figs. 17A-17C), the drop in neurofilament light chain level is significant in patients who had a baseline of 8-16 pg neurofilament light chain per ml serum (Fig. 17B); and in patients who had a baseline of greater than 8 pg neurofilament light chain per ml serum (Fig. 17C). The droop becomes more pronounced at 6 months after baseline (NFLM6 in Figs. 17A-17C) and 12 months after baseline (NFLY1 in Figs. 17A-17C). In patients with elevated sNfL levels, PEG-IFN-beta-1a treatment effect was observed as early as 3 months post treatment initiation ($P<0.05$).

[000176] Data from the ADVANCE study (on RRMS patients, treated with PEG-IFN-beta-1a) also shows that short-term sNfL change on treatment associated with longer-term MRI and clinical outcomes. In patients with elevated baseline sNfL levels (>16 pg/mL), reduction in sNfL levels below 16 pg/mL at month 6 was associated with better outcomes at 2-4 years. See FIG. 24.

[000177] This early detection is key as the appearance of physical changes (e.g., new Gd+ or T2 lesions) or loss of function may not be noticed within the first 3 months following treatment. By measuring changes in serum neurofilament levels, and *not* detecting a decrease when the patient is on treatment, a caregiver (e.g., physician or nurse) can identify that the therapeutic (e.g., PEG-IFNβ-1a) is not efficacious to that patient prior to the appearance of physical change or loss of function and switch that patient to another therapeutic (e.g., natalizumab) prior to the appearance of physical change or loss of function.

[000178] Additionally, the sNfL profile was tracked in a single patient on placebo and then PEG-IFN-beta-1a. See FIG. 25. The data herein show that sNfL levels >16 pg/mL (e.g., measured using methods described herein) were associated with a high probability of disease activity and worse short- and long-term outcome (e.g., determined by clinical, MRI, and OCT measurements). Also, DMTs (e.g., therapies described herein) lowered sNfL levels. Without wishing to be bound by any particular theory, the results

provided herewith support the use of serum neurofilament protein levels as a marker to determine if a therapeutic a patient is on is actually efficacious for that patient. For example, if a patient is previously on a non-efficacious therapeutic and still has relapses, measuring the serum neurofilament levels may give advance notice, before relapses occur, that the therapeutic is not efficacious and should be switched.

Example 5: sNfL levels in healthy subjects and sNfL association with rate of thinning of GCIPL and pRNFL in subjects with MS

[000179] sNfL levels were determined in various healthy (e.g., not having any symptoms of disease, e.g., neurodegenerative disease or neuromuscular disease, e.g., MS) subjects. sNfL levels were relatively stable up to age 40-45, followed by a steady increase. Normative cut-offs were defined based on the 97.5th percentile of sNfL values in the healthy controls. See FIG. 22.

[000180] sNfL levels were determined and compared to thinning of subjects' ganglion cell layer and inner plexiform layer (GCIPL) and peri-papillary retinal nerve fiber layer (pRNFL). 298 multiple sclerosis patients had serial optical coherence tomography (OCT) within 30 days of blood sampling. Patients with high sNfL (>97.5th percentile of healthy controls) exhibited ~2x faster short-term (1-3 years) rates of GCIPL and pRNFL thinning. See FIGs. 23A-23B. Thus, elevated sNfL was associated with accelerated rates of GCIPL and pRNFL thinning in subjects with MS.

[000181] The embodiments of the invention described above are intended to be merely exemplary; numerous variations and modifications will be apparent to those skilled in the art. All such variations and modifications are intended to be within the scope of the present invention as defined in any appended claims.

Claims

1. A method for alleviating symptoms in a patient with multiple sclerosis, comprising administering an efficacious therapeutic that is efficacious to the patient, the efficacious therapeutic, when administered to the patient, resulting in a level of a neurofilament protein in serum of the patient that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the efficacious therapeutic.

2. A method for determining if a therapeutic administered to a patient with multiple sclerosis is an efficacious therapeutic that is efficacious to the patient, comprising obtaining or having obtained a blood or serum sample from the patient administered with the therapeutic; and measuring or having measured a level of a neurofilament protein in blood or serum of the patient to obtain a post-treatment level, wherein a post-treatment level that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the therapeutic identifies the therapeutic as being efficacious to the patient.

3. A method for identifying a efficacious therapeutic that will alleviate the symptoms of multiple sclerosis in a patient with multiple sclerosis, comprising administering a candidate therapeutic to the patient and measuring a level of a neurofilament protein in serum of the patient after the patient has been administered for X weeks with the candidate therapeutic to obtain a post-treatment level, wherein a post-treatment level that is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the candidate therapeutic, identifies the candidate therapeutic as an efficacious therapeutic that will alleviate the symptoms of multiple sclerosis in the patient.

4. The method of claim 3, wherein X is 48.

5. A method for alleviating symptoms in a patient with multiple sclerosis by administration of an efficacious therapeutic, comprising:

(a) determining whether symptoms of multiple sclerosis in the patient will be alleviated by a candidate therapeutic by:

(i) obtaining or having obtained a blood or serum sample from the patient following administration of the candidate therapeutic; and

(ii) measuring or having measured a level of a neurofilament protein in blood or serum to obtain a post-treatment level; and if the post-treatment level is lower than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or is lower than a level of neurofilament protein in a patient with multiple sclerosis not administered the candidate therapeutic, then the candidate therapeutic is an efficacious therapeutic and

(b) administering the efficacious therapeutic to the patient, wherein administration of the efficacious therapeutic will alleviate the symptoms of multiple sclerosis in the patient.

6. The method of claim 3 or 5 wherein the patient is currently being administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the candidate therapeutic.

7. A method for alleviating symptoms in a patient with multiple sclerosis by administration of an efficacious therapeutic, comprising measuring a level of a neurofilament protein in blood or serum of the patient, the patient currently being administered with a therapeutic that is (a) a non-efficacious therapeutic or (b) different than the efficacious therapeutic and, if the level is greater than an amount equivalent to 16 pg serum neurofilament light chain per ml serum or is greater than a level of neurofilament protein in a patient with multiple sclerosis not administered the efficacious therapeutic, then stopping administration of the therapeutic to the patient and starting administration of the efficacious therapeutic to the patient.

8. The method of claim 1, 2, 3, 5, or 7, wherein the neurofilament protein is a neurofilament light chain.

9. The method of claim 1, 2, 3, 5, or 7, wherein the neurofilament protein is a neurofilament heavy chain.

10. The method of claim 1, 2, 3, 5, or 7, wherein the patient with multiple sclerosis has relapsing remitting multiple sclerosis.

11. The method of claim 1, 2, 3, 5, or 7, wherein the patient with multiple sclerosis has secondary progressive multiple sclerosis.

12. The method of claim 1, 2, 3, 5, or 7, wherein the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic is at least 10% lower than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

13. The method of claim 1, 2, 3, 5, or 7, wherein the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least three months is at least 10% lower than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

14. The method of claim 1, 2, 3, 5, or 7, wherein the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least six months is at least 10% lower than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

15. The method of claim 1, 2, 3, 5, or 7, wherein the level of the neurofilament protein in the serum of the patient administered the efficacious therapeutic for at least twelve months is at least 10% lower than the level of the neurofilament protein in the serum of a patient not administered the efficacious therapeutic.

16. The method of claim 1, 2, 3, 5, or 7, wherein the efficacious therapeutic is a very late antigen 4 (VLA-4)-binding agent.

17. The method of claim 16, wherein the VLA-4-binding agent is natalizumab.

18. The method of claim 1, 2, 3, 5, or 7, wherein the efficacious therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate,

alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine and dimethyl fumarate.

19. The method of claim 1, 2, 3, 5, or 7, wherein the administration of the efficacious therapeutic slows the progression of the MS disease in the patient.

20. The method of claim 1 or 2 wherein the patient is currently being administered with or has formerly been administered with (a) a non-efficacious therapeutic or (b) a therapeutic that is different than the efficacious therapeutic.

21. A method of treating multiple sclerosis in a human subject in need thereof, comprising:

administering one or more doses of a therapeutic to the human subject;

measuring a serum neurofilament light chain level in a sample obtained from the human subject; and

if the measured serum neurofilament light chain level is greater than an amount equivalent to 16 pg per ml, then administering one or more further doses of the therapeutic to the human subject, and

if the measured serum neurofilament light chain level is equal to or lower than an amount equivalent to 16 pg per ml, then further measuring serum neurofilament light chain levels in the human subject one or more times prior to further administering the therapeutic.

22. A method of treating multiple sclerosis in a human subject in need thereof, comprising:

(i) administering one or more doses of a therapeutic to the human subject;

(ii) measuring a serum neurofilament light chain level that is equal to or lower than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and

(iii) further measuring the serum neurofilament light chain level in the human subject, wherein the therapeutic is not further administered to the human subject in the interval between the measuring in step (ii) and the further measuring in step (iii).

23. A method of treating multiple sclerosis in a human subject in need thereof, comprising:

administering initial doses of a therapeutic to the human subject, wherein each of the initial doses is in the same amount and is administered at the same dosing interval between doses;

measuring a serum neurofilament light chain level that is equal to or lower than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and

administering further doses of the therapeutic to the human subject, wherein each of the further doses is in the same or lesser amount and at the same or lengthened dosing interval as compared to the initial doses.

24. A method of treating multiple sclerosis in a human subject in need thereof, comprising:

administering initial doses of a therapeutic to the human subject, wherein each of the initial doses is in the same amount and is administered at the same dosing interval between doses;

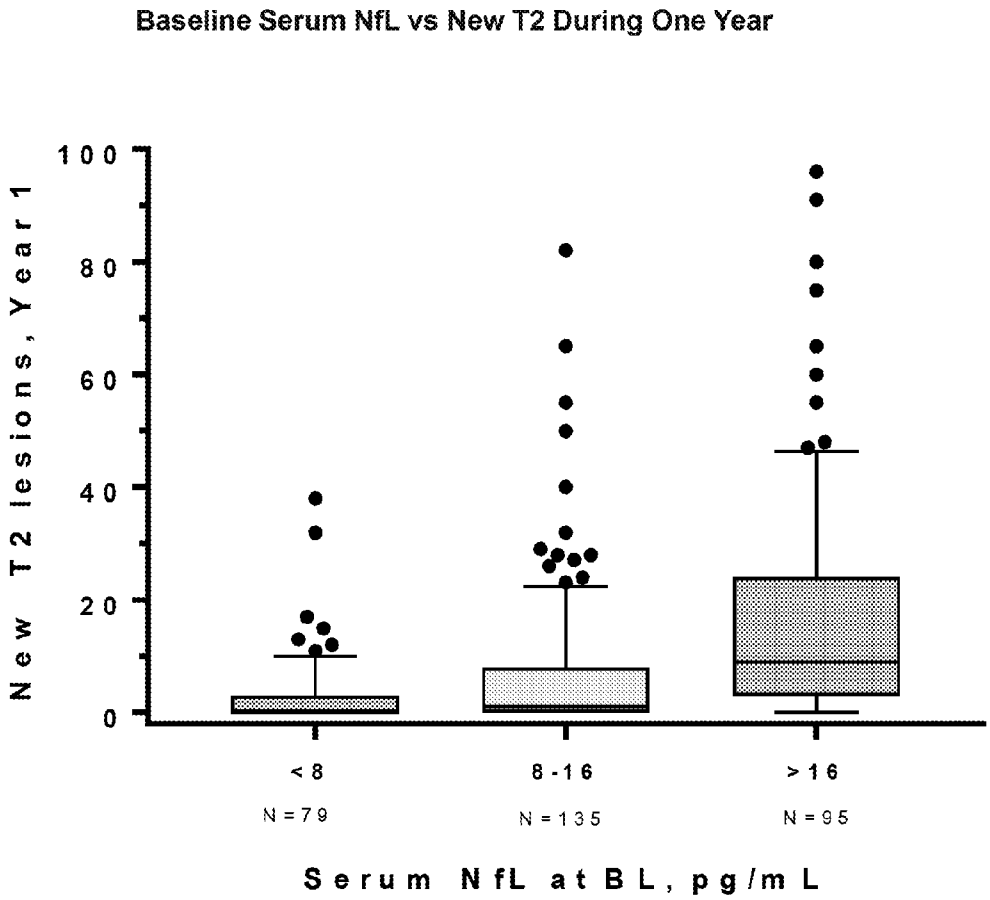
measuring a serum neurofilament light chain level that is greater than an amount equivalent to 16 pg per ml in a sample obtained from the human subject; and

administering further doses of the therapeutic to the human subject, wherein each of the further doses is in an increased amount and/or at a shortened dosing interval as compared to the initial doses.

25. The method of any one of claims 21 to 24, wherein the therapeutic is a very late antigen 4 (VLA-4)-binding agent.

26. The method of claim 25, wherein the VLA-4-binding agent is natalizumab.

27. The method of any one of claims 21 to 24, wherein the therapeutic is selected from the group consisting of interferon beta-1a, interferon beta-1b, peginterferon beta-1a, glatiramer acetate, alemtuzumab, mitoxantrone, opicinumab, ocrelizumab, teriflunomide, fingolimod, dalfampridine, dimethyl fumarate, diroximel fumarate, and monomethyl fumarate.



