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(54) Title: METHOD FOR TREATING MULTIPLE SCLEROSIS

(57) Abstract: The present invention concerns methods for treating multiple sclerosis (MS) in a patient, and an article of manufacture with instructions for such use.

METHOD FOR TREATING MULTIPLE SCLEROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/238,103, filed October 6, 2015, U.S. Provisional Patent Application No. 62/238,674, filed October 7, 2015, U.S. Provisional Patent Application No. 62/271,985, filed December 28, 2015, U.S. Provisional Patent Application No. 62/296,049, filed February 16, 2016, U.S. Provisional Patent Application No. 62/322,734, filed April 14, 2016, U.S. Provisional Patent Application No. 62/342,633, filed May 27, 2016, and U.S. Provisional Patent Application No. 62/355,299, filed June 27, 2016, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392035245SEQLISTING.txt, date recorded: October 4, 2016, size: 41 KB).

FIELD OF THE INVENTION

[0003] The present invention concerns methods for treating multiple sclerosis (MS) in a patient, and an article of manufacture with instructions for such use.

BACKGROUND OF THE INVENTION

[0004] Multiple Sclerosis (MS) is an inflammatory and demyelinating degenerative disease of the human central nervous system (CNS). It is a worldwide disease that affects approximately 300,000 persons in the United States; it is a disease of young adults, with 70%–80% having onset between 20 and 40 years old (Anderson *et al. Ann Neurology* 31(3):333–6 (1992); Noonan *et al. Neurology* 58:136–8 (2002)). MS is a heterogeneous disorder based on clinical course, magnetic resonance imaging (MRI) scan assessment, and pathology analysis of biopsy and autopsy material (Lucchinetti *et al. Ann Neurol* 47:707–17 (2000)). The disease manifests itself in a large number of possible combinations of deficits, including spinal cord, brainstem, cranial nerve, cerebellar, cerebral, and cognitive syndromes. Progressive disability is the fate of most patients with MS, especially when a 25-year perspective is included. Half of MS patients require a cane to walk within 15 years of disease onset. MS is a major cause of neurologic disability in young and middle-aged adults and, until the past decade, has had no known beneficial treatments. MS is difficult to diagnose because of the non-specific clinical findings, which led to the development of highly structured diagnostic criteria that include several technological advances, consisting of

MRI scans, evoked potentials, and cerebrospinal fluid (CSF) studies. All diagnostic criteria rely upon the general principles of scattered lesions in the central white matter occurring at different times and not explained by other etiologies such as infection, vascular disorder, or autoimmune disorder (McDonald *et al. Ann Neurol* 50:121–7 (2001)). MS has four patterns of disease: relapsing-remitting MS (RRMS; 80%–85% of cases at onset), primary progressive MS (PPMS; 10%–15% at onset), progressive relapsing MS (PRMS; 5% at onset); and secondary progressive MS (SPMS) (Kremenchutzky *et al. Brain* 122 (Pt 10):1941–50 (1999); Confavreux *et al. N Engl J Med* 343(20):1430–8 (2000)). An estimated 50% of patients with RRMS will develop SPMS in 10 years, and up to 90% of RRMS patients will eventually develop SPMS (Weinshenker *et al. Brain* 112(Pt 1):133–46 (1989)).

[0005] Several disease modifying drugs in five classes are approved in the United States for the treatment of RRMS, whereas no drugs have been approved for PPMS. The RRMS treatments include the following: interferon class, IFN-beta-1a (REBIF®, Extavia, AVONEX® and PLEGRIDY™ and IFN-beta-1b (BETASERON®); glatiramer acetate (COPAXONE®), a polypeptide; natalizumab (TYSABRI®), alemtuzumab (LEMTRADA®, both monoclonal antibodies; dimethyl fumarate (TECFIDERA®) and fingolimod (GILENYA®) both small molecules, and mitoxantrone (NOVANTRONE®), a cytotoxic agent; teriflunomide (AUBAGIO®). Other drugs with an aim of disease modification have been used with varying degrees of success, including methotrexate, cyclophosphamide, azathioprine, and intravenous (IV) immunoglobulin. Current therapies lack sufficient efficacy to quell early disease or are not used in early MS due to associated side effects (Hartung *et al.* (2011) *Expert Rev Neurother.* 11, 351-62; Freedman et al., *Mult. Scler. Relat. Disord.* 3(2):147-55, 2014). Thus, there remains a need for highly effective therapies with an acceptable benefit-risk profile that can be given early in the course of disease to decrease the long-term consequences of accumulating disability and improve the quality of life of patients.

[0006] All references cited herein are incorporated by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0007] Provided herein are methods for treating multiple sclerosis in a human patient comprising administering an effective amount of an anti-CD20 antibody. Any of the anti-CD20 antibodies described herein may be administered. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the

amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6.

[0008] In one aspect, provided herein is a method of improving functional ability in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the patient has improvement in functional ability after treatment; wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, functional ability is improved in the patient after treatment. In some embodiments, the patient has 12-week confirmed disability improvement after treatment. In some embodiments, the patient has 24-week confirmed disability improvement after treatment. In some embodiments, the improvement in functional ability in the patient is sustained for at least 12 weeks. In some embodiments, the improvement in functional ability in the patient is sustained for at least 24 weeks. In some embodiments, the improvement in functional ability is measured by the Timed 25-Foot Walk (T-25FW) test or EDSS score. In some embodiments, the improvement in functional ability is measured by the Timed 25-Foot Walk (T-25FW) test and EDSS score.

[0009] In some embodiments, the patient has T1 gadolinium staining lesions at baseline. In some embodiments, the patient does not have T1 gadolinium staining lesions at baseline.

[0010] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0011] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody,

and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0012] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0013] In some embodiments, the patient has improved functional ability after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0014] In another aspect, provided is a method of suppressing composite disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the administration results in reduction of 12-week confirmed composite disability progression. In some embodiments, the administration results in reduction of 24-week confirmed composite disability progression. In some embodiments, the confirmed composite disability progression is determined by Expanded Disability Status Scale (EDSS) score, Timed 25-Foot Walk (T25-FW), or 9-Hole Peg Test (9-HPT). In some embodiments, the confirmed composite disability progression is determined by Expanded Disability Status Scale (EDSS) score, Timed 25-Foot Walk (T25-FW), and 9-Hole Peg Test (9-HPT).

[0015] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0016] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0017] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0018] In some embodiments, the composite disability progression is suppressed in the patient after one or two, three, and/or four exposures of the anti-CD20 antibody. In some embodiments, the patient has delayed onset or reduced risk of 12-week confirmed disability progression after one, two, three, and/or four exposures of the anti-CD20 antibody. In some embodiments, the onset 24-week confirmed disability progression is delayed in the patient of after one, two, three, and/or four exposures of the anti-CD20 antibody. In some embodiments, the risk 24-week confirmed disability progression is reduced in the patient of after one, two, three, and/or four

exposures of the anti-CD20 antibody. In some embodiments, the confirmed disability progression is determined by Expanded Disability Status Scale (EDSS) score.

[0019] In another aspect, provided herein is a method of suppressing disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the administration results in reduction of 12-week confirmed disability progression. In some embodiments, the administration results in reduction of 24-week confirmed disability progression. In some embodiments, the confirmed disability progression is determined by Expanded Disability Status Scale (EDSS) score. In some embodiments the confirmed disability progression is determined by an increase in EDSS.

[0020] In some embodiments, the confirmed disability progression is determined by a change in Timed 25-foot walk (T25-FW). In some embodiments, the confirmed disability progression is determined by percent change in MRI total T2 lesion volume. In some embodiments, the confirmed disability progression is determined by percent change in MRI total brain volume. In some embodiments, the confirmed disability progression is determined by a change in Short Form-36 (SF-36) physical component score.

[0021] In some embodiments, the patient has T1 gadolinium staining lesions at baseline. In some embodiments, the patient does not have T1 gadolinium staining lesions at baseline.

[0022] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0023] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the

interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0024] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0025] In some embodiments, the patient has suppressed composite disability progression after one or two, three, or four exposures of the anti-CD20 antibody.

[0026] In another aspect, provided herein is a method of delaying onset of confirmed disability progression or reducing the risk of confirmed disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the administration results in delayed onset or reduced risk of 12-week confirmed disability progression. In some embodiments, the administration results in delayed onset or reduced risk of 24-week confirmed disability progression.

[0027] In some embodiments, the patient has T1 gadolinium staining lesions at baseline. In some embodiments, the patient does not have T1 gadolinium staining lesions at baseline.

[0028] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0029] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0030] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14^{th} day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0031] In some embodiments, the patient has delayed onset of confirmed disability progression or reduced risk of confirmed disability progression after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0032] Provided is a method of reducing T2 lesion volume in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11,

and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In certain embodiments, the patient has T1 gadolinium staining lesions at baseline. In certain embodiments, the patient does not have T1 gadolinium staining lesions at baseline.

[0033] In another aspect, provided herein is a method of slowing or preventing reduction in brain volume in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the brain volume reduction is slowed or prevented in the patient; and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, further reduction in brain volume in a patient who has experienced brain volume loss is slowed or prevented.

[0034] Also provided herein is a method of reducing brain atrophy in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein brain atrophy is slowed or prevented, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In certain embodiments, further brain atrophy in a patient who has experienced brain atrophy is slowed or prevented.

[0035] Provided herein is a method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in no evidence of disease activity (NEDA) for at least 12 weeks, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In

certain embodiments, treatment results in no evidence of disease activity (NEDA) for at least 24 weeks.

[0036] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0037] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0038] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14^{th} day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0039] In some embodiments, treatment results in NEDA for at least 12 weeks after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0040] Also provided herein is a method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in the patient achieving lesion-free status after 24, 48, or 96 weeks of treatment,

wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In certain embodiments, treatment results in the patient achieving lesion-free status after 48 weeks of treatment. In certain embodiments, treatment results in the patient achieving lesion-free status after 24 weeks of treatment. In certain embodiments, treatment results in the patient achieving lesion-free status after 48 weeks of treatment. In certain embodiments, treatment results in the patient achieving lesion-free status after 96 weeks of treatment. In certain embodiments, treatment results in the patient being free of gadolinium staining lesions. In certain embodiments, treatment results in the patient being free of T2 lesions.

[0041] Provided herein is a method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in one or more of:

a) the patient being relapse-free at 96 weeks;

b) the patient having no confirmed disability progression events at 96 weeks;

c) the patient being without T1 gadolinium-enhancing lesions at 96 weeks;

d) the patient being without new and/or enlarging T2 lesions at 96 weeks;

wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and 2) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6.

[0042] Provided herein is a method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in the patient having no confirmed disability progression events at 96 weeks, wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and 2) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2

having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6.

[0043] In certain embodiments, treatment results in one or more of:

a) the patient being relapse-free at 96 weeks;

b) the patient being without T1 gadolinium-enhancing lesions at 96 weeks; and

c) the patient being without new and/or enlarging T2 lesions at 96 weeks.

[0044] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0045] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0046] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0047] In some embodiments, treatment results in the patient being free of confirmed disability progression events at 96 weeks after one, two, three, and/or four exposures of the anti-CD20 antibody. In certain embodiments, treatment further results in one or more of:

a) the patient being relapse-free at 96 weeks;

b) the patient being without T1 gadolinium-enhancing lesions at 96 weeks; and/or

c) the patient being without new and/or enlarging T2 lesions at 96 weeks after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0048] Provided herein is a method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in one or more of:

a) the patient being relapse-free;

b) the patient having no confirmed disability progression events;

c) the patient being without T1 gadolinium-enhancing lesions;

d) the patient being without new and/or enlarging T2 lesions;

wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and 2) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6, and wherein treatment results any one or more of a)-d) after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0049] In another aspect, provided herein is a method of treating a human patient with highly active multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the patient with highly active multiple sclerosis is an inadequate responder to other therapy for multiple sclerosis. In some embodiments, the patient with highly active multiple sclerosis has not been previously treated with other therapy for

multiple sclerosis. In some embodiments, the other therapy for multiple sclerosis is an interferon or glatiramer acetate. In some embodiments, the administration of the anti-CD20 antibody is effective in one or more of the following: (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and (4) improvement of function ability. In some embodiments, the method further comprises performing an MRI scan and determining if the patient has highly active multiple sclerosis before administering the anti-CD20 antibody to the patient.

[0050] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0051] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0052] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14^{th} day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0053] In some embodiments, patient with highly active multiple sclerosis has (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and/or (4) improvement of function ability after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0054] In another aspect, provided herein is a method of treating a human patient with early stage multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the method further comprises diagnosing a patient having early stage multiple sclerosis before administering the anti-CD20 antibody to the patient. In some embodiments, the patient has been diagnosed as having multiple sclerosis but has not received treatment for at least two years prior to administration of the anti-CD20 antibody. In certain embodiments early stage multiple sclerosis is a first clinical presentation of multiple sclerosis in a patient. In certain embodiments, the first clinical presentation of multiple sclerosis is a first clinical demyelinating event (FCDE) (also known as clinical isolated syndrome (CIS)), *i.e.*, a first episode of neurologic symptoms that lasts at least 24 hours and is caused by inflammation or demyelination in the central nervous system. In certain embodiments, the FDCE affects the optic nerve, brainstem, subcortical white matter, or spinal cord. In certain embodiments, early stage multiple sclerosis refers to a diagnosis of clinically definite multiple sclerosis (CDMS), wherein a patient experiences an FCDE followed by a second clinical attack.

[0055] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0056] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6

months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0057] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0058] In another aspect, provided herein is a method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the treatment results in no evidence of disease activity (NEDA) in the patient, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In certain embodiments, NEDA is defined as: no protocol-defined relapses, no CDP events, no new or enlarging T2 lesions, and no Gd-enhancing T1 lesions.

[0059] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0060] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0061] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0062] In some embodiments, treatment results in NEDA after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0063] In certain embodiments, treatment or administration of the anti-CD20 antibody to human patients with a relapsing form of multiple sclerosis results in one or more of the following:

a) about any of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% reduction in annualized relapse rate over time (for example, over a period of at least about 1 year, 1.5 years, or two years), compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

b) about any of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% reduction in risk of confirmed disability progression for at least 12 weeks compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

c) about any of 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% reduction in risk of confirmed disability progression for at least 24 weeks compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

d) about any of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% reduction in total number of T1 Gadolinium^{+} lesions compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

e) about any of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% reduction in the mean number of T1 Gadolinium^{+} lesions at Week 24, Week 48, and/or Week 96 compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

f) about any of 70%, 71%, 72% 73% 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, or 85% reduction in the number of new and/or enlarging T2 hyperintense lesions compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

g) about any of 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% reduction in the mean number of new and/or enlarging T2 hyperintense lesions at Week 24, Week 48, and/or Week 96 compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

h) about any of 10%, 10.5%, 11%, 11.5%, 12%, 12.5%, 13%, 13.5%, 14%, 14.5%, 15%, 15,5%, 16%, 16.5%, 17%, 17.5%, 18%, 18.5%, 19%, 19.5%, or 20% reduction in the rate of whole brain volume loss compared to patient(s) receiving no treatment, including any range in between these values;

i) about any of 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%. 59%, 60%, 61%, 62%, 63%, 64%, or 65% improvement in confirmed disability improvement sustained for at least 12 weeks compared to patient(s)

receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values;

j) about any of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, or 75% reduction in the number of new T1 hypointense lesions compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values; and

k) about any of 55%, 56%, 57%. 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%,70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, or 85% increase in the chance of reaching NEDA (no evidence of disease activity),

wherein NEDA is defined as no protocol-defined relapses, no CDP events, no new or enlarging T2 lesions, and no Gd-enhancing T1 lesions, compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values.

[0064] In certain embodiments, treatment may additionally or alternatively result in about any of 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, or 25% reduction in brain atrophy compared to patient(s) receiving treatment with interferon beta-1a (such as REBIF®), including any range in between these values.

[0065] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0066] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about

600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0067] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0068] In some embodiments, treatment results in one or more of a) – k) after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0069] In certain embodiments, treatment or administration of the anti-CD20 antibody to a patient with primary progressive multiple sclerosis results in one or more of the following:

a) about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% reduction in risk of confirmed disability progression for at least 12 weeks compared to patient(s) receiving no treatment, including any range in between these values;

b) about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% reduction in risk of confirmed disability progression for at least 24 weeks compared to patient(s) receiving no treatment, including any range in between these values;

c) about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, or 35% reduction in the progression rate of walking time, as measured by the Timed 25-Foot Walk, compared to patient(s) receiving no treatment, including any range in between these values;

d) about any of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% reduction in T2 lesion volume compared to patient(s) receiving no treatment, including any range in between these values;

e) about any of 1%, 1.5%, 2%, 2.5%, 3%, 3.4%, 3.5%, 4%, 4.5% or 5% reduction in hyperintense T2 lesion volume from baseline at about any of Week 20, Week 24, Week 28, Week

32, Week 36 Week 40, Week 44, Week 48, Week 52, Week 56, Week 60, Week 64, Week 68, Week 72, Week 76, Week80, Week 84, Week 88, Week 92, Week 96, Week 100, Week 104, Week 108, Week 112, Week 116, and Week 120; and

f) about any of 12%, 12.5%, 13%, 13.5%, 14%, 14.5%, 15%, 15.5%, 16%, 16.5%, 17%, 17.5%, 18%, 18.5%, 19%, 19.5%, or 20% reduction in the rate of whole brain volume loss compared to patient(s) receiving no treatment, including any range in between these values.

[0070] In certain embodiments, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0071] In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0072] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0073] In some embodiments, treatment results in one or more of a) – f) after one, two, three, and/or four exposures of the anti-CD20 antibody.

[0074] In certain embodiments, provided is a method of reducing the risk of confirmed disability progression for at least 12 weeks in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% (including any range in between these values) reduction in risk of confirmed disability progression for at least 12 weeks in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0075] In certain embodiments, provided is a method of reducing the risk of confirmed disability progression for at least 24 weeks in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% (including any range in between these values) reduction in risk of confirmed disability progression for at least 24 weeks in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0076] In certain embodiments, provided is a method of reducing the progression rate of walking time, as measured by the Timed 25-Foot Walk, in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in about any of 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, or 35% (including any range in between these values) reduction in the progression rate of walking time in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0077] In certain embodiments, provided is a method of reducing T2 lesion volume in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in

about any of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% (including any range in between these values) reduction in T2 lesion volume in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0078] In certain embodiments, provided is a method of reducing hyperintense T2 lesion volume in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in about any of 1%, 1.5%, 2%, 2.5%, 3%, 3.4%, 3.5%, 4%, 4.5% or 5% reduction in hyperintense T2 lesion volume from baseline at about any of Week 20, Week 24, Week 28, Week 32, Week 36 Week 40, Week 44, Week 48, Week 52, Week 56, Week 60, Week 64, Week 68, Week 72, Week 76, Week80, Week 84, Week 88, Week 92, Week 96, Week 100, Week 104, Week 108, Week 112, Week 116, and Week 120 in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0079] In certain embodiments, provided is a method of reducing the rate of whole brain volume loss in a patient having primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein administration of the anti-CD20 antibody results in about any of 12%, 12.5%, 13%, 13.5%, 14%, 14.5%, 15%, 15.5%, 16%, 16.5%, 17%, 17.5%, 18%, 18.5%, 19%, 19.5%, or 20% (including any range in between these values) reduction in the rate of whole brain volume loss in the patient, compared to patient(s) receiving no treatment, and wherein the antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months. In some embodiments, the anti-CD20 antibody is administered to the patient to provide a fourth anti-

CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0080] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0081] In certain embodiments, a) risk of confirmed disability progression for at least 12 weeks, b) risk of confirmed disability progression for at least 24 week, c) progression rate of walking time, as measured by the Timed 25-Foot Walk, d) T2 lesion volume, e) hyperintense T2 lesion volume, and/or f) rate of whole brain volume loss is reduced after one, two, three, and/or four exposures of the anti-CD20 antibody. In certain embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0082] In certain embodiments of any of the methods, the patient maintains the ability to mount a humoral response to an antigen during treatment. In certain embodiments, the antigen is a mumps antigen, a rubella antigen, a varicella antigen, an S. pneumonia antigen, a tetanus toxoid antigen, a pneumococcal antigen, or an influenza antigen.

[0083] In certain embodiments, the patient is premedicated prior to infusion with the anti-CD20 antibody. In certain embodiments, the patient is premedicated with methylprednisolone (or an equivalent) approximately 30 minutes prior to each infusion of anti-CD20 antibody. In certain embodiments, the patient is premedicated with 100 mg IV methylprednisolone (or an equivalent) approximately 30 minutes prior to each infusion of anti-CD20 antibody. In certain embodiments, the patient is additionally (or alternatively) premedicated with an antihistaminic drug (e.g. diphenhydramine) approximately 30-60 minutes before each infusion of anti-CD20 antibody. In certain embodiments, the patient is additionally (or alternatively) premedicated with an antipyretic (e.g. acetaminophen/paracetamol).

[0084] In some embodiments of the methods described above and herein, a second medicament is administered with the initial exposure or later exposures, wherein the anti-CD20 antibody is a first medicament. In some embodiments, the second medicament is selected from the group consisting of an interferon, glatiramer acetate, a cytotoxic agent, chemotherapeutic agent, mitoxantrone, methotrexate, cyclophosphamide, chlorambucil, azathioprine, gamma globulin, Campath, anti-CD4, cladribine, corticosteroid, mycophenolate mofetil (MMF), cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone replacement drug, a TNF inhibitor, disease modifying anti-rheumatic drug (DMARD), non-steroidal anti-inflammatory drug (NSAID), levothyroxine, cyclosporin A, somatastatin analogue, cytokine or cytokine receptor antagonist, antimetabolite, immunosuppressive agent, integrin antagonist or antibody, LFA-l antibody, efalizumab, alpha 4 integrin antibody, natalizumab, and another B-cell surface marker antibody.

[0085] In some embodiments of the methods described above and herein, the multiple sclerosis is a relapsing form of multiple sclerosis. In some embodiments, the relapsing form of multiple sclerosis is a relapsing remitting multiple sclerosis (RRMS). In some embodiments, the relapsing form of multiple sclerosis is a secondary progressive multiple sclerosis with superimposed relapses (rSPMS). In some embodiments, the multiple sclerosis is a progressive multiple sclerosis. In some embodiments, the multiple sclerosis is a primary progressive multiple sclerosis (PPMS).

[0086] In certain embodiments according to (or as applied to) any of the embodiments above, the patient is selected for treatment based upon having a relapsing form of multiple sclerosis (such as RRMS or rSPMS). In certain embodiments according to (or as applied to) any of the embodiments above, treatment is based upon the patient having a relapsing form of multiple sclerosis (such as RRMS or rSPMS). In certain embodiments according to (or as applied to) any of the embodiments above, the patient is diagnosed with a relapsing form of multiple sclerosis (such as RRMS or rSPMS) prior to treatment.

[0087] In certain embodiments according to (or as applied to) any of the embodiments above, the patient is selected for treatment based upon having a progressive form of multiple sclerosis (such as PPMS). In certain embodiments according to (or as applied to) any of the embodiments above, treatment is based upon the patient having a progressive form of multiple sclerosis (such as PPMS). In certain embodiments according to (or as applied to) any of the embodiments above, the patient is diagnosed with a progressive form of multiple sclerosis (such as PPMS) prior to treatment.

[0088] In some embodiments of the methods described above and herein, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by one or more additional anti-CD20 antibody exposures, wherein each exposure is about 600 mg of the antibody, and each exposure is provided to the patient as one or two doses of the anti-CD20 antibody, and wherein the interval between each exposure is about 20-24 weeks or about 5-6 months. In some embodiments, "about 20-24 weeks" refers to a time point between 20 weeks and 24 weeks. In some embodiments, "about 20-24 weeks" refers to a variation of a week or 7 days before or after the 24th week. In some embodiments, "about 5-6 months" refers to a time point between 5 and 6 months.

[0089] In some embodiments, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days).

[0090] In certain embodiments according to (or as applied to) any of the embodiments above, the anti-CD20 antibody comprises a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In certain embodiments according to (or as applied to) any of the embodiments above, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:15 or SEQ ID NO: 26 or SEQ ID NO: 27, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

[0091] In certain embodiments according to (or as applied to) any of the embodiments above, the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months. In certain embodiments according to (or as applied to) any of the embodiments above, the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second

exposure and the third exposure is about 20-24 weeks or about 5-6 months. In certain embodiments according to (or as applied to) any of the embodiments above, the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

[0092] In some embodiments according to (or as applied to) any of the embodiments above, the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are administered intravenously. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses each comprise 250 ml of anti-CD20 antibody at a concentration of about 1.2 mg/ml. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are each infused at a rate of 30 ml/hour. In certain embodiments according to (or as applied to) any of the embodiments above, the infusion rate of the first and second doses can be increased in 30 ml/hour increments to a maximum rate of 180 ml/hr. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are each be given over approximately 2.5 hours.

[0093] In some embodiments, the second, third, and/or fourth exposures comprise a single dose of about 600 mg. In certain embodiments according to (or as applied to) any of the embodiments above, the second, third, and/or fourth exposures are administered intravenously. In certain embodiments according to (or as applied to) any of the embodiments above, the second, third, and/or fourth exposures each comprise 500 ml of anti-CD20 antibody at a concentration of about 1.2 mg/ml. In certain embodiments according to (or as applied to) any of the embodiments above, the second, third, and/or fourth exposures are each infused at a rate of 40 ml/hour. In certain embodiments according to (or as applied to) any of the embodiments above, the infusion rate of the second, third, and/or fourth exposures can be increased in 40 ml/hour increments to a maximum rate of 200 ml/hr. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are each be given over approximately 3.5 hours.

[0094] In some embodiments, the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about

14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are administered intravenously. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses each comprise 250 ml of anti-CD20 antibody at a concentration of about 1.2 mg/ml. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are each infused at a rate of 30 ml/hour. In certain embodiments according to (or as applied to) any of the embodiments above, the infusion rate of the first and second doses can be increased in 30 ml/hour increments to a maximum rate of 180 ml/hr. In certain embodiments according to (or as applied to) any of the embodiments above, the first and second doses are each be given over approximately 2.5 hours.

[0095] In certain embodiments, the patient receives at least 2, 3, 4, or more than 4 anti-CD20 antibody exposures.

[0096] In some embodiments, the anti-CD20 antibody is administered to the patient to provide one or more additional anti-CD20 antibody exposure after the fourth exposure, wherein the one or more additional exposure is about 600 mg of the antibody, and wherein the interval between the fourth exposure and the additional exposure is about 20-24 weeks or about 5-6 months. In some embodiments, "about 20-24 weeks" refers to a time point between 20 weeks and 24 weeks. In some embodiments, "about 20-24 weeks" refers to a variation of a week or 7 days before or after the 24th week. In some embodiments, "about 5-6 months" refers to a time point between 5 and 6 months. In some embodiments, the interval between each of the additional exposures following the fourth exposure is 20-24 weeks or about 5-6 months. In some embodiments, the one or more exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. In some embodiments, the one or more exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about 14 days (such as 13 days or 15 days).

[0097] In some embodiments of the methods described above and herein, the anti-CD20 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In some embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:26 or SEQ ID NO: 15 or SEQ ID NO: 27 and a light

chain comprising the amino acid sequence of SEQ ID NO:13. In some embodiments, the anti-CD20 antibody is ocrelizumab. In some embodiments, the anti-CD20 antibody is in a pharmaceutically acceptable composition. In some embodiments, the anti-CD20 antibody is in a formulation comprising 30 mg/mL antibody, 20 mM Sodium Acetate, 106 mM Trehalose, 0.02% polysorbate 20, pH 5.3. In some embodiments, the antibody in the formulation is stored at about 2-8°C at 300 mg/vial. In some embodiments, the antibody is diluted in saline (0.9% sodium chloride) in an IV bag for administration by infusion. In some embodiments, the anti-CD20 antibody is an antigen binding fragment thereof.

[0098] In some embodiments of the methods described above and herein, the anti-CD20 antibody is administered intravenously. In some embodiments, the anti-CD20 antibody is administered intravenously for each antibody exposure. In some embodiments of the methods described above and herein, the antibody is administered subcutaneously. In some embodiments, the anti-CD20 antibody is administered subcutaneously for each antibody exposure.

[0099] In some embodiments, provided is a composition comprising an anti-CD20 antibody that comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6 for use in the treatment of multiple sclerosis in a patient according to any of the methods described above.

[0100] In some embodiments, provided is an anti-CD20 antibody that comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6 for use in the manufacture of a medicament for the treatment of multiple sclerosis in a patient according to any of the methods described above.

[0101] In certain embodiments of any of the methods above and/or herein, a reduction or decrease or improvement after administration of the anti-CD20 antibody can be compared to a baseline level, to a level in untreated patient(s), and/or to a level in patient(s), e.g., such as mean, average, or median level of a group of patients, receiving a different treatment (such as interferon beta-1a or REBIF®).

[0102] In another aspect, provided herein is an article of manufacture comprising: (a) a container comprising an anti-CD20 antibody (e.g., ocrelizumab); and (b) a package insert with instructions for treating multiple sclerosis in a patient according to any one of the methods described above and herein.

[0103] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0104] **FIG. 1A** is a sequence alignment comparing the amino acid sequences of the light chain variable domain (V_L) of each of murine 2H7 (SEQ ID NO:1), humanized 2H7.v16 variant (SEQ ID NO:2), and the human kappa light chain subgroup I (SEQ ID NO:3). The CDRs of V_L of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:5), and CDR3 (SEQ ID NO:6).

[0105] **FIG. 1B** is a sequence alignment comparing the amino acid sequences of the heavy chain variable domain (V_H) of each of murine 2H7 (SEQ ID NO:7), humanized 2H7.v16 variant (SEQ ID NO:8), and the human consensus sequence of the heavy chain subgroup III (SEQ ID NO:9). The CDRs of V_H of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12).

[0106] In **FIG. 1A** and **FIG. 1B**, the CDR1, CDR2 and CDR3 in each chain are enclosed within brackets, flanked by the framework regions, FR1-FR4, as indicated. 2H7 refers to the murine 2H7 antibody. The asterisks in between two rows of sequences indicate the positions that are different between the two sequences. Residue numbering is according to Kabat *et al.* *Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), with insertions shown as a, b, c, d, and e.

[0107] **FIG. 2** shows the amino acid sequence of the mature 2H7.v16 light chain (SEQ ID NO:13)

[0108] **FIG. 3** shows the amino acid sequence of the mature 2H7.v16 heavy chain (SEQ ID NO:14).

[0109] **FIG. 4** shows the amino acid sequence of the mature 2H7.v31 heavy chain (SEQ ID NO:15). The L chain of 2H7.v31 is the same as for 2H7.v16.

[0110] **FIG. 5** shows an alignment of the mature 2H7.v16 and 2H7.v511 light chains (SEQ ID NOS. 13 and 16, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

[0111] **FIG. 6** shows an alignment of the mature 2H7.v16 and 2H7.v511 heavy chains (SEQ ID NOS. 14 and 17, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

[0112] **FIG. 7** shows an overview of the study design for two identical Phase III studies (i.e., STUDY I and STUDY II) of ocrelizumab in comparison with interferon beta-1a (REBIF®) in patients with relapsing multiple sclerosis.

[0113] **FIG. 8** shows the hierarchical statistical analysis plan for STUDY I and STUDY II.

[0114] **FIG. 9** shows the patient disposition in STUDY I and STUDY II.

[0115] **FIG. 10A** shows the Annualized Relapse Rate (ARR) at 96 weeks for patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY I.

[0116] **FIG. 10B** shows the Annualized Relapse Rate (ARR) at 96 weeks for patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY II.

[0117] **FIG. 11** shows time to onset of Confirmed Disability Progression (CDP) for at least 12 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a (pooled data from STUDY I and STUDY II).

[0118] **FIG. 12A** shows time to onset of Confirmed Disability Progression for at least 12 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY I.

[0119] **FIG. 12B** shows time to onset of Confirmed Disability Progression for at least 12 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY II.

[0120] **FIG. 13** shows time to onset of Confirmed Disability Progression for at least 24 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a (pooled data from STUDY I and STUDY II).

[0121] **FIG. 14A** shows time to onset of Confirmed Disability Progression for at least 24 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY I.

[0122] **FIG. 14B** shows time to onset of Confirmed Disability Progression for at least 24 weeks over a 96 week period in patients treated with ocrelizumab compared to patients treated with interferon beta-1a in STUDY II.

[0123] **FIG. 15A** shows proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 12 weeks in patients receiving ocrelizumab compared to patients receiving interferon beta-1a (the pooled data from STUDY I and STUDY II).

[0124] **FIG. 15B** shows proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 24 weeks in patients receiving ocrelizumab compared to patients receiving interferon beta-1a (the pooled data from STUDY I and STUDY II).

[0125] **FIG. 16A** shows proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 12 weeks in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0126] **FIG. 16B** shows the proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 12 weeks was in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0127] **FIG. 16C** shows the change in Multiple Sclerosis Functional Composite score from baseline to Week 96 patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0128] **FIG. 16D** shows the change in Multiple Sclerosis Functional Composite score from baseline to Week 96 patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0129] **FIG. 17A** shows the total number of T1 gadolinium-enhancing lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0130] **FIG. 17B** shows the total number of T1 gadolinium-enhancing lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0131] **FIG. 18A** shows the mean total number of T1 gadolinium-enhancing lesions in patients receiving ocrelizumab at Week 24, at Week 48, and at week 96 compared to patients receiving IFN β-1a in STUDY I.