- If serum NfL levels >16 pg/mL, highly likely new T2 lesions over next year

Figure 1A

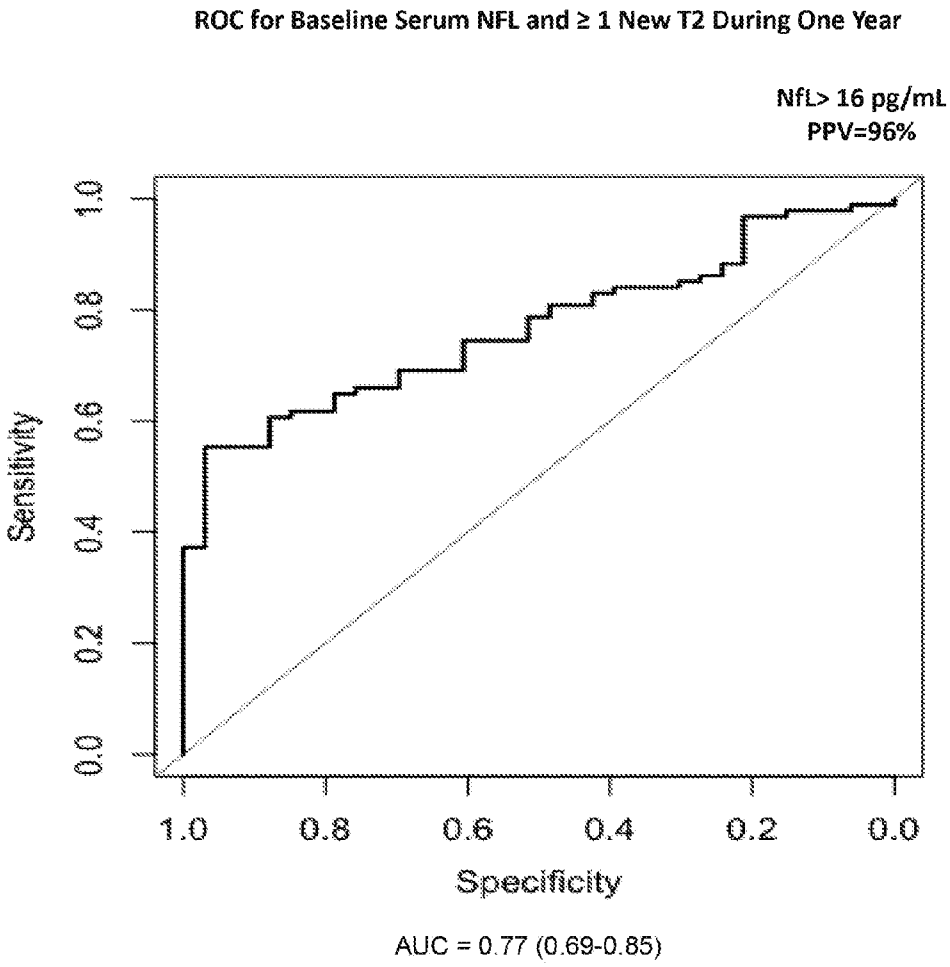

ROC for Baseline Serum NFL and ≥ 1 New T2 During One Year
NfL> 16 pg/mL
PPV=96%
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.0
0.8
0.6
0.4
0.2
0.0
Specificity
AUC = 0.77 (0.69-0.85)

Figure 1B

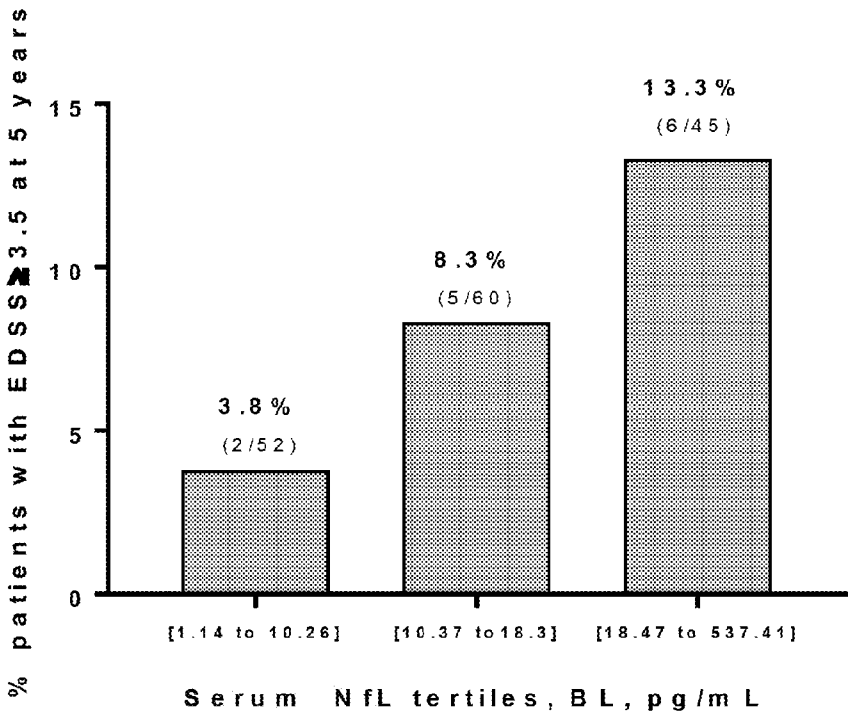

% patients with EDSS≥3.5 at 5 years
15
10
5
0
3.8%
(2/52)
8.3%
(5/60)
13.3%
(6/45)
[1.14 to 10.26]
[10.37 to 18.3]
[18.47 to 537.41]
Serum NfL tertiles, BL, pg/mL

Figure 2A

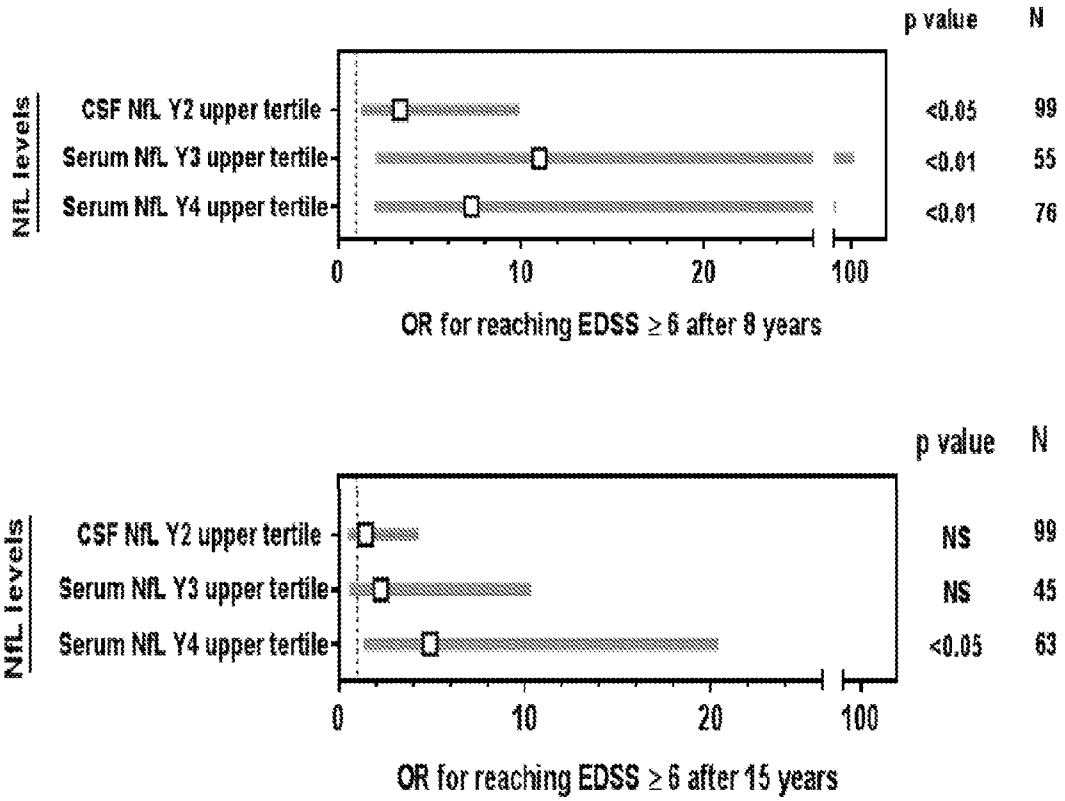

p value
N
NfL levels
CSF NfL Y2 upper tertile
Serum NfL Y3 upper tertile
Serum NfL Y4 upper tertile
<0.05
<0.01
<0.01
99
55
76
0
10
20
100
OR for reaching EDSS ≥ 6 after 8 years
p value
N
NfL levels
CSF NfL Y2 upper tertile
Serum NfL Y3 upper tertile
Serum NfL Y4 upper tertile
NS
NS
<0.05
99
45
63
0
10
20
100
OR for reaching EDSS ≥ 6 after 15 years

Figure 2B

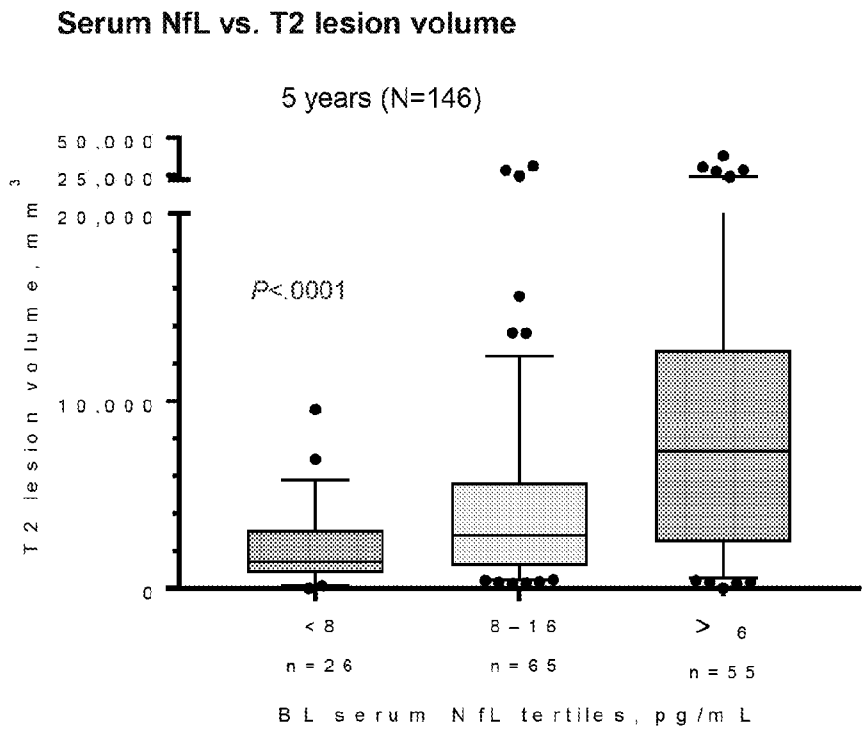

Serum NfL vs. T2 lesion volume
5 years (N=146)
50,000
25,000
20,000
10,000
0
T2 lesion volume, mm3
P<.0001
< 8
n = 26
8 – 16
n = 65
> 16
n = 55
BL serum NfL tertiles, pg/mL

Figure 2C

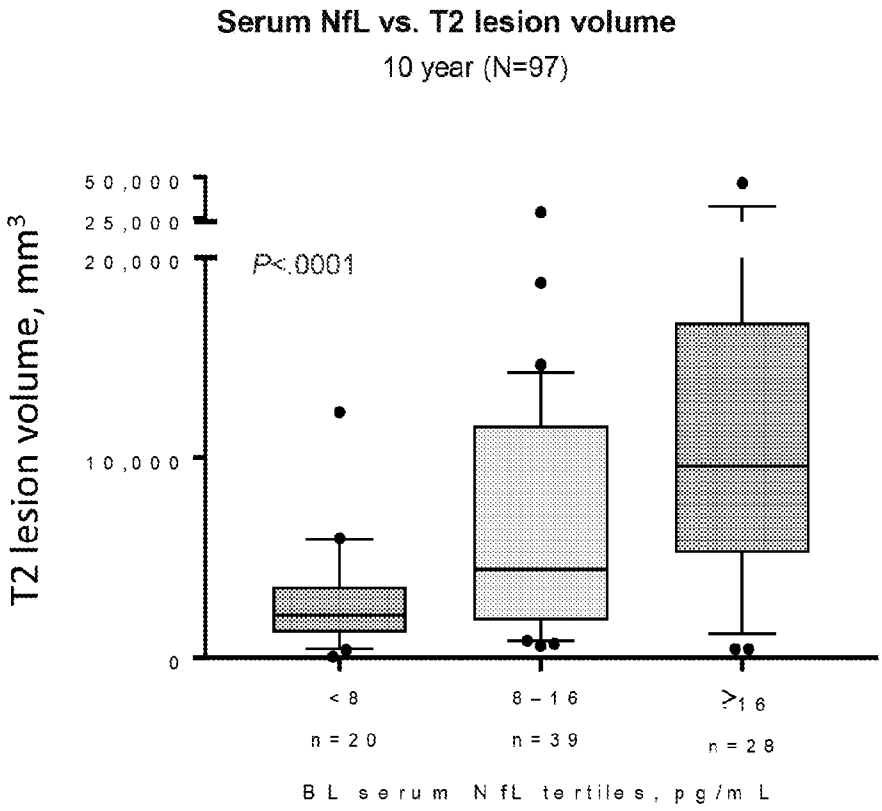

Serum NfL vs. T2 lesion volume
10 year (N=97)
T2 lesion volume, mm3
50,000
25,000
20,000
10,000
0
P<.0001
< 8
n = 20
8 – 16
n = 39
>16
n = 28
BL serum NfL tertiles, pg/mL

Figure 2D

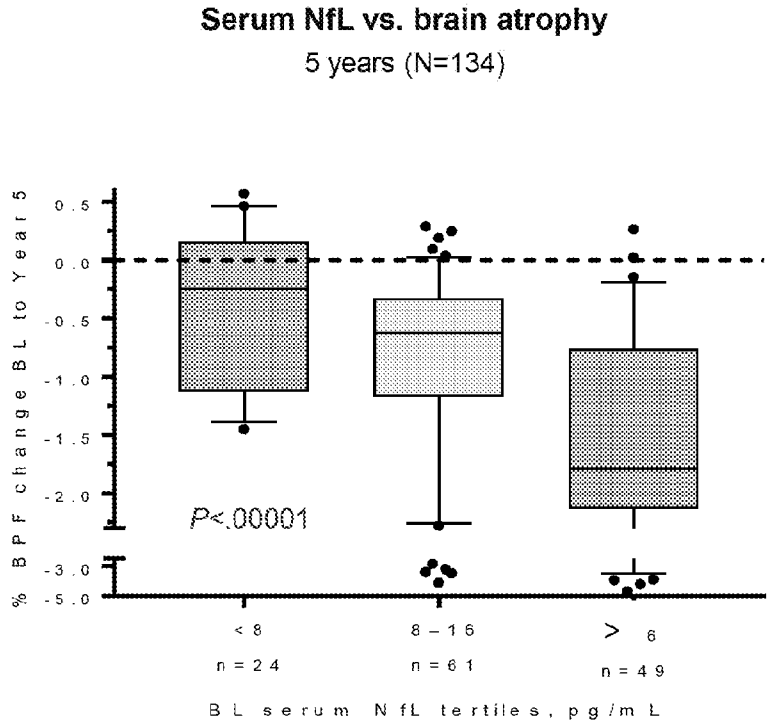

Serum NfL vs. brain atrophy
5 years (N=134)
% BPF change BL to Year 5
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-3.0
-5.0
P<.00001
< 8
n = 24
8 – 16
n = 61
> 6
n = 49
BL serum NfL tertiles, pg/mL