[0132] **FIG. 18B** shows the mean total number of T1 gadolinium-enhancing lesions in patients receiving ocrelizumab at Week 24, at Week 48, and at week 96 compared to patients receiving IFN β-1a in STUDY II.

[0133] **FIG. 19A** shows the total number of new and/or enlarging hyperintense T2 lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I and STUDY II.

[0134] **FIG. 19B** shows the total number of new and/or enlarging hyperintense T2 lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0135] **FIG. 19C** shows the mean number of new and/or enlarging hyperintense T2 lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0136] **FIG. 19D** shows the mean number of new and/or enlarging hyperintense T2 lesions detected at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0137] **FIG. 20A** shows the rate of brain volume loss from Week 24 to Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0138] **FIG. 20B** shows the rate of brain volume loss from Week 24 to Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0139] **FIG. 21A** shows the rate of brain volume loss from baseline to Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0140] **FIG. 21B** shows the rate of brain volume loss from baseline to Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0141] **FIG. 22A** shows the total number of new T1 hypointense lesions per MRI scan at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY I.

[0142] **FIG. 22B** shows the total number of new T1 hypointense lesions per MRI scan at Week 24, Week 48, and Week 96 in patients receiving ocrelizumab compared to patients receiving interferon beta-1a in STUDY II.

[0143] **FIG. 23A** shows infusion related reactions for patients receiving IFN β-1a in STUDY I and STUDY II (pooled).

[0144] **FIG. 23B** shows infusion related reactions for patients receiving ocrelizumab in STUDY I and STUDY II (pooled).

[0145] **FIG. 24** shows the study design for a Phase III study of Ocrelizumab in patients with primary progressive multiple sclerosis (PPMS).

[0146] **FIG. 25** provides a statistical hierarchy of the Phase III study of Ocrelizumab in patients with PPMS.

[0147] **FIG. 26** shows patient disposition at the clinical cut-off date for the Phase III PPMS study.

[0148] **FIG. 27** shows time to onset of Confirmed Disability Progression for at least 12 weeks in patients treated with ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0149] **FIG. 28** shows time to onset of Confirmed Disability Progression for at least 24 weeks in patients treated with ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0150] **FIG. 29** shows the rate of decline in walking speed, as measured by the Timed 25-Foot Walk, in patients receiving 600mg ocrelizumab compared to patients receiving placebo from baseline to Week 120 for the Phase III PPMS study.

[0151] **FIG. 30** shows the rate of decline in walking speed at Week 120 relative to baseline in patients receiving 600mg ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0152] **FIG. 31** shows the percent change of whole brain volume from Week 24 to Week 96 in patients receiving 600mg ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0153] **FIG. 32** shows the change in T2 lesion volume in in patients receiving 600mg ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0154] **FIG. 33** shows infusion related reactions (IRR) in patients receiving 600mg ocrelizumab compared to patients receiving placebo by exposure and severity until the clinical cut-off date for the Phase III PPMS study.

[0155] **FIG. 34A** shows the proportion of patients with no evidence of disease activity (NEDA) taking IFN β-1a compared to the proportion of patients with NEDA taking ocrelizumab in STUDY I.

[0156] **FIG. 34B** shows the proportion of patients with no evidence of disease activity (NEDA) taking IFN β-1a compared to the proportion of patients with NEDA taking ocrelizumab in STUDY II.

[0157] **FIG. 35A** shows the reduction in the percent change from baseline walking time in the timed 25-Foot Walk test to Week 120 in patients with Gd^+ lesions at baseline receiving ocrelizumab vs. patients with Gd^+ lesions at baseline receiving placebo.

[0158] **FIG. 35B** shows the reduction in the percent change from baseline walking time in the timed 25-Foot Walk test to Week 120 in patients without Gd^+ lesions at baseline receiving ocrelizumab vs. patients without Gd^+ lesions at baseline receiving placebo.

[0159] **FIG. 35C** shows the reduction in the percent change from baseline walking time in the timed 25-Foot Walk test to Week 120 in patients in the overall study population receiving ocrelizumab vs. patients in the overall study population receiving placebo.

[0160] **FIG. 36A** shows the difference of whole brain volume loss from Week 24 to Week 120 in ocrelizumab-treated patients with T1 Gd^+ lesions at baseline as compared to patients with T1 Gd^+ lesions at baseline receiving placebo.

[0161] **FIG. 36B** shows the difference of whole brain volume loss from Week 24 to Week 120 in ocrelizumab-treated patients without T1 Gd^+ lesions at baseline as compared to patients without T1 Gd^+ lesions at baseline receiving placebo.

[0162] **FIG. 36C** shows the rate of whole brain volume loss from Week 24 to Week 120 in ocrelizumab-treated patients in the overall study population as compared to patients in the overall study population receiving placebo.

[0163] **FIG. 37A** shows the difference in T2 lesion volume from baseline to week 120 in patients receiving ocrelizumab vs. patients receiving placebo.

[0164] **FIG. 37B** shows the difference in T2 lesion volume from baseline to week 120 in patients with T1 Gd^+ lesions at baseline receiving ocrelizumab vs. patients with T1 Gd^+ lesions at baseline receiving placebo.

[0165] **FIG. 37C** shows the difference in T2 lesion volume from baseline to week 120 in patients without T1 Gd^+ lesions at baseline receiving ocrelizumab vs. patients without T1 Gd^+ lesions at baseline receiving placebo.

[0166] **FIG. 38** shows the difference in reported improvement in change in quality of life between patients receiving ocrelizumab and patients receiving IFN β-1a, as measured by Short Form-36 (SF-36) Physical Component Summary (PCS).

[0167] **FIG. 39A** shows the difference between changes in EDSS score from baseline and Week 96 in patients receiving ocrelizumab vs. patients receiving IFN β-1a in STUDY I.

[0168] **FIG. 39B** shows the difference between changes in EDSS score from baseline and Week 96 in patients receiving ocrelizumab vs. patients receiving IFN β-1a in STUDY II.

[0169] **FIG. 40A** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients in the overall study population receiving 600mg ocrelizumab compared to patients in the overall study population receiving placebo.

[0170] **FIG. 40B** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients with T1 gadolinium-enhancing lesions at baseline receiving 600mg ocrelizumab compared to patients with T1 gadolinium-enhancing lesions at baseline receiving placebo.

[0171] **FIG. 40C** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients without T1 gadolinium-enhancing lesions at baseline receiving 600mg ocrelizumab compared to patients without T1 gadolinium-enhancing lesions at baseline receiving placebo.

[0172] **FIG. 41** shows time to onset of Confirmed Composite Disability Progression for at least 12 weeks in patients treated with ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0173] **FIG. 42** shows time to onset of Confirmed Composite Disability Progression for at least 24 weeks in patients treated with ocrelizumab compared to patients receiving placebo for the Phase III PPMS study.

[0174] **FIG. 43** shows the annualized protocol-defined relapse rate by Week 96 in the following subgroups: active inadequate responders, active treatment-naïve patients, highly active inadequate responders, and highly active treatment naïve patients.

[0175] **FIG. 44** shows the time to onset of CDP for at least 12 weeks in the following subgroups: active inadequate responders, active treatment-naïve patients, highly active inadequate responders, and highly active treatment naïve patients.

[0176] **FIG. 45** shows the time to onset of CDP for at least 24 weeks in the following subgroups: active inadequate responders, active treatment-naïve patients, highly active inadequate responders, and highly active treatment naïve patients.

[0177] **FIG. 46** shows the proportion of patients in the following subgroups who have CDI for at least 12 weeks: active inadequate responders, active treatment-naïve patients, highly active inadequate responders, and highly active treatment naïve patients.

[0178] **FIG. 47** shows the total number of T1 gadolinium-enhancing lesions as detected by brain MRI in the following subgroups: active inadequate responders, active treatment-naïve patients, highly active inadequate responders, and highly active treatment naïve patients.

Detailed Description of the Invention

I. Definitions

[0179] A "B-cell" is a lymphocyte that matures within the bone marrow, and includes a naive B cell, memory B cell, or effector B cell (plasma cells). The B-cell herein may be a normal or non-malignant B cell.

[0180] A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antibody that binds thereto. Exemplary B-cell surface markers include the CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers (for descriptions, *see* The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Other B-cell surface markers include RP105, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest herein is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface marker herein is CD20.

[0181] The "CD20" antigen, or "CD20," is an about 35-kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is present on both normal B cells as well as malignant B cells, but is not expressed on stem cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al. Proc. Natl. Acad. Sci.* (USA) 82:1766 (1985), for example.

[0182] An "antibody antagonist" herein is an antibody that, upon binding to a B cell surface marker on B cells, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antibody antagonist preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC), inhibition of B-cell proliferation and/or induction of B-cell death *(e.g.* via apoptosis).

[0183] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) *(e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

[0184] "Human effector cells" are leukocytes that express one or more FcRs and perform effector functions. In some embodiments, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred.

[0185] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. In some embodiments, the FcR is a native sequence human FcR.

Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (*see* Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al., Immunomethods* 4:25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim *et al., J. Immunol.* 24:249 (1994)).

[0186] "Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule *(e.g.* an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996) may be performed.

[0187] "Growth inhibitory" antibodies are those that prevent or reduce proliferation of a cell expressing an antigen to which the antibody binds. For example, the antibody may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

[0188] Antibodies that "induce apoptosis" are those that induce programmed cell death, *e.g.* of a B cell, as determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies).

[0189] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies *(e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

[0190] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0191] For the purposes herein, an "intact antibody" is one comprising heavy and light variable domains as well as an Fc region.

[0192] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0193] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see* Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0194] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0195] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0196] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0197] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0198] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0199] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0200] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable

domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0201] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature*, 256:495 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature,* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991), for example.

[0202] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate *(e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

[0203] "Humanized" forms of non-human *(e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0204] The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" *(e.g.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" *(e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

[0205] A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

[0206] Purely for the purposes herein and unless indicated otherwise, "humanized 2H7" refers to a humanized antibody that binds human CD20, or an antigen-binding fragment thereof, wherein the antibody is effective to deplete primate B cells *in vivo*, the antibody comprising in the

H chain variable region (V_H) thereof at least a CDR H3 sequence of SEQ ID NO:12 (Fig. 1B) from an anti-human CD20 antibody and substantially the human consensus framework (FR) residues of the human heavy- chain subgroup III (V_HIII). In some embodiments, this antibody further comprises the H chain CDR H1 sequence of SEQ ID NO:10 and CDR H2 sequence of SEQ ID NO:11, and, in some embodiments, further comprises the L chain CDR L1 sequence of SEQ ID NO:4, CDR L2 sequence of SEQ ID NO:5, CDR L3 sequence of SEQ ID NO:6 and substantially the human consensus framework (FR) residues of the human light chain kappa subgroup I (V_κI), wherein the V_H region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3. In some embodiments, such antibody comprises the V_H sequence of SEQ ID NO:8 (v16, as shown in Fig. 1B), optionally also comprising the V_L sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), which may have the amino acid substitutions of D56A and N100A in the H chain and S92A in the L chain (v96). In some embodiments, the antibody is an intact antibody comprising the light and heavy chain amino acid sequences of SEQ ID NOS: 13 and 14, respectively, as shown in Figs. 2 and 3. In some embodiments, the antibody is 2H7.v31 comprising the light and heavy chain amino acid sequences of SEQ ID NOS: 13 and 15, respectively, as shown in Figs. 2 and 4. The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC and/or CDC activity, such as one wherein the amino acid substitutions are S298A/E333A/K334A, and in some embodiments, the 2H7.v31 having the heavy chain amino acid sequence of SEQ ID NO: 15 (as shown in Fig. 4). Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that decreases CDC activity, for example, comprising at least the substitution K322A. *See* U.S. Patent No. 6,528,624B1 (Idusogie *et al.).*

[0207] The term "ocrelizumab" (CAS Registration No. 637334-45-3) herein refers to the genetically engineered humanized monoclonal antibody directed against the CD20 antigen and comprising (a) a light chain comprising the amino acid sequence of SEQ ID NO: 13 and (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:14, including fragments thereof that retain the ability to bind CD20. Ocrelizumab is available from Genentech.

[0208] An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 95% by weight of

antibody as determined by the Lowry method, and in some embodiments, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, in some embodiments, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0209] A "subject" or "patient" herein is a human subject or patient. Generally, the subject or patient is eligible for treatment for multiple sclerosis. For the purposes herein, such eligible subject or patient is one who is experiencing, has experienced, or is likely to experience, one or more signs, symptoms or other indicators of multiple sclerosis; has been diagnosed with multiple sclerosis, whether, for example, newly diagnosed (with "new onset" MS), previously diagnosed with a new relapse or exacerbation, previously diagnosed and in remission, etc; and/or is at risk for developing multiple sclerosis. One suffering from or at risk for suffering from multiple sclerosis may optionally be identified as one who has been screened for elevated levels of CD20-positive B cells in serum, cerebrospinal fluid (CSF) and/or MS lesion(s) and/or is screened for using an assay to detect autoantibodies, assessed qualitatively, and preferably quantitatively. Exemplary such autoantibodies associated with multiple sclerosis include anti-myelin basic protein (MBP), anti-myelin oligodendrocytic glycoprotein (MOG), anti-ganglioside and/or anti-neurofilament antibodies. Such autoantibodies may be detected in the subject's serum, cerebrospinal fluid (CSF) and/or MS lesion. By "elevated" autoantibody or B cell level(s) herein is meant level(s) of such autoantibodies or B cells which significantly exceed the level(s) in an individual without MS.

[0210] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), delay or slowing the progression of the disease, ameliorating the disease state, decreasing the dose of one or more other medications required to treat the disease, and/or increasing the quality of life.

[0211] As used herein, "delaying" or "slowing" the progression of multiple sclerosis means to prevent, defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This

delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated.

[0212] As used herein, "at the time of starting treatment" refers to the time period at or prior to the first exposure to a multiple sclerosis drug, such as an anti-CD20 antibody. In some embodiments, "at the time of starting treatment" is about any of one year, nine months, six months, three months, second months, or one month prior to a multiple sclerosis drug, such as an anti-CD20 antibody. In some embodiments, "at the time of starting treatment" is immediately prior to coincidental with the first exposure to a multiple sclerosis drug, such as an anti-CD20 antibody.

[0213] As used herein, "based upon" includes (1) assessing, determining, or measuring the patient characteristics as described herein (and preferably selecting a patient suitable for receiving treatment; and (2) administering the treatment(s) as described herein.

[0214] A "symptom" of MS is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the subject and indicative of MS.

[0215] "Multiple sclerosis" refers to the chronic and often disabling disease of the central nervous system characterized by the progressive destruction of the myelin. There are four internationally recognized forms of MS, namely, primary progressive multiple sclerosis (PPMS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS).

[0216] "Progressive multiple sclerosis" as used herein refers to primary progressive multiple sclerosis (PPMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS). In some embodiments, progressive multiple sclerosis is characterized by documented, irreversible loss of neurological function persisting for $\geq$ 6 months that cannot be attributed to clinical relapse.

[0217] "Primary progressive multiple sclerosis" or "PPMS" is characterized by a gradual progression of the disease from its onset with rare superimposed relapses and remissions. There may be periods of a leveling off of disease activity and there may be good and bad days or weeks. PPMS differs from RRMS and SPMS in that onset is typically in the late thirties or early forties, men are as likely women to develop it, and initial disease activity is often in the spinal cord and not in the brain. PPMS disease activity can also be observed (or found) in the brain. PPMS is the sub-type of MS that is least likely to show inflammatory (gadolinium enhancing) lesions on MRI scans. The Primary Progressive form of the disease affects between 10 and 15% of all people

with multiple sclerosis. PPMS may be defined according to the criteria in Polman *et al. Ann Neurol* 69:292-392 (2010). The subject with PPMS treated herein is usually one with probable or definitive diagnosis of PPMS.

[0218] "Relapsing-remitting multiple sclerosis" or "RRMS" is characterized by relapses (also known as exacerbations) during which time new symptoms can appear and old ones resurface or worsen. The relapses are followed by periods of remission, during which time the person fully or partially recovers from the deficits acquired during the relapse. Relapses can last for days, weeks or months and recovery can be slow and gradual or almost instantaneous. The vast majority of people presenting with MS are first diagnosed with RRMS. This is typically when they are in their twenties or thirties, though diagnoses much earlier or later are known. Twice as many women as men present with this sub-type of MS. During relapses, myelin, a protective insulating sheath around the nerve fibers (neurons) in the white matter regions of the central nervous system (CNS), may be damaged in an inflammatory response by the body's own immune system. This causes a wide variety of neurological symptoms that vary considerably depending on which areas of the CNS are damaged. Immediately after a relapse, the inflammatory response dies down and a special type of glial cell in the CNS (called an oligodendrocyte) sponsors remyelination - a process whereby the myelin sheath around the axon may be repaired. It is this remyelination that may be responsible for the remission. Approximately 50% of patients with RRMS convert to SPMS within 10 years of disease onset. After 30 years, this figure rises to 90%. At any one time, the relapsing-remitting form of the disease accounts around 55% of all people with MS.

[0219] "Secondary progressive multiple sclerosis" or "SPMS" is characterized by a steady progression of clinical neurological damage with or without superimposed relapses and minor remissions and plateaux. People who develop SPMS will have previously experienced a period of RRMS which may have lasted anything from two to forty years or more. Any superimposed relapses and remissions there are, tend to tail off over time. From the onset of the secondary progressive phase of the disease, disability starts advancing much quicker than it did during RRMS though the progress can still be quite slow in some individuals. After 10 years, 50% of people with RRMS will have developed SPMS. By 25 to 30 years, that figure will have risen to 90%. SPMS tends to be associated with lower levels of inflammatory lesion formation than in RRMS but the total burden of disease continues to progress. At any one time, SPMS accounts around 30% of all people with multiple sclerosis.

[0220] "Progressive relapsing multiple sclerosis" refers to "PRMS" is characterized by a steady progression of clinical neurological damage with superimposed relapses and remissions.

There is significant recovery immediately following a relapse but between relapses there is a gradual worsening of symptoms. PRMS affects around 5% of all people with multiple sclerosis. Some neurologists believe PRMS is a variant of PPMS. The expression "effective amount" refers to an amount of the antibody (or other drug) that is effective for ameliorating or treating the multiple sclerosis. Such an effective amount will generally result in an improvement in the signs, symptoms or other indicators of MS, such as reducing relapse rate, preventing disability, reducing number and/or volume of brain MRI lesions, improving timed 25-foot walk, slow or delay the progression of the disease such as extending the time to disease progression *(e.g.* using Expanded Disability Status Scale, EDSS), etc.

[0221] "Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses administered over a period of time of about 1-20 days. The doses may be given at one time or at fixed or irregular time intervals over this period of exposure. Initial and later *(e.g.* second or third) antibody exposures are separated in time from each other as described in detail herein.

[0222] As used herein, an "interval" between antibody exposures refers to time period between an earlier antibody exposure and a later antibody exposure. An antibody exposure of the present disclosure may include one or two doses. In cases where the antibody exposures contain one dose, an interval between two antibody exposures refers to the amount of time elapsed between the dose of one antibody exposure (*e.g.,* Day 1) and the dose of the next antibody exposure. If one antibody exposure includes two doses and the next antibody exposure includes one dose, an interval between the two antibody exposures refers to the amount of time elapsed between the first of the two doses of the first antibody exposure (*e.g.,* Day 1) and the dose of the next antibody exposure. In cases where each of the two antibody exposures contain two doses, an interval between to the antibody exposures refers to the amount of time elapsed between the first of the two doses of the first antibody exposure (*e.g.,* Day 1) and the first dose of the two doses of the second antibody exposure. For example, if a method of the present disclosure includes a first antibody exposure with two doses and a second antibody exposure with two doses, and the second antibody exposure is not provided until about 24 weeks or 6 months after the first antibody exposure, then the interval between the first dose of the first antibody exposure and the first dose of the second antibody exposure is about 24 weeks or 6 months. In certain embodiments, the second antibody exposure is not provided until about 20-24 weeks or about 5-6 months after the first antibody exposure. In some embodiments, the interval between the first dose of the first antibody exposure and the first dose of the second antibody exposure is about 20-

24 weeks or about 5-6 months. In some embodiments, "about 20-24 weeks" refers to a time point between 20 weeks and 24 weeks. In some embodiments, "about 20-24 weeks" refers to a variation of a week or 7 days before or after the 24th week. In some embodiments, "about 5-6 months" refers to a time point between 5 and 6 months.

[0223] The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, down-regulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (*see* U.S. Pat. No. 4,665,077); nonsteroidal anti-inflammatory drugs (NSAIDs); ganciclovir, tacrolimus, glucocorticoids such as cortisol or aldosterone, anti-inflammatory agents such as a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist; purine antagonists such as azathioprine or mycophenolate mofetil (MMF); alkylating agents such as cyclophosphamide; bromocryptine; danazol; dapsone; glutaraldehyde (which masks the MHC antigens, as described in U.S. Pat. No. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); hydroxycloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antagonists including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor-alpha antibodies (infliximab or adalimumab), anti-TNF-alpha immunoahesin (etanercept), anti-tumor necrosis factor-beta antibodies, anti-interleukin-2 antibodies and anti-IL-2 receptor antibodies; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 90/08187 published 7/26/90); streptokinase; TGF-beta; streptodornase; RNA or DNA from the host; FK506; RS-61443; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, U.S. Pat. No. 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294;Janeway, *Nature*, 341: 482 (1989); and WO 91/01133); and T cell receptor antibodies (EP 340,109) such as T10B9.

[0224] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu),

chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0225] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (*see*, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic

acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0226] Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0227] The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

[0228] The term "hormone" refers to polypeptide hormones, which are generally secreted by glandular organs with ducts. Included among the hormones are, for example, growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); prolactin, placental lactogen, mouse gonadotropin-associated peptide, inhibin; activin; mullerian-inhibiting substance; and thrombopoietin. As used herein, the term hormone includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence hormone, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

[0229] The term "growth factor" refers to proteins that promote growth, and include, for example, hepatic growth factor; fibroblast growth factor; vascular endothelial growth factor; nerve growth factors such as NGF-β; platelet-derived growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; and colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF). As used herein, the term growth factor includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence growth factor, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

[0230] The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells and apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits, called

alpha and beta that are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include Alpha6beta1, Alpha3beta1, Alpha7beta1, LFA-1, alpha 4 integrin etc. As used herein, the term integrin includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence integrin, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

[0231] Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody; an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen Idec/Elan Pharmaceuticals, Inc.; diazacyclic phenylalanine derivatives (WO 2003/89410); phenylalanine derivatives (WO 2003/70709, WO 2002/28830, WO 2002/16329 and WO 2003/53926); phenylpropionic acid derivatives (WO 2003/10135); enamine derivatives (WO 2001/79173); propanoic acid derivatives (WO 2000/37444); alkanoic acid derivatives (WO 2000/32575); substituted phenyl derivatives (US Pat. Nos. 6,677,339 and 6,348,463); aromatic amine derivatives (US Pat. No. 6,369,229); and ADAM disintegrin domain polypeptide (US2002/0042368), antibodies to alphavbeta3 integrin (EP 633945); aza-bridged bicyclic amino acid derivatives (WO 2002/02556) etc.

[0232] For the purposes herein, "tumor necrosis factor alpha (TNF-alpha)" refers to a human TNF-alpha molecule comprising the amino acid sequence as described in Pennica *et al., Nature,* 312:721 (1984) or Aggarwal *et al., JBC*, 260:2345 (1985).

[0233] A "TNF-alpha inhibitor" herein is an agent that inhibits, to some extent, a biological function of TNF-alpha, generally through binding to TNF-alpha and neutralizing its activity. Examples of TNF inhibitors specifically contemplated herein are Etanercept (ENBREL®), Infliximab (REMICADE®) and Adalimumab (HUMIRA™).

[0234] Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methrotrexate), azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, Staphylococcal protein A immunoadsorption, including salts and derivatives thereof, etc.

[0235] Examples of "nonsteroidal anti-inflammatory drugs" or "NSAIDs" are acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, including salts and derivatives thereof, etc.

[0236] "Corticosteroid" refers to any one of several synthetic or naturally occurring substances with the general chemical structure of steroids that mimic or augment the effects of the naturally occurring corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone), dexamethasone, glucocorticoid and betamethasone.

[0237] A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products, etc.

[0238] A "label" is used herein to refer to information customarily included with commercial packages of pharmaceutical formulations including containers such as vials and package inserts, as well as other types of packaging.

[0239] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X."

[0240] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise. It is understood that aspects and variations of the invention described herein include "consisting" and/or "consisting essentially of" aspects and variations.

[0241] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

[0242] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

II. Methods of Treatment

[0243] In certain embodiments, provided is a method of providing an improvement in functional ability in a patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the patient has improvement in functional ability after treatment. In some embodiments, the method further comprises a step of measuring the patient's functional ability (e.g., using methods described elsewhere herein, such as measuring EDSS score and/or the Timed 25-Foot Walk (T25-FW)) after 1, 2, 3, 4, or more than 4 exposures of anti-CD20 antibody. In certain embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID

NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[0244] In certain embodiments, the patient has at least about a 12-week confirmed disability improvement after treatment. In certain embodiments, the patient has at least about a 24-week confirmed disability improvement after treatment. In certain embodiments, the confirmed disability improvement is determined by Expanded Disability Status Scale (EDSS) score. In certain embodiments, the patient's EDSS score decreases by at least about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0 or more than about 1.0 points (such as about 1.1, about 1.2, about 1.3, about 1.4, or about 1.5 points).

[0245] In certain embodiments, the improvement in functional ability is sustained for at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 9 weeks, at least about 10 weeks, at least about 11 weeks, at least about12 weeks, at least about 13 weeks, at least about 14 weeks, at least about 15 weeks, at least about 16 weeks, at least about 17 weeks, at least about 18 weeks, at least about 19 weeks, at least about 20 weeks, at least about 21 weeks, at least about 22 weeks, at least about 23 weeks, including any range in between these values. In certain embodiments, the improvement in functional ability is sustained for at least about 24 weeks, at least about 25 weeks, at least about 26 weeks, at least about 27 weeks, at least about 28 weeks, at least about 29 weeks, at least about 30 weeks, at least about 35 weeks, at least about 40 weeks, at least about 45 weeks, at least about 50 weeks, at least about 55 weeks, at least about 60 weeks, at least about 65 weeks, at least about 70 weeks, at least about 75weeks, or more than about 75 weeks, including any range in between these values.

[0246] In certain embodiments, the improvement in functional ability is measured by the Timed 25-Foot Walk (T25-FW) test. In certain embodiments, the time to walk 25 feet following the start of treatment is reduced by about 5 seconds, about 10 seconds, about 30 seconds, about

60 seconds, about 90 seconds, about 2 minutes, about 2.5 minutes, about 3 minutes, about 3.5 minutes, about 4 minutes, about 4.5 minutes, about 5 minutes, about 5.5 minutes, about 6 minutes, about 6.5 minutes, about 7 minutes, about 7.5 minutes, about 8 minutes, about 8.5 minutes, about 9 minutes about 9.5 minutes, or about 10 minutes relative to the time to walk 25 feet immediately prior to starting treatment.

[0247] In certain embodiments, improvement in functional activity is demonstrated by no evidence of disease activity (NEDA). In certain embodiments, NEDA is demonstrated by the absence of new or enlarging T2 lesions or T1 gadolinium-enhancing lesions on magnetic resonance imaging. In certain embodiments, NEDA is demonstrated by absence of relapse. In certain embodiments, NEDA is demonstrated by the absence of progression. In certain embodiments, NEDA is demonstrated by lack of worsening of EDSS. In certain embodiments, NEDA is defined as: no protocol-defined relapses, no CDP events, no new or enlarging T2 lesions, and no gadolinium-enhancing T1 lesions. In certain embodiments, NEDA is sustained for about least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 9 weeks, at least about 10 weeks, at least about 11 weeks, at least about12 weeks, at least about 13 weeks, at least about 14 weeks, at least about 15 weeks, at least about 16 weeks, at least about 17 weeks, at least about 18 weeks, at least about 19 weeks, at least about 20 weeks, at least about 21 weeks, at least about 22 weeks, at least about 23 weeks, including any range in between these values. In certain embodiments, the improvement in functional ability is sustained for at least about 24 weeks, at least about 25 weeks, at least about 26 weeks, at least about 27 weeks, at least about 28 weeks, at least about 29 weeks, at least about 30 weeks, at least about 35 weeks, at least about 40 weeks, at least about 45 weeks, at least about 50 weeks, at least about 55 weeks, at least about 60 weeks, at least about 65 weeks, at least about 70 weeks, at least about 75 weeks, or more than about 75 weeks, including any range in between these values.

[0248] In certain embodiments, the patient has T1 gadolinium staining lesions at baseline (i.e., before starting treatment). In certain embodiments, the patient does not have T1 gadolinium staining lesions at baseline (i.e., before starting treatment).

[0249] In certain embodiments, provided is a method of suppressing composite disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID

NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, the administration results in reduction of 12-week confirmed composite disability progression. In certain embodiments, the administration results in reduction of 24-week confirmed composite disability progression.

[0250] In certain embodiments, provided is a method of delaying onset of composite disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, the administration results in reduction of 12-week confirmed composite disability progression. In certain embodiments, the administration results in reduction of 24-week confirmed composite disability progression.

[0251] In certain embodiments, the confirmed composite disability progression is determined by Expanded Disability Status Scale (EDSS) score. In certain embodiments, confirmed composite disability progression is defined as EDSS progression (i.e., an increase in EDSS score). In certain embodiments, the confirmed composite disability progression is determined by

Timed 25-Foot Walk (T25-FW). In certain embodiments, the confirmed composite disability progression is defined as at least 20% in T25-FW. In certain embodiments, the confirmed composite disability progression is determined by 9-Hole Peg Test (9-HPT). In certain embodiments, confirmed composite disability progression is defined as at least 20% increase in 9-hole peg test (9-HPT) time. In certain embodiments, the confirmed composite disability progression is determined by EDSS progression, Timed 25-Foot Walk, and 9-Hole Peg Test.

[0252] In certain embodiments, provided is a method of suppressing disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events. In certain embodiments, the reduction of confirmed disability progression events is observed after 1, 2, 3, 4, or more than 4 exposures of anti-CD20 anitbody. In certain embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, the administration results in reduction of a 12-week confirmed disability progression. In certain embodiments, the administration results in reduction of a 24-week confirmed disability progression. In certain embodiments, the administration results in reduction in risk for a 12-week confirmed disability progression. In certain embodiments, the administration results in reduction in risk for a 24-week confirmed disability progression.

[0253] In certain embodiments, the patient has T1 gadolinium staining lesions at baseline (i.e., before starting treatment). In certain embodiments, the patient does not have T1 gadolinium staining lesions at baseline (i.e., before starting treatment).

[0254] In certain embodiments, provided is a method of delaying onset of confirmed disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a

heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[0255] In certain embodiments, the confirmed disability progression is determined by Expanded Disability Status Scale (EDSS) score. In certain embodiments, the patient's EDSS score increases by at least about 1.0 point from a baseline EDSS score of about 5.5 or less. In certain embodiments, the patient's EDSS score increases by about 0.5 point from a baseline EDSS score over 5.5. In certain embodiments, an increase in EDSS is confirmed at least 12 weeks after the initial neurological worsening.

[0256] In certain embodiments, the patient has T1 gadolinium staining lesions at baseline (i.e., before starting treatment). In certain embodiments, the patient does not have T1 gadolinium staining lesions at baseline (i.e., before starting treatment).

[0257] In certain embodiments, provided is a method of reducing T2 lesion volume in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, the

patient has T1 gadolinium staining lesions at baseline. In certain embodiments, the patient does not have T1 gadolinium staining lesions at baseline.

[0258] In certain embodiments, provided is a method of slowing or preventing reduction in brain volume in a patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the brain volume reduction is slowed or prevented in the patient; and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, brain volume reduction is slowed. In certain embodiments, brain volume reduction is slowed or prevented in a patient that has not experienced brain volume loss. In certain embodiments, further reduction in brain volume is slowed or prevented in a patient who has experienced brain volume loss.

[0259] In certain embodiments, provided is a method of slowing or preventing brain atrophy in a patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the brain atrophy is slowed or prevented in the patient; and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and

CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, brain atrophy is slowed. In certain embodiments, brain atrophy is slowed or prevented in a patient that has not experienced brain atrophy. In certain embodiments, further brain atrophy is slowed or prevented in a patient who has experienced brain atrophy.

[0260] In certain embodiments, provided is a method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in no evidence of disease activity (NEDA) for at least 12 weeks, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, treatment results in no evidence of disease activity (NEDA) for at least 24 weeks.

[0261] A method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in the patient achieving lesion-free status after 96 weeks of treatment, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, treatment results in the patient achieving

lesion-free status after 48 weeks of treatment. In certain embodiments, treatment results in the patient achieving lesion-free status after 24 weeks of treatment. In certain embodiments, treatment results in the patient being free of gadolinium staining lesions. In certain embodiments, treatment results in the patient being free of T2 lesions.

[0262] In certain embodiments, provided is a method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in one or more of: a) the patient being relapse-free at 96 weeks; b) the patient having no confirmed disability progression events at 96 weeks; c) the patient being without T1 gadolinium-enhancing lesions at 96 weeks; d) the patient being without new and/or enlarging T2 lesions at 96 weeks; wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and 2) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[0263] In certain embodiments, the patient has not been previously treated with other therapy for multiple sclerosis (i.e., a "naïve patient"). In certain embodiments, the naïve patient experienced at least 2 relapses in 2 years prior to starting treatment. In certain embodiments, the naïve patient experienced at least 1 relapse in the last year prior to starting treatment.

[0264] In certain embodiments, the patient is an inadequate responder to other therapy for multiple sclerosis. In certain embodiments, the patient who is an inadequate responder has been previously treated with interferon beta-1a or glatiramer acetate for at least 1 year. In certain embodiments, the patient who is an inadequate responder has experienced at least one relapse or experienced at least 1 baseline gadolinium-enhancing lesion while being treated with another therapy for multiple sclerosis.

[0265] In certain embodiments, provided is a method of treating a human patient with highly active multiple sclerosis comprising administering to the patient an effective amount of an anti-

CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[0266] In certain embodiments, the patient with highly active multiple sclerosis has not been previously treated with other therapy for multiple sclerosis (i.e., a "naïve patient"). In certain embodiments, the naïve patients with highly active multiple sclerosis has experienced at least 2 relapses in the last year prior to randomization, and either (a) at least 1 baseline gadolinium lesion or (b) increase in T2 lesion count at baseline visit (changing categorically from 0-5 to 6-9 lesions or from 6-9 lesions to > 9 lesions), as compared to a prior MRI.

[0267] In certain embodiments, the patient with highly active multiple sclerosis is an inadequate responder to other therapy for multiple sclerosis. In certain embodiments, the patient with highly active multiple sclerosis who is an inadequate responder has been previously treated with interferon beta-1a or glatiramer acetate for at least 1 year. In certain embodiments, the patient with highly active multiple sclerosis who is an inadequate responder has experienced at least one relapse and either (a) had at least nine T2-lesions or (b) had at least one gadolinium lesion at baseline while being treated with another therapy for multiple sclerosis.

[0268] In certain embodiments, administration of the anti-CD20 antibody to the patient with highly active multiple sclerosis is effective in one or more of the following: (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and (4) improvement of function ability. In certain embodiments, the method of treating a patient with highly active multiple sclerosis further comprising performing an MRI scan and determining if the patient has highly active multiple sclerosis before administering the anti-CD20 antibody to the patient.

[0269] In certain embodiments, provided is a method of treating a human patient with early stage multiple sclerosis comprising administering to the patient an effective amount of an anti-

CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6. In certain embodiments, the method further comprises diagnosing a patient having early stage multiple sclerosis before administering the anti-CD20 antibody to the patient.

[0270] In certain embodiments, provided is a method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the treatment results in no evidence of disease activity in the patient, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:10, CDR2 having the amino acid SEQ ID NO:11, and CDR3 having the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 having the amino acid sequence of SEQ ID NO:4, CDR2 having the amino acid sequence of SEQ ID NO:5, and CDR3 having the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:10, CDR2 comprising he amino acid SEQ ID NO:11, and CDR3 comprising the amino acid sequence of SEQ ID NO:12, and b) a light chain variable region comprising CDR1 comprising the amino acid sequence of SEQ ID NO:4, CDR2 comprising the amino acid sequence of SEQ ID NO:5, and CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[0271] In certain embodiments, the patient has a relapsing form of multiple sclerosis. In some embodiments, a relapsing form of MS (RMS) refers to a patient population typically composed of both RRMS and SPMS with superimposed relapses (also commonly referred to as "relapsing SPMS"). In certain embodiments, the relapsing form of multiple sclerosis is relapsing remitting multiple sclerosis (RRMS). In certain embodiments, the relapsing form of multiple sclerosis is a secondary progressive multiple sclerosis with superimposed relapses (rSPMS). In certain

embodiments, the patient is less than 18 years of age. In certain embodiments, the patient is between 18 and 55 years of age. In certain embodiments, the patient is over 55 years of age. In certain embodiments, the patient has a diagnosis of multiple sclerosis in accordance with the 2010 revised McDonald criteria (Polman *et al.* (2011) "Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria." *Ann Neurol* 69, 292-302). Additionally or alternatively, in certain embodiments, the patient has an Expanded Disability Status Scale (EDSS, see world-wide-web.neurostatus.org) score of 0 to 5.5 at screening. Additionally or alternatively, in certain embodiments, the patient has had at least two documented clinical attacks within the previous 2 years or one clinical attack occurring within the year. Additionally or alternatively, in certain embodiments, the patient has documented MRI of brain with abnormalities consistent with multiple sclerosis.

[0272] In certain embodiments, the patient with a relapsing form of multiple sclerosis does not have a diagnosis of primary progressive multiple sclerosis. Additionally or alternatively, in certain embodiments, the patient has not had previous treatment with any B-cell targeted therapies, systemic corticosteroids and/or immunosuppressants, Additionally or alternatively, in certain embodiments, the patient does not have a history of primary or secondary immunodeficiency, active infection, or presence of recurrent or chronic infection (e.g. hepatitis B or C, HIV, syphilis, tuberculosis), or history of progressive multifocal leukoencephalopathy.

[0273] In certain embodiments, the patient has progressive multiple sclerosis. In certain embodiments, the progressive multiple sclerosis is primary progressive multiple sclerosis. In certain embodiments, the patient is less than 18 years of age. In certain embodiments, the patient is between 18 and 55 years of age. In certain embodiments, the patient is over 55 years of age. In certain embodiments, the patient has a diagnosis of Primary Progressive Multiple Sclerosis in accordance with the 2005 revised McDonald criteria (Polman *et al.* (2011) "Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald criteria.'" *Ann Neurol* 58, 840-846). Additionally or alternatively, in certain embodiments, the patient has an Expanded Disability Status Scale (EDSS) score of 3 to 6.5 points. Additionally or alternatively, in certain embodiments, the patient has a score of at least 2.0 on the pyramidal functions component of the Functional Systems Scale (FSS). Additionally or alternatively, in certain embodiments, the patient has a documented history or presence at screening of elevated IgG index in a cerebrospinal fluid (CSF) specimen and/or one or more IgG oligoclonal bands detected by isoelectric focusing in a cerebrospinal fluid (CSF) specimen. Additionally or alternatively, in certain embodiments, the patient has no history of relapse-remitting multiple sclerosis (RRMS).

Additionally or alternatively, in certain embodiments, the patient has no history of secondary progressive multiple sclerosis (SPMS). Additionally or alternatively, in certain embodiments, the patient has history of progressive relapsing multiple sclerosis (PRMS).

[0274] In certain embodiments, the patient has had previous treatment with B-cell targeted therapies (*e.g.* rituximab, ocrelizumab, atacicept, belimumab, or ofatumumab). In certain embodiments, the patient has not had previous treatment with B-cell targeted therapies (*e.g.* rituximab, ocrelizumab, atacicept, belimumab, or ofatumumab).

[0275] In some embodiments, "Confirmed Disability Progression" or "CDP" refers to an increase of at least 1.0 point from the baseline EDSS score in patients with a baseline score of 5.5 or less, or an increase of a 0.5 point in patients with a baseline score over 5.5, during the 96 weeks, wherein increases in the EDSS were confirmed at a regularly scheduled visit at least 12 weeks after the initial neurologic worsening.

[0276] In some embodiments, "Confirmed Disability Improvement" or "CDI" refers to a reduction in EDSS score of at least 1.0 compared to baseline in patients with a baseline EDSS score of 5.5 or less, or a reduction of a 0.5 point in patients with a baseline EDSS score above 5.5.

[0277] In some embodiments, brain atrophy refers to one or more of the following: axonal loss in the brain, tissue loss within gray matter lesions or white matter lesions, Wallerian degeneration in pathways related to the lesions, or lesion burden. In certain embodiments, brain atrophy refers to a decrease in whole brain volume. In certain embodiments, brain atrophy refers to a decrease in volume of one or more of the structures of the brain (including, but not limited to, the cerebrum, the cerebellum, the thalamus, frontotemporal neocortex, brainstem, hippocampus, parietal lobe, and/or the hypothalamus). In certain embodiments, brain atrophy refers to cortical thinning in precentral gyrus, superior frontal gyrus, thalamus and/or putamen. In certain embodiments, brain atrophy refers to a loss of at least about 0.4%, at least about 0.5%, at least about 0.6%, or at least 0.7% brain volume per year. Further details regarding brain atrophy are detailed in, *e.g.*, Riley et al. (2012) *Expert Rev Neurother* 12(3), 323-333.

[0278] In some embodiments, the patient or subject has highly active multiple sclerosis. In some embodiments, "highly active multiple sclerosis" in treatment naïve patients refers to patients who have not been previously treated with other therapy for multiple sclerosis and who have experienced at least 2 relapses in the last year prior to randomization, and either (a) at least 1 baseline gadolinium lesion or (b) increase in T2 lesion count at baseline visit (changing categorically from 0-5 to 6-9 lesions or from 6-9 lesions to > 9 lesions) as compared to a prior MRI. In some embodiments, "highly active multiple sclerosis" in patients who have been

previously treated with other therapy for multiple sclerosis and who have had at least one relapse in the previous year and either (a) have at least nine T2-lesions or (b) have at least one gadolinium lesion at baseline.

[0279] In certain embodiments, a baseline level in a patient refers to the level prior to administration of or treatment with an anti-CD20 antibody to the patient, for example, about 2 months, about 1.5 months, about 1 month, about 30 days, about 25 days, about 21 days, about 14 days, about 7 days, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day prior to administration of or treatment with an anti-CD20 antibody to the patient.

[0280] In certain embodiments, the patient maintains the ability to mount a humoral immune response to an antigen during treatment. In certain embodiments, the antigen is a mumps antigen, a rubella antigen, a varicella antigen, an S. pneumonia antigen, a tetanus toxoid antigen, a pneumococcal antigen, or an influenza antigen.

[0281] The methods described herein may encompass any combination of the embodiments described herein.

III. Dosages

[0282] According to some embodiments of any of the methods or articles of manufacture described herein, the method or instructions comprises administering an effective amount of an anti-CD20 antibody to the multiple sclerosis patient to provide an initial antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams) followed by a second antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams), the second antibody exposure not being provided until from about 16 to about 60 weeks from the initial antibody exposure. For purposes of this invention, the second antibody exposure is the next time the patient is treated with the anti-CD20 antibody after the initial antibody exposure, there being no intervening anti-CD20 antibody treatment or exposure between the initial and second exposures. In some embodiments, the initial antibody exposure and/or the second antibody exposure is about any of 0.3 grams, 0.4 grams, 0.5 grams, 0.6 grams, 0.7 grams, 0.8 grams, 0.9 grams, or 1.0 grams.

[0283] The interval between the initial and second or subsequent antibody exposures can be measured from the first dose of the initial antibody exposure.

[0284] In some embodiments, the antibody exposures are approximately 24 weeks or 6 months apart; or approximately 48 weeks or 12 months apart. In some embodiments, the antibody

exposures are approximately are about 20-24 weeks or about 5-6 months. In some embodiments, "about 20-24 weeks" refers to a time point between 20 weeks and 24 weeks. In some embodiments, "about 20-24 weeks" refers to a variation of a week or 7 days before or after the 24th week. In some embodiments, "about 5-6 months" refers to a time point between 5 and 6 months.

[0285] In one embodiment, the second antibody exposure is not provided until about 20 to about 30 weeks from the initial exposure, optionally followed by a third antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams), the third exposure not being administered until from about 46 to 60 weeks (preferably from about 46 to 54 weeks) from the initial exposure, and then, in some embodiments, no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure. In some embodiments, the third antibody exposure is about any of 0.3 grams, 0.4 grams, 0.5 grams, 0.6 grams, 0.7 grams, 0.8 grams, 0.9 grams, or 1.0 grams.

[0286] In an alternative embodiment, the second antibody exposure is not provided until about 46 to 60 weeks from the initial exposure, and subsequent antibody exposures, if any, are not provided until about 46 to 60 weeks from the previous antibody exposure.

[0287] According to some embodiments of any of the methods or articles of manufacture described herein, the method or instructions comprises administering an effective amount of an anti-CD20 antibody to the multiple sclerosis patient to provide an initial antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams) followed by a second antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams), the second antibody exposure not being provided until from about 20 to about 30 weeks from the initial antibody exposure, followed by a third antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams), the third antibody exposure not being provided until from about 46 to about 54 weeks from the initial exposure, followed by a fourth antibody exposure of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams), the fourth antibody exposure not being provided until from about 70 to about 75 weeks from the initial exposure.

[0288] In certain embodiments the fourth antibody exposure is followed by one or more antibody exposures of about 0.3 to about 4 grams (preferably about 0.3 to about 1.5 grams, such as about 0.6 grams or about 1.0 grams). In certain embodiments each subsequent antibody exposure is about 20 to about 30 weeks from the previous exposure.

[0289] For purposes of this invention, the each subsequent exposure is the next time the patient is treated with the anti-CD20 antibody after the initial antibody exposure, there being no intervening anti-CD20 antibody treatment or exposure between, e.g., the initial and second exposures, the second and third exposures, or the third and fourth exposures, etc. In some embodiments, the initial, second, third, fourth, and/or subsequent antibody exposure is about any of 0.3 grams, 0.4 grams, 0.5 grams, 0.6 grams, 0.7 grams, 0.8 grams, 0.9 grams, or 1.0 grams.

[0290] Any one or more of the antibody exposures herein may be provided to the patient as a single dose of antibody, or as two separate doses of the antibody (*i.e.*, constituting a first and second dose). The particular number of doses (whether one or two) employed for each antibody exposure may be dependent, for example, on the type of MS treated, the type of antibody employed, whether and what type of second medicament is employed, and the method and frequency of administration. Where two separate doses are administered, the second dose is preferably administered from about 3 to 17 days, more preferably from about 6 to 16 days, and most preferably from about 13 to 16 days from the time the first dose was administered. In some embodiments, where two separate doses are administered, the second dose is about 14 days (such as 13 days or 15 days). In some embodiments "about 14 days" refers to a variation of 1 day before or after the 14th day. Where two separate doses are administered, the first and second dose of the antibody is preferably about 0.3 to 1.5 grams, more preferably about 0.3 to about 1.0 grams. In some embodiments, where two separate doses are administered, the first and second dose of the antibody is about any of 0.3 grams, 0.4 grams, 0.5 grams, or 0.6 grams. In some embodiments, the initial ocrelizumab exposure comprises a first dose and a second dose of ocrelizumab, wherein the first dose and second dose of ocrelizumab is about 0.3 grams. In some embodiments, the second ocrelizumab exposure comprises a single dose of ocrelizumab, wherein the single dose of ocrelizumab is 0.6 grams.

[0291] In one embodiment, the patient is provided at least about three, at least about four, or at least about five exposures of the antibody, for example, from about 3 to 60 exposures, and more particularly about 3 to 40 exposures, most particularly, about 3 to 20 exposures. In some embodiments of any of the methods, the methods further comprising providing between about one to about three subsequent ocrelizumab exposures. In some embodiments, such exposures are administered at intervals each of approximately 24 weeks or 6 months, or 48 weeks or 12 months. In certain embodiments, an interval is shortened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day. In certain embodiments,

more than 1 interval is shortened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day. In certain embodiments, an interval is lengthened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day. In certain embodiments, more than one interval is lengthened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day. In certain embodiments, more than 1 interval is shortened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day or lengthened by approximately 4 weeks, about 3.5 weeks, about 3 weeks, about 2.5 weeks, about 2 weeks, about 1.5 weeks, about 1 week, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days or about 1 day.

[0292] In one embodiment, each antibody exposure is provided as a single dose of the antibody. In an alternative embodiment, each antibody exposure is provided as two separate doses of the antibody. In some embodiments, some exposures are provided as a single dose or as two separate doses.

[0293] The antibody may be a naked antibody or may be conjugated with another molecule such as a cytotoxic agent such as a radioactive compound. In some embodiments, the antibody is Rituximab, humanized 2H7 (*e.g.* comprising the variable domain sequences in SEQ ID NOS. 2 and 8) or humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 23 and 24, or huMax-CD20 (Genmab). In some embodiments, the antibody is ocrelizumab (*e.g.*, comprising (a) a light chain comprising the amino acid sequence of SEQ ID NO: 13 and (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:14).

[0294] In one embodiment, the patient has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the multiple sclerosis and/or has never been previously treated with an antibody to a B-cell surface marker *(e.g.* never previously treated with a CD20 antibody).

[0295] The antibody is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (*see*, *e.g.*, US Patent

Appln No. 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antibody. In some embodiments, the dosing is given intravenously, subcutaneously or intrathecally. In some embodiments, the dosing is given by intravenous infusion(s).

[0296] In certain embodiments, the patient is premedicated prior to infusion with the anti-CD20 antibody. In certain embodiments, the patient is premedicated with methylprednisolone (or an equivalent) approximately 30 minutes prior to each infusion of anti-CD20 antibody. In certain embodiments, the patient is premedicated with 100 mg IV methylprednisolone (or an equivalent) approximately 30 minutes prior to each infusion of anti-CD20 antibody. In certain embodiments, the patient is additionally (or alternatively) premedicated with an antihistaminic drug (e.g. diphenhydramine) approximately 30-60 minutes before each infusion of anti-CD20 antibody. In certain embodiments, the patient is additionally (or alternatively) premedicated with an antipyretic (e.g. acetaminophen/paracetamol).

[0297] While the CD20 antibody may be the only drug administered to the patient to treat the multiple sclerosis, one may optionally administer a second medicament, such as a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody *(e.g.* an LFA-1 antibody, or an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen Idec/Elan Pharmaceuticals, Inc) etc, with the antibody that binds a B cell surface marker *(e.g.* with the CD20 antibody).

[0298] In some embodiments of combination therapy, the antibody is combined with an interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting therapy *(e.g.*, mitoxantrone, cyclophosphamide, alemtuzumab (Campath®, LEMTRADA™), anti-CD4, cladribine, total body irradiation, bone marrow transplantation); corticosteroid *(e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid), including systemic corticosteroid therapy; non–lymphocyte-depleting immunosuppressive therapy *(e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class, which includes cerivastatin (BAYCOL®), fluvastatin (LESCOL®), atorvastatin (LIPITOR®), lovastatin (MEVACOR®), pravastatin (PRAVACHOL®), Simvastatin (ZOCOR®); estradiol; testosterone (optionally at elevated dosages; Stuve *et al. Neurology* 8:290–301 (2002)); hormone

replacement therapy; treatment for symptoms secondary or related to MS *(e.g.,* spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); plasmapheresis; levothyroxine; cyclosporin A; somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; rehabilitative surgery; radioiodine; thyroidectomy; another B-cell surface antagonist/antibody; etc.

[0299] The second medicament is administered with the initial exposure and/or later exposures of the CD20 antibody, such combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

[0300] Aside from administration of antibodies to the patient, the present application contemplates administration of antibodies by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression administering an "effective amount" of an antibody. *See*, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

[0301] There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes that are implanted into the patient (*see*, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

[0302] In some embodiments, the *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane

protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins that bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols *see* Anderson *et al.*, *Science* 256:808-813 (1992). *See also* WO 93/25673 and the references cited therein.

IV. Antibodies and their Production

[0303] The methods and articles of manufacture of the present invention use, or incorporate, an antibody that binds to a B-cell surface marker, especially one that binds to CD20. Accordingly, methods for generating such antibodies will be described here.

[0304] In some embodiments, the anti-CD20 antibody used in the methods described here is produced by a method comprising expressing a nucleic acid encoding a humanized antibody comprising the heavy and light chain amino acid sequences of SEQ ID NO:14 or 13, respectively, in a host cell, and recovering the humanized antibody or an antigen-binding fragment thereof expressed in the host cell. In some embodiments, the host cell is a mammalian cell (e.g., a CHO cell), an insect cell, or a plant cell. In some embodiments the host cell is a bacterial cell. Methods of producing an anti-CD20 are described in further detail in, e.g., U.S. Patent No. 7,799,900.

[0305] The B cell surface marker to be used for production of, or screening for, antibodies may be, *e.g.*, a soluble form of the marker or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing the marker at their cell surface can be used to generate, or screen for, antibodies. Other forms of the B cell surface marker useful for generating antibodies will be apparent to those skilled in the art.

[0306] A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention.

(i) Polyclonal antibodies

[0307] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized,

e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[0308] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. In some embodiments, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

[0309] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

[0310] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0311] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

[0312] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0313] In some embodiments, the myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, in some embodiments, the myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0314] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. In some embodiments, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[0315] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

[0316] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[0317] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0318] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures *(e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In some embodiments, the hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al., Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

[0319] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al., Nature*, 348:552-554 (1990). Clackson *et al., Nature*, 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al., Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al., Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0320] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[0321] Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) Humanized antibodies

[0322] Methods for humanizing non-human antibodies have been described in the art. In some embodiments, a humanized antibody has one or more amino acid residues introduced into it from

a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0323] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al., J. Immunol.*, 151:2296 (1993); Chothia *et al., J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter *et al., Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al., J. Immunol.*, 151:2623 (1993)).

[0324] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, in some embodiments of the methods, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved.

In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0325] In some embodiments, the anti-CD20 antibody is a humanized 2H7 antibody. In some embodiments, the humanized 2H7 antibody preferably comprises one, two, three, four, five or six of CDR sequences as shown in FIG. 1A and 1B. In some embodiments, the humanized 2H7 antibody preferably comprises one, two, three, four, five or six of the following CDR sequences:

CDR L1 sequence RASSSVSYXH wherein X is M or L (SEQ ID NO: 18), for example RASSSVSYMH (SEQ ID NO: 4) (Fig. 1A),

CDR L2 sequence APSNLAS (SEQ ID NO: 5) (Fig. 1A),

CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID NO: 19), for example QQWSFNPPT (SEQ ID NO: 6) (Fig. 1A),

CDR H1 sequence GYTFTSYNMH (SEQ ID NO: 10) (Fig. 1B),

CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID NO: 20), for example AIYPGNGDTSYNQKFKG (SEQ ID NO: 11) (Fig. 1B), and

CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W or D, and the X as position 7 is S or R (SEQ ID NO: 21), for example VVYYSNSYWYFDV (SEQ ID NO: 12) (Fig. 1B).

[0326] The CDR sequences above are generally present within human variable light and variable heavy framework sequences, such as substantially the human consensus FR residues of human light chain kappa subgroup I (V_L6I), and substantially the human consensus FR residues of human heavy chain subgroup III (V_HIII). *See also* WO 2004/056312 (Lowman *et al.*).

[0327] In some embodiments, the variable heavy region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3, including native sequence and variant constant regions.

[0328] In some embodiments, such antibody comprises the variable heavy domain sequence of SEQ ID NO:8 (v16, as shown in Fig. 1B), optionally also comprising the variable light domain sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), which optionally comprises one or more amino acid substitution(s) at positions 56, 100, and/or 100a, *e.g.* D56A, N100A or N100Y, and/or S100aR in the variable heavy domain and one or more amino acid substitution(s) at positions 32 and/or 92, *e.g.* M32L and/or S92A, in the variable light domain. In some embodiments, the antibody is an intact antibody comprising the light chain amino acid sequences of SEQ ID NOs.

13 or 16, and heavy chain amino acid sequences of SEQ ID NO. 14, 15, 17, 22 or 25. In some embodiments, the humanized 2H7 antibody is ocrelizumab (Genentech).

[0329] In the embodiments, the humanized 2H7 is an intact antibody or antibody fragment comprising the variable light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and the variable heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY PGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSN SYWYFDVWGQGTLVTVSS (SEQ ID NO:8).

[0330] In some embodiments, the humanized 2H7 antibody is an intact antibody, in some embodiments, it comprises the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

and the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY PGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSN SYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY PGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSN

SYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:26)

or the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY
PGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSN
SYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15)

or the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY
PGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSN
SYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:27).

[0331] In some embodiments, the amino acid K at C-terminus of the heavy chain is removed.

[0332] In some embodiments, the humanized 2H7 antibody comprises 2H7.v511 variable light domain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR
(SEQ ID NO:23)

and 2H7.v511 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY
PGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSY
RYWYFDVWGQGTLVTVSS (SEQ ID NO:24).

[0333] In some embodiments, the humanized 2H7.v511 antibody is an intact antibody, it may comprise the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLA
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:16)

and the heavy chain amino acid sequence of SEQ ID NO:17 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIY
PGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSY
RYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQD
WLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:25).

[0334] In some embodiments, the antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC activity, such as one wherein the amino acid substitutions are at positions 298, 333, and 334, preferably S298A, E333A, and K334A, using Eu numbering of heavy chain residues. *See also* US Patent No. 6,737,056B1, Presta. Any of these antibodies may comprise at least one substitution in the Fc region that improves FcRn binding or serum half-life, for example a substitution at heavy chain position 434, such as N434W. *See also* US Patent No. 6,737,056B1, Presta. Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that increases CDC activity, for

example, comprising at least a substitution at position 326, preferably K326A or K326W. *See also* US Patent No. 6,528,624B1 (Idusogie *et al.*).

[0335] In some embodiments, the humanized 2H7 variants are those comprising the variable light domain of SEQ ID NO:2 and the variable heavy domain of SEQ ID NO:8, including those with or without substitutions in an Fc region (if present), and those comprising a variable heavy domain with alteration N100A; or D56A and N100A; or D56A, N100Y, and S100aR; in SEQ ID NO:8 and a variable light domain with alteration M32L; or S92A; or M32L and S92A; in SEQ ID NO:2. M34 in the variable heavy domain of 2H7.v16 has been identified as a potential source of antibody stability and is another potential candidate for substitution.

[0336] In some embodiments of the invention, the variable region of variants based on 2H7.v16 comprise the amino acid sequences of v16 except at the positions of amino acid substitutions that are indicated in **Table 1** below. Unless otherwise indicated, the 2H7 variants will have the same light chain as that of v16.

Table 1: Exemplary Humanized 2H7 Antibody Variants

2H7 Version	**Heavy chain (V_H) changes**	**Light chain (V_L) changes**	**Fc changes**
16 for reference			-
31	-	-	S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N100A	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K334A, K322A
138	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
477	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
375	-	-	K334L
588	-	-	S298A, E333A, K334A, K326A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, E333A, K334A, K326A

(iv) Human antibodies

[0337] As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals *(e.g.*, mice) that are capable, upon immunization,

of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See*, *e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); Bruggermann *et al., Year in Immuno.*, 7:33 (1993); and US Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

[0338] Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review *see*, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). *See also*, US Patent Nos. 5,565,332 and 5,573,905.

[0339] Human antibodies may also be generated by *in vitro* activated B cells (*see* US Patents 5,567,610 and 5,229,275).

(v) Antibody fragments

[0340] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see*, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al., Science*, 229:81 (1985)). However, these fragments can now be produced directly

by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). *See* WO 93/16185; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific antibodies

[0341] Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the B cell surface marker. Other such antibodies may bind the B cell surface marker and further bind a second different B-cell surface marker. Alternatively, an anti-B cell surface marker binding arm may be combined with an arm that binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule *(e.g.* CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the B cell. Bispecific antibodies may also be used to localize cytotoxic agents to the B cell. These antibodies possess a B cell surface marker-binding arm and an arm that binds the cytotoxic agent *(e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments *(e.g.* $F(ab')_2$ bispecific antibodies).

[0342] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al., Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al., EMBO J.*, 10:3655-3659 (1991).

[0343] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain

sequences. In some embodiments, the fusion is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the first heavy chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0344] In some embodiments of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al., Methods in Enzymology*, 121:210 (1986).

[0345] According to another approach described in US Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are recovered from recombinant cell culture. In some embodiments, the interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains *(e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones *(e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0346] Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360,

WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

[0347] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al., Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0348] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al., J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) by a linker that is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber *et al., J. Immunol.*, 152:5368 (1994).

[0349] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991).

V. Conjugates and Other Modifications of the Antibody

[0350] The antibody used in the methods or included in the articles of manufacture herein is optionally conjugated to a cytotoxic agent. For instance, the antibody may be conjugated to a drug as described in WO2004/032828.

[0351] Chemotherapeutic agents useful in the generation of such antibody-cytotoxic agent conjugates have been described above.

[0352] Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (US Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one embodiment of the invention, the antibody is conjugated to one or more maytansine molecules *(e.g.* about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me, which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al. Cancer Research* 52: 127-131 (1992)) to generate a maytansinoid-antibody conjugate.

[0353] Alternatively, the antibody is conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics is capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin that may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)).

[0354] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. *See*, for example, WO 93/21232 published October 28, 1993.

[0355] The present invention further contemplates antibody conjugated with a compound with nucleolytic activity *(e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0356] A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

[0357] Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate

(SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See* WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used.

[0358] Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

[0359] In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" *(e.g.* avidin) that is conjugated to a cytotoxic agent *(e.g.* a radionucleotide).

[0360] The antibodies of the present invention may also be conjugated with a prodrug-activating enzyme that converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, *see* WO81/01145) to an active anti-cancer drug. *See*, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

[0361] The enzyme component of such conjugates includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

[0362] Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and

neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (*see*, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

[0363] The enzymes of this invention can be covalently bound to the antibody by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (*see*, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

[0364] Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. In some embodiments, the antibody fragments, such as Fab', are linked to one or more PEG molecules.

[0365] The antibodies disclosed herein may also be formulated as liposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[0366] Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of an antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. *See* Gabizon *et al. J. National Cancer Inst.* 81(19)1484 (1989).

[0367] Amino acid sequence modification(s) of the antibody are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the

antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0368] A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified *(e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0369] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme, or a polypeptide that increases the serum half-life of the antibody.

[0370] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by different residue. The sites of greatest interest for substitutional mutagenesis of antibody antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in **Table 2** under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in **Table 2**, may be introduced and the products screened.

Table 2

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0371] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

[0372] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0373] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0374] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0375] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites *(e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity *(e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques

elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0376] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. Such altering includes deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0377] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0378] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0379] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 A1 (Presta, L.); *see also* US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd) concerning a CD20 antibody composition. Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO03/011878, Jean-Mairet *et al.* and US Patent No. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO97/30087 (Patel *et al.*); *see also* WO98/58964 (Raju, S.) and WO99/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof.

[0380] In some embodiments, the glycosylation variant herein comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or

334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US Pat. Appl. No. US 2003/0157108 A1, Presta, L; WO 00/61739A1; WO01/29246A1; US2003/0115614A1; US2002/0164328A1; US2004/0093621A1; US2004/0132140A1; US2004/0110704A1; US2004/0110282A1; US2004/0109865A1; WO03/085119A1; WO03/084570A1; WO2005/035778; WO2005/035586 (describing RNA inhibition (RNAi) of fucosylation); Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,*knockout CHO cells (Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004)).

[0381] Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

[0382] It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cyotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of an antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). *See* Caron *et al., J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. *See* Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

[0383] WO00/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof. In some embodiments, the antibody with improved ADCC comprises

substitutions at positions 298, 333, and/or 334 of the Fc region. In some embodiments, the altered Fc region is a human IgG1 Fc region comprising or consisting of substitutions at one, two or three of these positions.

[0384] Antibodies with altered C1q binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof.

[0385] To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule *(e.g.,* IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO00/42072 (Presta, L.).

[0386] Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 A1, Miller *et al.*).

VI. Pharmaceutical Formulations

[0387] Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions

such as sodium; metal complexes *(e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0388] Exemplary anti-CD20 antibody formulations are described in WO98/56418. This publication describes a liquid multidose formulation comprising 40 mg/mL Rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8BC. Another anti-CD20 formulation of interest comprises 10mg/mL Rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5.

[0389] Lyophilized formulations adapted for subcutaneous administration are described in US Pat No. 6,267,958 (Andya *et al.*). Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

[0390] Crystalized forms of the antibody or antibody are also contemplated. *See*, for example, US 2002/0136719A1 (Shenoy *et al.).*

[0391] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, in some embodiments, those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent; chemotherapeutic agent; immunosuppressive agent; cytokine; cytokine antagonist or antibody; growth factor; hormone; integrin; integrin antagonist or antibody *(e.g.* an LFA-1 antibody, or an alpha 4 integrin antibody such as natalizumab/TYSABRI®) available from Biogen Idec/Elan Pharmaceuticals, Inc.); interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug *(e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug *(e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; testosterone; hormone replacement therapy; drug that treats symptoms secondary or related to MS *(e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid *(e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A; somatastatin analogue; cytokine antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody *(e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4

integrin antibody such as natalizumab); or another B-cell surface antagonist/antibody; etc in the formulation. The type and effective amounts of such other agents depend, for example, on the amount of antibody present in the formulation, the type of multiple sclerosis being treated, and clinical parameters of the patients. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0392] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0393] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0394] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0395] In some embodiments, the formulation comprises one or more of the group consisting of a histidine buffer, trehalose, sucrose, and polysorbate 20. In some embodiments, the histidine buffer is a histidine-acetate buffer, pH 6.0. Examples of formulations suitable for the administration of the anti-CD20 antibody are found in Andya et al., US2006/0088523, which is incorporated by reference in its entirety with respect to formulations.