Figure 2E

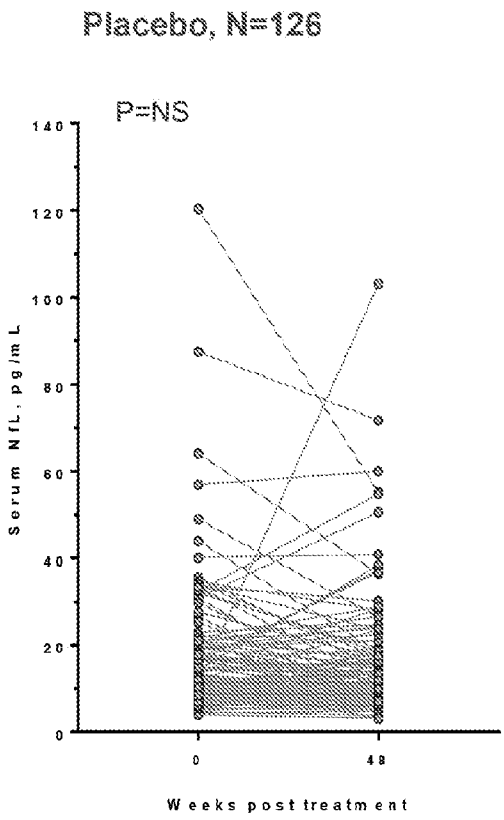


Figure 3A

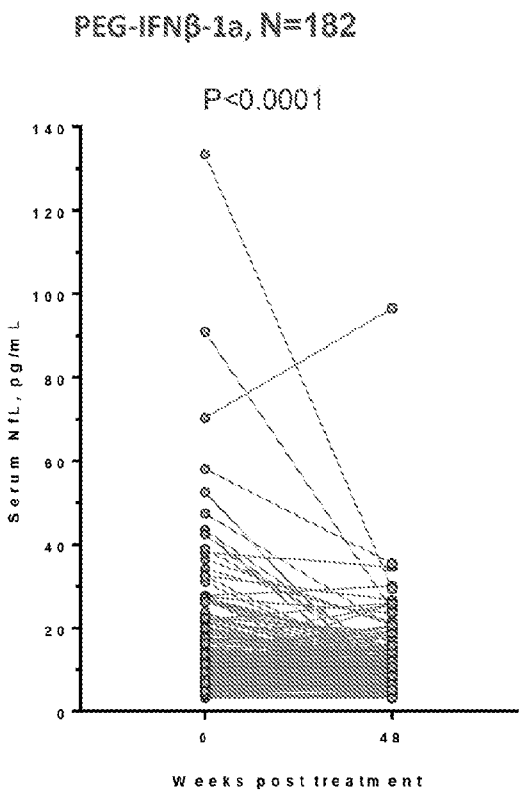


Figure 3B

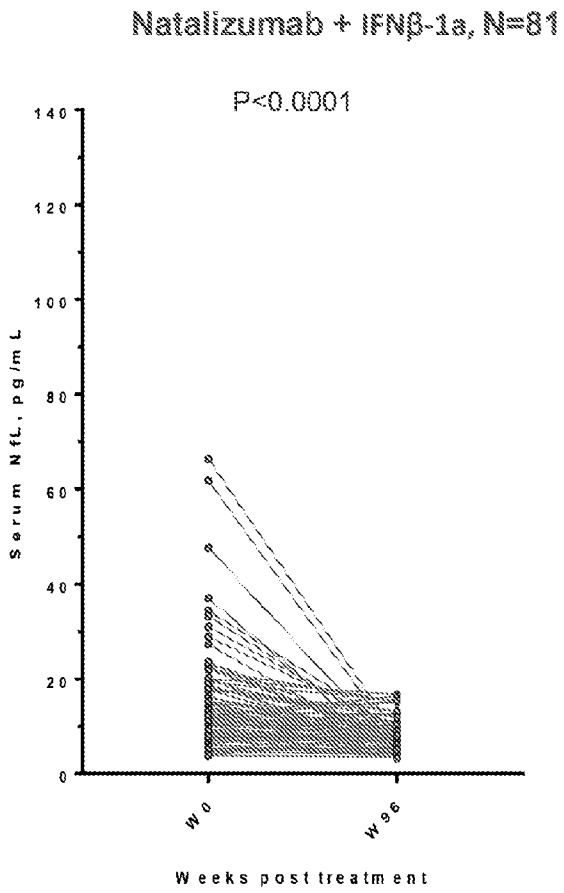

Natalizumab + IFNβ-1a, N=81
P<0.0001
Serum NfL, pg/mL
140
120
100
80
60
40
20
0
W 0
W 96
Weeks post treatment

Figure 3C

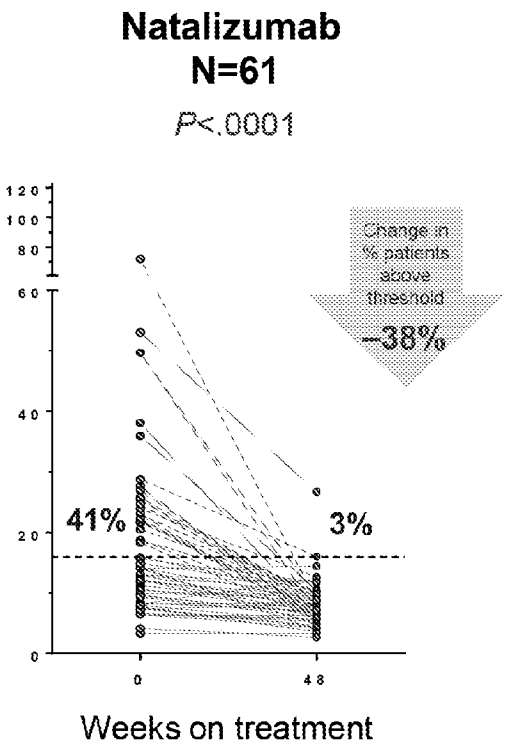


Figure 3D

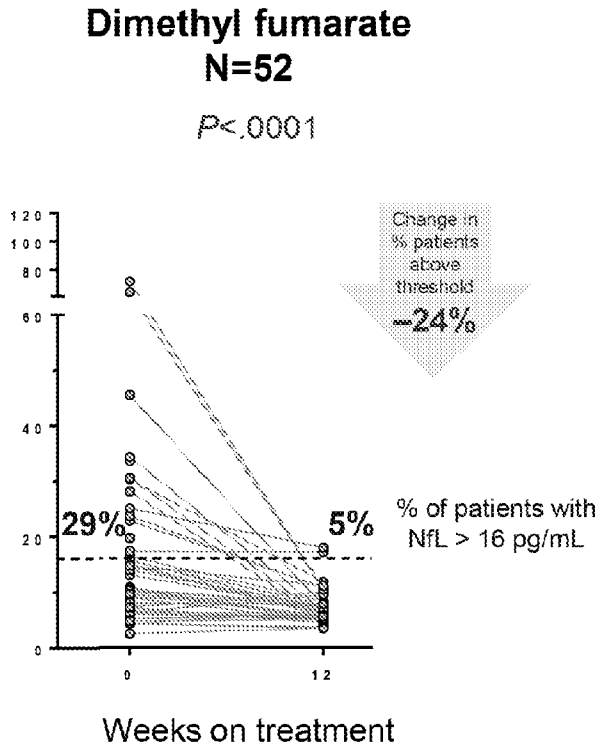


Figure 3E

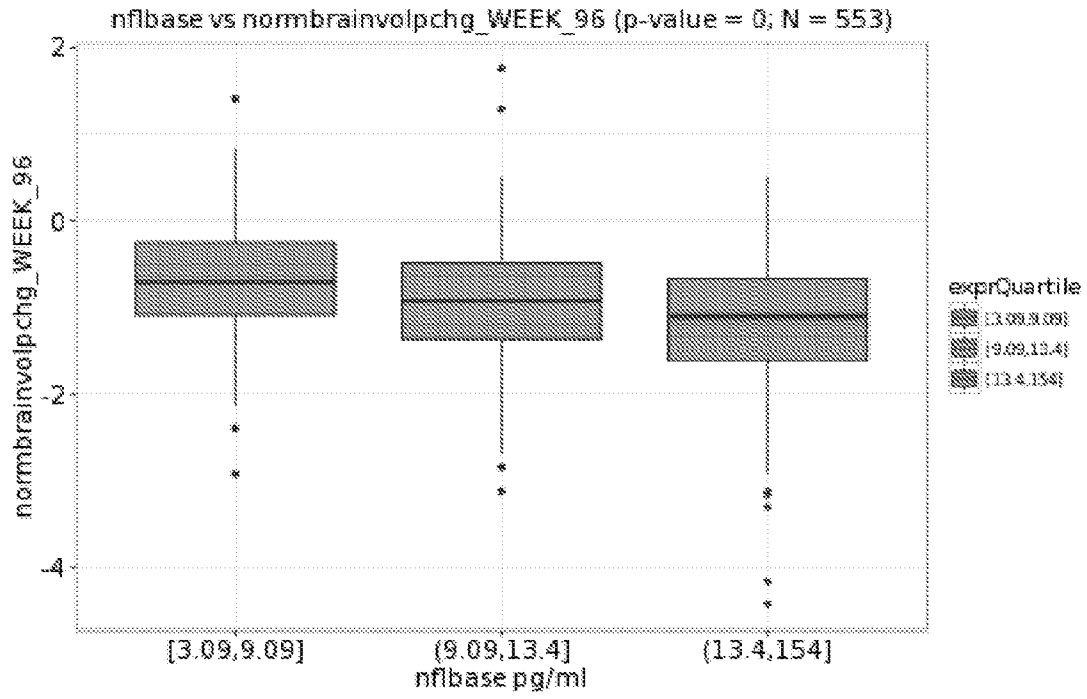

nflbase vs normbrainvolpchg_WEEK_96 (p-value = 0; N = 553)
normbrainvolpchg_WEEK_96
2
0
-2
-4
[3.09,9.09]
(9.09,13.4]
(13.4,154]
nflbase pg/ml
exprQuartile
[3.09,9.09]
(9.09,13.4]
(13.4,154]

Figure 4A

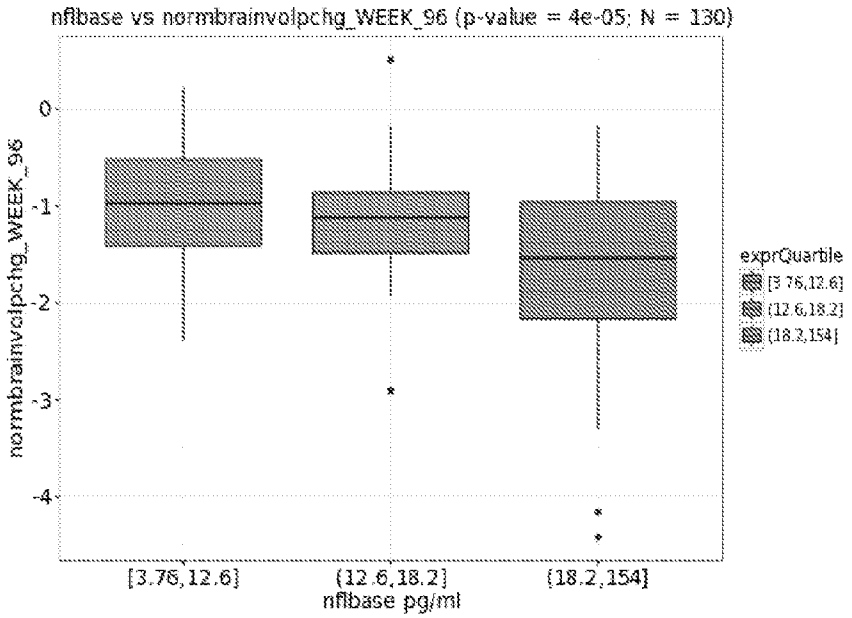

nflbase vs normbrainvolpchg_WEEK_96 (p-value = 4e-05; N = 130)
normbrainvolpchg_WEEK_96
0
-1
-2
-3
-4
[3.76,12.6]
(12.6,18.2]
(18.2,154]
nflbase pg/ml
exprQuartile
[3.76,12.6]
(12.6,18.2]
(18.2,154]

Fig. 4B

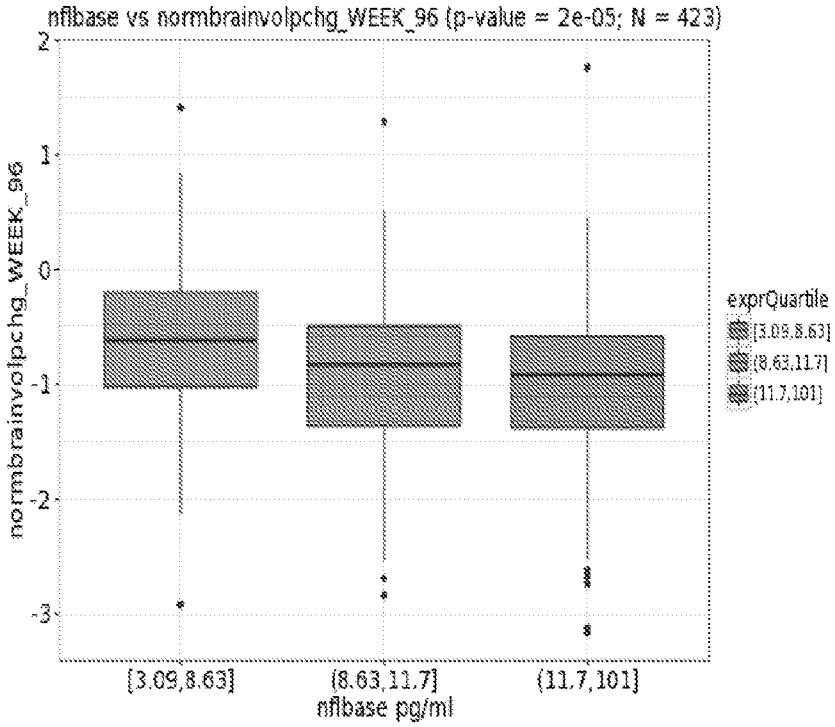

nflbase vs normbrainvolpchg_WEEK_96 (p-value = 2e-05; N = 423)
normbrainvolpchg_WEEK_96
2
1
0
-1
-2
-3
[3.09,8.63]
(8.63,11.7]
(11.7,101]
nflbase pg/ml
exprQuartile
[3.09,8.63]
(8.63,11.7]
(11.7,101]