[0396] Exemplary anti-CD20 antibody formulations are described in Andya et al., US2006/0088523 and WO98/56418, which are incorporated by reference in its entirety. In some embodiments, formulation is a liquid multidose formulation comprising the anti-CD20 antibody at 40 mg/mL, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. In some embodiments, anti-

CD20 formulation of interest comprises 10mg/mL antibody in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5. In some embodiments, the anti-CD20 antibody is in an aqueous pharmaceutical formulation comprising 10-30 mM sodium acetate from about pH 4.8 to about pH 5.5, preferably at pH5.5, polysorbate as a surfactant in a an amount of about 0.01-0.1% v/v, trehalose at an amount of about 2-10% w/v, and benzyl alcohol as a preservative (U.S. 6,171,586, which is incorporated by reference in its entirety). Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801, which is incorporated by reference in its entirety. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

[0397] In some embodiments, the humanized 2H7 variants formulation is antibody at 12-14 mg/mL in 10 mM histidine, 6% sucrose, 0.02% polysorbate 20, pH 5.8. In a specific embodiment, 2H7 variants and in particular 2H7.v16 is formulated at 20mg/mL antibody in 10mM histidine sulfate, 60mg/ml sucrose., 0.2 mg/ml polysorbate 20, and Sterile Water for Injection, at pH5.8. In a specific embodiment, one IV formulation of humanized 2H7 v16 is: 30mg/ml antibody in 20mM sodium acetate, 4% trehalose dihydrate, 0.02% polysorbate 20 (Tween 20™), pH 5.3. In some embodiments, the humanized 2H7.v511 variant formulation is 15-30mg/ml antibody, preferably 20mg/mL antibody, in 10mM histidine sulfate, 60mg/ml sucrose (6%), 0.2 mg/ml polysorbate 20 (0.02%), and Sterile Water for Injection, at pH5.8. In yet another embodiment, the formulation for 2H7 variants and in particular 2H7.v511 is 20 mg/ml 2H7, 20 mM sodium acetate, 4% trehalose dihydrate, 0.02% polysorbate 20, pH 5.5, for intravenous administration. In some embodiments, 2H7.v 114 formulation is antibody at 15-25 mg/ml, preferably 20mg/ml, in 20mM Sodium Acetate, 240mM (8%) trehalose dihydrate, 0.02% Polysorbate 20, pH 5.3. In some embodiments, the anti-CD20 antibody (e.g., 2H7.v16) is in a formulation comprising 30 mg/mL antibody, 20 mM Sodium Acetate, 106 mM Trehalose, 0.02% polysorbate 20, and pH 5.3. The liquid formulation containing the antibody may be in 300 mg/vial, and may be stored at 2-8°C, protected from light. In some embodiments, prior to administration, the antibody formulation is diluted with normal saline (0.9% Sodium Chloride) in an IV bag for administration by infusion.

VII. Articles of Manufacture and Kits

[0398] The invention further provides articles of manufacture or kits (such as kits-of parts) containing materials useful for the treatment of progressive multiple sclerosis described herein. In

some embodiments, the article of manufacture comprising, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting that the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with multiple sclerosis and provides an improvement in functional ability in patients having multiple sclerosis.

[0399] In some embodiments, the article of manufacture or kit comprises, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with multiple sclerosis and suppresses disability progression in patients having multiple sclerosis.

[0400] In some embodiments, the article of manufacture or kit comprises, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with multiple sclerosis and delays onset of confirmed disability progression in patients having multiple sclerosis. In some embodiments, the confirmed disease progression is an increase in EDSS that is sustained for twelve weeks. In some embodiments, the confirmed disease progression is an increase in EDSS that is sustained for twenty-four weeks.

[0401] In some embodiments, the article of manufacture or kit comprises, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with multiple sclerosis and slows or prevents brain volume loss in patients having multiple sclerosis.

[0402] In some embodiments, the article of manufacture or kit comprises, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with highly active multiple sclerosis. In some embodiments, the label further denotes that the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with highly active multiple sclerosis that have not been previously treated with other therapy for multiple sclerosis. In some embodiments, the label further denotes that the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with highly active multiple sclerosis that have been previously treated with other therapy for multiple sclerosis. In some embodiments, the label further denotes that the anti-CD20 antibody or pharmaceutical

composition is indicated for treating patients with highly active multiple sclerosis that are inadequate responders to other therapy for multiple sclerosis.

[0403] In some embodiments, the article of manufacture or kit comprises, packaged together, a pharmaceutical composition comprising an anti-CD20 antibody and a pharmaceutically acceptable carrier and a label denoting the anti-CD20 antibody or pharmaceutical composition is indicated for treating patients with multiple sclerosis and that the anti-CD20 antibody or pharmaceutical composition is effective in one or more of the following: (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and (4) improvement of function ability.

[0404] In some embodiments of any of the articles of manufacture or kits, the multiple sclerosis is progressive multiple sclerosis. In certain embodiments, the progressive multiple sclerosis is primary progressive multiple sclerosis. In some embodiments, the multiple sclerosis is a relapsing form of multiple sclerosis. In certain embodiments, the relapsing form if multiple sclerosis is relapsing remitting multiple sclerosis. In certain embodiments, the relapsing form of multiple sclerosis is secondary progressive multiple sclerosis with superimposed relapses (rSPMS).

[0405] In some embodiments of any of the articles of manufacture or kits, the anti-CD20 antibody comprises: a) a heavy chain variable region comprising SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and b) a light chain variable region comprising SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6. In some embodiments, the anti-CD20 antibody is ocrelizumab.

[0406] In certain embodiments, the packet insert has instructions denoting (*i.e.*, indicating) that an amount of ocrelizumab is administered to the patient that is effective to provide an initial ocrelizumab exposure of between about 0.3 to about 0.6 grams followed by a second ocrelizumab exposure of between about 0.3 to about 0.6 grams, the second exposure not being administered until from about 16 to 60 weeks from the initial exposure, and each of the ocrelizumab exposures is provided to the patient as one or two doses of ocrelizumab. In some embodiments, the initial ocrelizumab exposure is about 0.6 grams. In some embodiments, the second ocrelizumab exposure is about 0.6 grams. In some embodiments, the second exposure is administered from about 20-24 weeks from the initial exposure. In some embodiments, "about 20-24 weeks" refers to a time point between 20 weeks and 24 weeks. In some embodiments, "about 20-24 weeks" refers to a variation of a week or 7 days before or after the 24th week. In some embodiments, one or more of the ocrelizumab exposures are provided to the patient as one dose of ocrelizumab. In some embodiments, one or more of the ocrelizumab exposures are provided to the patient as two

doses of ocrelizumab. In some embodiments, the two doses of ocrelizumab comprise about 0.3 grams of ocrelizumab.

[0407] In certain embodiments, the article of manufacture or kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for treating the multiple sclerosis and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the antibody. In some embodiments, the container comprises between about 0.3 to about 4.0 grams of the anti-CD20 antibody. In some embodiments, the container comprises between about 0.3 to about 1.5 grams of the anti-CD20 antibody.

[0408] The label or package insert indicates that the composition is used for treating multiple sclerosis in a patient suffering therefrom with specific guidance regarding dosing amounts and intervals of antibody and any other drug being provided. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0409] Optionally, the article of manufacture or kit provided herein further comprises a container comprising an agent other than the antibody for treatment and further comprising instructions on treating the patient with such agent, such agent preferably being a chemotherapeutic agent or immunosuppressive agent, interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug *(e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug *(e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; hormone replacement therapy; drug that treats symptoms secondary or related to MS *(e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid *(e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A;

somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody *(e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4 integrin antibody such as natalizumab); and another B-cell surface marker antibody; etc.

EXAMPLES

Example 1: Phase III Studies of Ocrelizumab in Comparison with Interferon Beta-1a (Rebif) in Patients with Relapsing Multiple Sclerosis

[0410] Multiple sclerosis is a heterogeneous disease with an unpredictable disease course and no cure (Scalfari *et al.* (2013) *JAMA Neurol.*70, 214-22; Tremlett *et al.* (2006) *Neurology*. 66, 172-7; Markowitz (2010) *Am J Manag Care.* 16, S211-8; Hauser *et al.* (2013) *Ann Neurol* . 74, 317-27). Despite various treatments for relapsing forms of multiple sclerosis becoming available in recent years, many patients continue to accrue neurologic disability, thus there remains an important unmet need for more efficacious and well-tolerated treatments (Markowitz (2010) *Am J Manag Care.* 16, S211-8; Rotstein *et al.* (2015) *JAMA Neurol.* 72, 152-8; Sorensen (2007) *J Neurol Sci.* 259,128-32). In addition, the risk profile of higher efficacy treatments has so far prevented their use early in the disease course (Markowitz (2010) *Am J Manag Care.* 16, S211-8; Hartung *et al.* (2011) *Expert Rev Neurother.* 11, 351-62; Hauser SL. (2015) *Mult Scler.* 21, 8-21).

[0411] B cells are a key contributor to the pathogenesis of multiple sclerosis (Monson (2005) *J Neuroimmunol.* 158, 170-81; Hauser SL. (2015) *Mult Scler.* 21, 8-21). B cells are rarely observed in the cerebrospinal fluid of healthy controls, but are frequently found at low percentages in the cerebrospinal fluid of patients with multiple sclerosis (Cepok (2005) *Brain.* 128, 1667-76; Cross *et al.* (2011) *Biochim Biophys Acta.* 1812, 231-8) elevated levels in the cerebrospinal fluid correlate with faster disease progression in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis (Cepok (2005) *Brain.* 128, 1667-76). B cells influence the underlying pathogenesis of multiple sclerosis via a number of functions: antigen presentation (Constant (1999) *J Immunol.* 162, 5695-703; Crawford *et al.* (2006) *J Immunol.* 176, 3498-506), autoantibody production (Bar-Or A (2010) *Ann Neurol.* 67, 452-61; Duddy (2007) *J Immunol.* 178, 6092-9), cytokine regulation (Genain *et al.* (1999) *Nat Med.* 5, 170-5; Storch *et al.* (1998) *Ann Neurol.* 43, 465-71), and formation of ectopic lymphoid follicle-like aggregates (Magliozzi *et al.* (2010) *Ann Neurol.* 68, 477-93; Serafini *et al.* (2004) *Brain Pathol.* 14:164-74. Interest in B cells has increased with proof-of-concept and observational studies and interest in their utility in multiple sclerosis has evolved over time (Hauser *et al.* (2008) *N Engl J Med.* 358, 676-88;

Kappos *et al.* (2011) *Lancet.* 378, 1779-87; Lehmann-Horn *et al.* (2013) *Ther Adv Neurol Disord.* 6, 161-73).

[0412] CD20 is a cell surface antigen found on pre-B cells, mature, and memory B cells, but it is not expressed on lymphoid stem cells and plasma cells (Stashenko *et al.* (1980) *J Immunol.* 125, 1678-85; Loken *et al.* (1987) *Blood.* 70, 1316-24; Tedder *et al.* (1994) *Immunol Today.* 15, 450-4). In the HERMES study, rituximab, an anti-CD20 chimeric monoclonal antibody, significantly reduced inflammatory brain lesions and clinical relapses compared with placebo in patients with relapsing-remitting multiple sclerosis; thus, providing evidence that selective depletion of $CD20^+$ B cells is a potentially effective treatment approach in multiple sclerosis (Kappos *et al.* (2011) *Lancet.* 378, 1779-87).

[0413] Ocrelizumab is a recombinant humanized monoclonal antibody that selectively depletes CD20-expressing B cells (Klein *et al.* (2013) *MAbs.* 5, 337-8; Genovese *et al.* (2008) *Arthritis Rheum.* 58, 2652-61) while preserving the capacity for B cell reconstitution and pre-existing humoral immunity (Martin *et al.* (2006) *Annu Rev Immunol.* 24, 467-96; DiLillo *et al.* (2008) *J Immunol.* 180, 361-71). Ocrelizumab binds to the large extracellular loop of CD20 with high affinity, selectively depleting B cells via several mechanisms including antibody-dependent cell-mediated phagocytosis, antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and induction of apoptosis (Klein *et al.* (2013) *MAbs.* 5, 22-33).

[0414] Two identical Phase 3, multicenter, randomized, double-blind, double-dummy, parallel-group trials were undertaken (STUDY I and STUDY II) to investigate the efficacy and safety of ocrelizumab compared with interferon (IFN) β-1a in patients with relapsing forms of multiple sclerosis. The results from these two studies are reported here.

Methods

Eligibility and Exclusion Criteria

[0415] Key eligibility criteria included: an age of 18 to 55 years; a diagnosis of multiple sclerosis in accordance with the 2010 revised McDonald criteria (Polman *et al.* (2011) "Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria." *Ann Neurol* 69, 292-302); an Expanded Disability Status Scale (EDSS, see world-wide-web.neurostatus.org) score of 0 to 5.5 at screening; at least two documented clinical attacks within the previous 2 years or one clinical attack occurring within the year prior to screening (but not within 30 days prior to screening); documented MRI of brain with abnormalities consistent with multiple sclerosis; neurologic stability for at least 30 days prior to both screening and baseline.

[0416] Key exclusion criteria included: a diagnosis of primary progressive multiple sclerosis; patients with a disease duration of more than 10 years in combination with an EDSS score of less than or equal to 2.0 at screening; known presence of other neurological disorders which may mimic multiple sclerosis; pregnancy or lactation; previous treatment with any B-cell targeted therapies or other contraindicated medications (i.e., requirement for chronic treatment with systemic corticosteroids or immunosuppressants during the course of the study, history of or currently active primary or secondary immunodeficiency, active infection, or history of or known presence of recurrent or chronic infection (e.g. hepatitis B or C, HIV, syphilis, tuberculosis), history of progressive multifocal leukoencephalopathy, contraindications to or intolerance of oral or intravenous corticosteroids, or contraindications to Rebif or incompatibility with Rebif use).

Study Design

[0417] Patients were randomized (1:1) to receive either ocrelizumab 600 mg by intravenous infusion every 24 weeks (administered as two 300-mg infusions on Days 1 and 15 for the first dose, and as a single 600 mg infusion on Day 1 for each 24-week treatment course thereafter), or subcutaneous IFN β-1a three times per week at a dose of 44 μg throughout the 96-week treatment period. *See* **FIG. 7**. Patients in the ocrelizumab group and the IFN β-1a group also received subcutaneous and intravenous placebos, respectively. All patients received intravenous methylprednisolone 100 mg (and optional analgesics/antipyretics and antihistamines) prior to infusion. Randomization was performed centrally by an independent provider. Patients were stratified by region (US/rest of world) and baseline EDSS score (less than 4/greater than or equal to 4).

[0418] To maintain concealment of the study-group assignments, each study center had separate treating investigators (neurologists experienced in the care of multiple sclerosis) and examining investigators (neurologists or other healthcare practitioners), all blinded throughout the course of the trial. The treating investigators had access to safety and blinded efficacy data and made treatment decisions based on the patient's clinical response and laboratory findings. The examining investigators conducted the neurologic assessments, including the EDSS scores (Kurtze (1983) "Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS)." *Neurology.* 33, 1444-52), the Functional System Scores (Kurtze (1983) "Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS)." *Neurology.* 33, 1444-52; Haber and LaRocca, eds. *Minimal Record of Disability for multiple sclerosis.* New York: National Multiple Sclerosis Society; 1985), the Multiple Sclerosis Functional Composite (MSFC) (Rudick *et al.* (2002) "The multiple sclerosis functional

composite: a new clinical outcome measure for multiple sclerosis trials." *Multiple sclerosis (Houndmills, Basingstoke, England)* 8, 359–65), Low Contrast Visual Acuity (LCVA) testing (Wieder *et al.* (2013) "Low contrast visual acuity testing is associated with cognitive performance in multiple sclerosis: a pilot study." *BMC Neurology.* 13, 167), the Symbol Digit Modalities Test (SDMT) (Smith A. (1982). *Symbol digit modalities test: Manual.* Los Angeles: Western Psychological Services), and the Karnofsky Performance Status Scale (Mor *et al.* "The Karnofsky Performance Status Scale. An examination of its reliability and validity in a research setting." *Cancer.* 53. 2002-2007). MRI assessments were analyzed independently by a central MRI reader blinded to treatment assignment.

[0419] Patients who completed the 96-week treatment period were eligible to enter the open-label extension phase of the study. Patients who discontinued prematurely or who did not want to participate in the open-label extension were entered into the 48-week safety follow-up phase, which included B cell monitoring.

Study Procedures

[0420] EDSS scores were determined at screening, baseline, and every 12 weeks; the MSFC, LCVA, and SDMT scores were determined at baseline and every 12 weeks; and Karnofsky Performance Status Scale was determined at baseline and every 24 weeks. MRI was performed at baseline and Weeks 24, 48, and 96. Adverse events were monitored throughout the study.

[0421] The primary endpoint was the annualized protocol-defined relapse rate at 96 weeks, wherein relapses were defined as new or worsening neurologic symptoms persisting for over 24 hours that are attributable to multiple sclerosis only. New or worsening neurologic symptoms must have been accompanied by objective neurologic worsening consistent with an increase of at least half a step on the EDSS, of 2 points in one EDSS functional system score, or 1 point in each of two or more EDSS functional system scores. Relapses were reclassified as protocol-defined relapses by an automated algorithm following the rules described above. The algorithm was written before database closure and unblinding of the data.

[0422] Key secondary endpoints included: time to onset of 12 week confirmed disability progression (i.e., CDP), defined in the study as an increase of at least 1.0 point from the baseline EDSS score in patients with a baseline score of 5.5 or less, or an increase of a 0.5 point in patients with a baseline score over 5.5, during the 96 weeks, wherein increases in the EDSS were confirmed at a regularly scheduled visit at least 12 weeks after the initial neurologic worsening; total number of T1 gadolinium-enhancing lesions at Weeks 24, 48, and 96; total number of new and/or enlarging T2 hyperintense lesions at Weeks 24, 48, and 96; proportion of patients who

have 12-week confirmed disability improvement (i.e., CDI) during the 96 weeks (only analyzed for the subgroup of patients with a baseline EDSS score of at least 2.0); time to onset of 24 week CDP, confirmed at 24 weeks after the initial neurologic worsening, during the 96 weeks; pharmacokinetics, immunogenicity, and pharmacodynamics of ocrelizumab; and safety and tolerability of ocrelizumab. CDI in the study is defined as a reduction in EDSS score of at least 1.0 compared to baseline in patients with a baseline EDSS score of 5.5 or less, or a reduction of a 0.5 point in patients with a baseline EDSS score above 5.5.

[0423] Proportion of patients with an EDSS score ≥2.0 who have no evidence of disease activity (NEDA) by Week 96; NEDA analysis in all patients was an exploratory endpoint. Percentage change in brain volume as detected by brain MRI from Week 24 to Week 96; analysis from baseline to Week 96 was also performed as an exploratory analysis.

Statistical Analysis

[0424] The statistical hierarchy is provided in **FIG. 8**. In order to achieve sufficient statistical power to test the effects of ocrelizumab on CDP and CDI, it was pre-specified that these endpoints would be pooled for both Phase III studies. All other endpoints were analyzed separately for each study. Primary and secondary efficacy analyses were performed on the intention-to-treat population.

[0425] All efficacy analyses were performed on the intent-to-treat population. Annualized relapse rate (ARR), the primary efficacy endpoint, was analyzed using a negative binomial model that included, for each patient, the onset between randomization data and date of early treatment-discontinuation/Week 96 in the statistical analysis to take into account the length of exposure, and treatment group, region (USA/rest of world) and baseline EDSS score (less than 4.0/greater than or equal to 4.0) as covariates. A significant result at a two-sided alpha of <0.05 would demonstrate a superior effect of ocrelizumab in reducing ARR compared with IFN β-1a.

[0426] The sample size for each of the studies was based on an estimated annualized relapse rate of 0.165 for the ocrelizumab group and 0.33 for the IFN β-1a group. Using a two-sided t-test, it was calculated that a sample of 400 patients per arm would provide 84% statistical power to maintain a type I error rate of 0.05 and detect a relative reduction of 50% on ocrelizumab compared with IFN β-1a (assuming a drop-out rate of approximately 20%). A per-protocol sensitivity analysis evaluated the effect of major protocol violations on the primary endpoint.

[0427] Ten secondary efficacy endpoints were tested in hierarchical order of decreasing clinical importance at a two-sided alpha of 0.05 (*see* **FIG. 8**; ARR = annualized relapse rate; CDI

= confirmed disability improvement; CDP = confirmed disability progression; Gd = gadolinium; MSFC = Multiple Sclerosis Functional Composite; NEDA = no evidence of disease activity; SF-36 PCS = Short-Form 36, Physical Component Summary). NEDA is defined as: no protocol-defined relapses, no CDP events, no new or enlarging T2 lesions, and no Gd-enhancing T1 lesions. Analyses of secondary efficacy endpoints at the individual study level is as follows:

[0428] • For the first secondary efficacy endpoint (the time to onset of confirmed disability progression for at least 12 weeks), the study-level p-value will be interpreted as non-confirmatory, due to inadequate statistical power at the study level to detect relevant treatment differences.

[0429] • The second secondary efficacy endpoint (total number of T1 Gd-enhancing lesions at Weeks 24, 48, and 96) will be tested in a confirmatory manner if and only if, in the analysis of both studies combined, the first secondary efficacy endpoint reaches a significance level of 0.05 (i.e., pooled analysis $p \leq 0.05$). If, in the analysis of the combined studies, the first secondary efficacy endpoint pooled analysis $p > 0.05$, then the second and subsequent secondary efficacy endpoint p-values within the hierarchy will be interpreted as non-confirmatory.

[0430] • The third secondary efficacy endpoint (total number of new and/or enlarging T2 hyperintense lesions at Weeks 24, 48, and 96) will be tested in a confirmatory manner if and only if the second secondary efficacy endpoint (total number of T1 Gd-enhancing lesions at Weeks 24, 48, and 96) reaches a significance level of 0.05 (i.e., $p \leq 0.05$). If the second secondary efficacy endpoint $p > 0.05$, then the third and subsequent secondary efficacy endpoint p-values within the hierarchy will be interpreted as non-confirmatory.

[0431] • For the fourth (proportion of patients who have confirmed disability improvement for at least 12 weeks) and fifth (time to onset of confirmed disability progression for at least 24 weeks) secondary efficacy endpoints, the study-level p-value will be interpreted as non-confirmatory, due to inadequate statistical power at the study level to detect relevant treatment differences.

[0432] • The sixth secondary efficacy endpoint (total number of new T1-hypo-intense lesions (chronic black holes) at Weeks 24, 48, and 96) will be tested in a confirmatory manner if and only if, in the analysis of the combined studies, the fifth secondary efficacy endpoint (time to onset of confirmed disability progression for at least 12 weeks) reaches a significance level of 0.05 (i.e., pooled analysis $p \leq 0.05$). If, in the analysis of both studies combined, the fifth secondary efficacy endpoint pooled analysis $p > 0.05$, then the sixth and subsequent secondary efficacy endpoint p-values within the hierarchy will be interpreted as non-confirmatory.

[0433] • The seventh (and subsequent) secondary efficacy endpoint will be tested in a confirmatory manner if and only if the sixth (or immediately previous) secondary efficacy endpoint reaches a significance level of 0.05 (i.e., $p \leq 0.05$). If the sixth (or immediately previous) secondary efficacy endpoint $p > 0.05$, then the seventh (or current) and all subsequent secondary efficacy endpoint p-values within the hierarchy will be interpreted as non-confirmatory.

[0434] Furthermore, for analyses of secondary efficacy endpoints where data from both studies are combined (so there is sufficient statistical power for all primary and secondary efficacy endpoint comparisons):

[0435] • The first secondary efficacy endpoint (the time to onset of confirmed disability progression for at least 12 weeks) will be tested in a confirmatory manner if and only if the primary efficacy endpoint (annualized protocol-defined relapse rate by 2 years), reaches a significance level of 0.05 (i.e., pooled analysis $p \leq 0.05$). If the primary efficacy endpoint pooled analysis $p > 0.05$, then all secondary efficacy endpoint pooled analysis p-values within the hierarchy will be interpreted as non-confirmatory.

[0436] • The second (and subsequent) secondary efficacy endpoint will be tested in a confirmatory manner if and only if the first (or immediately previous) secondary efficacy endpoint reaches a significance level of 0.05 (i.e., pooled analysis $p \leq 0.05$). If the first (or immediately previous) secondary efficacy endpoint pooled analysis $p > 0.05$, then the second (or current) and all subsequent secondary efficacy endpoint pooled analysis p-values within the hierarchy will be interpreted as non-confirmatory.

[0437] The safety population was used for all analyses of safety data and included all patients who have received any study treatment.

Results

Patients

[0438] Overall, 1656 patients were enrolled into the first (N=821) and second (N=835) Phase III studies (intention-to-treat population). Baseline demographics and disease characteristics were similar among the populations within each study and between the two studies (*see* **Table 3**).

Table 3. Baseline demographics and disease characteristics.

CHARACTERISTIC	STUDY I		STUDY II	
	IFN β-1a 44 µg (n=411)	Ocrelizumab 600 mg (n=410)	IFN β-1a 44 µg (n=418)	Ocrelizumab 600 mg (n=417)
Age, yr, mean (SD)	36.9 (9.3)	37.1 (9.3)	37.4 (9.0)	37.2 (9.1)
Female sex, n (%)	272 (66.2)	270 (65.9)	280 (67.0)	271 (65.0)
Time since onset, years, mean (SD)	6.3 (6.0)	6.7 (6.4)	6.7 (6.1)	6.7 (6.1)
Time since diagnosis years, mean (SD)	3.7 (3.6)	3.8 (4.8)	4.1 (5.1)	4.2 (5.0)
Relapses in previous 12 months, mean (SD)	1.3 (0.6)	1.3 (0.7)	1.3 (0.7)	1.3 (0.7)
Previously untreated, no. (%)†	292 (71.4)	301 (73.8)	314 (75.3)	304 (72.9)
Mean score on EDSS	2.8 (1.3)	2.9 (1.2)	2.8 (1.4)	2.8 (1.3)
T1 gadolinium-enhancing lesions - no. (%)				
0	252 (61.9)	233 (57.5)	243 (58.6)	252 (61.0)
1	52 (12.8)	64 (15.8)	62 (14.9)	58 (14.0)
2	30 (7.4)	30 (7.4)	38 (9.2)	33 (8.0)
3	16 (3.9)	16 (3.9)	14 (3.4)	15 (3.6)
≥4	57 (14.0)	57 (14.0)	58 (14.0)	55 (13.3)
T2 lesions – no.	51.06±39.91	51.04±39.00	51.01±35.69	49.26±38.59
Volume of T2 lesions - cm^3	9.74±11.28	10.84±13.90	10.61±12.30	10.73±14.28
Normalized brain volume – cm^3	1499.18±87.68	1500.93±84.10	1501±90.98	1503±92.63
Patients with no Gd^+ lesions, n (%)	252 (61.9)	233 (57.5)	243 (58.6)	252 (61.0)

Patients with Gd^+ lesions, n (%)	155 (38.1)	172 (42.5)	172 (41.4)	161 (39.0)
Number Gd^+ T1 lesions, mean (SD)	1.9 (5.2)	1.7 (4.2)	2.0 (4.9)	1.8 (5.0)
Number T2 lesions, mean (SD)	51.1 (39.9)	51.0 (39.0)	51.0 (35.7)	49.3 (38.6)

†Patients untreated with disease modifying therapy in the 2 years prior to study entry

[0439] A total of 366 (89%) and 340 (83%) patients in the ocrelizumab and IFN β-1a arms, respectively, completed the first study (*see* **FIG. 9**), and 360 (86%) and 320 (77%) in the ocrelizumab and IFN β-1a arms in the second study (*see* **FIG. 9**). Over 85% of the patients in the ocrelizumab arms completed STUDY I and STUDY II. All randomized patients were included in the ITT population. Patients that withdrew prematurely from the studies for any reason and patients for whom assessments were not performed for any reason were still included in the ITT (intent-to-treat) analysis.

Efficacy

[0440] The clinical and MRI outcomes from the first and second studies are summarized in **Table 4**. The frequency of relapses of multiple sclerosis was reduced by ocrelizumab in both studies, with an adjusted annualized relapse (ARR) rate at 96 weeks (primary endpoint) of 0.156 for ocrelizumab (versus 0.292 for IFN β-1a) in STUDY I and 0.155 for ocrelizumab (versus 0.290 for IFN β-1a) in STUDY II (*see* **Table 4** and **FIGs. 10A** and **10B**). Annualized relapse rates (based on protocol-defined relapses) in patients treated with ocrelizumab were reduced by 46% and 47% versus IFN β-1 in STUDY I and STUDY II, respectively ($p<0.0001$ for both comparisons). Annualized relapse rates (based on protocol-defined relapses) in patients treated with ocrelizumab were reduced by 46% versus IFN β-1 (STUDY I and STUDY II, pooled data; $p=0.0001$). Adjusted ARR was calculated by binomial regression and adjusted for baseline EDSS score (<4.0 vs. ≥ 4.0) and geographic location (US vs. rest of world). Annualized relapse rates (based on all clinical relapses) in patients treated with ocrelizumab were reduced by 42% ($p=0.001$) and 47% ($p<0.0001$) versus IFN β-1 in STUDY I and STUDY II, respectively.

*Table 4. Clinical and MRI endpoints during the 96-week study (ITT population)**

Endpoint	Pooled STUDY I and II		STUDY I		STUDY II	
	IFN β-1a 44 µg (n=829)	Ocrelizumab 600 mg (n=827)	IFN β-1a 44 µg (n=411)	Ocrelizumab 600 mg (n=410)	IFN β-1a 44 µg (n=418)	Ocrelizumab 600 mg (n=417)
Clinical endpoints						
Annualized relapse rate at 96-weeks						
Rate (95% CI)			0.292 (0.235-0.361)	0.156 (0.122-0.200)	0.290 (0.234-0.361)	0.155 (0.121-0.198)
Risk reduction vs. IFN β-1a – % (95% CI; p-value)		46 (p<0.0001)		46 (p<0.0001)		47 (p<0.0001)
Confirmed disability progression for at least 12 weeks†						
Patients with event – %	**13.6**	**9.1**				
Hazard ratio vs. IFN β-1a (95% CI)		**0.60 (0.45–0.81)**		**0.57 (0.37–0.90)**		**0.63 (0.42–0.92)**
Risk reduction vs. IFN β-1a – % (p-value)		**40 (p=0.0006)**		**43 (p=0.0139)**		**37 (p<0.0169)**
Confirmed disability progression for at least 24 weeks†						
Patients with event – %	**10.5**	**6.9**				
Hazard ratio vs. IFN β-1a (95% CI)		**0.60 (0.43–0.84)**		**0.57 (0.34–0.95)**		**0.63 (0.40–0.98)**
Risk reduction vs. IFN β-1a – % (p-value)		**40 (p=0.0025)**		**43 (p=0.0278)**		**37 (p=0.0370)**
Confirmed disability improvement for at least 12 weeks†,‡						
No. of patients evaluated	**614**	**628**				
Patients with improvement sustained for 12 weeks – %	**15.6**	**20.7**				
Relative improvement vs. IFN β-1a – % (p-value)		**33 (p=0.0194)**		**61 (p=0.0106)**		**14 (p=0.4019)**

Multiple Sclerosis Functional Composite score Mean change in MSFC score from baseline to Week 96 Difference in adjusted mean (95% CI; p-value)			0.174±0.031	0.213±0.031) 0.039±0.039 (-0.039-0.166; p = 0.3261)	0.169±0.029	0.276±0.028 0.107±0.037 (0.034-0.180; p=0040)
NEDA by Week 96[‡,§] No. of patients evaluated Patients with NEDA – % Relative improvement vs. IFN β-1a – % (p-value)			291 27.1	289 47.4 74 (p<0.0001)	270 24.1	289 43.9 81 (p<0.0001)
NEDA components by Wk 96[‡,§] – % Patients relapse free Patients free from confirmed disability progression Patients free from T1 gadolinium-enhancing lesions Patients free from new and/or enlarging T2 hyperintense lesions			66.7 87.8 69.8 38.7	80.4 92.4 91.7 61.7	64.3 84.9 63.9 38.0	78.9 89.4 90.2 60.9
MRI endpoints						
T1 gadolinium-enhancing lesions at Weeks 24, 48, and 96[¶] Patients with any T1 gadolinium-enhancing lesions Mean no. of lesions (95% CI) Risk reduction vs. IFN β-1a – % (p-value)			30.2 0.286 (0.200–0.409)	8.3 0.016 (0.0009–0.030) 94 (p<0.0001)	36.1 0.416 (0.309–0.561)	9.8 0.021 (0.012–0.036) 95 (p<0.0001)

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New and/or enlarging T2 hyperintense lesions at Weeks 24, 48, and 96¶ Patients with any new and/or enlarging T2 hyperintense lesions – % Mean no. of lesions (95% CI) Rate Ratio (95% CI) Risk reduction vs. IFN β-1a – % (p-value)			61.3 1.413 (1.123–1.777)	38.3 0.323 (0.256–0.407) 0.229 (0.174–0.300) 77 (P<0.0001)	62.0 1.904 (1.536–2.359)	39.1 0.325 (0.259–0.409) 0.171 (0.130–0.225) 83 (P<0.0001)
T1 hypointense lesions at Weeks 24, 48, and 96¶ Mean no. of lesions (95% CI) Rate Ratio (95% CI) Risk reduction vs. IFN β-1a – % (p-value)			0.982 (0.780–1.237)	0.420 (0.337–0.524) 0.428 (0.328–0.557) 57 (P<0.0001)	1.255 (1.003–1.571)	0.449 (0.357–0.560) 0.357 (0.272–0.470) 64 (P<0.0001)
Brain volume from Week 24–96 Mean change p-value Mean difference in adjusted means (95% CI)			-0.741±0.046	-0.572±0.044 P=0.0042 0.168 (0.053–0.283)	-0.750±0.051	0.638±0.049 P=0.0900 0.112 (-0.018–0.241)
Brain volume from baseline to Wk 96** Mean percentage change p-value Mean difference in adjusted means (95% CI)			-1.233±0.055	-0.943±0.054 p<0.0001 0.290 (0.148–0.432)	-1.354±0.064	-1.032±0.062 p<0.0001 0.322 (0.156–0.488)

Patient-reported outcomes						
Short-form-36 physical component summary from baseline to Week 96 Mean p-value Difference in adjusted means			45.399±0.529	45.065±0.507 P=0.2193 0.693 (-0.414–1.800)	44.552±0.544	44.307±0.541 P=0.0404 1.159 (0.051–2.269)

*Plus–minus values are means ± standard deviation

†Bold text indicates pre-specified pooled endpoints

‡In patients with baseline EDSS score at least 2.0

§NEDA is defined as: no protocol-defined relapses, no CDP events, no new or enlarging T2 lesions, and no gadolinium-enhancing lesions

Adjusted by baseline lesion count, EDSS (<4.0 / ≥4.0), and geographical region (US / ROW)

Disability

[0441] As compared with IFN β-1a, ocrelizumab reduced the risk of confirmed disability progression (i.e., CDP) that was sustained for 12 weeks by 40% over the 96-week study period (STUDY I and STUDY II, pooled data; p=0.0006; **FIG. 11**, CI = confidence interval; HR = hazard ratio). Unpooled data for STUDY I and STUDY II showing that ocrelizumab reduced the risk of confirmed disability progression (*i.e.*, CDP) that was sustained for 12 weeks as compared to IFN β-1a are shown in **FIG. 12A** (STUDY I) and **FIG. 12B** (STUDY II). Ocrelizumab also reduced the risk of CDP that was sustained for 24 weeks by 40% versus IFN β-1a over the 96-week study period (STUDY I and STUDY II, pooled data; p=0.0025; **FIG. 13**). Unpooled data for STUDY I and STUDY II showing that ocrelizumab reduced the risk of confirmed disability progression (i.e., CDP) that was sustained for 24 weeks as compared to IFN β-1a is shown in **FIG. 14A** and **FIG. 14B**. The proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 12 weeks was 20.7% in patients receiving ocrelizumab (n=628) versus 15.6% in patients receiving IFN β-1a (n=614), representing a risk improvement of 33% with ocrelizumab (STUDY I and STUDY II, pooled data; p=0.0194). *See* **FIG. 15A**. The proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement (i.e., CDI) for at least 12 weeks was 15.6% in patients receiving ocrelizumab (n=628) versus 11.6% in patients receiving IFN β-1a (n=614), representing a risk improvement of 36% with ocrelizumab (STUDY I and STUDY II, pooled data; p=0.0343). *See* **FIG. 15B**. For patients with a baseline EDSS score ≥ 2.0 and ≤ 5.5, disability improvement was defined as a reduction in EDSS score ≥ 1.0 point compared to baseline EDSS score. For patients with a baseline EDSS score > 5.5, disability improvement was defined as a reduction in EDSS score ≥ 0.5 point p value for relative improvement is from the Cochran-Mantel-Haenszel Chi-Squared test, stratified by study, baseline EDSS score (<4.0 vs ≥4.0), and geographic region (US vs rest of world), and includes stratification factors. Patients with missing EDSS or no confirmation after onset of disability improvement are counted as not having CDI.

[0442] Unpooled data for the proportion of patients (with baseline EDSS score of 2.0 or more) with confirmed disability improvement for at least 12 weeks in STUDY I and STUDY II are provided in **FIG. 16A** and **FIG. 16B**. Unpooled data for Multiple Sclerosis Functional Composite score outputs are provided in **FIGs. 16C** and **16D**. **FIGs. 16C** and **16D** include patients with

assessment at baseline and at least one post-baseline value. Estimates are from analysis based on mixed-effect model of repeated measures (MMRM) using unstructured covariance matrix: Change = Baseline MSFCS Score + Geographic Region (US vs Rest of World) + Baseline EDSS (<4.0 vs >=4.0) + Week + Treatment + Treatment*Week (repeated values over week) + Baseline MSFCS Score*Week. 15.6% of ocrelizumab-treated patients achieved CDI at 24 weeks (vs. 11.6% for IFNβ-1a-treated patients), representing a 36% relative improvement (relative risk 1.36 [p=0.0343]).

[0443] In pooled analyses, a higher proportion of ocrelizumab-treated patients had improved EDSS scores (20.2% [n=146]) as compared to patients treated with IFNβ-1a (15.0% [n=98]) (adjusted odds ratio [aOR] 1.288 [0.964, 1.72]; p=0.0866]). In pooled analyses, significantly fewer patients in the ocrelizumab group had worsened EDSS scores (10.1% [n=73] compared with IFNβ-1a (16.6% [n=109]) (aOR 0.575 [0.414-0.797], p=0.0009). See **FIG. 39**.

[0444] Worsened disability (measured as an increase in EDSS score of >0.5 at Week 96 compared with baseline) was reduced by 44% in ocrelizumab-treated patients vs. patients receiving IFN β-1a in STUDY I (p=0.0242) and by 44% in ocrelizumab-treated patients vs. patients receiving IFN β-1a in STUDY II (p=0.121). (p value for relative decrease in worsening is from multinomial logistic regression, adjusted for baseline EDSS score (<4.0 vs ≥4.0), and geographic region (US vs rest of world).) Worsened disability (measured as an increase in EDSS score of >0.5 at Week 96 compared with baseline) was reduced by 43% in ocrelizumab-treated patients vs. patients receiving IFN IFN β-1a (STUDY I and STUDY II, pooled data; p=0.0009).

MRI-related endpoints

[0445] As compared with IFN β-1a, ocrelizumab reduced the total number of T1 gadolinium-enhancing lesions at Weeks 24, 48, and 96 by 94% in STUDY I and 95% in STUDY II (p<0.0001 for both studies; *see* **Table 4** and **FIGs. 17A** and **17B**). Ocrelizumab reduced mean total number of T1 gadolinium-enhancing lesions by 91% at Week 24, by 98% at Week 48, and by 95% at week 96 compared to IFN β-1a in STUDY I (p<0.0001 for all time points, *see* **FIG. 18A**). Ocrelizumab reduced mean total number of T1 gadolinium-enhancing lesions by 92% at Week 24, by 96% at Week 48, and by 97% at week 96 compared to IFN β-1a in STUDY II (p<0.0001 for all time points, *see* **FIG. 18B**). The results in **FIGs. 17A-B and 18A-B** were adjusted by means calculated by negative binomial regression and adjusted for baseline T1 Gd lesion (present or not) baseline EDSS

(<4.0 vs. ≥ 4.0), and geographical location (US vs. rest of world). The cumulative number of T1 gadolinium-enhancing lesions was reduced by 94% in patients receiving ocrelizumab vs. patients receiving IFN β-1a (STUDY I and STUDY II, pooled data; p=0.0001).

[0446] Ocrelizumab also reduced the total number of new/enlarging T2 hyperintense lesions at Weeks 24, 48, and 96 by 77% compared with IFN β-1a in STUDY I and 83% compared with IFN β-1a in STUDY II (p<0.0001 for both comparisons; *see* **Table 4** and **FIGs. 19A** and **19B**). As shown in **FIG. 19C**, ocrelizumab reduced the mean number of new/enlarging T2 hyperintense lesions at Week 24 by 41%; at Week 48 by 94%; and by Week 96 by 98% compared with IFN β-1a in STUDY I. As shown in **FIG. 19D**, ocrelizumab reduced the mean number of new/enlarging T2 hyperintense lesions at Week 24 by 61%; at Week 48 by 96%; and by Week 97 by 97% compared with IFN β-1a in STUDY II. (Adjusted by means calculated by negative binomial regression and adjusted for baseline T2 lesion count, baseline EDSS (<4.0 vs ≥4.0) and geographical region (US vs ROW).) EDSS, Expanded Disability Status Scale; IFN, interferon; MRI, magnetic resonance imaging; ROW, rest of the world. Ocrelizumab reduced the emergence of more chronic or growing areas of MS-related brain injury (T2 hyperintense lesions) at 24, 48, and 96 week by about 80% compared to IFN β-1a (pooled STUDY I and STUDY II). The results in **FIGs. 19A-D** were adjusted by means calculated by negative binomial regression and adjusted for baseline T2 lesion count, baseline EDSS (<4.0 vs. ≥ 4.0), and geographical location (US vs. rest of world). The cumulative number of new and/or enlarging T2 lesions was reduced by 80% in patients receiving ocrelizumab vs. patients receiving IFN β-1a (STUDY I and STUDY II, pooled data; p=0.0001).

[0447] Ocrelizumab slowed brain volume loss from Week 24 to Week 96 as compared with IFN β-1a (STUDY I, p=0.0042; STUDY II, p=0.0900; **FIGs. 20A** and **20B**). Ocrelizumab reduced the rate of brain volume loss by 18.8% as compared with IFN β-1a (STUDY I and STUDY II, pooled data; p=0.0015; difference in adjusted means (0.140; 95% CI: 0.054, 0.226). Analysis based on ITT population with week 24 and at least one post-week 24 assessment; p-value based on mixed effect model repeat measurement (MMRM) at 96 week visit adjusted by study, for week 24 brain volume, geographic region and age).

[0448] Ocrelizumab slowed also brain volume loss from baseline to Week 96 as compared with IFN β-1a (STUDY I, p=<0.0001; STUDY II, p=0.0001; **FIGs. 21A** and **21B**). Endpoints in **FIGs.**

20A-B and **21A-B** were compared using the Cochran-Mantel-Haenszel test stratified by geographic region (US vs rest of world) and baseline EDSS score (<4.0 vs. Endpoints in **FIGs. 20A-B** and **21A-B** were compared using the Cochran-Mantel-Haenszel test stratified by geographic region (US vs rest of world) and baseline EDSS score (<4.0 vs. >4.0). Ocrelizumab reduced the rate of brain volume loss from Week 24 to Week 96 by 22.8% in STUDY I (p=0.0042) and 14.9% in STUDY II (p= 0.0900) compared with IFN β-1a. *See* **FIGs. 20A** and **B**. In STUDY I, brain volume loss (from baseline to week 96) in ocrelizumab-treated patients was reduced by was reduced by 23.5% vs. IFNβ-1a-treated patients (*P*<0.0001). In STUDY II, brain volume loss (from baseline to week 96) in ocrelizumab-treated patients was reduced by was reduced by 23.8% vs. IFNβ-1a-treated patients (*P*=0.0001). *See* **FIGs. 21A** and **B**.

[0449] Ocrelizumab-treated patients showed an 18.8% reduction in brain atrophy as compared to IFN β-1a-treated patients.

[0450] Ocrelizumab reduced the total number of new T1 hypointense lesions by 57% compared to IFN β-1a in STUDY I and by 64% compared to IFN β-1a in STUDY II. *See* **FIGs. 22A** and **22B**. The cumulative number of new T1 hypointense lesions was reduced by 62% in patients receiving ocrelizumab vs. patients receiving IFN β-1a (STUDY I and STUDY II, pooled data; p=0.0001). Ocrelizumab reduced the number of new T1 hypointense lesions at Week 24 by 27%; at Week 48 by 95%; and by Week 96 by 99% compared with IFN β-1a in STUDY I. Ocrelizumab reduced the number of new T1 hypointense lesions at Week 24 by 33%; at Week 48 by 95%; and by Week 97 by 96% compared with IFN β-1a in STUDY II. (The total number of new T1 hypointense lesions for all patients in the treatment group at each time point (either Week 24, 48 or 96) was divided by the total number of brain MRI scans at that time point. Adjusted by means calculated by negative binomial regression and adjusted for baseline T1 hypointense lesion count, baseline EDSS (<4.0 vs ≥4.0) and geographical region (US vs rest of world).

[0451] The efficacy endpoints for STUDY I and STUDY II are summarized in **Table 5**.

Table 5. Summary of Efficacy for STUDY I and STUDY II

	STUDY I	STUDY II	Pooled STUDIES
Endpoint	Risk reduction, p value	Risk reduction, p value	Risk reduction, p value
ARR	46%, < 0.0001	47%, < 0.0001	46%, <0.0001
Time to CDP 12 week	43%, 0.0139	37%, 0.0169	40%, 0.0006
Total T1 Gd lesions (week 24, 48, 96)	94%, <0.0001	95%, <0.0001	94%, <0.0001
Total new / enlarging T2 lesions (week 24, 48, 96)	77%, <0.0001	83%, <0.0001	80%, <0.0001
CDI 12 week	61% improvement, 0.0106	14% improvement, 0.4019	33% improvement, 0.0194
Time to CDP 24 weeks	43%, 0.0278	37%, 0.0370	40%, 0.0025
Total T1 hypointense lesions (week 24, 48, 96)	57%, <0.0001	64%, <0.0001	62%, <0.0001
Difference in mean MSFC (baseline to week 96)	p=0.3261	p=0.0040	
Difference in mean brain volume (week 24 to week 96)	p=0.0042	p=0.090	

Change in SF-36 PCS (baseline to week 96)	p=0.2193	p=0.0404	
NEDA by Week 96 in patients with EDSS ≥ 2.0	74% improvement, <0.0001	81% improvement, <0.0001	75% improvement, <0.0001

[0452] In STUDY I, 80.4% of ocrelizumab-treated patients were without relapses at 96 weeks, vs. 66.7% of IFNβ-1a-treated patients. In STUDY II, 78.9% of ocrelizumab-treated patients were without relapses at 96 weeks, vs. 64.5% of IFNβ-1a-treated patients.

[0453] In STUDY I, 92.4% of ocrelizumab-treated patients were without CDP at 96 weeks, vs. 87.8% of IFNβ-1a-treated patients. In STUDY II, 89.4% of ocrelizumab-treated patients were without CDP at 96 weeks, vs. 84.9% of IFNβ-1a-treated patients.

[0454] In STUDY I, 92.3% of ocrelizumab-treated patients had improved/stable disability vs. 86.1% IFNβ-1a-treated patients. In STUDY II, 87.5% of ocrelizumab-treated patients had improved/stable disability vs. 80.4% IFNβ-1a-treated patients.

[0455] In STUDY I, 7.7% OCR-treated patients had worsened disability vs 13.9% IFNβ-1a treated patients (adjusted odds ratio 0.559; p=0.0242). In STUDY II, 12.5% OCR-treated patients had worsened disability vs 19.6% IFNβ-1a treated patients (adjusted odds ratio 0.577; p=0.0121).

[0456] In STUDY I, 91.7% of ocrelizumab-treated patients were without gadolinium-enhancing T1 lesions at 96 weeks, vs. 69.8% of IFNβ-1a-treated patients. In STUDY II, 90.2% of ocrelizumab -treated patients were without gadolinium-enhancing T1 lesions at 96 weeks, vs. 63.9% of IFNβ-1a-treated patients.

[0457] In STUDY I, 61.7% of ocrelizumab-treated patients were without new/enlarging T2 lesions at 96 weeks, vs. 38.7% of IFNβ-1a-treated patients. In STUDY II, 60.9% of ocrelizumab-treated patients were without new/enlarging T2 lesions at 96 weeks, vs. 38.0% of IFNβ-1a-treated patients.

[0458] After week 24, ≥96.0% of all OCR-treated patients were without new/enlarging T2 lesions, compared with 60.8–70.9% of IFNβ1-a-treated patients.

[0459] More patients receiving ocrelizumab reported improvement in change in quality of life, as measured by Short Form-36 (SF-36) Physical Component Summary (PCS) than patients receiving IFNβ-1a. See **FIG. 38**.

Safety

Adverse events

[0460] The overall incidence of adverse events was 83.3% and 83.3% for patients treated with IFN β-1a and ocrelizumab, respectively, in STUDY I and STUDY II (pooled). (*See* **Table 6A**).

Table 6A. Adverse events (safety population).

n (%)	POOLED STUDY I AND STUDY II	
	IFN β-1a 44 µg (n=826)	**Ocrelizumab 600 mg (n=825)**
Total number of patients with ≥ 1 adverse event (AE)	**688 (83.3)**	**687 (83.3)**
Total number of patients with ≥ 1 AE occurring at relative frequency ≥ 5%	**539 (65.3)**	**544 (65.9)**
Injury, Poisoning, and Procedural Complications	**155 (18.8)**	**333 (40.4)**
Infusion-related reaction	80(9.7)	283 (34.3)
General Disorders and Administration-Site Conditions	**396 (47.9)**	**173 (21.0)**
Influenza-like illness	177 (21.4)	38 (4.6)
Injection-site erythema	127 (15.4)	1 (0.1)
Fatigue	64 (7.7)	64 (7.8)
Injection-site reaction	45 (5.4)	2 (0.2)
Infections and Infestations	**433 (52.4)**	**482 (58.4)**
Upper respiratory tract infection	87 (10.5)	125 (15.2)
Nasopharyngitis	84 (10.2)	122 (14.8)
Urinary tract infection	100 (12.1)	96 (11.6)
Sinusitis	45 (5.4)	46 (5.6)
Bronchitis	29 (3.5)	42 (5.1)
Nervous system disorders	**252 (30.5)**	**224 (27.2)**
Headache	124 (15.0)	93 (11.3)
Psychiatric disorders	**144 (17.4)**	**149 (18.1)**
Depression	54. (6.5)	64 (7.8)
Insomnia	38 (4.6)	46 (5.6)
Musculoskeletal and connective tissue disorders	**207 (25.1)**	**204 (24.7)**
Back pain	37 (4.5)	53 (6.4)
Arthralgia	51 (6.2)	46 (5.6)

Table 6A includes only pooled AEs occurring in ≥5% of patients in at least one treatment group and the corresponding system organ class.

Table 6B. Total Serious Adverse Events

n (%)	IFN β-1a 44μg (n = 826)	Ocrelizumab 600 mg (n = 825)
Overall patients with ≥ SAE	72 (8.7)	57 (6.9)
Infections and infestations	24 (2.9)	11 (1.3)
Nervous system disorders	11 (1.3)	8 (1.0)
Injury, poisoning, and procedural complications	10 (1.2)	6 (0.7)

[0461] Serious adverse events were reported in 8.7% of IFN β-1a–treated patients and 6.9% of ocrelizumab-treated patients in STUDY I and STUDY II (pooled). (*See* **Table 6B**).

Infections

[0462] The incidence of infection was 52.8% with IFN β-1a and 56.6% with ocrelizumab in STUDY I and 52.0% with IFN β-1a and 60.2% with ocrelizumab in STUDY II. The most common infections (reported in at least 10% of patients in at least one treatment arm) were urinary tract infections, upper respiratory tract infections, and nasopharyngitis. The incidence of serious infection in both studies was low (1.2 to 2.9% across all treatment arms in both studies). No serious opportunistic infections were reported in any group during the 96-week study. Overall, herpes-virus infections were reported in 28 patients treated with IFN β-1a and 50 patients treated with ocrelizumab. All cases were mild or moderate with the exception of one case of herpes simplex in a patient treated with ocrelizumab in STUDY I, graded as severe.

Infusion-related reactions

[0463] The number of patients with at least one infusion-related reaction (IRR) was higher in patients treated with ocrelizumab versus IFN β-1a (30.9% for ocrelizumab versus 7.3% for IFN β-1a in STUDY I; 37.6% for ocrelizumab versus 12.0% for IFN β-1a in STUDY II; 34.3% for ocrelizumab versus 9.7% for IFN β-1 in both STUDY I and STUDY II (pooled)). One OCR-treated patient had a serious IRR at first infusion, with life-threatening bronchospasm; despite event resolution, subsequent treatment was withdrawn per protocol. Withdrawal from treatment due to

IRRs during the first infusion occurred in 11 patients (1.3%) in the ocrelizumab group only. IRR incidence with ocrelizumab-treated patients was highest with the first infusion (27.5%) and markedly decreased with subsequent dosing (13.7% at Dose 2). The majority of infusion-related reactions were Grade 1 or 2 and were reported at the first infusion of dose one (*see* **FIGs. 23A** and **23B**, pooled data for STUDY I and STUDY II). Numbers in columns represent the proportion of patients experiencing a grade of IRR. Grading per Common Terminology Criteria (CTCAE): **Grade 1** Mild; asymptomatic or mild symptoms; **Grade 2** Moderate; minimal, local or noninvasive intervention indicated; **Grade 3** Severe or medically significant but not immediately life-threatening; **Grade 4** Life-threatening consequences; urgent intervention indicated; **Grade 5** Death related to AE. Note: All received 100-mg i.v. methylprednisolone. Over 96 weeks, 9.7% of IFNβ-1a patients (n=80/826) and 34.3% of ocrelizumab-treated patients (n=283/825) had at least 1 IRR; most were mild to moderate in severity (99% [n=79] and 93% [n=262], respectively). Most IRR symptoms with ocrelizumab-treated included pruritus, rash, throat irritation and flushing. IRRs mostly occurred during infusion (IFNβ-1a, 46.3%; ocrelizumab, 80.6%) and were managed with infusion adjustments and symptomatic treatment (42.5% of patients that had IRRs in IFNβ-1a group and 65.4% in the ocrelizumab group received treatment).

Malignancies

[0464] In total, two malignancies were reported with IFN β-1a (one mantle cell carcinoma and one squamous cell carcinoma), and four malignancies were reported in patients treated with ocrelizumab (two cases of invasive breast ductal breast carcinoma, one renal cell carcinoma, and one malignant melanoma).

Laboratory assessments

[0465] In laboratory assessments, by Week 2, mean CD19 levels had decreased to negligible in patients treated with ocrelizumab. There was no impact on CD3-, CD4, CD8-, CD16-, and CD56-positive cells, supporting that innate immunity and total T-cell numbers are not affected by ocrelizumab. There was no impact of ocrelizumab on existing antibody titers for mumps, rubella, varicella, and pneumococcus.

[0466] While only a direct comparison of ocrelizumab to IFN β-1a was measured in STUDY I and STUDY II, these efficacy and safety results suggest the benefit/risk profile of ocrelizumab over a 2

year period is superior to all disease-modifying therapies (DMTs) available for the treatment of RMS patients.

Effects of ocrelizumab on humoral immunity markers

[0467] Prior to study enrollment, physicians were advised to review patient immunization status and follow local guidance for vaccination; immunizations were to be completed ≥6 weeks prior to treatment. Measurements of antibody (Ab) titers against mumps, rubella, varicella, and *Streptococcus pneumoniae* were taken at baseline and at Weeks 12, 24, 48, 72, and 96. The proportion of patients with Ab levels that could be considered protective was assessed for each treatment group over time.

[0468] **Mumps:** 94.1% of IFNβ-1a-treated patients and 93.6% of ocrelizumab-treated patients had positive levels of mumps Ab at baseline. (This proportion ranged (min–max) 92.7–94.8% (IFNβ-1a) and 91.8–93.5% (ocrelizumab) over the six measurements taken during the 96-week study treatment period.)

[0469] **Rubella:** 87.9% of IFNβ-1a- treated patients and 89.0% of ocrelizumab-treated patients had positive levels of rubella Ab, and ranged (min–max) 89.8–90.8% (IFNβ-1a) and 88.7–89.4% (ocrelizumab) over the six measurements taken during the treatment period.

[0470] **Varicella:** 95.5% of both treatment groups had positive levels of varicella Ab at baseline, and ranged (min–max) 96.2–97.5% (IFNβ-1a) and 94.8–95.6% (ocrelizumab) over the six measurements taken during the treatment period.

[0471] ***S. pneumonia:*** Among the evaluable patients, the mean (standard deviation) level of *S. pneumonia* Ab was 53.67 (54.13) mg/mL for IFNβ-1a-treated patients and 55.35 (67.00) mg/mL for ocrelizumab-treated patients at baseline. At week 96, the mean (standard deviation) level of *S. pneumonia* Ab was 51.74 (42.50) mg/mL for IFNβ-1a-treated patients and 54.06 (80.98) mg/mL for ocrelizumab-treated patients. At 96 weeks the mean change from baseline was -1.13 (40.25) mg/mL for IFNβ-1a-treated patients and -1.99 (59.60) mg/mL for ocrelizumab-treated patients.

Effects of ocrelizumab on immune responses

[0472] Infections can lead to MS disease exacerbation and may cause treatment complications with currently employed therapies. Accordingly, vaccinations against infections are a part of the management of patients with MS. This study evaluates whether ocerlizumab-treated patients can

mount protective immune responses against clinically relevant vaccines. This study uses the following vaccines to evaluate different immune response pathways:

a) Tetanus toxoid (TT)-containing vaccination to assess the T-cell dependent anamnestic humoral response;

b) 23-valent pneumococcal polysaccharide vaccine (23-PPV) to assess a mostly T-cell independent or pure B-cell humoral response;

c) Keyhole limpet haemocyanin (KLH) to explore the B cell dependent immune response to neo-antigen;

d) Booster 13-valent conjugate pneumococcal vaccine (13-PCV) to assess the clinical efficacy of the 23-PPV vaccine followed by the booster 13-PCV compared to 23-PPV vaccine alone;

e) Influenza vaccine to test the ability to mount a humoral response to a clinically relevant vaccine.

[0473] In a Phase III, open-label Study to evaluate the effect of ocrelizumab on immune responses in patients with relapsing Multiple Sclerosis, approximately 100 patients are randomized (2:1) to receive ocrelizumab 600mg as two 300mg intravenous infusions on Day 1 and Day 15. **Group A** patients receive ocrelizumab prior to immunization. **Group B** patients remain treatment-naive/continue with interferon treatment during immunization.

[0474] **Group A** patients are immunized $\geq$ 85 days/ 12 weeks after the first ocrelizumab administration. Briefly, **Group A** are immunized with tetanus toxoid (TT)–containing adsorbed vaccine at Week 12, with 23-valent pneumococcal polysaccharide vaccine (23-PPV) at Week 16, and with keyhole limpet hemocyanin (KLH) at Weeks 12, 16, and 20. **Group A** patients are further subdivided to either receive:

- booster 13-pneumococcal conjugate vaccine (13-PCV; **Group A1**); or
- influenza vaccine (**Group A2**).

[0475] **Group B** patients are immunized with TT on Day 1, with 23-PPV on Day 28, with (KLH) on Days 1, 28, and 56, and with influenza vaccine during Weeks 1-12.

[0476] The key inclusion criteria for this study are: RMS diagnosis (2010 revised McDonald criteria, Polman *et al. Ann Neurol* 2011;69:292–302); age between 18–55 years; receipt of ≥1 previous immunization against TT or tetanus and diphtheria (DT/Td), or tetanus, diphtheria, and acellular pertussis (DTaP/Tdap); EDSS score of 0.0–5.5. The key exclusion criteria are: known hypersensitivity to any component of the TT-containing adsorbed vaccine, pneumococcal polysaccharide/conjugate vaccine, or influenza vaccine; receipt of any PPV <5 years prior to screening or a live vaccine <6 weeks prior to randomization; previous exposure to KLH or immunization with any tetanus-containing vaccine <2 years prior to screening.

[0477] All groups undergo post-immunization assessments until the end of the study period (**Group A:** Day 169/Week 24; **Group B:** Day 84/Week 12). Key eligibility criteria include at least 1 previous immunization against TT, tetanus or diphtheria; or tetanus, diphtheria and acellular pertussis. The primary outcome measure compares the proportion of patients in Groups A1 and A2 with a positive TT response (IgG) 8 weeks post-immunization with patients in Group B. A positive response to the booster immunisation is defined as an antibody titre ≥ 0.2 IU/mL (pre-immunisation titres <0.1 IU/mL) or a 4-fold increase in antibody titre (pre-immunisation titres ≥ 0.1 IU/mL).

Key secondary outcome measures are listed below:

TT response

- The proportion of patients treated with ocrelizumab (Groups A1 and A2) vs. the proportion of patients not treated with ocrelizumab (Group B) with a positive response (IgG) to TT vaccine at 4 weeks post-immunization;
- The proportion of patients treated with ocrelizumab (Groups A1 and A2) vs. the proportion of patients not treated with ocrelizumab (Group B) with a 2-fold increase in tetanus antibody titres (pre-immunisation titres ≥0.1 IU/mL), or with tetanus antibody titres ≥0.2 IU/mL (pre-immunisation titres <0.1 IU/mL) at 4 weeks post-immunisation;
- Mean levels of anti-tetanus antibody in patients treated with OCR (Groups A1 and A2) and not treated with OCR (Group B) measured immediately prior to and 4 weeks after a booster vaccine

23 PPV

- The proportion of patients treated with ocrelizumab (Groups A1 and A2) vs. the proportion of patients not treated with ocrelizumab (Group B) with a positive response against individual anti-pneumococcal antibody serotype at 4 weeks post–23-PPV. A positive response is defined as developing a 2-fold increase in level or a >1μg/mL rise in level compared with pre-immunisation levels.

KLH

- Mean levels of anti-KLH antibody (IgG) in patients treated with ocrelizumab (Groups A1 and A2) vs. the proportion of patients not treated with ocrelizumab (Group B) measured immediately prior to the first administration and 4 weeks after the last administration of KLH.

Pneumococcal conjugate booster response in Groups A1 and B

- The proportion of patients treated with ocrelizumab (Group A1) in Group A1 vs. the proportion of patients not treated with ocrelizumab (Group B) with positive responses against an individual anti-pneumococcal antibody serotype (23 serotypes) measured 4 weeks after the booster 13-PCV vaccine. A positive response is defined as developing a 2-fold increase in level or a >1μg/mL rise in level compared with pre-immunisation levels.

Influenza vaccine response

- The proportion of patients treated with ocrelizumab (Group A2) who achieve seroprotection (specific haemagglutination inhibition titres >1:40) at 4 weeks post-immunisation compared with patients not treated with ocrelizumab (Group B).

Immunophenotyping outcome measures

- Measures of humoral and cellular immunity including: (a) total B cells ($CD19^+$); (b) B-cell subsets (memory; naive; plasma); (c) total T cells ($CD3^+$); (d) T helper cells ($CD3^+CD4^+$); (e) cytotoxic T lymphocytes ($CD3^+CD8^+$); and (f) natural killer cells ($CD3^-CD16/56^+$).

Open-Label Extension Phase

[0478] Following completion of the double-blind, controlled treatment phases STUDY I and STUDY II, patients may be eligible to enter open-label extension (OLE) phases to evaluate long-term safety, tolerability, and efficacy of ocrelizumab. The design of the open-label STUDY I and STUDY II extension studies, which evaluates the long-term safety and efficacy of OCR in relapsing MS, is described below.

[0479] Patients entering the OLE phase enter the OLE screening phase, lasting up to 4 weeks. During OLE screening, patients receive IFN β-1A or IFN β-1A placebo until first infusion of dose 5. The OLE phase lasts until ocrelizumab is commercially available in the patient's country, as per local regulations or until the sponsor decides to terminate the ocrelizumab MS program. The OLE phase does not exceed 4 years (208 weeks). OLE is not mandatory. Patients unwilling to proceed to the OLE phase are entered into the safety follow-up phase, which is performed in 12-week intervals starting from the date of the patient's latest visit. Continued monitoring occurs if B cells are not repleted.

[0480] Key inclusion criteria for the OLE phase include: completion of the blinded treatment period; consent from investigators who determine that the patient may benefit from OCR treatment based on the assessments performed during the treatment period; written informed consent from the patient to enter the OLE phase; and meeting ocrelizumab treatment/retreatment criteria, in which the patient is free from the following conditions and laboratory abnormalities: (a) life-threatening (CTCAE Grade 4) infusion-related event that occurred during a previous OCR infusion; (b) any significant or uncontrolled medical condition or treatment-emergent , clinically significant laboratory abnormality; (c) active infection; (d) absolute neutrophil count $<1.5 \times 10^3/\mu L$; (e) CD4 cell count $<250/\mu L$; and (f) hypogammaglobulinemia immunoglobulin G <3.3 g/L.

[0481] The schedule of key safety and efficacy assessments are shown in **Table 6C** below.

Table 6C

	OLE phase											Delayed dosing visit[a]	Unscheduled visit[b]	Withdrawal from treatment
Cycle	**5**			**6**		**7**		**8**		**N**				
Visit	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**...**	**...**			

OLE week	0	2 (±2)	12 (±7)	22 (±7)	24 (±5)	46 (±7)	48 (±5)	70 (±7)	72 (±5)	n–2 wk (±7)	n (±7)			
Neurological exam[c]	x		x		x		x		x		x		x	x
EDSS[d]	x		x		x		x		x		x		x	x
Potential relapses[e]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
EQ-5D[f]	x					x					(x)			
MRI[g]						x					(x)			
Routine safety lab[h]	x		x	x		x		x		x			x	x
Concomitant treatments[i]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Antibody titers[j]	x		x	x		x		x		x				x

[a]A delayed dosing visit is performed when dosing cannot be administered at the scheduled dosing visit. At this time, other tests/assessments may be performed as appropriate.

[b]During an unscheduled visit (non-dosing) assessments are performed according to the clinical needs of the patient. At this time, other tests/assessments may be performed as appropriate.

[c]Neurological examination is performed at every planned and unscheduled visit. All patients with new neurological symptoms suggestive of MS worsening or of a relapse should have EDSS performed by examining investigator.

[d]EDSS score is performed in all patients by the examining investigator every 12 weeks. Additional EDSS assessments may be performed between visits (i.e. during an MS relapse, neurological worsening, etc). Disability progression is defined as a $\geq$1.0 point (where baseline EDSS is $\leq$5.5) or $\geq$0.5 point (where baseline EDSS is >5.5) increase from the baseline EDSS score that is not attributable to another etiology.