Figure 4C

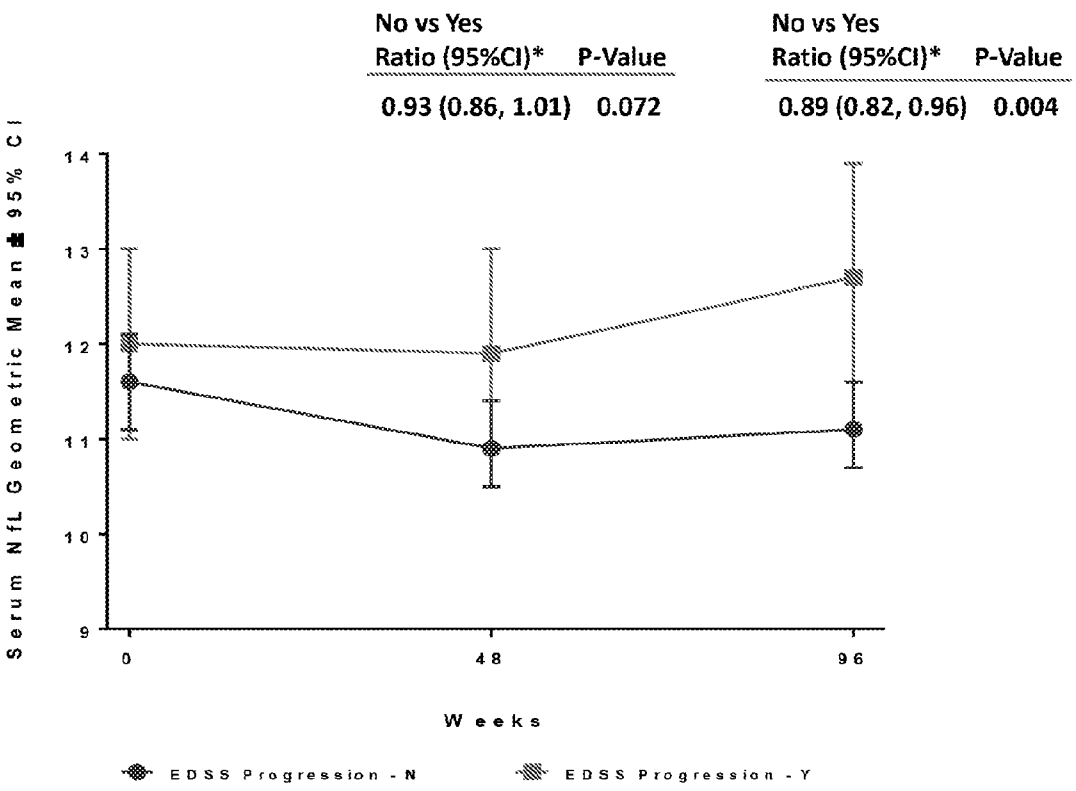

No vs Yes
Ratio (95%CI)* P-Value
0.93 (0.86, 1.01) 0.072
No vs Yes
Ratio (95%CI)* P-Value
0.89 (0.82, 0.96) 0.004
Serum NfL Geometric Mean ± 95% CI
14
13
12
11
10
9
0
48
96
Weeks
EDSS Progression - N
EDSS Progression - Y

Figure 5

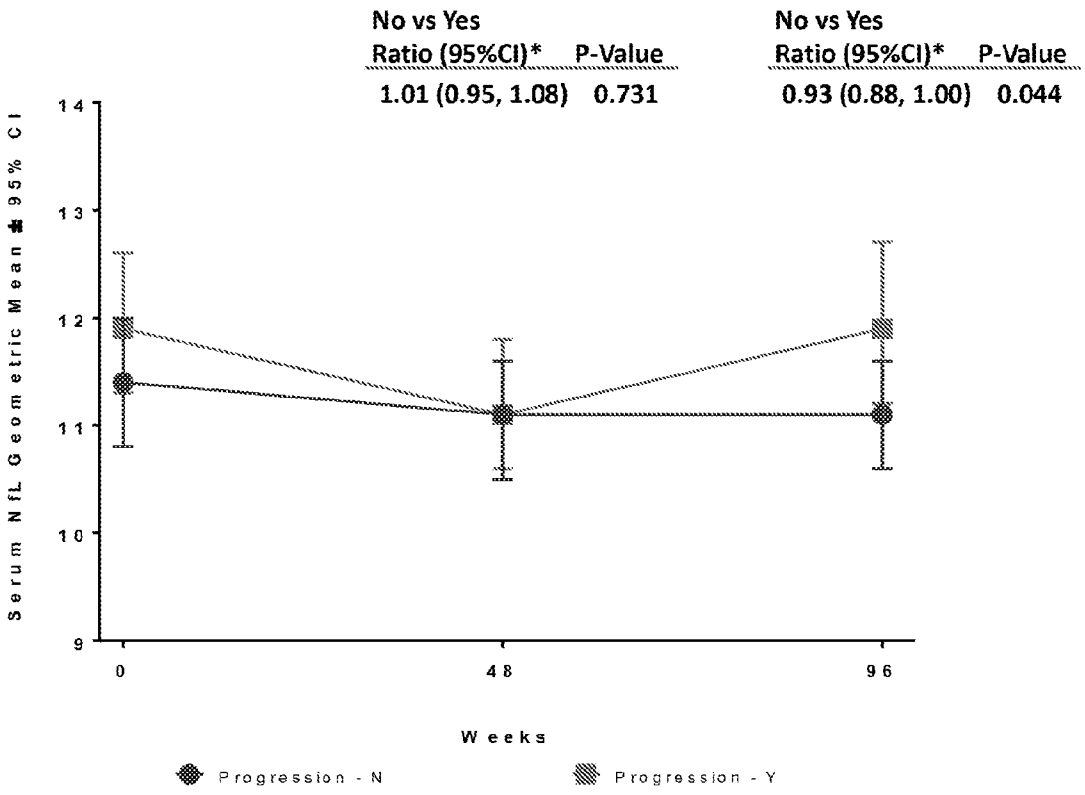

No vs Yes
Ratio (95%CI)* P-Value
1.01 (0.95, 1.08) 0.731
No vs Yes
Ratio (95%CI)* P-Value
0.93 (0.88, 1.00) 0.044
Serum NfL Geometric Mean ± 95% CI
14
13
12
11
10
9
0
48
96
Weeks
Progression - N
Progression - Y

Figure 6

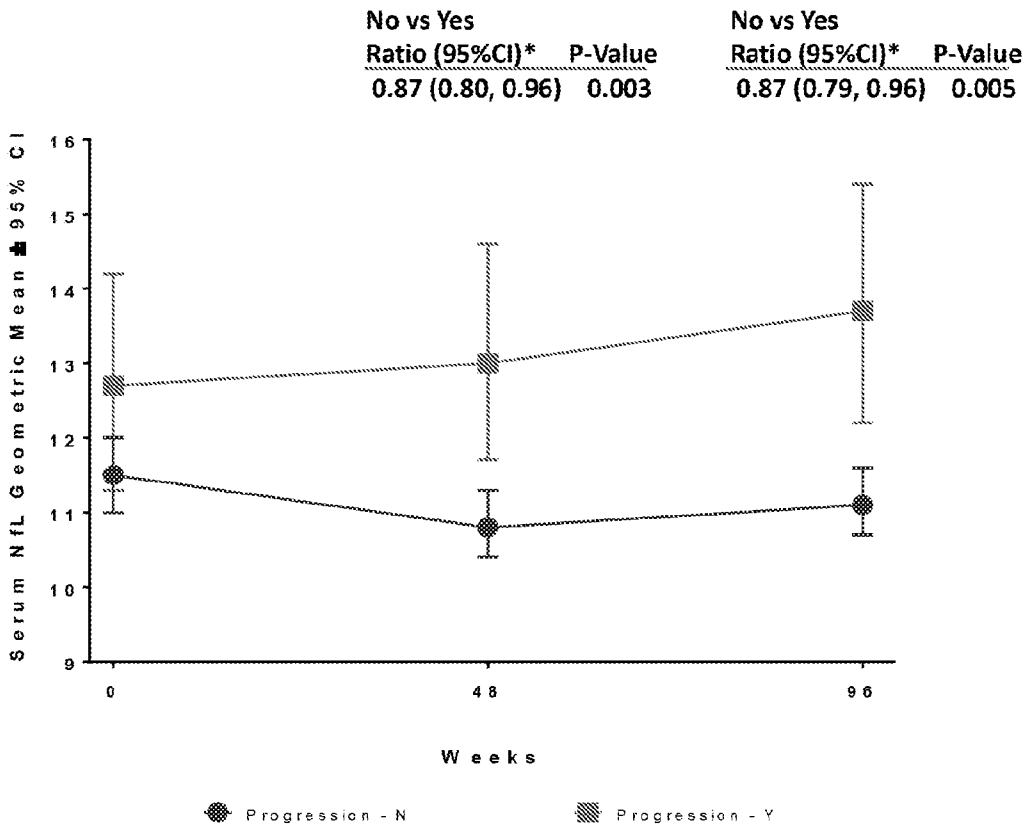

No vs Yes
Ratio (95%CI)* P-Value
0.87 (0.80, 0.96) 0.003
No vs Yes
Ratio (95%CI)* P-Value
0.87 (0.79, 0.96) 0.005
Serum NfL Geometric Mean ± 95% CI
16
15
14
13
12
11
10
9
0
48
96
Weeks
Progression - N
Progression - Y

Figure 7

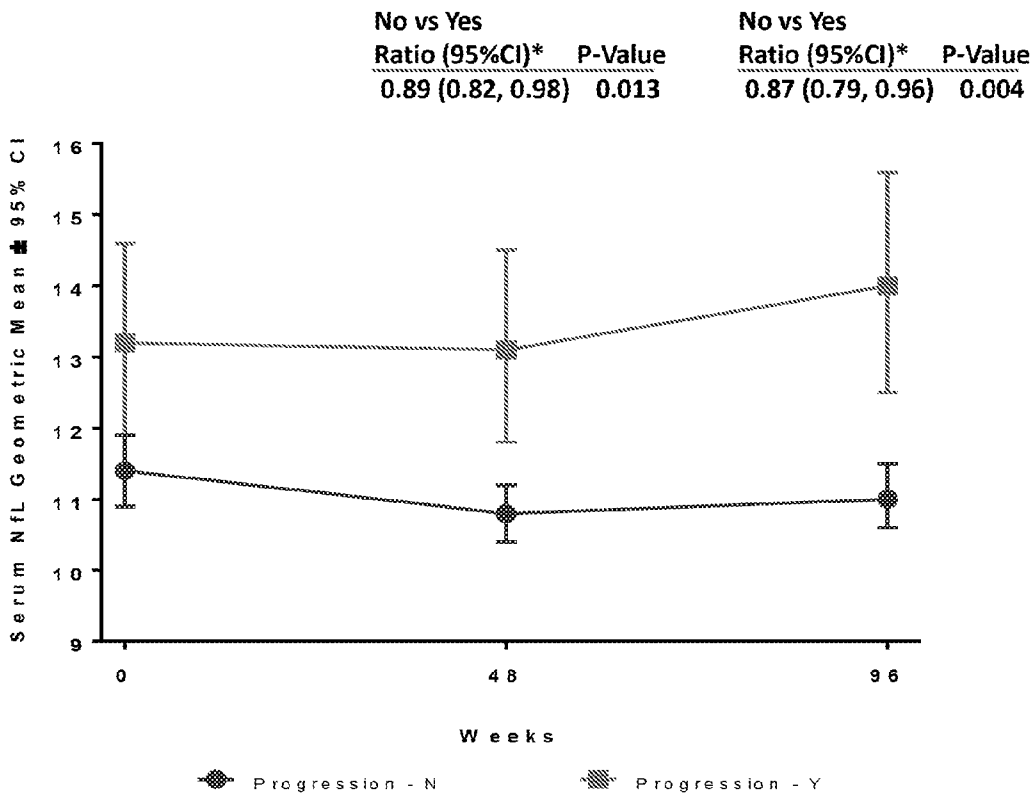

No vs Yes
Ratio (95%CI)* P-Value
0.89 (0.82, 0.98) 0.013
No vs Yes
Ratio (95%CI)* P-Value
0.87 (0.79, 0.96) 0.004
Serum NfL Geometric Mean ± 95% CI
16
15
14
13
12
11
10
9
0
48
96
Weeks
Progression - N
Progression - Y

Figure 8

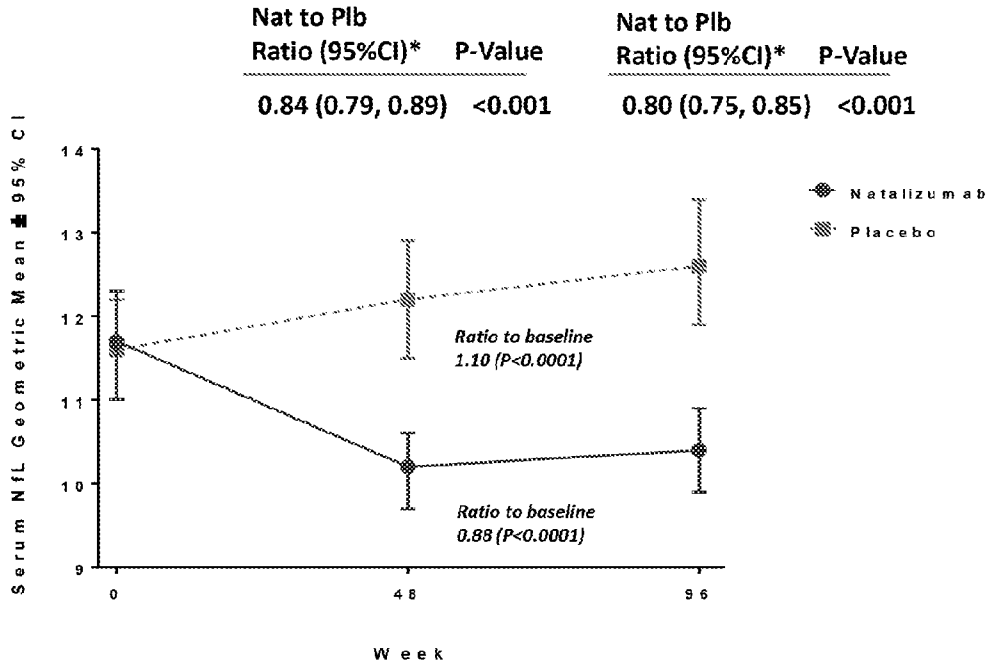

Nat to Plb
Ratio (95%CI)* P-Value
0.84 (0.79, 0.89) <0.001
Nat to Plb
Ratio (95%CI)* P-Value
0.80 (0.75, 0.85) <0.001
Natalizumab
Placebo
Ratio to baseline
1.10 (P<0.0001)
Ratio to baseline
0.88 (P<0.0001)
Serum NfL Geometric Mean ± 95% CI
14
13
12
11
10
9
0
48
96
Week