[e]Patients is evaluated for relapses by the treating investigator at each scheduled visit and, if necessary, at unscheduled visits to confirm relapses occurring between the visits. If a relapse is suspected, the EDSS should also be performed in addition to completing the MS relapse electronic case report form.

[f]The EQ-5D (EuroQol five dimensions questionnaire) assessments is performed annually.

[g]Annual brain MRI scans is performed at the start of the OLE phase (if none were taken in the previous 12 weeks), within (±) 4 weeks of scheduled visits and at OLE withdrawal visits (if none were taken in the previous 4 weeks).

[h]Routine safety laboratory assessments include hematology, chemistry, and urinalysis.

[i]Concomitant medications and procedures is reported at each scheduled and unscheduled visit.

[j]Antibody titers against common antigens (mumps, rubella, varicella, and *Streptococcus pneumoniae*) is performed.

Summary

[0482] STUDY I and STUDY II showed that in patients with relapsing forms of MS, ocrelizumab significantly reduced the annualized relapse rate and the risk of 12- and 24-week confirmed disability progression compared with IFN β-1a. Ocrelizumab is the first treatment for multiple sclerosis to significantly reduce both 12- and 24-week confirmed disability progression in two separate Phase III studies and against an active comparator, demonstrating a consistency of effect. The proportion of patients achieving confirmed disability improvement for at least 12 weeks was also significantly increased in patients receiving ocrelizumab compared with IFN β-1a. These clinical findings are supported by the MRI endpoints whereby ocrelizumab significantly reduced the number of T1 gadolinium-enhancing and new/enlarging T2 lesions in the brain compared with IFN β-1a. The effect on T1 gadolinium-enhancing and new/enlarging T2 lesions is of such magnitude that it suggests focal inflammation and disease activity is largely stopped/suspended.

[0483] In exploratory analyses, ocrelizumab reduced brain volume loss (see **FIGs. 21A-D**) as compared to IFN β-1a. In further exploratory analyses, ocrelizumab increased the proportion of patients with no evidence of disease activity (NEDA) in the ITT population with an EDSS $\geq$ 2.0 (see **Tables 4** and **5**) as compared to IFN β-1a. Ocrelizumab increased the proportion of patients with no evidence of disease activity (NEDA) in all patients in the ITT population as compared to IFN β-1a (see **FIGs. 34A** and **34B**). In **FIGs. 34A** and **34B**, the proportion of patients with NEDA was compared using the Cochran–Mantel–Haenszel X2 test stratified by geographic region (United States vs rest of world) and baseline EDSS score (<4.0 vs. ≥4.0).

[0484] These efficacy results, which demonstrate reduced worsening of disability and in some patients increased functioning, support the hypothesis that ocrelizumab has effects on markers of inflammation and neuronal damage. The efficacy demonstrated by ocrelizumab on both inflammatory as well as degenerative outcomes suggests that treatment with ocrelizumab has potent anti-inflammatory effects and may also have neuroprotective effects.

[0485] Benefits with ocrelizumab in STUDY I and STUDY II were observed early and were sustained over the 96-week treatment period; based on earlier experience, the risk of rebound is not expected upon treatment discontinuation (Kappos *et al.* (2012) "Long-term safety and efficacy of ocrelizumab in patients with relapsing-remitting multiple sclerosis: Week 144 results of a phase II,

randomized, multicentre trial." *28th Congress of the European Committee for Treatment and Research in Multiple Sclerosis*).

[0486] Adverse events observed in patients receiving ocrelizumab were consistent with those reported in the Phase II study of ocrelizumab (Kappos *et al.* (2011) *Lancet.* 378, 1779-87). There were no cases of serious opportunistic infections reported during the 96-week studies. The results to date suggest ocrelizumab has no apparent impact on immune surveillance. Overall, ocrelizumab had a similar safety profile compared with IFN β-1a over 96 weeks in both STUDY I and STUDY II.

[0487] As a class of drugs, $CD20^+$ targeted treatments have a low risk of progressive multifocal leukoencephalopathy (<1 in 25,000) (Clifford *et al.* (2011) *Arch Neurol.* 68, 1156-64). No cases of progressive multifocal leukoencephalopathy have been reported in the STUDY studies to date.

[0488] As expected, infusion-related reactions were more commonly seen with ocrelizumab. Consistent with the Phase II trial with the 600 mg dose of ocrelizumab, these reactions were largely observed with first dose and decreased with subsequent doses (Hauser *et al.* (2008) *N Engl J Med.* 358, 676-88). This supports the rationale for splitting the first 600 mg dose into two separate infusions of 300 mg separated by approximately two weeks. Infusion-related reactions were manageable with pre-medication, infusion adjustments and symptomatic treatment.

[0489] The clinical and MRI data in aggregate suggest that ocrelizumab is at least as effective as any other tested treatment for multiple sclerosis, including those which are considered to be high-efficacy therapies. While only a direct comparison to IFN β-1a was measured in STUDY I and STUDY II, these efficacy and safety results suggest the benefit / risk profile of ocrelizumab over a 2 year period is superior to all disease modifying therapies available for the treatment of RMS patients. The high efficacy is not associated with an increase in safety risk compared with IFN β-1a. Other benefits include the early start of treatment benefit and no evidence of rebound upon discontinuation of ocrelizumab.

[0490] Data obtained from STUDY I and STUDY II show that targeting $CD20^+$ B cells may be effective in treatment of relapsing MS.

Subgroup Analysis

[0491] To evaluate the relative benefit/risk with varying degrees of disease activity and prior response to treatment before study entry, four additional subgroups were identified:

[0492] **Active treatment naïve;** defined with no prior treatment experiencing at least 2 relapses in the previous 2 year, and at least 1 relapse in the last year prior to randomization.

[0493] **Active inadequate responders;** defined as patients previously treated with IFN β-1a or glatiramer acetate for at least 1 year, and either (a) experienced at least one relapse in the previous year or (b) experienced at least 1 baseline gadolinium-enhancing lesion

[0494] **Highly active treatment naïve patients;** defined as patients naïve to treatment who have experienced at least 2 relapses in the last year prior to randomization, and either (a) at least 1 baseline gadolinium lesion or (b) increase in T2 lesion count at baseline visit (changing categorically from 0-5 to 6-9 lesions or from 6-9 lesions to > 9 lesions), as compared to a prior MRI.

[0495] **Highly active inadequate responders;** defined as patients previously treated with interferon or glatiramer acetate for at least 1 year, had at least one relapse in the previous year, and either (a) have at least nine T2-lesions or (b) have at least one Gd lesion at baseline.

[0496] STUDY I and STUDY II were pooled to increase the power to detect differences across the 4 subgroups. There were at least 100 patients in each subgroup to enable adequate detection of treatment differences.

[0497] Annualized relapse rate per arm/group was interrogated to determine whether trends in inadequate responders were driven by ocrelizumab IFN β-1a absolute outcomes. Overall, ocrelizumab showed a significant effect in reducing the annualized relapse rate in all subgroups compared with IFN β-1a (*see* **FIG. 43**). Inadequate responders in the interferon arm had a higher ARR than the ITT group, whereas ocrelizumab patients in the same group had a lower ARR than the ITT group. The active treatment naïve group had a higher ARR in both interferon and ocrelizumab groups.

[0498] Across all 4 subgroups, ocrelizumab had a greater treatment effect compared with IFN β-1a for ARR, CDP for at least 12 weeks, CDP for at least 24 weeks, CDI, and MRI (see **FIGs. 43-47** above). For ARR and MRI the difference was statistically significant across all 4 subgroups. For

CDP for at least 12 weeks, there were statistically significant effects in both active and highly active inadequate responders demonstrating ocrelizumab has consistent treatment effects compared with IFN β-1a. For CDP for at least 24 weeks, these groups demonstrated trends towards significance (p=0.075 and p = 0.082 respectively). When comparing on objective measures like T1 Gd-enhancing lesions (indicative of acute inflammatory effects, consistent with the mechanism of action for OCR), the robust and consistent effects of ocrelizumab compared with IFN β-1a is evident across all subgroups

[0499] Similarly, analysis of safety outcomes for the 4 subgroups was undertaken, and compared to the overall safety population. Due to the comparatively small size of the groups, comparison between the groups has been limited in order to not draw inappropriate conclusions based on small numbers of events. Safety outcomes in the 4 subgroups were consistent with each other, and similar with the overall population ("safety population", defined as any patient with any exposure to OCR), with in general a low proportion of patients who experienced any AE, SAEs and AEs leading to withdrawal. Consistent with observations in the safety population, there were more infections in the ocrelizumab group compared with the interferon group, whereas the proportion of patients experiencing serious infections in the ocrelizumab arm was similar to or lower than interferon in all subgroups.

[0500] Overall ocrelizumab efficacy and safety outcomes were comparable to the ITT/safety population in every subgroup. Ocrelizumab has thus demonstrated a favorable benefit/risk profile in all subgroups. The consistency of data across subgroups further supports the observed effects in the total population.

Example 2: A Phase III Study of Ocrelizumab in Patients with Primary Progressive Multiple Sclerosis

[0501] A randomized, parallel group, double-blind, placebo controlled study was performed to evaluate the efficacy and safety of ocrelizumab compared with placebo in patients with primary progressive multiple sclerosis.

[0502] Clinical trials in progressive forms of MS are typically > 2 years in duration due to the heterogeneity in disease progression rates, which have at times been historically lower than anticipated resulting in inability to show a treatment effect. This study had an event-driven design,

meaning that if the projected number of confirmed disability progression events were not reached by week 120 due to slow disease progression, the treatment period was been extended until sufficient progression events have occurred to maintain statistical power to detect a treatment difference. As a result, the blinded treatment period was a minimum of 120 weeks from the last patient enrolled; the average treatment period was 3.5 years. Most patients continue to be observed for a 3- to 4-year period in the blinded treatment phase.

Methods

Eligibility and Exclusion Criteria

[0503] Key eligibility criteria included: an age of 18 to 55 years; a diagnosis of Primary Progressive Multiple Sclerosis in accordance with the 2005 revised McDonald criteria (Polman *et al.* (2011) "Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald criteria.'" *Ann Neurol* 58, 840-846); an Expanded Disability Status Scale (EDSS) score of 3 to 6.5 points at screening; a score of at least 2.0 on the pyramidal functions component of the Functional Systems Scale (FSS) documented history or presence at screening of elevated IgG index in a cerebrospinal fluid (CSF) specimen and/or one or more IgG oligoclonal bands detected by isoelectric focusing in a cerebrospinal fluid (CSF) specimen; no history of relapse-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) or progressive relapsing multiple sclerosis (PRMS); disease duration from the onset of MS symptoms of (a) less than 15 years in patients with an EDSS at screening > 5.0 or (b) less than 10 years in patients with an EDSS at screening $\leq$ 5.0; and no treatment with other multiple sclerosis disease modifying treatments at screening.

[0504] Key exclusion criteria included: a history of relapsing remitting multiple sclerosis, secondary progressive, or progressive relapsing multiple sclerosis at screening; contraindications for Magnetic Resonance Imaging (MRI); known presence of other neurologic disorders; known active infection or history of or presence of recurrent or chronic infection; history of cancer, including solid tumors and hematological malignancies (except for basal cell, *in situ* squamous cell carcinomas of the skin and in situ carcinoma of the cervix that have been excised and resolved); previous treatment with B-cell targeted therapies (*e.g.* rituximab, ocrelizumab, atacicept, belimumab, or ofatumumab); any previous treatment with lymphocyte trafficking blockers (e.g. natalizumab, FTY720), any previous treatment with alemtuzumab (CAMPATH®, LEMTRADA™), anti-CD4, cladribine,

cyclophosphamide, mitoxantrone, azathioprine, mycophenolate mofetil, cyclosporine, methotrexate, total body irradiation, or bone marrow transplantation; treatment with β interferons, glatiramer acetate, i.v., immunoglobulin, plasmapheresis, or other immunomodulatory therapies within 12 weeks prior to randomization; systemic corticosteroid therapy within 4 weeks prior to screening; and any concomitant disease that may require chronic treatment with systemic corticosteroids or immunosuppressants during the course of the study. The screening period may be extended (but cannot exceed 8 weeks) for patients who have used systemic corticosteroids for their MS before screening. For a patient to be eligible, systemic corticosteroids should not have been administered between screening and baseline.

Study Design

[0505] Patients were randomized 2:1 to receive either to receive either ocrelizumab (300 mg intravenously, 2 infusions separated by 14 days in each treatment cycle (i.e., exposure) or placebo. The patients were stratified by age (≤ 45 vs. > 45) and region (United States vs. rest of the world).

[0506] The study consisted of the following periods (summarized in **FIG. 24**):

[0507] Screening for up to 4 weeks.

[0508] **Blinded Treatment Period:** All patients underwent at least 120 weeks of study treatment representing five treatment cycles, each cycle 24 weeks in duration. As patients were recruited over a 12–18 month period, this blinded treatment period extended up to 3.5-4 years for the first group of patients enrolled into the study. As shown in **FIG. 24**, Ocrelizumab doses were administered as dual intravenous infusions of 300 mg x 2 separated by 14 days. Thirty minutes prior to the start of each ocrelizumab or placebo infusion, patients received 100 mg methylprednisolone intravenously. Preinfusion treatment with an oral analgesic/antipyretic (e.g., acetaminophen), and an oral antihistamine (e.g., diphenhydramine) was also recommended. The blinded treatment period was designed to end when approximately 253 events were reached, based on the original sample size assumptions. If the number of events had not been reached by week 120, the study would continue until the target number of events had been reached.

[0509] **Open-label Treatment Period:** Following the primary unblinding, patients who can benefit from further treatment receive open-label ocrelizumab. Patients randomized to the

ocrelizumab group continue open-label treatment with a dual ocrelizumab infusion (300 mg i.v. infusions administered 14 days apart) every 24 weeks. Patients randomized to placebo receive a dual ocrelizumab infusion for the first open-label treatment cycle, *i.e.*, two i.v. infusions administered 14 days apart, beginning with the next regularly scheduled visit following the interim database lock and primary analysis.

Study Procedures

[0510] The primary efficacy endpoint was time to onset of confirmed disability progression over the treatment period, defined as an increase in EDSS that was sustained for at least 12 weeks, based on regularly scheduled visits. Confirmed disability progression (CDP) refers to an at least 12-week sustained increase of at least 1.0 from baseline EDSS if the baseline score was no greater than 5.5, or an increase of at least 0.5 if the baseline score was greater than 5.5.

[0511] Secondary endpoints included: time to onset of confirmed disability progression over the treatment period, defined as an increase in EDSS that was sustained for at least 24 weeks (i.e., 24-week CDP), based on regularly scheduled visits; change in Timed 25-foot walk (T25-FW) from baseline to Week 120; percent change in MRI total T2 lesion volume from baseline to Week 120; percent change in MRI total brain volume from Week 24 to Week 120; and change in Short Form-36 (SF-36) physical component score from baseline to Week 120, and safety and tolerability of ocerlizumab.

[0512] Exploratory endpoints included time to onset of 12-week and 24-week confirmed composite disability progression (defined as the first confirmed occurrence of EDSS progression, or at least 20% increase in timed 25-foot walk, and/or at least 20% increase in 9-hole peg test (9-HPT) time), time to sustained progression of at least 20% in timed 25-foot walk, time to sustained progression of at least 20% in 9-hole peg test during the treatment period, and total number of new or enlarging T2 lesions as detected by brain MRI from baseline to Week 120. The 9-HPT is a standardized, quantitative test of upper extremity function and fine manual dexterity. It is the second component of the MSFC (Multiple Sclerosis Functional Composite).

[0513] Additional sensitivity analysis was consistent with the primary results and was performed to evaluate an influence of on-study relapses on confirmed disability progression data by excluding patients who experienced physician-reported clinical relapses including protocol-defined relapses

(**Table 12A**). Protocol-defined relapses were reported for 11% of patients in the placebo group and 5% in the ocrelizumab group. A subgroup analysis of key clinical and MRI efficacy endpoints in patients with and without T1 gadolinium-enhancing lesions at baseline was consistent with the overall study population (**Tables 12C-12E** below).

Table 12A: Sensitivity Analysis of Primary Endpoint and First Secondary Endpoint Exploring Influences of Clinical Relapses

	Excluding patients with clinical relapses	
Endpoints	**Placebo (n=204)**	**Ocrelizumab 600 mg (n=456)**
Primary endpoint		
Confirmed disability progression for at least 12 wks Hazard ratio vs. placebo (95% CI) P-value	0.74 (0.56–0.98) P=0.0324	
Secondary endpoint		
Confirmed disability progression for at least 24 wks Hazard ratio vs. placebo (95% CI) P-value	0.71 (0.53–0.95) P=0.0188	

Statistical Analysis

[0514] The statistical hierarchy is provided in **FIG. 25**.

Results

Patients

[0515] As shown in **Table 12B**, patients' multiple sclerosis disease histories and characteristics were well balanced.

Table 12B. Patient Demographics and Baseline Disease Characteristics

	Placebo n=244	Ocrelizumab 600 mg N= 488
Age, yr, mean (SD) **Median (range)**	44.4 (8.3) 46.0 (18–56)	44.7 (7.9) 46.0 (20–56)
Female, n (%)	124 (50.8)	237 (48.6)
Time since symptom onset, yr, mean (SD) **Median (range)**	6.1 (3.6) 5.5 (0.9–23.8)	6.7 (4.0) 6.0 (1.1–32.9)
Time since diagnosis, yr, mean (SD) **Median (range)**	2.8 (3.3) 1.3 (0.1–23.8)	2.9 (3.2) 1.6 (0.1–16.8)
MS disease modifying treatment naive, n (%)	214 (87.7)	433 (88.7)
EDSS, mean (SD) **Median (range)**	4.7 (1.2) 4.5 (2.5–6.5)	4.7 (1.2) 4.5 (2.5–7.0)
MRI findings		
Patients with 0 Gd^+ T1 lesions, n (%)	183 (75.3)	351 (72.5)
Patients with Gd^+ T1 lesions, n (%)	60 (24.7%)	133 (27.5)
1 lesion	29 (11.9)	62 (12.8)
2 lesions	15 (6.2)	22 (4.5)
3 lesions	5 (2.1)	17 (3.5)
≥ 4 lesions	11 (4.5)	32 (6.6)
Number of Gd^+ T1 lesions, mean (SD)	0.6 (1.6)	1.2 (5.1)
Number of T2 lesions, mean (SD) **Median (range)**	48.15 (39.31) 43 (0-208)	48.71 (38.16) 42.0 (0-249)
T2 lesion volume, cm^3, mean (SD) **Median (range)**	10.9 (13.0) 6.17 (0-81.1)	12.7 (15.1) 7.31 (0-90.3)
Normalised brain volume, cm^3, mean (SD) **Median** **(range)**	1469.9 (88.7) 1464.51 (1216.3-1701.7)	1462.9 (83.9) 1462.23 (1214.3-1711.1)

[0516] As shown in **FIG. 26**, the characteristics of the intent-to-treat population were well balanced. At database lock for primary analysis 390 (80%) treated patients in the ocrelizumab group versus 159 (67%) in the placebo group remained on treatment (median study duration was 2.8 years

with placebo and 2.9 years with ocrelizumab). Of patients who withdrew from the study before the database lock, 61 (64%) in the ocrelizumab group versus 45 (56%) in the placebo group entered safety follow-up.

Disability

[0517] The time to confirmed disability progression that was sustained for at least 12 weeks in patients receiving 600mg ocrelizumab compared to patients receiving placebo is provided in **FIG. 27** (HR – hazard ratio). The time to confirmed disability progression that was sustained for at least 24 weeks in patients receiving 600mg ocrelizumab compared to patients receiving placebo is provided in **FIG. 28**. ITT patients with initial disability progression who discontinued treatment early with no confirmatory EDSS assessment were considered as having confirmed disability progression, p-value based on log-rank test stratified by geographic region and age. Ocrelizumab reduced the risk of CDP sustained for $\geq$ 12 weeks by 24% versus placebo and reduced the risk of CDP sustained for $\geq$ 24 weeks by 25% versus placebo. Analyses in **FIGs. 27** and **28** were based on ITT population; p-value based on log rank test stratified by geographic region and age. Patients with initial disability progression who discontinued treatment early with no confirmatory EDSS assessment were considered as having confirmed disability progression.

[0518] Risk of 12-week CDP was reduced by 35% in ocrelizumab-receiving patients with T1 Gd^+ lesions at baseline (hazard ratio, 0.65; 95% CI, 0.40-1.06; p=0.0826) vs. 16% in ocrelizumab-receiving patients without T1 Gd^+ lesions at baseline (hazard ratio, 0.84; 95% CI, 0.62-1.13; p=0.2441). *See* **Table 12C** below.

Table 12C

	Total	**Placebo (N=244)**		**Ocrelizumab 600mg (N=488)**		**Hazard Ratio**	**95% CI**	**p value**
	n	**n**	**Events**	**n**	**Events**			
Overall population	731	244	96	487	160	0.76	(0.59, 0.98)	731
T1 Gd^+-enhancing lesions at baseline	193	60	27	133	43	0.65	(0.40, 1.06)	0.0826
No T1 Gd^+-enhancing lesions at baseline	533	183	68	350	115	0.84	(0.62, 1.13)	0.2441

[0519] Risk of 24-week CDP was reduced by 33% in ocrelizumab-receiving patients with T1 Gd^+ lesions at baseline (hazard ratio, 0.67; 95% CI, 0.40-1.14; p=0.1417) vs. 19% in ocrelizumab-receiving patients without T1 Gd^+ lesions at baseline (hazard ratio, 0.81; 95% CI, 0.59-1.10; p=0.1783). *See* **Table 12D** below.

Table 12D

	Total	**Placebo (N=244)**		**Ocrelizumab 600mg (N=488)**		**Hazard Ratio**	**95% CI**
	n	**n**	**Events**	**n**	**Events**		
Overall population	731	244	87	487	144	0.75	(0.58, 0.98)
T1 Gd-enhancing lesions at baseline	193	60	23	133	39	0.67	(0.40, 1.14)
No T1 Gd-enhancing lesions at baseline	533	183	63	350	103	0.81	(0.59, 1.10)

[0520] Additional subgroup analyses of key clinical and MRI endpoints in patients with or without gadolinium-enhancing lesions on MRI scan at baseline are summarized in **Table 12E** below.

Table 12E

Endpoints	**Placebo (n=244)**		**Ocrelizumab 600 mg (n=488)**			
	Gd lesions	**No Gd lesions**	**Gd lesions**	**P value**	**No Gd lesions**	**P value**
Primary endpoint						
Confirmed disability progression for at least 12 wks Patients with event – n Hazard ratio vs. placebo (95% CI)	27/60	68/183	43/133 0.65 (0.40–1.06)	P=0.0826	115/350 0.84 (0.62–1.13)	P=0.2441
Secondary endpoints						
Confirmed disability progression for at least 24 wks Patients with event – n Hazard ratio vs. placebo (95% CI)	23/60	63/183	39/133 0.67 (0.40–1.14)	P=0.1417	103/350 0.81 (0.59–1.10)	P=0.1783

T25FW from baseline to wk 120 No. in group Ratio of adjusted geometric means (95% CI)	39	134	106 0.859 (0.655–1.112)	P=0.2464	288 0.922 (0.801–1.060)	P=0.2536
T2 lesion volume from baseline to wk 120 No. in group Ratio of adjusted geometric means (95% CI)	39	144	107 0.859 (0.818–0.901)	P<0.0001	291 0.913 (0.885–0.943	P<0.0001
Whole brain volume from wk 24–120 No. in group Difference in adjusted mean vs. placebo – % (95% CI)	31	119	83 18.1 (–21.1 to 57.2)	P=0.3625	241 20.6 (3.3 to 37.9)	P=0.0198
Exploratory endpoints						
12-week confirmed composite disability progression* Patients with event – n Hazard ratio vs. placebo (95% CI)	43/60	127/183	78/133 0.77 (0.52–1.13)	P=0.1824	206/351 0.75 (0.60–0.94)	P=0.0109
12-week confirmed progression in T25FW by at least 20% Patients with event – n (%) Hazard ratio vs. placebo (95% CI)	38/60	106/183	65/133 0.67 (0.45–1.02)	P=0.0591	170/351 0.78 (0.61–0.99)	P=0.0431
12-week confirmed progression in 9HPT by at least 20% Patients with event – n (%) Hazard ratio vs. placebo (95% CI)	22/60	43/183	24/133 0.42 (0.23–0.76)	P=0.0044	58/351 0.64 (0.43–0.95)	P=0.0254

[0521] **FIG. 29** shows the rate of decline in walking speed (i.e., reduction in the progression rate of walking time) as measured by the Timed 25-Foot Walk, in patients receiving 600mg ocrelizumab compared to patients receiving placebo from baseline to Week 120. Ocrelizumab reduced the rate of

walking speed decline by 29% versus placebo. Analysis was based on the ITT population; p-value was based on ranked ANCOVA at 120 week visit adjusted for baseline 25-foot timed walk, geographic region and age with missing values imputed by last observation carried forward (*i.e.*, LOCF). Points estimates and 95% CIs were based on log-transformed data. Analysis in **FIG. 19** is based on ITT (intent-to-treat population); p-value base on ranked ANCOVA at Week 120 visit adjusted for baseline Timed 25-Foot Walk, geographic regions, and age , with missing values imputed by LOCF (last observation carried forward). Point estimates and 95% CI (confidence interval) was based on MMRM analysis and log-transformed data adjusted for baseline timed 25-foot walk, geographic region, and age. The reduction in the percent change from baseline walking time to Week 120 with ocrelizumab vs placebo in patients with Gd^+ lesions at baseline (*see* **FIG. 35A**) and without T1 Gd^+ lesions at baseline (*see* **FIG. 35B**) was consistent with the overall study population (*see* **FIG. 35C**). The analysis in **FIGs. 35A-C** were based on intent-to-treat population; p-value based on ranked ANCOVA at 120-week visit adjusted for baseline timed 25-foot walk, geographic region and age with missing values imputed by LOCF (*i.e.*, last observation carried forward). Point estimates and 95% confidence intervals based on MMRM analysis on log-transformed data adjusted for baseline timed 25-foot walk, geographic region and age.

[0522] **FIG. 30** shows the rate of decline in walking speed at Week 120 relative to baseline in patients receiving 600mg ocrelizumab compared to patients receiving placebo. Ocrelizumab reduced the rate of decline in walking speed by 10% versus placebo (p=0.404).

[0523] **FIG. 40A** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients in the overall study population receiving 600mg ocrelizumab compared to patients in the overall study population receiving placebo. **FIG. 40B** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients with T1 gadolinium-enhancing lesions at baseline receiving 600mg ocrelizumab compared to patients with T1 gadolinium-enhancing lesions at baseline receiving placebo. **FIG. 40C** shows the change in SF-36 Physical Component Summary score from base line to Week 120 in patients without T1 gadolinium-enhancing lesions at baseline receiving 600mg ocrelizumab compared to patients without T1 gadolinium-enhancing lesions at baseline receiving placebo.

MRI-related endpoints

[0524] **FIG. 31** shows the percent change of whole brain volume from Week 24 to Week 96 in patients receiving 600mg ocrelizumab compared to patients receiving placebo. Ocrelizumab reduced the rate of whole brain volume loss from Week 24 to Week 96 by 17.5% as compared to placebo (p=0.0206). Analysis was based on ITT population with Week 24 and at least one post-Week 24 assessment; p-value was based on MMRM at Week 120 visit adjusted for Week 24 brain volume, geographic region and age. The rate of whole brain volume loss from Week 24 to Week 120 in OCR-treated patients with T1 Gd^+ lesions at baseline (*see* **FIG. 36A**) and without T1 Gd^+ lesions at baseline (*see* **FIG. 36B**) was consistent with the overall population data (*see* **FIG. 36C**). Analyses in **FIGs. 36A-C** were based on intent-to-treat population with week 24 and at least one post-week 24 assessment; p-value based on MMRM at 120 week visit adjusted for week 24 brain volume, geographic region and age.

[0525] Patients receiving 600mg ocrelizumab showed substantial reduction in T2 lesion volumes from baseline to Week 120 compared to patients receiving placebo (p<0.0001). As shown in **FIG. 32**, T2 lesion volume increased by 7.4% in patients in the placebo arm, whereas T2 lesion volume decreased by 3.4% in patients receiving ocrelizumab. Analysis was based on intent-to-treat population; p-value was based on ranked ANCOVA at 120 week visit adjusted for baseline T2 lesion volume, geographic region and age with missing values imputed by LOCF (last observation carried forward). Point estimates and 95% CIs (confidence intervals) based on MMRM analysis on log-transformed data adjusted for baseline T2 lesion volume, geographic region, and age.

[0526] Ocrelizumab treatment reduced the total volume of brain T2 hyperintense lesions from baseline to Week 120 in patients with and without T1 Gd^+ lesions at baseline, whereas the total lesion volume increased in patients with and without T1 Gd^+ lesions at baseline in the placebo group. *See* **FIG. 37A**.

[0527] In patients having T1 Gd^+ lesions at baseline, total T2 lesion volume was reduced by 3.8% in ocrelizumab-treated patients (95% CI, –7.0 to –0.5). By contrast, total T2 lesion volume increased by12.0% in patients receiving placebo (95% CI, 7.2-17.1; p<0.001). *See* **FIG. 37B**. In patients without T1 Gd^+ lesions at baseline, total T2 lesion volume was reduced by 3.1% in ocrelizumab-treated patients (95% CI, –5.0 to –1.1). By contrast, total T2 lesion volume increased by 6.1% in

patients receiving placebo (95% CI, 3.3-9.0; p<0.001). *See* **FIG. 37C**. Analyses in **FIGs. 37A-C** were based on ITT population; p-value based on ranked ANCOVA at 120-week visit adjusted for baseline T2 lesion volume, geographic region and age with missing values imputed by LOCF (last observation carried forward). Point estimates and 95% CIs based on MMRM analysis on log-transformed data adjusted for baseline T2 lesion volume, geographic region and age.

[0528] Patients receiving 600mg ocrelizumab showed a 97.6% reduction of T1 gadolinium enhancing lesions compared to patients receiving placebo (p<0.0001). Patients receiving 600mg ocrelizumab also showed a 91.9% reduction of new and/or enlarging T2 lesions compared to patients receiving placebo (p<0.0001). The total number of T1 Gd^+ lesions and total number of new and/or enlarging T2 lesions was divided by the sum of the individual number of lesions at Weeks 24, 48 and 120) for all patients in the treatment group by the total number of brain MRI scans. Adjusted by means calculated by negative binomial regression and adjusted for baseline *T1 Gd^+ lesion (present or not) or †T2 lesion count, baseline age (≤45 vs >45 years) and geographical region (US vs rest of world).

Efficacy

[0529] The efficacy endpoints for this study are summarized in **Table 13A**. Clinical and MRI endpoints (ITT population) are summarized in **Table 13B**.

Table 13A: Summary of Efficacy

Endpoint	Risk Reduction	p value
Time to CDP 12 week	24%	0.0321
Time to CDP 24 week	25%	0.0365
Change in Timed 25-Foot Walk (baseline to Week 120)	29%	0.0404
Percent change in MRI total T2 lesion volume (baseline to Week 120)	placebo: 7.4% increase ocrelizumab: 3.4% decrease	<0.0001
Percent change in MRI total brain volume (Week 24 to Week 120)	17.5%	0.0206

Change in SF-36 PCS (baseline to Week 120)		

Table 13B: Clinical and MRI endpoints (ITT population)

Endpoints	**Placebo (n=244)**		**Ocrelizumab 600 mg (n=488)**	
Clinical endpoints				
Primary endpoint				
Confirmed disability progression for at least 12 wks Patients with event: n (%) Hazard ratio vs. placebo (95% CI) P-value	96 (39.3)		160 (32.9) 0.76 (0.59–0.98) P=0.0321	
Secondary endpoints				
Confirmed disability progression for at least 24 weeks Patients with event – n (%) Hazard ratio vs. placebo (95% CI) P-value	87 (35.7)		144 (29.6) 0.75 (0.58–0.98) P=0.0365	
T25FW from baseline to Week 120 Mean percentage change Relative reduction vs. placebo – % (95% CI) P-value	55.10		39.93 29.337 (–1.618 to 51.456) P=0.0404	
Exploratory endpoints	**12-week CDP**	**24-week CDP**	**12-week confirmed**	**24-week confirmed**
Composite disability progression* Patients with event – n (%) Hazard ratio vs. placebo (95% CI) P-value	171 (70.1)	155 (63.5)	287 (58.8) 0.74 (0.61–0.89) P=0.0014	251 (51.4) 0.71 (0.58–0.87) P=0.0008
Progression in T25FW by at least 20%				

Patients with event – n (%) Hazard ratio vs. placebo (95% CI) P-value	145 (59.4)	127 (52.0)	238 (48.8) 0.75 (0.61–0.92) P=0.0053	202 (41.4) 0.73 (0.59–0.91) P=0.0055
Progression in 9HPT by at least 20% Patients with event – n (%) Hazard ratio vs. placebo (95% CI) P-value	66 (27.0)	57 (23.4)	83 (17.0) 0.56 (0.41–0.78) P=0.0004	69 (14.1) 0.55 (0.38–0.77) P=0.0006
MRI endpoints				
Secondary endpoints				
T2 lesion volume from baseline to Wk 120 Adjusted geometric mean percentage change (95% CI) Ratio of adjusted geometric mean vs. placebo (95% CI) P-value	7.426 (4.967–9.942)		-3.366 (-4.987 to -1.718) 0.900 (0.876–0.924) P<0.0001	
Total brain volume from Wk 24–120 Mean percentage change Relative reduction vs. placebo – % (95% CI) P-value	–1.093±0.072		–0.902±0.052 17.475 (3.206–29.251) P=0.0206	
Exploratory endpoint				
New and/or enlarging T2 lesions Mean no. of lesions per MRI scan (95% CI)‡ Percent change (95% CI)‡ P-value	3.880 (2.841–5.299)		0.313 (0.246–0.397) 0.081 (0.058–0.111) -91.9 (-88.9 to -94.2) P<0.0001	
Patient-reported outcomes				
Secondary endpoint				
SF-36 physical component summary score from baseline to Week 120 Adjusted mean change (95% CI) Difference in adjusted means (95% CI)	–1.108 (–2.394 to 0.177)		–0.731 (–1.655 to 0.193) 0.377 (–1.048 to 1.802) P=0.6034	

P-value		

[0530] At the time of the primary analysis, compared with placebo, ocrelizumab significantly reduced the risk of 12-week and 24-week confirmed disability progression by 24% (HR = 0.76, 95% CI [0.59, 0.98]; P=0.0321; **Table 13B**; **FIG. 27**) and by 25% (0.75, 95% CI [0.58, 0.98]; P=0.0365; **Table 13B**; **FIG. 28**), respectively. At Week 120, ocrelizumab reduced the progression rate of timed 25-foot walk by 29% (P=0.0404) compared with placebo (**Table 13B**). Compared with placebo, ocrelizumab also significantly reduced the risk of 12-week and 24-week confirmed composite disability progression (*i.e.* EDSS progression, or at least 20% progression in timed 25-foot walk test, and/or at least 20% progression in 9-hole peg test) by 26% (HR=0.74 [0.61–0.89], P=0.0014; **Table 13B**; **FIG. 41**), and by 29% (HR=0.71 [0.58–0.87], P=0.0008; **Table 13B**; **FIG. 42**), respectively, by the end of the double-blind treatment period. Regarding individual components of composite disability progression assessment, ocrelizumab consistently and significantly decreased the risk of 12-week and 24-week confirmed 20% progression on timed 25-foot walk (**Table 13B**) and on 9-hole peg test compared with placebo (**Table 13B**).