Figure 9

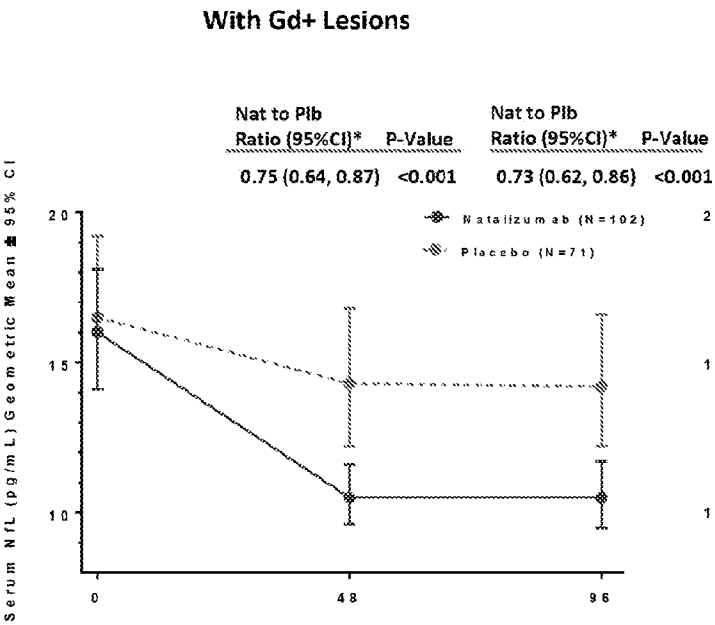


Figure 10A

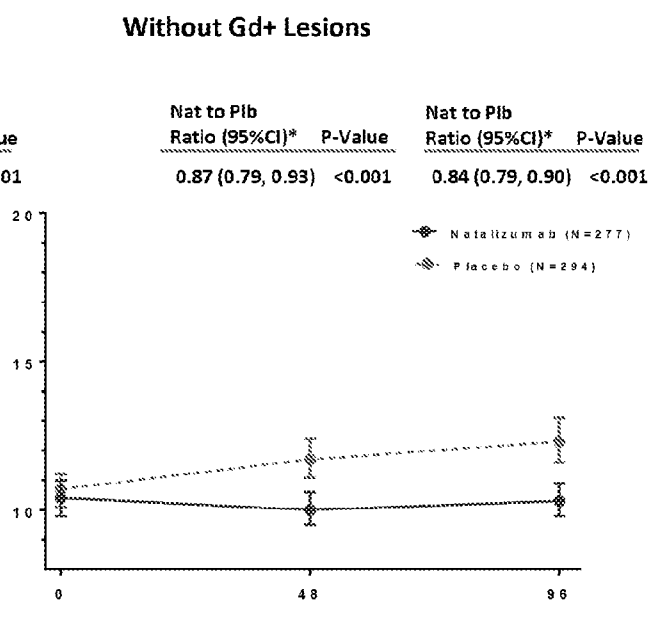


Figure 10B

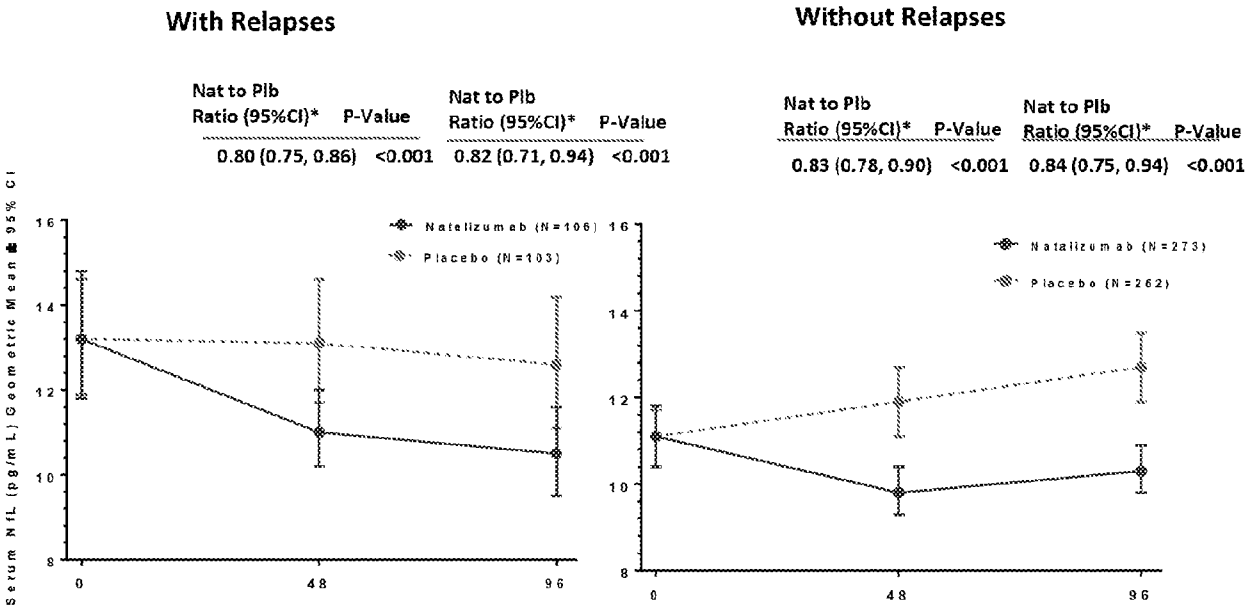
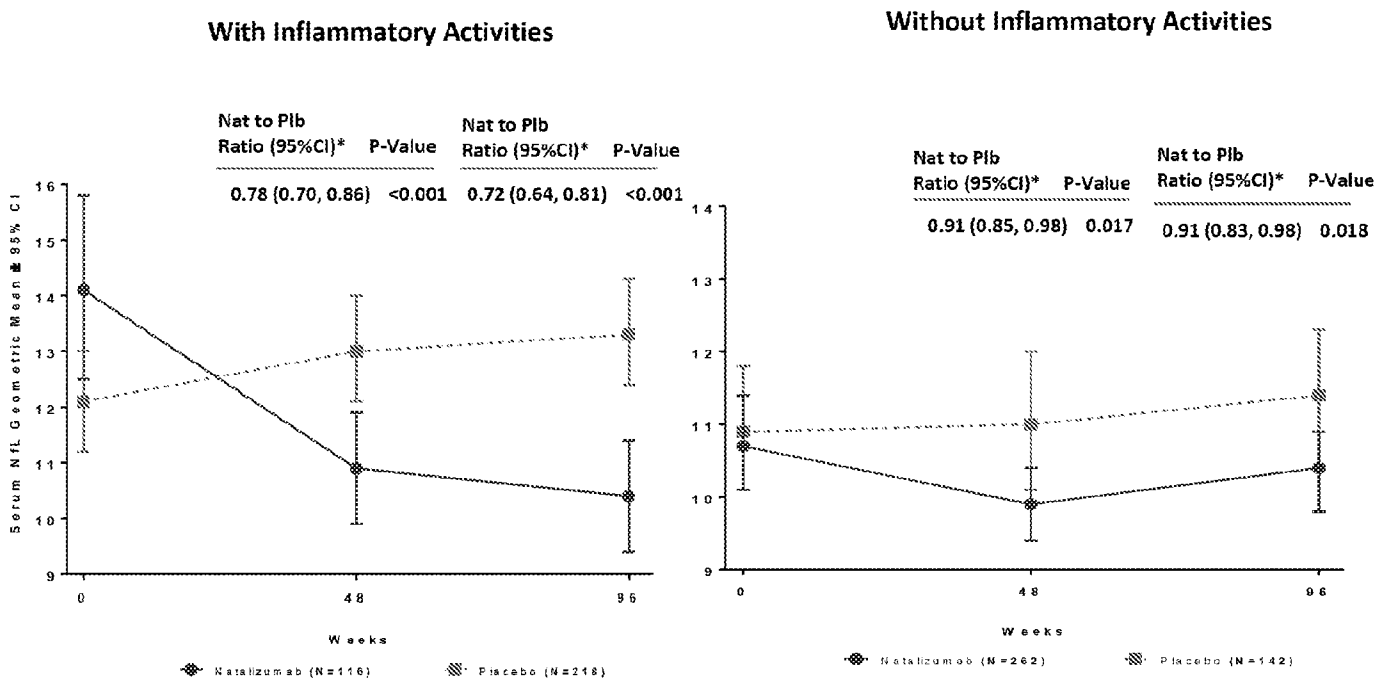


Figure 11A

Figure 11B

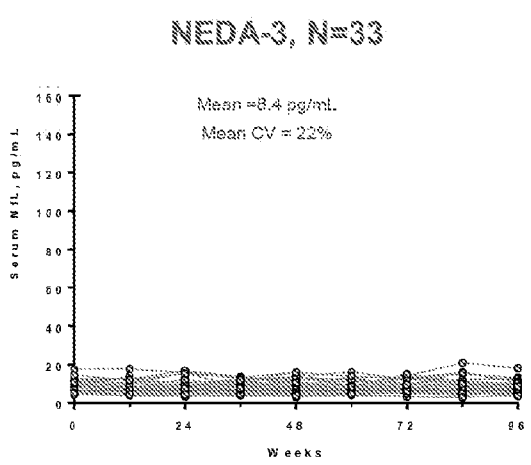


** Gd+ or new or newly enlarging T2 lesions on the visit and relapse any time during treatment

Figure 12A

Figure 12B

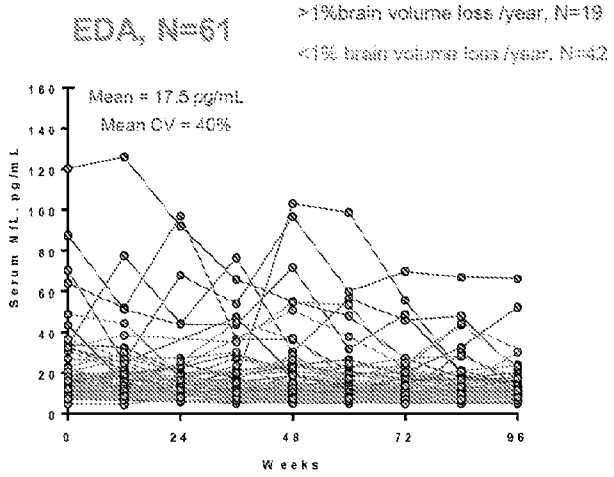
No Evident Disease



Patients with NEDA have consistently low and stable NfL levels over 2 years

Figure 13A

Activity Active Disease



Patients with EDA have higher($p<0.0001$) and more variable serum NfL levels

Figure 13B

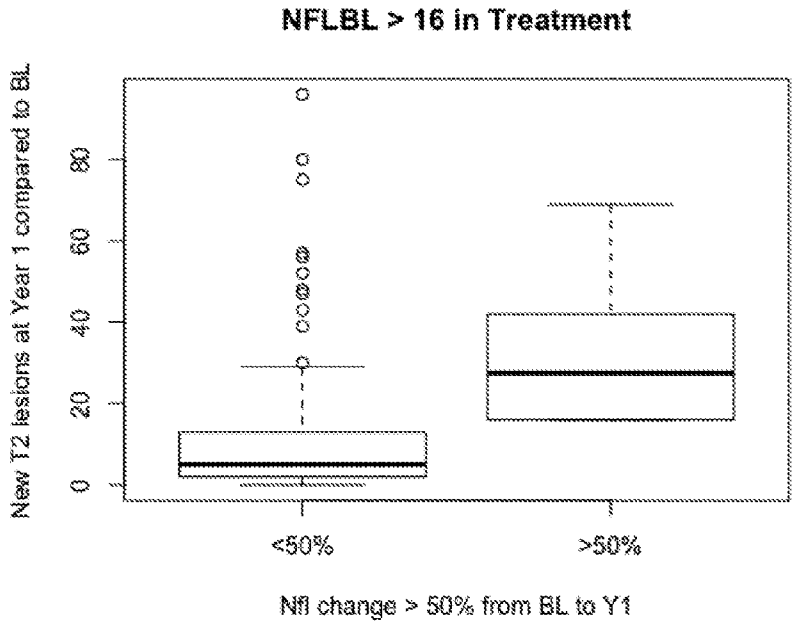

NFLBL > 16 in Treatment
New T2 lesions at Year 1 compared to BL
0
20
40
60
80
<50%
>50%
Nfl change > 50% from BL to Y1

Figure 14A

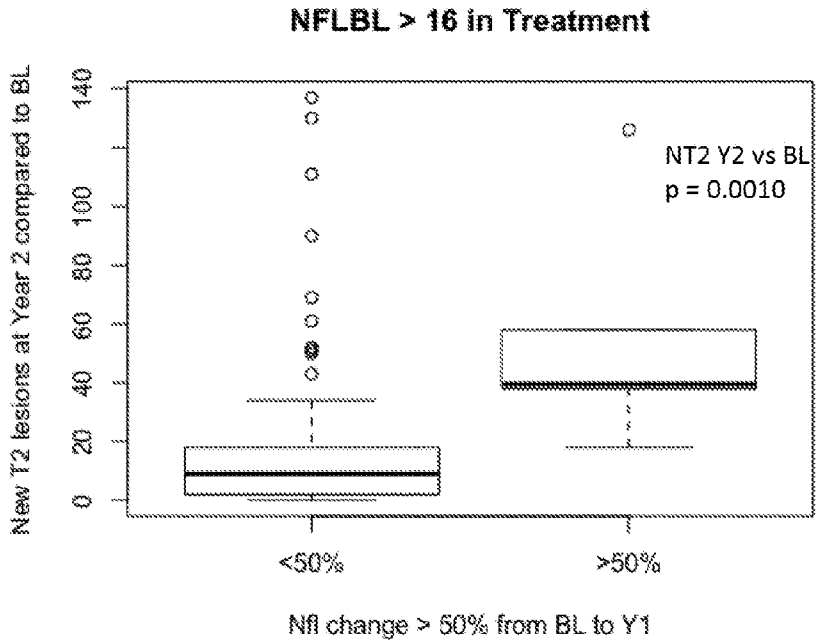

NFLBL > 16 in Treatment
New T2 lesions at Year 2 compared to BL
0
20
40
60
80
100
140
NT2 Y2 vs BL
p = 0.0010
<50%
>50%
Nfl change > 50% from BL to Y1