[0531] Additional sensitivity analysis was consistent with the primary results and was performed to evaluate an influence of on-study relapses on confirmed disability progression data by excluding patients who experienced physician-reported clinical relapses including protocol-defined relapses (**Table S1**). Protocol-defined relapses were reported for 11% of patients in the placebo group and 5% in the ocrelizumab group. A subgroup analysis of key clinical and MRI efficacy endpoints in patients with and without T1 gadolinium-enhancing lesions at baseline was consistent with the overall study population (**Table S2**). However, the ORATORIO study was not powered to demonstrate efficacy differences between these subgroups.

Safety

Infusion-Related Reactions

[0532] 725 patients were included in the safety analysis (i.e., 239 in the placebo group and 486 in the ocrelizumab group). Throughout a mean treatment duration of approximately 3 years, the proportion of patients with at least one infusion-related reaction (IRR) was 25.5% (n=61) for patients given placebo and 39.9% (n=194) for ocrelizumab-treated patients; most were mild-to-moderate in

severity (93.4% [n=57] and 96.9% [n=188], respectively). The most common IRR symptoms included skin and subcutaneous tissue disorders (45.9% [n=89] for ocrelizumab-treated patients and 13.1% [n=8] for patients given placebo). There were no life-threatening or fatal IRRs. The incidence of IRRs in ocrelizumab-treated patients was highest with the first infusion (27.4%) and decreased markedly with subsequent dosing (11.6% at first infusion of dose 2). Overall, IRRs mostly occurred during infusion in ocrelizumab-treated patients (61.3% compared with 37.7% in patients given placebo group). One patient (0.2%) withdrew ocrelizumab treatment due to an IRR during the first infusion.

Adverse Events

[0533] Adverse events reported by $\geq$ 10% of the patients in each treatment arm until clinical cut off date are provided in Table **14A**.

Table 14A.

n (%)	**Placebo (n=239)**	**Ocrelizumab 600 mg (n=486)**
Overall patients with ≥1 AE	215 (90.0)	462 (95.1)
Infections and Infestations*	162 (67.8)	339 (69.8)
Nasopharyngitis	65 (27.2)	110 (22.6)
Urinary tract infection	54 (22.6)	96 (19.8)
Influenza	21 (8.8)	56 (11.5)
Upper respiratory tract infection	14 (5.9)	53 (10.9)
Bronchitis	12 (5.0)	30 (6.2)
Gastroenteritis	12 (5.0)	20 (4.1)
Injury, Poisoning and Procedural Complications	104 (43.5)	263 (54.1)
Musculoskeletal and Connective Tissue Disorders	98 (41.0)	181 (37.2)
Nervous System Disorders	79 (33.1)	174 (35.8)
General Disorders and Administration-site Conditions	60 (25.1)	130 (26.7)
Gastrointestinal Disorders	60 (25.1)	126 (25.9)
Psychiatric Disorders	59 (24.7)	89 (18.3)
Skin and Subcutaneous Tissue Disorders	44 (18.4)	99 (20.4)
Respiratory, Thoracic and Mediastinal Disorders	35 (14.6)	87(17.9)

Metabolism and Nutrition disorders	28 (11.7)	56 (11.5)
Renal and Urinary Disorders	30 (12.6)	51 (10.5)
Vascular Disorders	26 (10.9)	54 (11.1)
Investigations	20 (8.4)	58 (11.9)

*For infections and infestations SOC only: events reported by at least 5% of patients in one treatment arm are presented.

[0534] The percent of overall patients experiencing more than 1 serious adverse event was 22.2% in the placebo arm as compared to 20.4% in the treatment arm. *See* **Table 14B**. Total serious adverse events were low and similar in both arms.

[0535] The most common adverse event associated with ocrelizumab was infusion-related reactions (39.9% vs. 25.5% for placebo). One patient (0.2%) withdrew from ocrelizumab treatment due to an IRR at the first infusion. As shown in **FIG. 33**, infusion related reactions (IRR) decreased in severity with each successive course and with each dose within the same course. Ocrelizumab has a similar safety profile to placebo.

Table 14B. Total Serious Adverse Events

n (%)	Placebo n = 239	Ocrelizumab n = 486
Overall patients with ≥1 SAE	53 (22.2)	99 (20.4)
Infections and Infestations	14 (5.9)	30 (6.2)
Injury, Poisoning, and Procedural Complications	11 (4.6)	19 (3.9)
Nervous System Disorders	9 (3.8)	18 (3.7)
Neoplasms: Benign, Malignant, and Unspecified (including cysts and polyps)	7 (2.9)	8 (1.6)
Gastrointestinal Disorders	3 (1.3)	10 (2.1)
Musculoskeletal and Connective Tissue Disorders	6 (2.5)	6 (1.2)
General Disorders and Administration Site Conditions	3 (1.3)	6 (1.2)
Renal and Urinary Disorders	3 (1.3)	5 (1.0)

[0536] Data obtained from this study show that B cells may play a role in PPMS pathophysiology. Ocrelizumab is the first investigational treatment showing a consistent, statistically significant clinical effect in PPMS in a large Phase III study compared to placebo. Ocrelizumab significantly reduced clinical disability progression sustained for 12 weeks (primary endpoint), clinical disability progression sustained for 24 weeks, change in Timed 25-foot walk, change in T2 lesion volume, and brain volume loss. Patients receiving ocrelizumab consistently met key secondary endpoints. Throughout the mean treatment duration of approximately 3 years, ocrelizumab showed a favorable safety profile. Overall incidence of adverse events associated with ocrelizumab was similar to placebo. The most common adverse events were mild-to-moderate infusion-related reactions. Incidence of serious adverse events associated with ocrelizumab, including serious infections, was also similar to placebo.

Open-Label Extension Phase

[0537] Following the randomized, parallel group, double-blind, placebo controlled study, patients may be eligible to enter open-label extension (OLE) phases to evaluate long-term safety, tolerability, and efficacy of ocrelizumab. The design of the open-label extension study, which will evaluate the long-term safety and efficacy of OCR in relapsing MS, is described below.

[0538] Following the primary database lock at the completion of the blinded treatment period, patients who can benefit from further treatment receive open-label ocrelizumab. Patients randomized to the ocrelizumab group continue open-label treatment with a dual ocrelizumab infusion (300 mg i.v. infusions administered 14 days apart) every 24 weeks. Patients randomized to placebo receive a dual ocrelizumab infusion for the first open-label treatment cycle, *i.e.*, two i.v. infusions administered 14 days apart, beginning with the next regularly scheduled visit following the interim database lock and primary analysis.

[0539] The OLE phase lasts until ocrelizumab is commercially available in the patient's country, as per local regulations or until the sponsor decides to terminate the ocrelizumab MS program. The OLE phase does not exceed 4 years (208 weeks). OLE is not mandatory. Patients unwilling to proceed to the OLE phase are entered into the safety follow-up phase, which is performed in 12-week intervals starting from the date of the patient's latest visit. Continued monitoring occurs if B cells are not repleted.

[0540] Key inclusion criteria for the OLE phase include: completion of the blinded treatment period; consent from investigators who determine that the patient may benefit from OCR treatment based on the assessments performed during the treatment period; written informed consent from the patient to enter the OLE phase; and meeting ocrelizumab treatment/retreatment criteria, in which the patient is free from the following conditions and laboratory abnormalities: (a) life-threatening (CTCAE Grade 4) infusion-related event that occurred during a previous OCR infusion; (b) any significant or uncontrolled medical condition or treatment-emergent , clinically significant laboratory abnormality; (c) active infection; (d) absolute neutrophil count $<1.5 \times 10^3/\mu L$; (e) CD4 cell count $<250/\mu L$; and (f) hypogammaglobulinemia immunoglobulin G <3.3 g/L.

[0541] The schedule of key safety and efficacy assessments are shown in **Table 15** below:

Table 15

Assessment	OLE phase cycles[a]			Delayed dosing visit[b]	Unscheduled visit[c]	Withdrawal from treatment visit
	Day 1 OLE cycle (±5 days)	Day 15 OLE cycle (±5 days)	12 weeks post-Day 1 infusion visit Day 1 OLE cycle (±7 days)			
Neurological exam[d]	x	x	x		x	x
EDSS[e]	x		x		x	x
Potential relapses[f]	x	x	x	x	x	x
EQ-5D[g]	x					x
MRI[h]	x					x
Routine safety lab[i]	x		x		x	x
Concomitant treatments[j]	x	x	x	x	x	x
Antibody titres[k]						x

[a]Patients continue with blinded 24-week treatment cycles until the last patient receives his/her final course of treatment scheduled at Week 98. At that point, all patients continue to the end of their current (24-week) cycle. If the

projected total number of confirmed disability progression events has not been reached at Week 120, then all patients continue with additional blinded 24-week treatment cycles until the projected number of confirmed disability events has been reached.

[b]A delayed dosing visit is performed when dosing cannot be administered at the scheduled dosing visit. At this time, other tests/assessments may be performed as appropriate.

[c]During an unscheduled visit (non-dosing) assessments are performed according to the clinical needs of the patient. At this time, other tests/assessments may be performed as appropriate.

[d]Neurological examination is performed at every planned and unscheduled visit. All patients with new neurological symptoms suggestive of MS worsening or of a relapse should have EDSS performed by examining investigator.

[e]Expanded Disability Status Scale score is performed in all patients by the examining investigator every 12 weeks. Additional EDSS assessments may be performed between visits (i.e. during an MS relapse, neurological worsening, etc). Disability progression is defined as a $\geq$1.0 point (where baseline EDSS is $\leq$5.5) or $\geq$0.5 point (where baseline EDSS is >5.5) increase from the baseline EDSS score that is not attributable to another aetiology.

[f]Patients are evaluated for relapses by the treating investigator at each scheduled visit and, if necessary, at unscheduled visits to confirm relapses occurring between the visits. If a relapse is suspected, the EDSS should also be performed in addition to completing the MS relapse electronic case report form.

[g]The EQ-5D (EuroQol five dimensions questionnaire) assessments will be performed annually.

[h]Annual brain MRI scans are performed at the start of the OLE phase (if none were taken in the previous 12 weeks), within (±) 4 weeks of scheduled visits and at OLE withdrawal visits (if none were taken in the previous 4 weeks).

[i]Routine safety laboratory assessments include haematology, chemistry and urinalysis.

[j]Concomitant medications and procedures are reported at each scheduled and unscheduled visit.

[k]Antibody titres against common antigens (mumps, rubella, varicella, and *Streptococcus pneumoniae*) are performed.

[0542] The examples, which are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way, also describe and detail aspects and embodiments of the invention discussed above. The foregoing examples and detailed description are offered by way of illustration and not by way of limitation.

CLAIMS

What is claimed is:

1. A method of improving functional ability in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the patient has improvement in functional ability after treatment; wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

2. The method of claim 1, wherein the patient has 12-week confirmed disability improvement after treatment.

3. The method of claim 1, wherein the patient has 24-week confirmed disability improvement after treatment.

4. The method of any one of claims 1-3, wherein the improvement in functional ability in the patient is sustained for at least 12 weeks.

5. The method of any one of claims 1-3, wherein the improvement in functional ability in the patient is sustained for at least 24 weeks.

6. The method of any one of claims 1-5, wherein the improvement in functional ability is measured by the Timed 25-Foot Walk (T-25FW) test or EDSS score.

7. The method of any one of claims 1-5, wherein the improvement in functional ability is measured by the Timed 25-Foot Walk (T-25FW) test and EDSS score.

8. The method of any one of claims 1-7, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

9. The method of claim 8, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the

antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

10. The method of claim 9, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

11. The method of any one of claims 8-10, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

12. The method of claim 11, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

13. The method of any one of claims 8-10, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

14. The method of any one of claims 8-13, wherein the patient has improved functional ability after one, two, three, and/or four exposures of the anti-CD20 antibody.

15. The method of any one of claims 1-14, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

16. A method of suppressing composite disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

17. The method of claim 16, wherein the administration results in reduction of 12-week confirmed composite disability progression.

18. The method of claim 16, wherein the administration results in reduction of 24-week confirmed composite disability progression.

19. The method of any one of claims 16-17, wherein the confirmed composite disability progression is determined by Expanded Disability Status Scale (EDSS) score, Timed 25-Foot Walk (T25-FW), and 9-Hole Peg Test (9-HPT).

20. The method of any one of claims 16-19, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

21. The method of claim 20, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

22. The method of claim 21, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

23. The method of any one of claims 20-22, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

24. The method of claim 23, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

25. The method of any one of claims 20-22, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

26. The method of any one of claims 20-25, wherein the composite disability progression is suppressed in the patient after one or two, three, or four exposures of the anti-CD20 antibody.

27. The method of any one of claims 16-26, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

28. A method of suppressing disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the administration results in reduction of confirmed disability progression events, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

29. The method of claim 28, wherein the administration results in reduction of 12-week confirmed disability progression.

30. The method of claim 28, wherein the administration results in reduction of 24-week confirmed disability progression.

31. The method of any one of claims 28-30, wherein the confirmed disability progression is determined by Expanded Disability Status Scale (EDSS) score.

32. The method of any one of claims 28-31, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

33. The method of claim 32, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

34. The method of claim 33, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the

antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

35. The method of any one of claims 32-34, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

36. The method of claim 35, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

37. The method of any one of claims 32-34, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

38. The method of any one of claims 32-37, wherein the composite disability progression is suppressed in the patient after one or two, three, or four exposures of the anti-CD20 antibody.

39. The method of any of any one of claims 28-38, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

40. A method of delaying onset of confirmed disability progression or reducing the risk of confirmed disability progression in a human patient having multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

41. The method of claim 40, wherein the administration results in delayed onset or reduced risk of 12-week confirmed disability progression.

42. The method of claim 40, wherein the administration results in delayed onset or reduced risk of 24-week confirmed disability progression.

43. The method of any one of claims 40-42, wherein the patient has T1 gadolinium staining lesions at baseline.

44. The method of any one of claims 40-42, wherein the patient does not have T1 gadolinium staining lesions at baseline.

45. The method of any one of claims 40-44, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

46. The method of claim 45, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

47. The method of claim 46, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about20-24 weeks or about 5-6 months.

48. The method of any one of claims 45-47, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

49. The method of claim 48, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

50. The method of any one of claims 45-47, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

51. The method of any one of claims 40-50, wherein the patient has delayed onset of confirmed disability progression or reduced risk of confirmed disability progression after one, two, three, and/or four exposures of the anti-CD20 antibody.

52. The method of claim 51, wherein the patient has delayed onset or reduced risk of 12-week confirmed disability progression after one, two, three, and/or four exposures of the anti-CD20 antibody.

53. The method of claim 51, wherein the patient has delayed onset or reduced risk of 24-week confirmed disability progression after one, two, three, and/or four exposures of the anti-CD20 antibody.

54. The method of any one of claims 51-53 wherein the confirmed disability progression is determined by Expanded Disability Status Scale (EDSS) score.

55. The method of any one of claims 40-54, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

56. A method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in no evidence of disease activity (NEDA) for at least 12 weeks, wherein the anti-CD20 antibody comprises: wherein the anti-CD20 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

57. The method of claim 56, wherein treatment results in no evidence of disease activity (NEDA) for at least 24 weeks.

58. The method of any one of claims 56-57, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

59. The method of claim 58, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

60. The method of claim 59, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

61. The method of any one of claims 58-59, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

62. The method of claim 61, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

63. The method of any one of claims 58-59, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

64. The method of any one of claims 58-63, wherein treatment results in NEDA for at least 12 weeks after one, two, three, and/or four exposures of the anti-CD20 antibody.

65. The method of any one of claims 56-64, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

66. A method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in the patient having no confirmed disability progression events at 96 weeks, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

67. The method of claim 66, wherein treatment further results in one or more of:

a) the patient being relapse-free at 96 weeks;

b) the patient being without T1 gadolinium-enhancing lesions at 96 weeks;

c) the patient being without new and/or enlarging T2 lesions at 96 weeks.

68. The method of claim 66 or 67, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

69. The method of claim 68, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

70. The method of claim 69, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

71. The method of any one of claims 68-70, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

72. The method of claim 71, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

73. The method of any one of claims 68-70, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

74. The method of any one of claims 68-73, wherein the patient has no confirmed disability progression events at 96 weeks after one, two, three, and/or four exposures of the anti-CD20 antibody.

75. The method of any one of claims 66-74, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

76. A method of treating a human patient with highly active multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

77. The method of claim 76, wherein the patient with highly active multiple sclerosis is an inadequate responder to other therapy for multiple sclerosis.

78. The method of claim 77, wherein the other therapy for multiple sclerosis is an interferon or glatiramer acetate.

79. The method of claim 76, wherein the patient with highly active multiple sclerosis has not been previously treated with other therapy for multiple sclerosis.

80. The method of any one of claims 76-79, wherein the administration of the anti-CD20 antibody is effective in one or more of the following: (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and (4) improvement of function ability.

81. The method of any one of claims 76-80, further comprising performing an MRI scan and determining if the patient has highly active multiple sclerosis before administering the anti-CD20 antibody to the patient.

82. The method of any one of claims 76-81, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

83. The method of claim 82, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

84. The method of claim 83, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the

antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

85. The method of any one of claims 82-84, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

86. The method of claim 85, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

87. The method of any one of claims 82-84, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

88. The method of any one of claims 82-87, wherein the patient has (1) reduction in number of lesions in the brain of the patient; (2) reduction in annualized relapse rate; (3) reduction of disability progression; and/or (4) improvement of function ability after one, two, three, and/or four exposures of the anti-CD20 antibody.

89. The method of any one of claims 76-88, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

90. A method of treating a human patient with early stage multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

91. The method of claim 90, further comprising diagnosing a patient having early stage multiple sclerosis before administering the anti-CD20 antibody to the patient.

92. The method of any one of claims 90-91, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody,

and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

93. The method of claim 92, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

94. The method of claim 93, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

95. The method of any one of claims 92-94, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

96. The method of claim 95, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

97. The method of any one of claims 92-94, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about 14 days.

98. The method of any one of claims 90-97, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

99. A method of treating a human patient with multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein the treatment results in no evidence of disease activity (NEDA) in the patient, and wherein the anti-CD20 antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

100. The method of claim 99, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

101. The method of claim 100, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

102. The method of claim 101, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

103. The method of any one of claims 100-101, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

104. The method of claim 103, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

105. The method of any one of claims 100-101, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

106. The method of any one of claims 100-105, wherein treatment results in NEDA after one, two, three, and/or four exposures of the anti-CD20 antibody.

107. The method of any one of claims 99-106, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

108. A method of treating a human patient with a relapsing form of multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in one or more of:

a) at least about 30% reduction in annualized relapse rate;

b) at least about 30% reduction in risk of confirmed disability progression for at least 12 weeks;

c) at least about 30% reduction in risk of confirmed disability progression for at least 24 weeks;

d) at least about 90% reduction in number of T1 gadolinium$^+$ lesions;

e) at least about 90% reduction in the mean number of T1 gadolinium$^+$ lesions at week 24, week 48, and/or week 96;

f) at least about 70% reduction in the number of new and/or enlarging T2 hyperintense lesions;

g) at least about 40% reduction in the mean number of new and/or enlarging T2 hyperintense lesions at week 24, week 48, and/or week 96;

h) at least about 10% reduction in the rate of whole brain volume loss;

i) at least about 10% improvement in confirmed disability improvement sustained for at least 12 weeks;

j) at least about 50% reduction in the number of new T1 hypointense lesions; and

k) at least about 55% improvement in NEDA (no evidence of disease activity);

wherein the reduction or improvement in a)-k) are compared to patient(s) receiving treatment with interferon beta-1a, and wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and 2) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

109. The method of claim 108, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure,

wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

110. The method of claim 109, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

111. The method of claim 110, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

112. The method of any one of claims 109-111, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

113. The method of claim 112, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

114. The method of any one of claims 109-111, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

115. The method of any one of claims 109-114, wherein treatment results in one or more of a) through k) after one, two, three, and/or four exposures of the anti-CD20 antibody.

116. The method of any one of claims 108-115, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

117. A method of treating a human patient with primary progressive multiple sclerosis comprising administering to the patient an effective amount of an anti-CD20 antibody, wherein treatment results in one or more of:

a) at least about 15 % reduction in the risk of confirmed disability progression for at least 12 weeks compared to patient(s) receiving no treatment;

b) at least about 15 % reduction in the risk of confirmed disability progression for at least 24 weeks compared to patient(s) receiving no treatment;

c) at least about 15%reduction in the progression rate of walking time, as measured by the Time 25-Foot Walk, compared to patient(s) receiving no treatment;

d) at least about 10% reduction in T2 lesion volume compared to patient(s) receiving no treatment;

e) at least about 3% reduction in T2 lesion volume from baseline to Week 24; and

f) at least about 12% reduction in the rate of whole brain volume loss compared to patient(s) receiving no treatment;

wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and 2) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

118. The method of claim 117, wherein the anti-CD20 antibody is administered to the patient to provide an initial anti-CD20 antibody exposure followed by a second anti-CD20 antibody exposure, wherein the first and second exposures are each about 600 mg of the antibody, and wherein the interval between the first exposure and the second exposure is about 20-24 weeks or about 5-6 months.

119. The method of claim 118, wherein the anti-CD20 antibody is administered to the patient to provide a third anti-CD20 antibody exposure, wherein the third exposure is about 600 mg of the antibody, and wherein the interval between the second exposure and the third exposure is about 20-24 weeks or about 5-6 months.

120. The method of claim 119, wherein the anti-CD20 antibody is administered to the patient to provide a fourth anti-CD20 antibody exposure, wherein the fourth exposure is about 600 mg of the antibody, and wherein the interval between the third exposure and the fourth exposure is about 20-24 weeks or about 5-6 months.

121. The method of any one of claims 118-120, wherein the first exposure comprises a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

122. The method of claim 121, wherein the second, third, and/or fourth exposures comprise a single dose of about 600 mg.

123. The method of any one of claims 118-120, wherein the initial exposure and second, third, and/or fourth additional exposures comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

124. The method of any one of claims 118-123, wherein treatment results in one or more of a) through f) after one, two, three, and/or four exposures of the anti-CD20 antibody.

125. The method of any one of claims 117-124, wherein the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO:13.

126. The method of any one of claims 1-125, wherein the patient maintains the ability to mount a humoral immune response to an antigen during treatment.

127. The method of claim 126, wherein the antigen is a mumps antigen, a rubella antigen, a varicella antigen, an S. pneumonia antigen, a tetanus toxoid antigen, a pneumococcal antigen, or an influenza antigen.

128. The method of any one of claims 1-127 wherein a second medicament is administered with the initial exposure or later exposures, wherein the anti-CD20 antibody is a first medicament.

129. The method of any of claims 1-116 and 126-128, wherein the multiple sclerosis is a relapsing form of multiple sclerosis.

130. The method of claim 129, wherein the relapsing form of multiple sclerosis is a relapsing remitting multiple sclerosis (RRMS).

131. The method of claim 129, wherein the relapsing form of multiple sclerosis is a secondary progressive multiple sclerosis with superimposed relapses (rSPMS).

132. The method of any one of claims 1-65 and 76-128, wherein the multiple sclerosis is a progressive multiple sclerosis.

133. The method of claim 132, wherein the multiple sclerosis is a primary progressive multiple sclerosis (PPMS).

134. The method of any one of claims 10, 22, 34, 47, 60, 70, 84, 94, 102, 111, and 120, wherein the anti-CD20 antibody is administered to the patient to provide one or more additional anti-CD20 antibody exposures after the fourth exposure, wherein the one or more additional exposures after the fourth exposure is about 600 mg of the antibody, and wherein the interval between the each exposure is about 20-24 weeks or about 5-6 months.

135. The method of claim 134, wherein the one or more additional anti-CD20 antibody exposures after the fourth exposure comprise a first dose and a second dose of the anti-CD20 antibody, wherein each dose is about 300 mg and the first dose and the second dose are separated by about two weeks or about 14 days.

136. The method of claim 134, wherein the one or more additional anti-CD20 antibody exposures after the fourth exposure comprise a single dose of about 600 mg.

137. The method of any one of the preceding claims, wherein the anti-CD20 antibody is ocrelizumab.

138. The method of any one of the preceding claims wherein the anti-CD20 antibody is an antigen binding fragment thereof.

139. The method of any one of the preceding claims, wherein the anti-CD20 antibody is in a pharmaceutically acceptable composition.

140. The method of any one of the preceding claims, wherein the anti-CD20 antibody is administered intravenously.

141. The method of claim 140, wherein the anti-CD20 antibody is administered intravenously for each antibody exposure.

142. The method of any one of claims 1-139 wherein the antibody is administered subcutaneously.

143. The method of claim 142, wherein the anti-CD20 antibody is administered subcutaneously for each antibody exposure.

144. An anti-CD20 antibody for use according to the method of any one of the preceding claims, wherein the anti-CD20 antibody comprises: wherein the anti-CD20 antibody comprises: 1) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8 and 2) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

145. An article of manufacture comprising: (a) a container comprising ocrelizumab; and (b) a package insert with instructions for treating multiple sclerosis in a patient according to any one of the preceding claims.

FIG. 1A

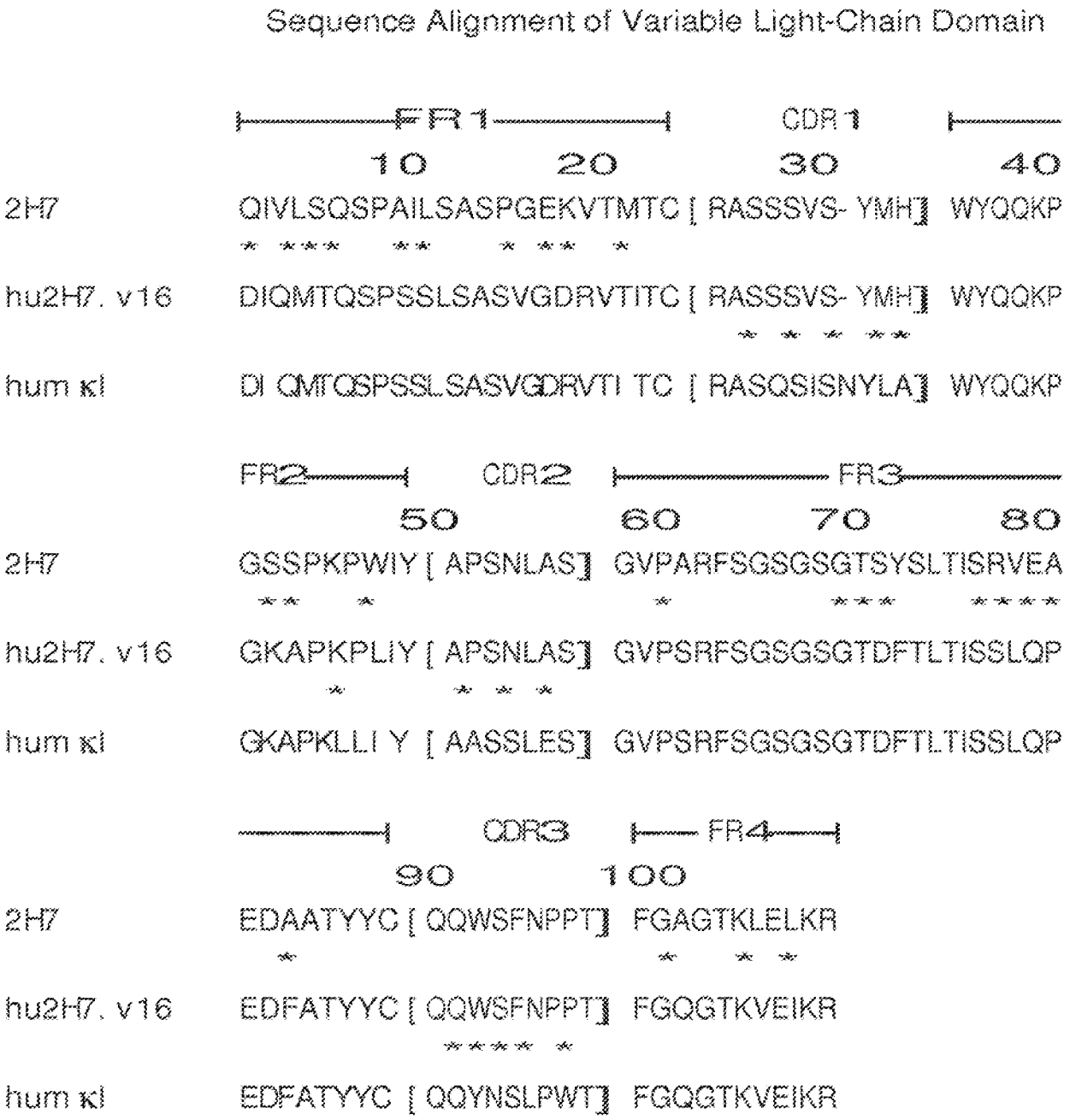

Sequence Alignment of Variable Light-Chain Domain
FR1
CDR1
10
20
30
40
2H7 QIVLSQSPAILSASPGEKVTMTC [RASSSVS- YMH] WYQQKP
hu2H7. v16 DIQMTQSPSSLSASVGDRVTITC [RASSSVS- YMH] WYQQKP
hum κI DI QMTQSPSSLSASVGDRVTI TC [RASQSISNYLA] WYQQKP
FR2
CDR2
FR3
50
60
70
80
2H7 GSSPKPWIY [APSNLAS] GVPARFSGSGSGTSYSLTISRVEA
hu2H7. v16 GKAPKPLIY [APSNLAS] GVPSRFSGSGSGTDFTLTISSLQP
hum κI GKAPKLLI Y [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP
CDR3
FR4
90
100
2H7 EDAATYYC [QQWSFNPPT] FGAGTKLELKR
hu2H7. v16 EDFATYYC [QQWSFNPPT] FGQGTKVEIKR
hum κI EDFATYYC [QQYNSLPWT] FGQGTKVEIKR

FIG. 1B

Sequence Alignment of Variable Heavy-Chain Domain

```
                    ├──────FR1──────┤          CDR1          ├──
                    10        20        30        40
2H7                 QAYLQQSGAELVRPGASVKMSCKAS [ GYTFTSYNMH] WVKQT
                    *** ** ** * * *** *                   * *
hu2H7.v16           EVQLVESGGGLVQPGGSLRLSCAAS [ GYTFTSYNMH] WVRQA
                                              * * * *
humIII              EVQLVESGGGLVQPGGSLRLSCAAS [ GFTFSSYAMS] WVRQA

                  ──FR2──┤        CDR2        ├──FR3──
                        50 a      60        70        80
2H7                 PRQGLEWIG [ AIYPGNGDTSYNQKFKG] KATLTVDKSSSTAYM
                    ** *                       ** ** ** * *
hu2H7.v16           PGKGLEWVG [ AIYPGNGDTSYNQKFKG] RFTISVDKSKNTLYL
                      * * ***** * * ****                * *
humIII              PGKGLEWVA [ VISGDGGSTYYADSVKG] RFTISRDNSKNTLTL

                    ├──────────────┤        CDR3        ├──FR4──┤
                    abc     90        100abcde        110
2H7                 QLSSLTSEDSAVYFCAR [ VVYYSNSYWYFDV] WGTGTTVTVSS
                    ** ** * *                         * *
hu2H7.v16           QMNSLRAEDTAVYYCAR [ VVYYSNSYWYFDV] WGQGTLVTVSS
                                      ******* *** *
humIII              QMNSLRAEDTAVYYCAR [ GRVGYSLY---DY] WGQGTLVTVSS
```