Fig. 14B

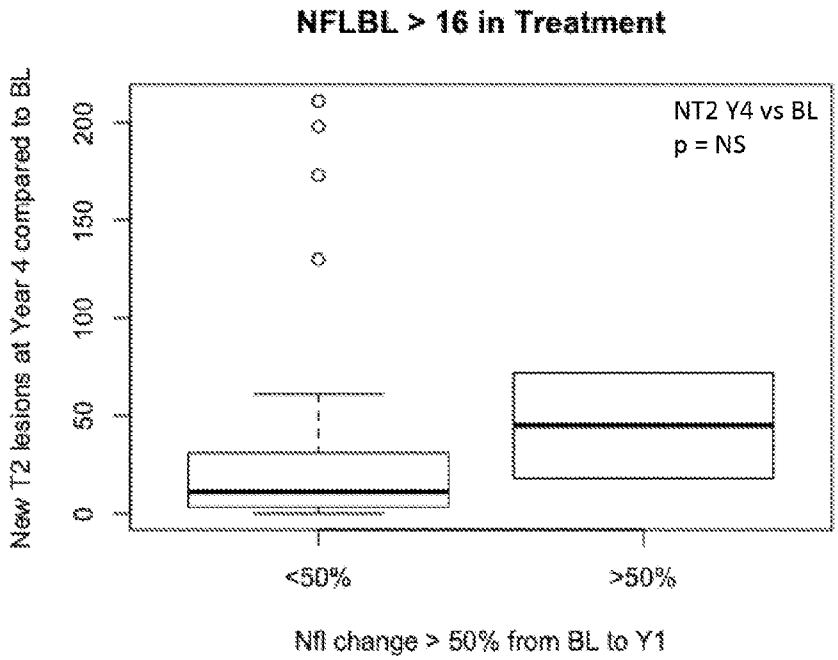

NFLBL > 16 in Treatment
New T2 lesions at Year 4 compared to BL
0
50
100
150
200
NT2 Y4 vs BL
p = NS
<50%
>50%
Nfl change > 50% from BL to Y1

Fig. 14C

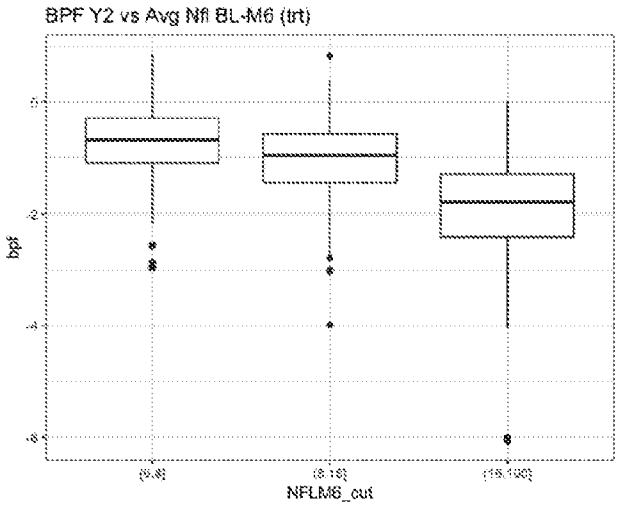


Figure 15A

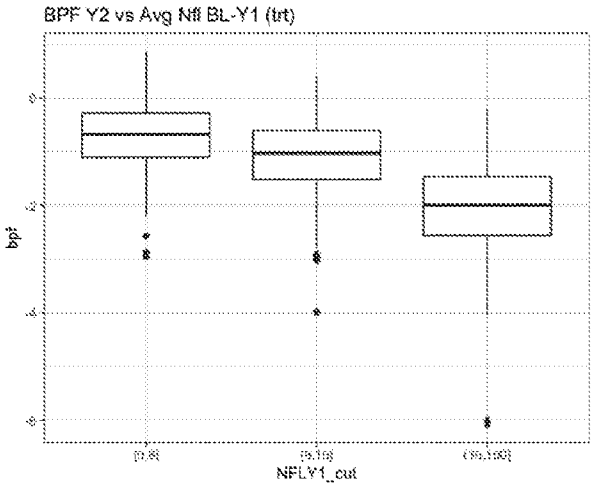


Figure 15B

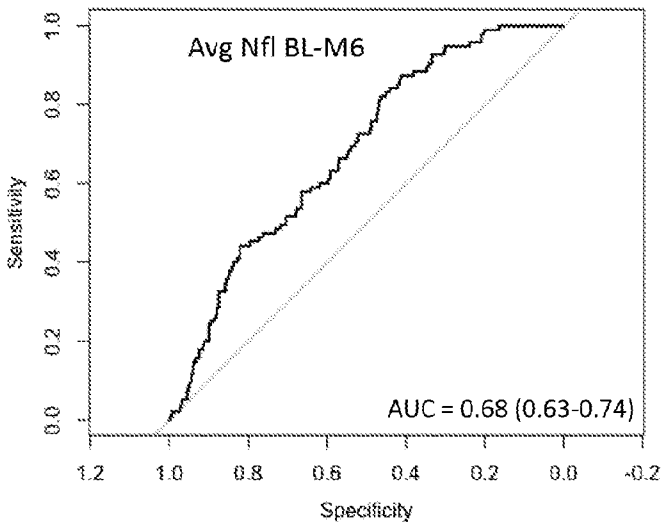


Fig. 16A

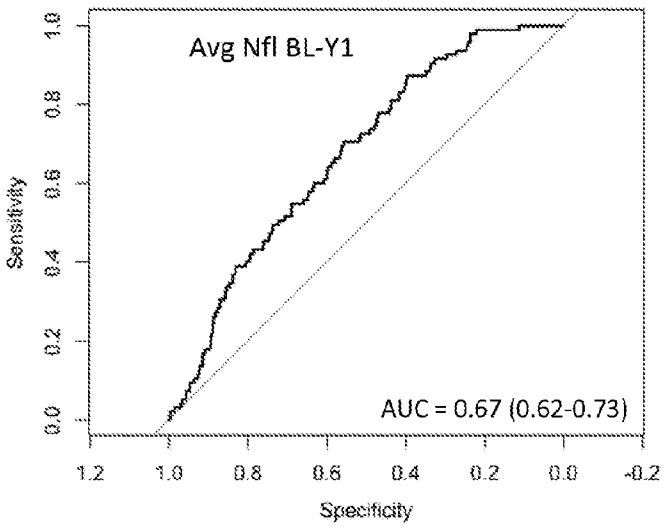


Fig. 16B

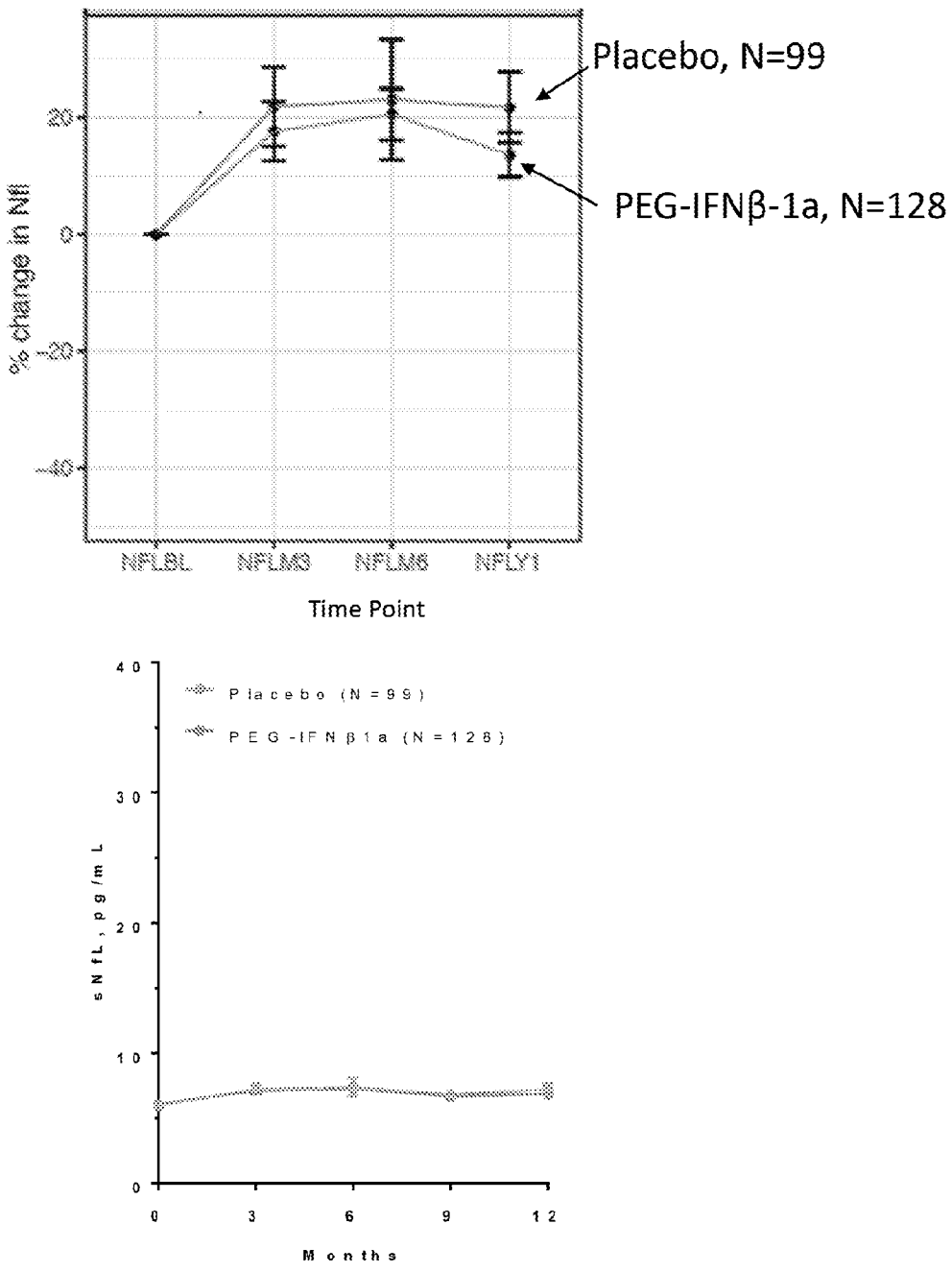
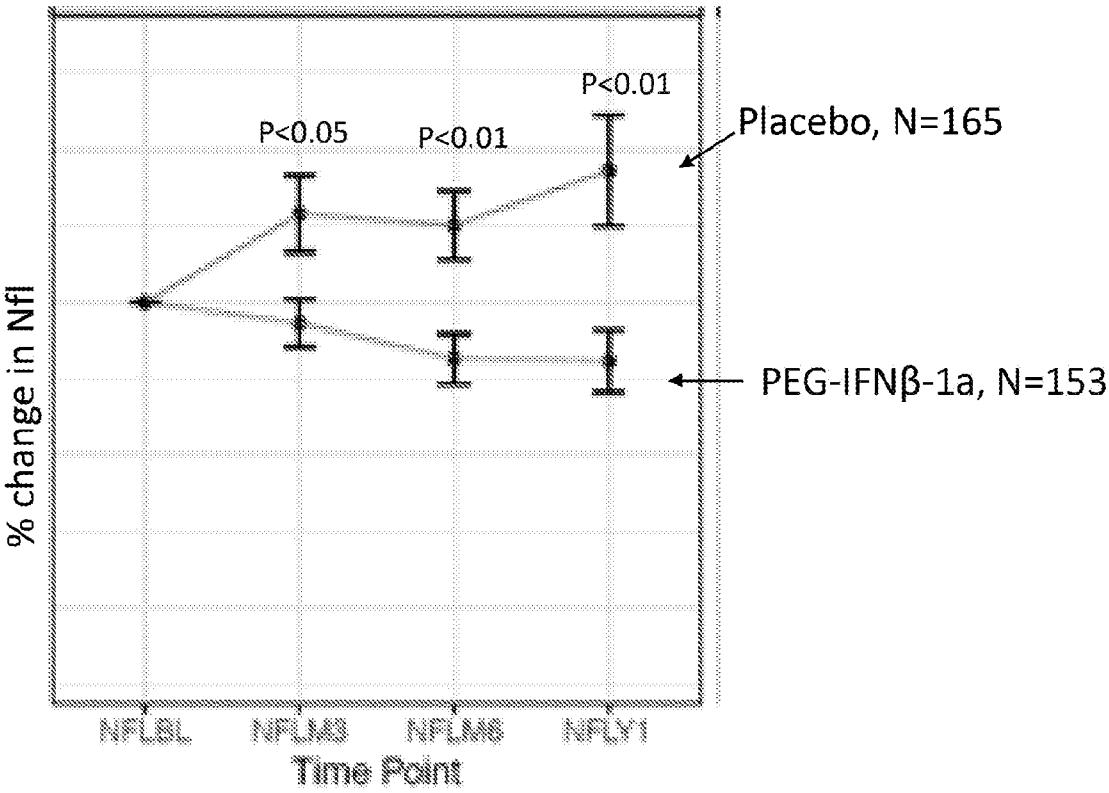


Figure 17A:
BL sNfL < 8pg/mL



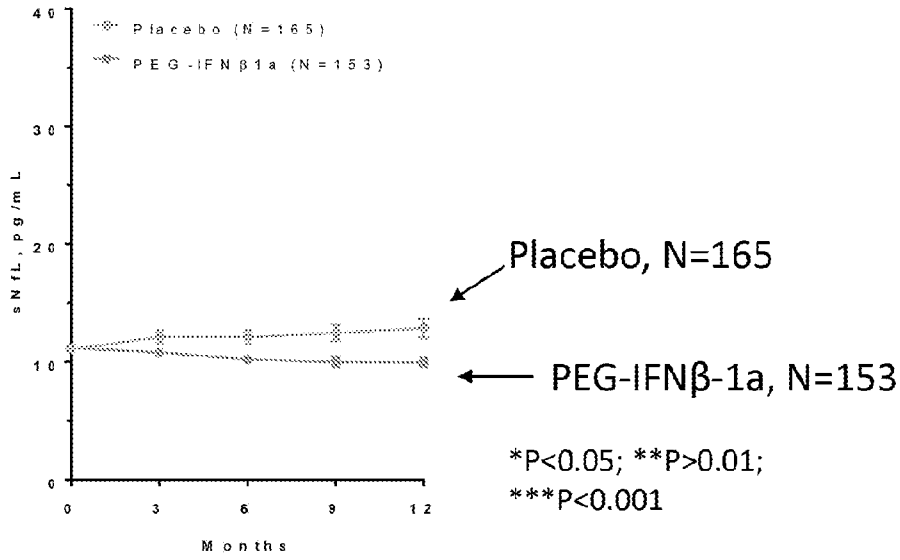
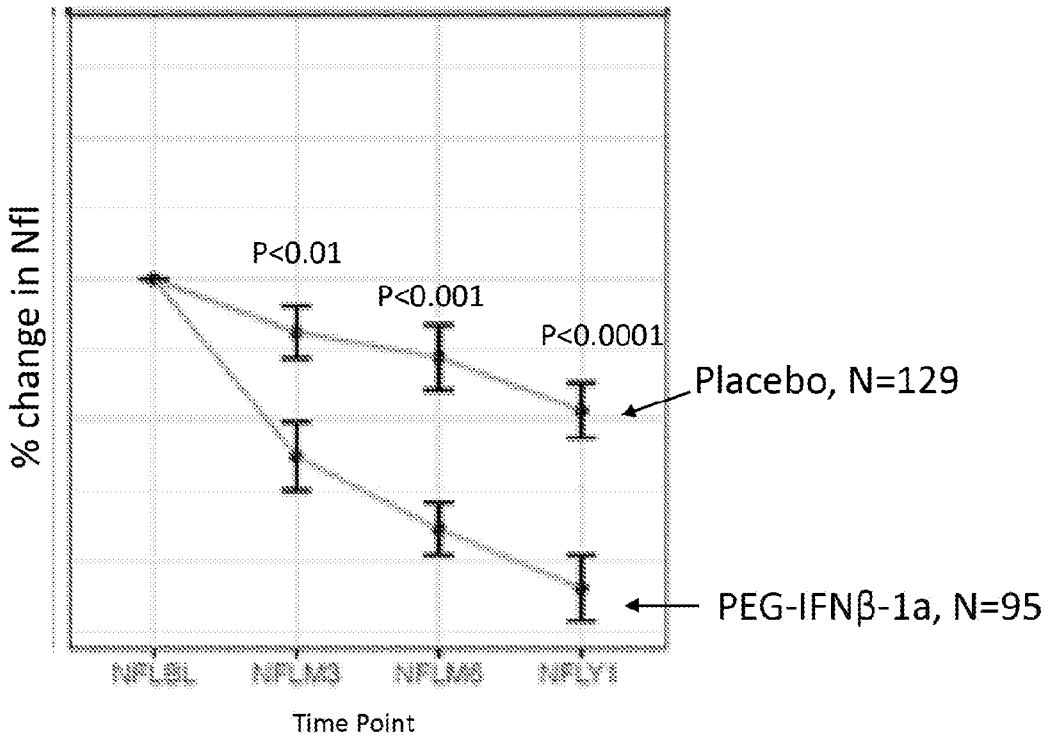


Figure 17B:
BL sNfL 8-16 pg/mL



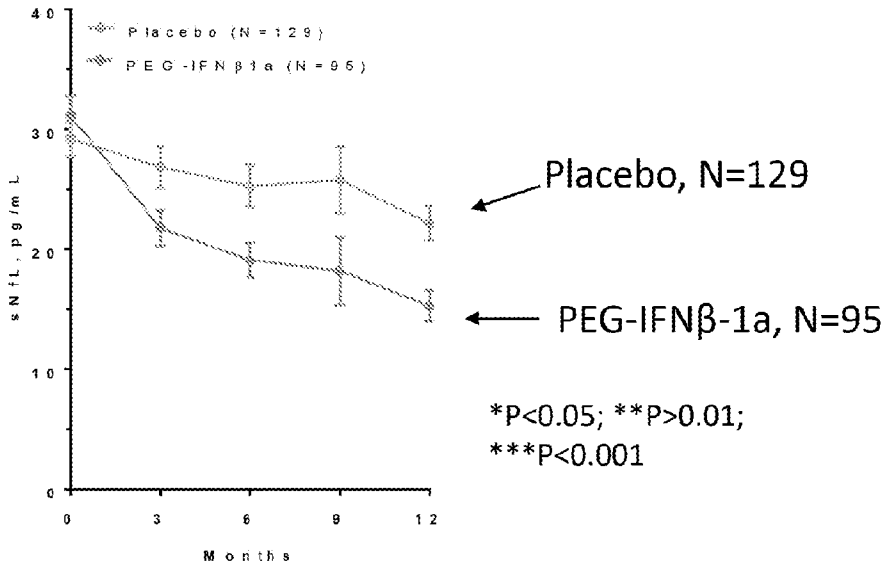


Figure 17C:
BL sNfL >16 pg/mL

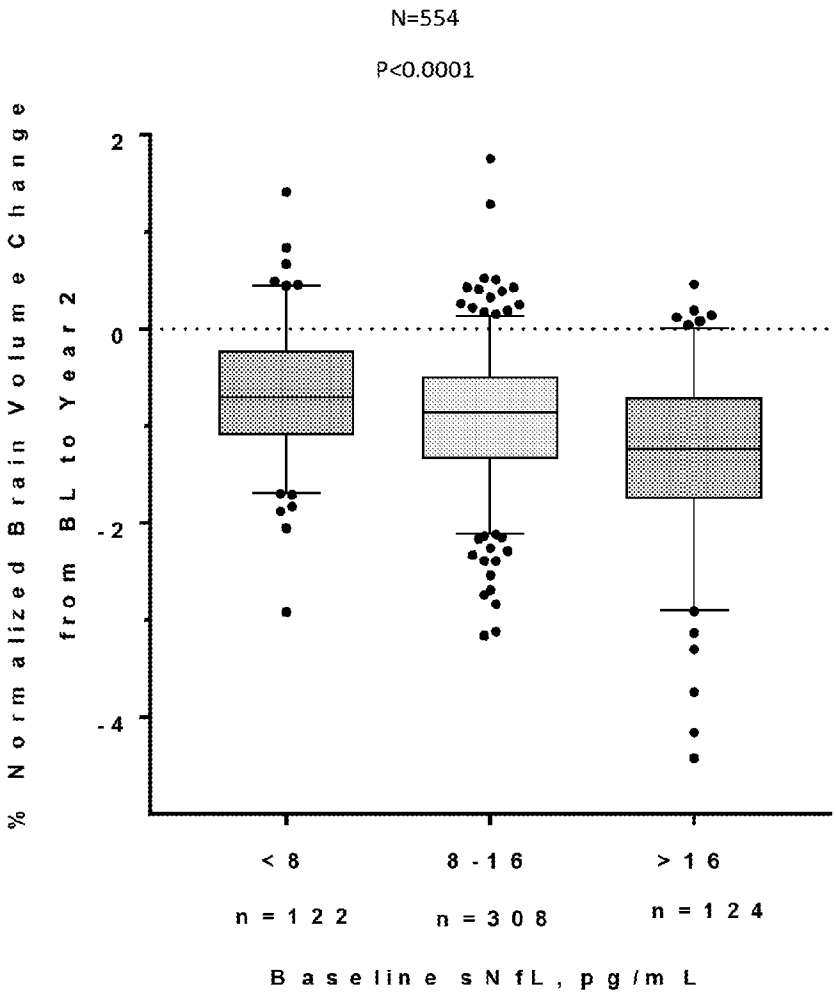

N=554
P<0.0001
% Normalized Brain Volume Change from BL to Year 2
2
0
-2
-4
< 8
8 - 16
> 16
n = 122
n = 308
n = 124
Baseline sNfL, pg/mL

FIG. 18

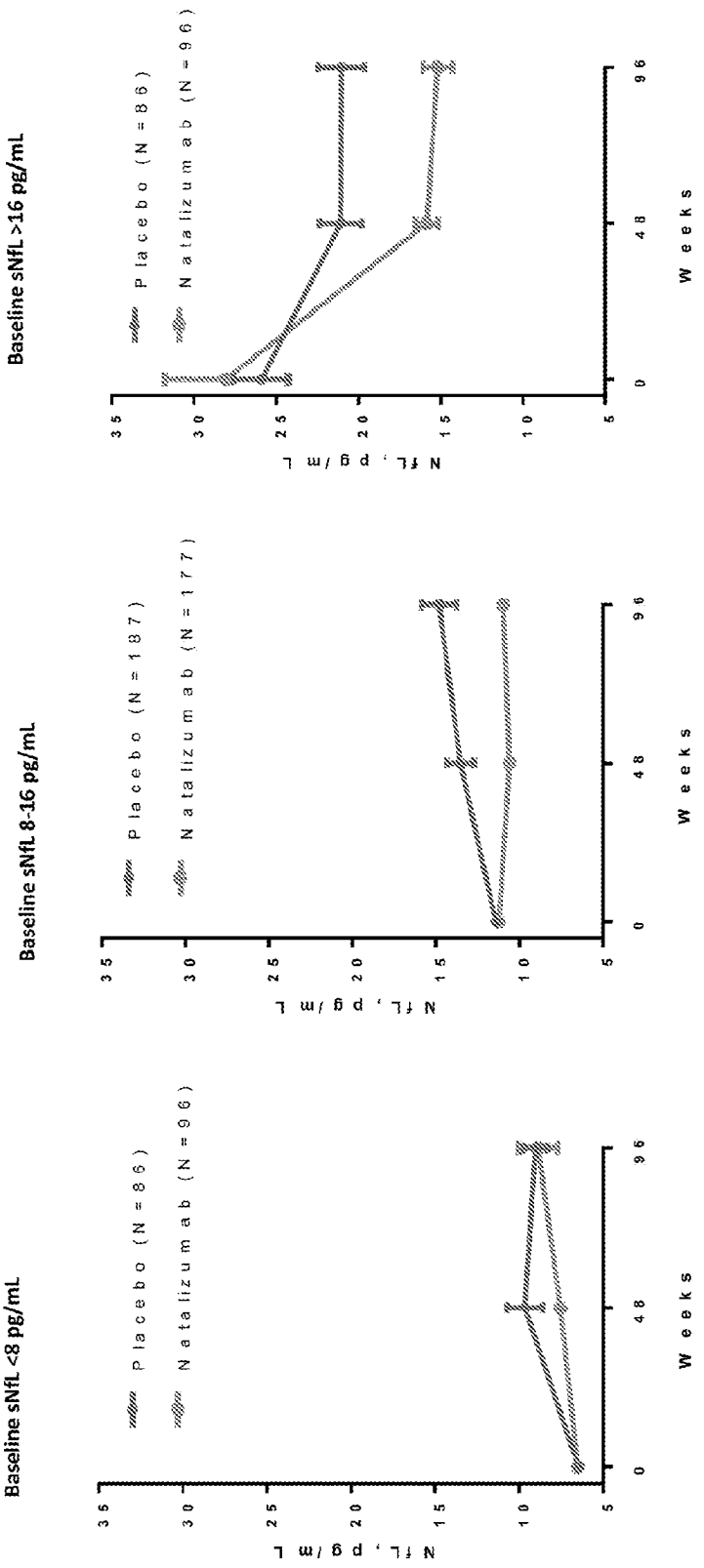

Baseline sNfL <8 pg/mL
Baseline sNfL 8-16 pg/mL
Baseline sNfL >16 pg/mL
Placebo (N = 86)
Natalizumab (N = 96)
Placebo (N = 187)
Natalizumab (N = 177)
Placebo (N = 86)
Natalizumab (N = 96)
sNfL, pg/mL
Weeks
0
48
96
5
10
15
20
25
30
35

FIG. 19

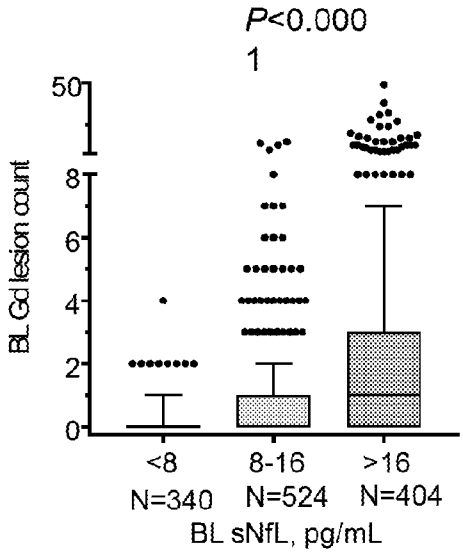


FIG. 20A

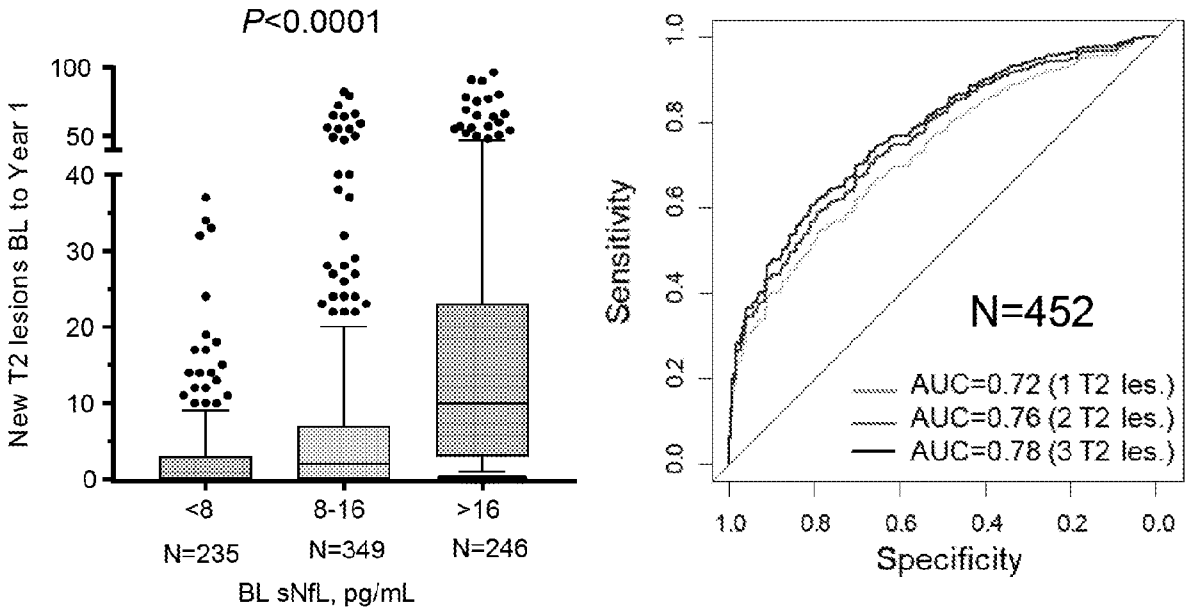


FIG. 20B

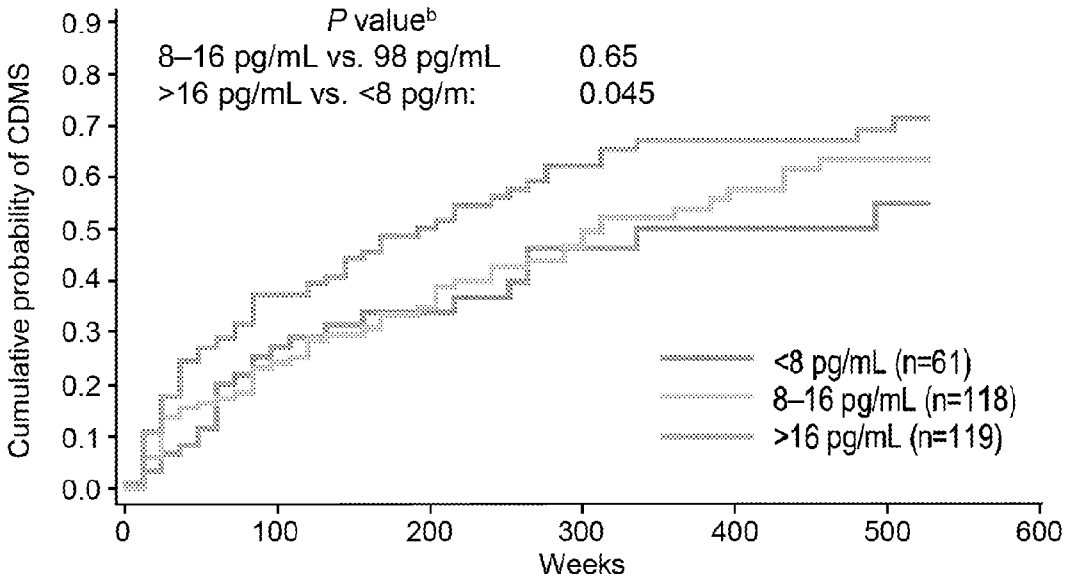


FIG. 21A

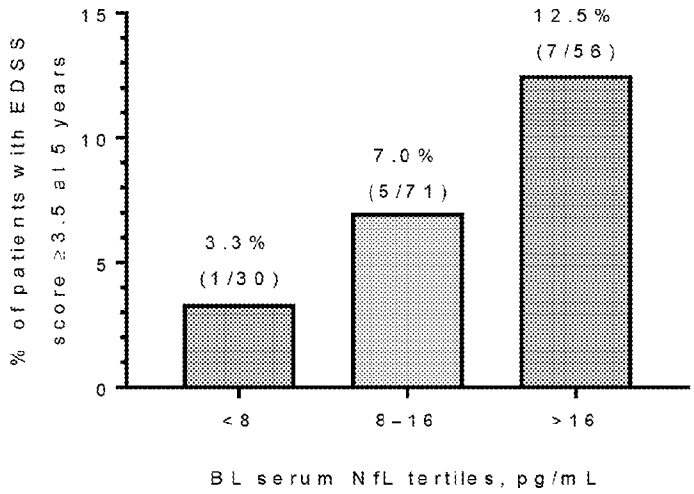


FIG. 21B

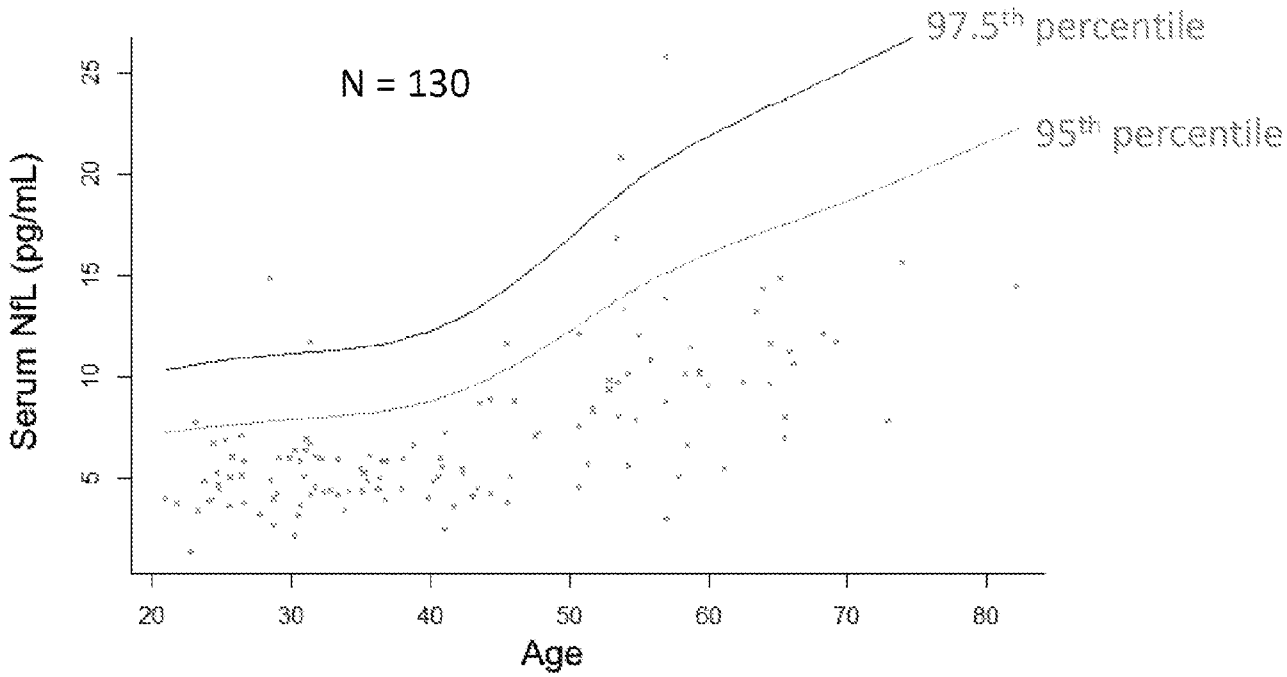

N = 130
97.5th percentile
95th percentile
Serum NfL (pg/mL)
5
10
15
20
25
20
30
40
50
60
70
80
Age

FIG. 22

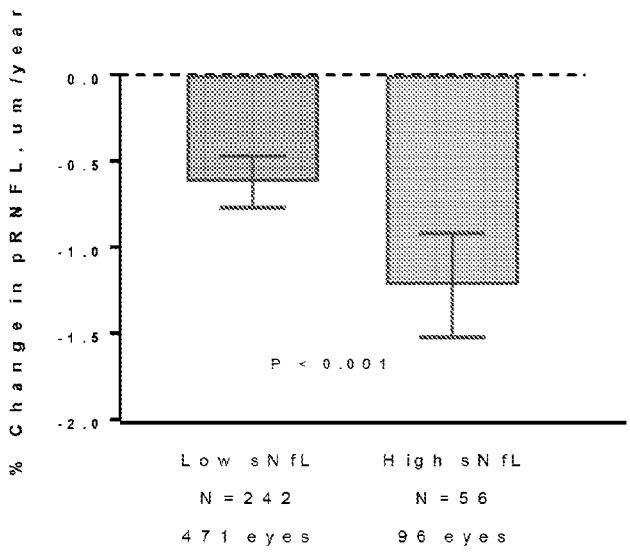


FIG. 23A

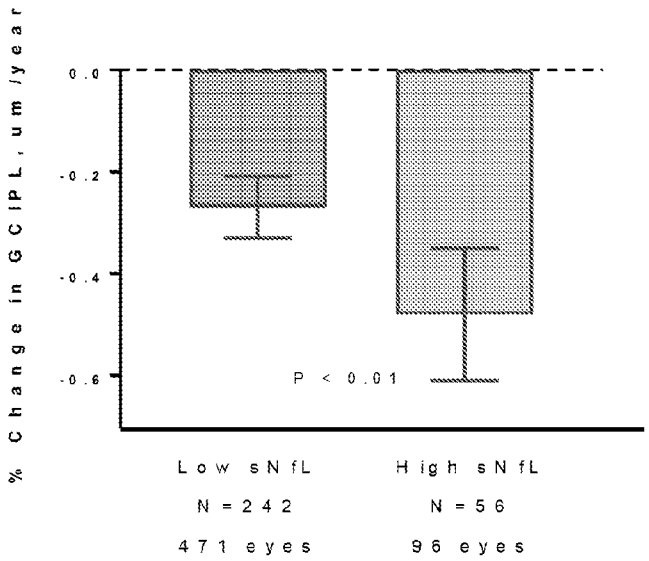


FIG. 23B

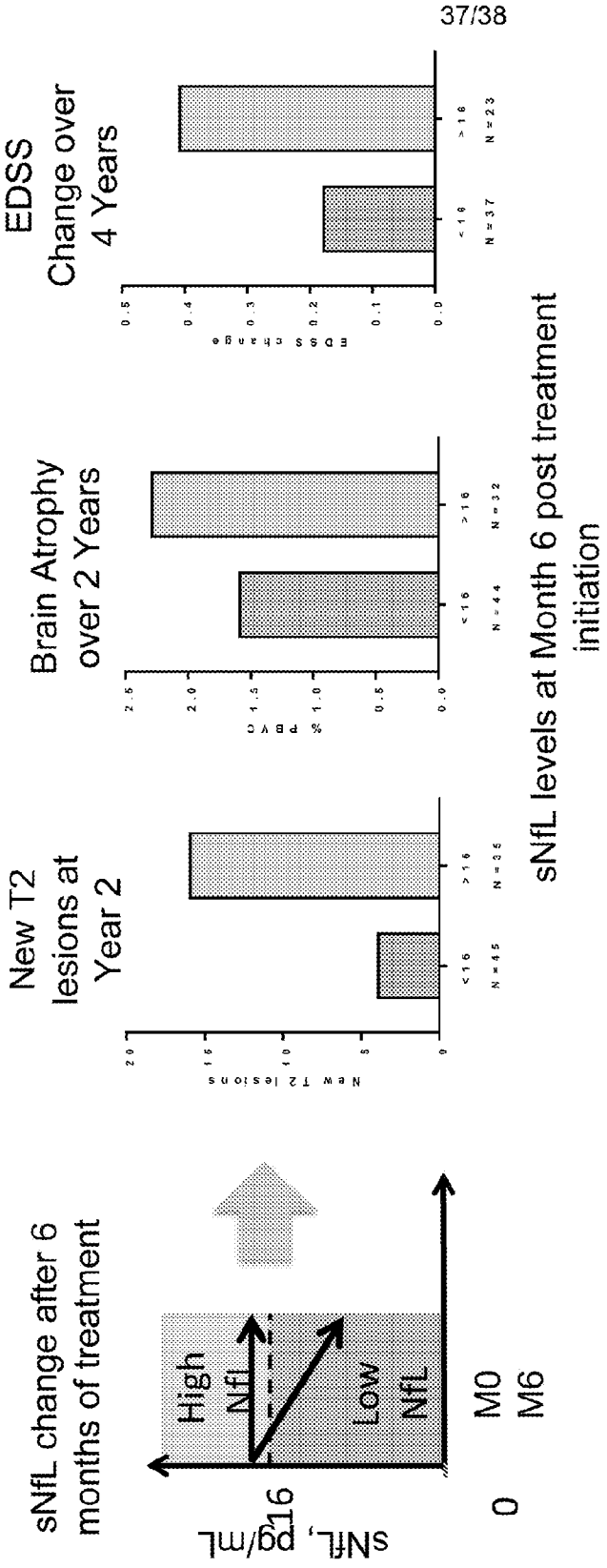
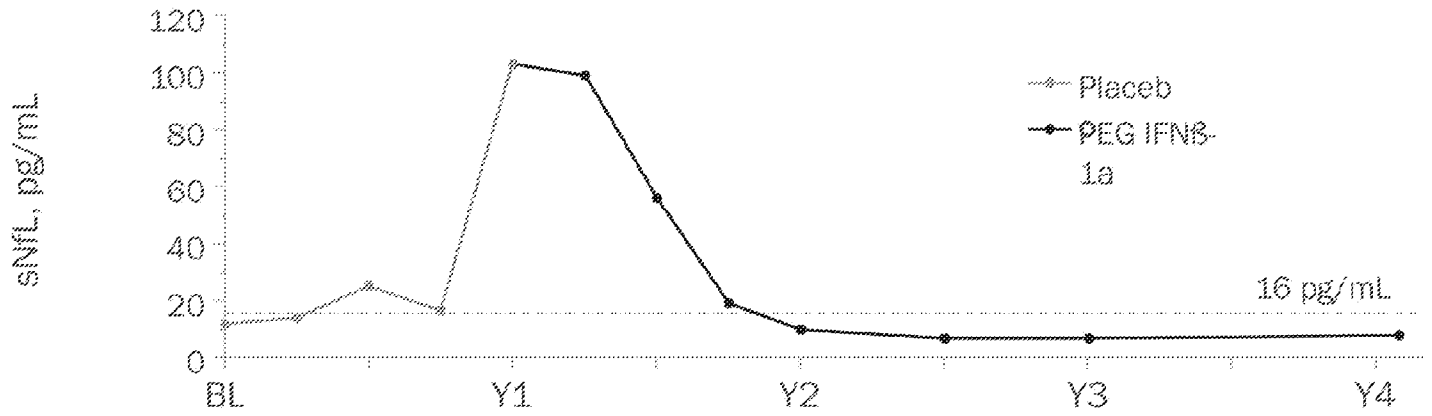

sNfL change after 6 months of treatment
High Nfl
Low NfL
sNfL, pg/mL
916
0
M0
M6
New T2 lesions at Year 2
Brain Atrophy over 2 Years
EDSS Change over 4 Years
sNfL levels at Month 6 post treatment initiation

FIG. 24



EDSS	0	0	2	2	2
New Gd+	0	13	0	0	0
New T2 from BL	-	28	48	49	49
SDMT	48	39	23	39	38

BL, baseline; DMT, disease modifying therapy; EDSS, expanded disability status scale; Gd+, gadolinium-enhancing lesions; New T2, new or newly enlarged T2 lesions, cumulative relative to BL; SDMT, Symbol Digit Modalities Test; PEG IFNß-1a, pegylated interferon beta-1a; sNfL, serum neurofilament light; Y, year.

FIG. 25