Humanized 2H7.v16 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG. 2

Humanized 2H7.v16 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

FIG. 3

Humanized 2H7.v31 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

FIG. 4

FIG. 5

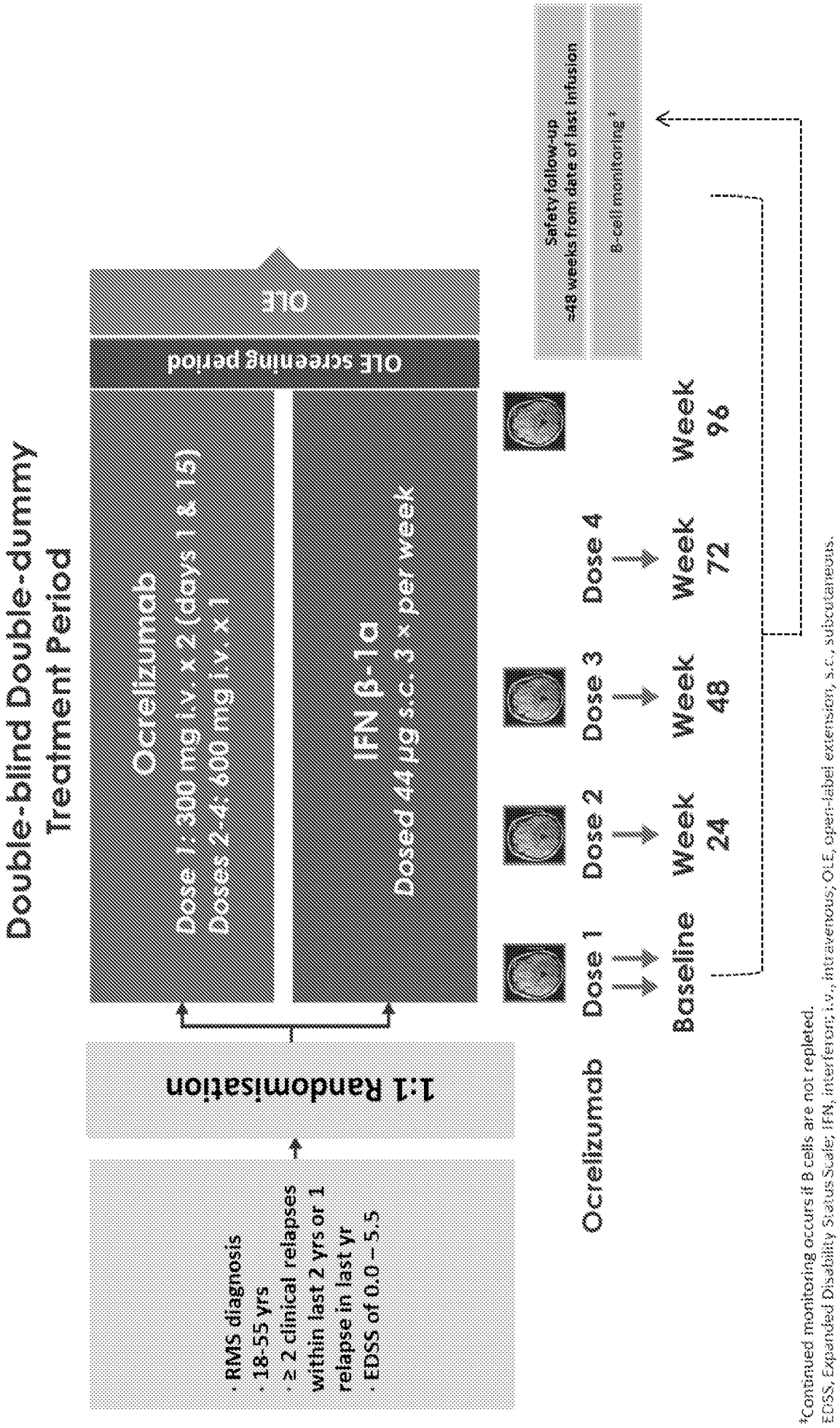
Light Chain Alignment
1
32
hu2H7.v16 DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP
hu2H7.v511 DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP
52
hu2H7.v16 SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG
hu2H7.v511 SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG
102
hu2H7.v16 TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
hu2H7.v511 TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
152
hu2H7.v16 NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
hu2H7.v511 NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
202
214
hu2H7.v16 SSPVTKSFNRGEC
hu2H7.v511 SSPVTKSFNRGEC

Heavy Chain Alignment

```
                1
hu2H7.v16       EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
                ************************************
hu2H7.v511      EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW

               37              52a                                82abc
hu2H7.v16       VRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSL
                ********************* ********************************
hu2H7.v511      VRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL

               83              100abcde          113
hu2H7.v16       RAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS
                ****************** ******************
hu2H7.v511      RAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS

              118
hu2H7.v16       ASTKGPSVFPLAPS
                **************
hu2H7.v511      ASTKGPSVFPLAPS

              132
hu2H7.v16       SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
                **************************************************
hu2H7.v511      SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS

              182
hu2H7.v16       LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
                **************************************************
hu2H7.v511      LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA

              232
hu2H7.v16       PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
                **************************************************
hu2H7.v511      PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG

              282
hu2H7.v16       VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
                ****************** ****************************** ******
hu2H7.v511      VEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAP

              332
hu2H7.v16       IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
                *  **************************************************
hu2H7.v511      IAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW

              382
hu2H7.v16       ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
                **************************************************
hu2H7.v511      ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

              432             447
hu2H7.v16       LHNHYTQKSLSLSPGK
                ****************
hu2H7.v511      LHNHYTQKSLSLSPGK
```

FIG. 6

FIG. 7



‡Continued monitoring occurs if B cells are not repleted.
EDSS, Expanded Disability Status Scale; IFN, interferon; i.v., intravenous; OLE, open-label extension; s.c., subcutaneous.

FIG. 8

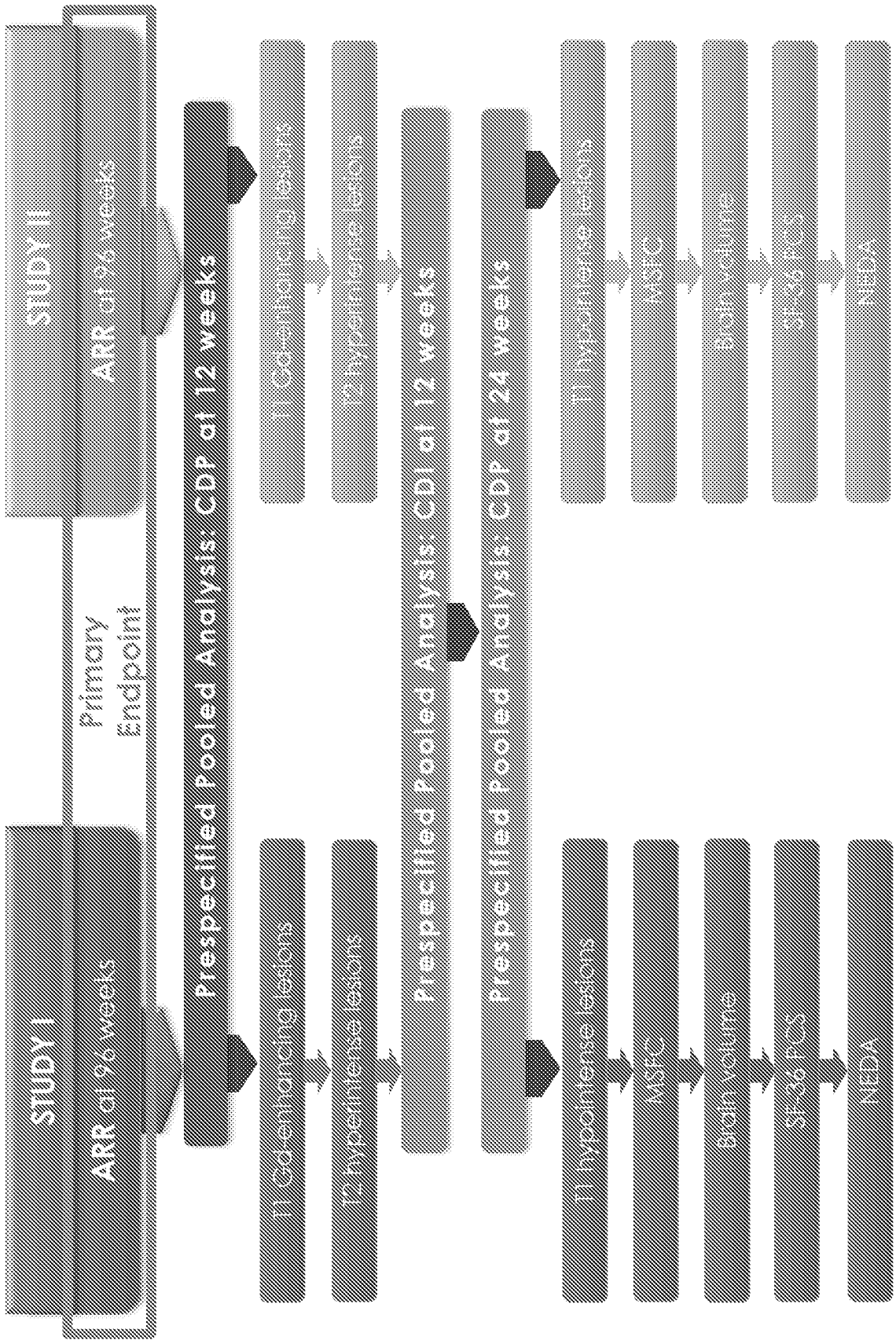

STUDY I
ARR at 96 weeks
STUDY II
ARR at 96 weeks
Primary Endpoint
Prespecified Pooled Analysis: CDP at 12 weeks
T1 Gadolinium-enhancing lesions
T2 hyperintense lesions
T1 hypointense lesions
MSFC
Brain volume
SPMS PCS
NEDA
Prespecified Pooled Analysis: CDP at 24 weeks
T1 Gadolinium-enhancing lesions
T2 hyperintense lesions
T1 hypointense lesions
MSFC
Brain volume
SPMS PCS
NEDA

FIG. 9

	STUDY I		STUDY II	
	IFN β-1a 44 µg	Ocrelizumab 600 mg	IFN β-1a 44 µg	Ocrelizumab 600 mg
ITT*, n	411	410	418	417
Treated, n	409	408	417	417
Withdrawn, n (%)	69 (17)	42 (10)	97 (23)	57 (14)
Withdrawn due to AE, n (%)	25 (6.1)	13 (3.2)	25 (6.0)	16 (3.8)
Entered safety follow-up, n (%)	42 (61)	24 (57)	39 (40)	16 (28)
Completed, n (%)	340 (83)	366 (89)	320 (77)	360 (86)
Entered safety follow-up, n (%)	12 (4)	10 (3)	17 (5)	9 (2)
Entered open-label extension, n (%)	326 (96)	352 (96)	297 (93)	350 (97)

FIG. 10

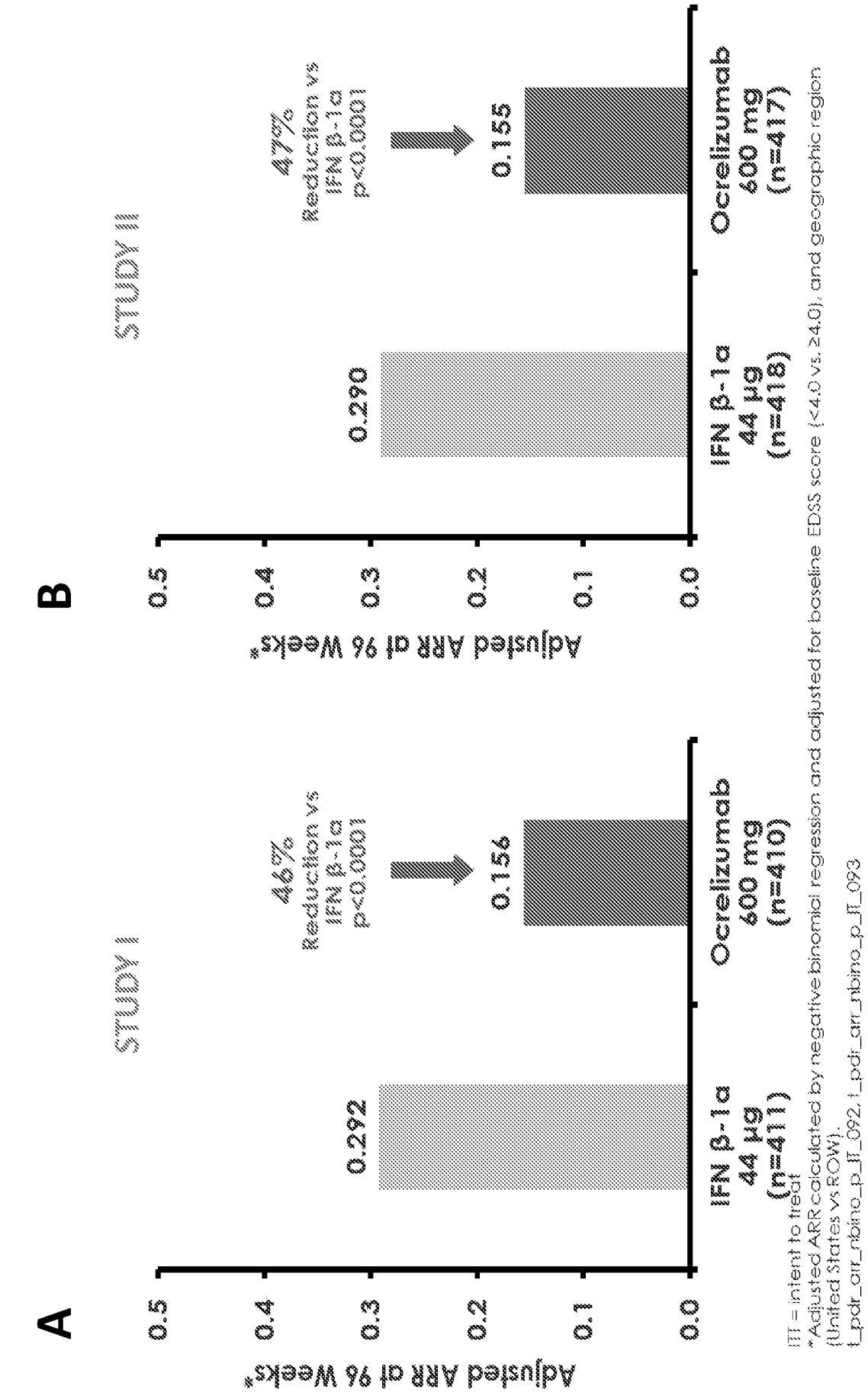

A
STUDY I
Adjusted ARR at 96 Weeks*
0.5
0.4
0.3
0.2
0.1
0.0
0.292
0.156
46%
Reduction vs
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=411)
Ocrelizumab
600 mg
(n=410)
B
STUDY II
Adjusted ARR at 96 Weeks*
0.5
0.4
0.3
0.2
0.1
0.0
0.290
0.155
47%
Reduction vs
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=418)
Ocrelizumab
600 mg
(n=417)
ITT = intent to treat
*Adjusted ARR calculated by negative binomial regression and adjusted for baseline EDSS score (<4.0 vs. ≥4.0), and geographic region (United States vs ROW).
t_pdr_arr_nbino_p_IT_092, t_pdr_arr_nbino_p_IT_093

FIG. 11

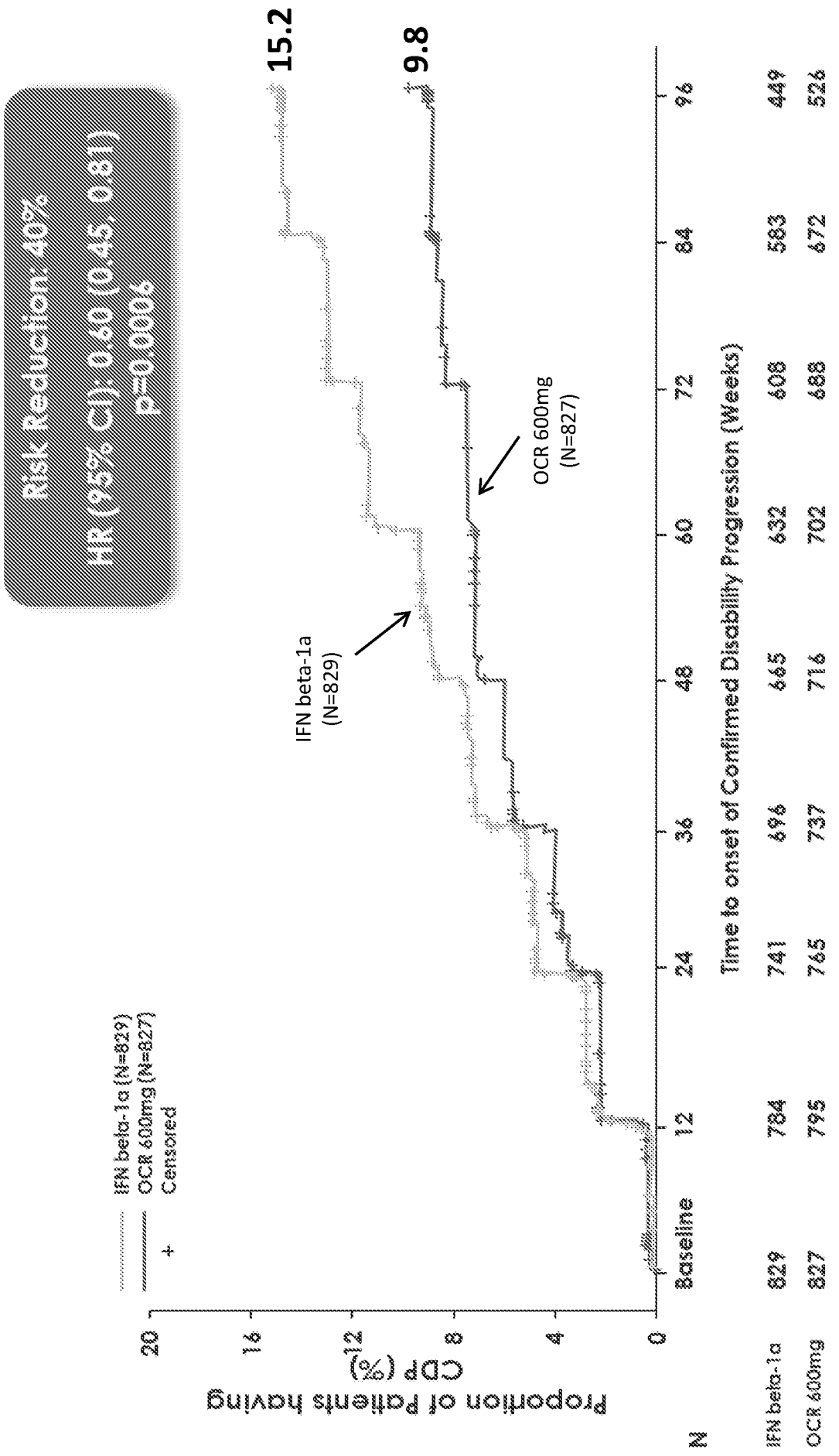

Risk Reduction: 40%
HR (95% CI): 0.60 (0.45, 0.81)
p=0.0006
15.2
9.8
IFN beta-1a (N=829)
OCR 600mg (N=827)
Proportion of Patients having CDP (%)
Time to onset of Confirmed Disability Progression (Weeks)
IFN beta-1a (N=829)
OCR 600mg (N=827)
Censored
N
IFN beta-1a 829 784 741 696 665 632 608 583 449
OCR 600mg 827 795 765 737 716 702 688 672 526
Baseline 12 24 36 48 60 72 84 96
0 4 8 12 16 20

FIG. 12

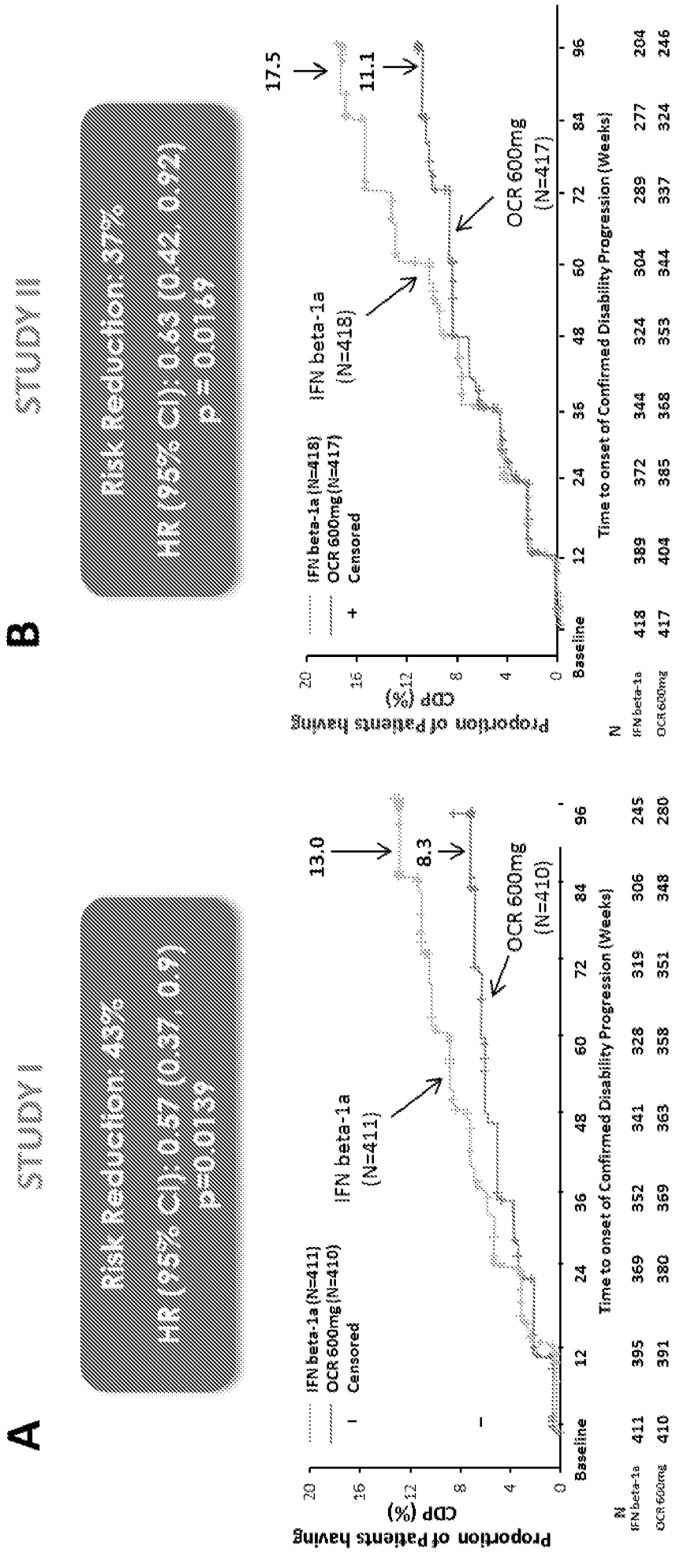

A
STUDY I
Risk Reduction 43%
HR (95% CI): 0.57 (0.37, 0.9)
p=0.0132
B
STUDY II
Risk Reduction 37%
HR (95% CI): 0.63 (0.42, 0.92)
p = 0.0169
IFN beta-1a (N=411)
OCR 600mg (N=410)
Censored
IFN beta-1a (N=418)
OCR 600mg (N=417)
Censored
IFN beta-1a (N=411)
OCR 600mg (N=410)
13.0
8.3
17.5
11.1
Proportion of Patients having CDP (%)
Time to onset of Confirmed Disability Progression (Weeks)
Baseline 12 24 36 48 60 72 84 96
IFN beta-1a 411 395 369 352 341 328 319 306 245
OCR 600mg 410 391 380 369 363 358 351 348 280
IFN beta-1a (N=418)
OCR 600mg (N=417)
Baseline 12 24 36 48 60 72 84 96
IFN beta-1a 418 389 372 344 324 304 289 277 204
OCR 600mg 417 404 385 368 353 344 337 324 246

FIG. 13

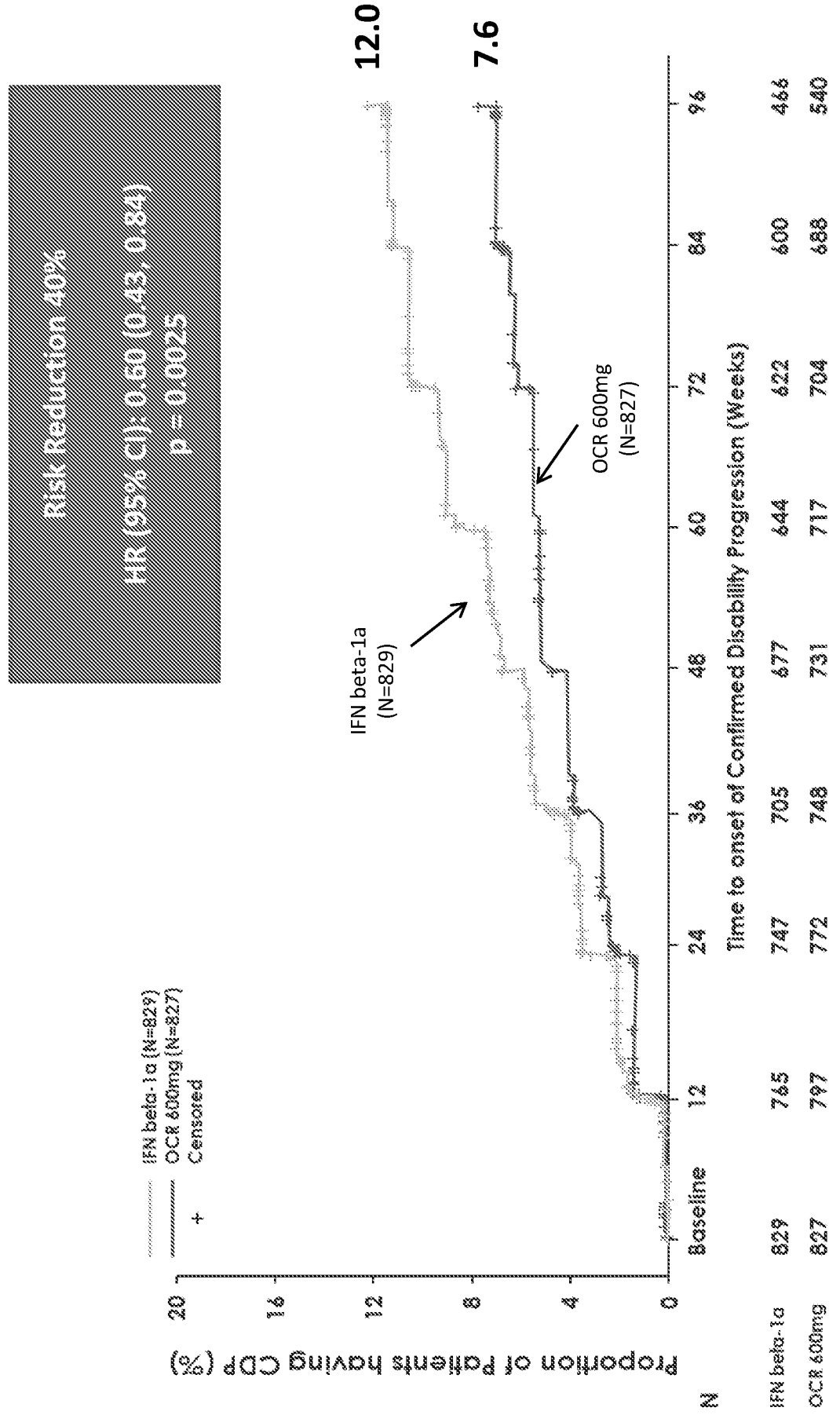

Risk Reduction 40%
HR (95% CI): 0.60 (0.43, 0.84)
p = 0.0025
12.0
7.6
IFN beta-1a (N=829)
OCR 600mg (N=827)
IFN beta-1a (N=829)
OCR 600mg (N=827)
+ Censored
Proportion of Patients having CDP (%)
0
4
8
12
16
20
Baseline
12
24
36
48
60
72
84
96
Time to onset of Confirmed Disability Progression (Weeks)
N
IFN beta-1a 829 765 747 705 677 644 622 600 466
OCR 600mg 827 797 772 748 731 717 704 688 540

FIG. 14

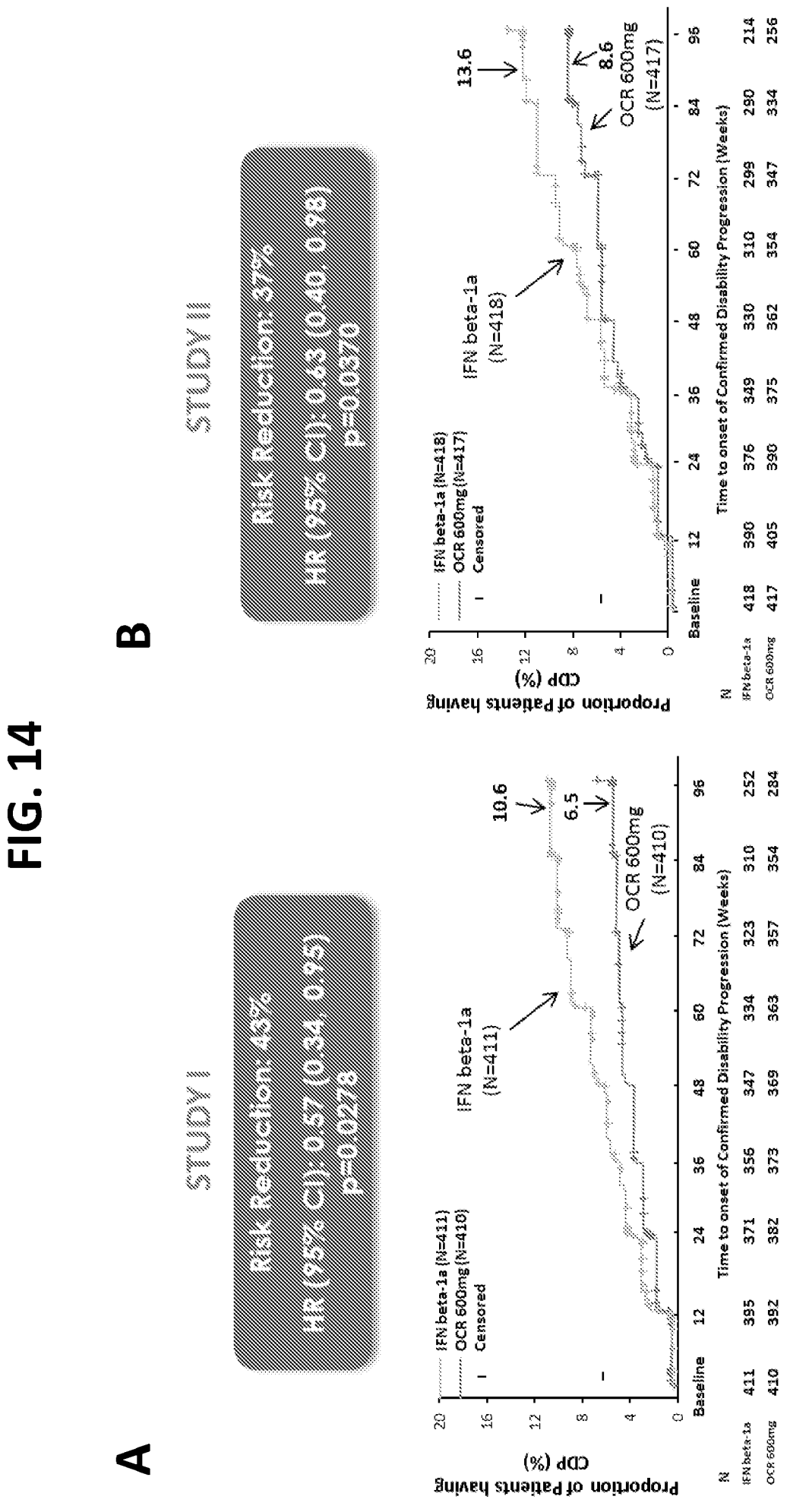

A
STUDY I
Risk Reduction: 46%
HR (95% CI): 0.57 (0.34, 0.95)
p=0.0278
IFN beta-1a (N=411)
OCR 600mg (N=410)
Censored
IFN beta-1a (N=411)
10.6
6.5
OCR 600mg (N=410)
Proportion of Patients having CDP (%)
Time to onset of Confirmed Disability Progression (Weeks)
N
IFN beta-1a 411 395 371 356 347 334 323 310 252
OCR 600mg 410 392 382 373 369 363 357 354 284
B
STUDY III
Risk Reduction: 37%
HR (95% CI): 0.63 (0.40, 0.98)
p=0.0370
IFN beta-1a (N=418)
OCR 600mg (N=417)
Censored
IFN beta-1a (N=418)
13.6
8.6
OCR 600mg (N=417)
Proportion of Patients having CDP (%)
Time to onset of Confirmed Disability Progression (Weeks)
N
IFN beta-1a 418 390 376 349 330 310 299 290 214
OCR 600mg 417 405 390 375 362 354 347 334 256

FIG. 15

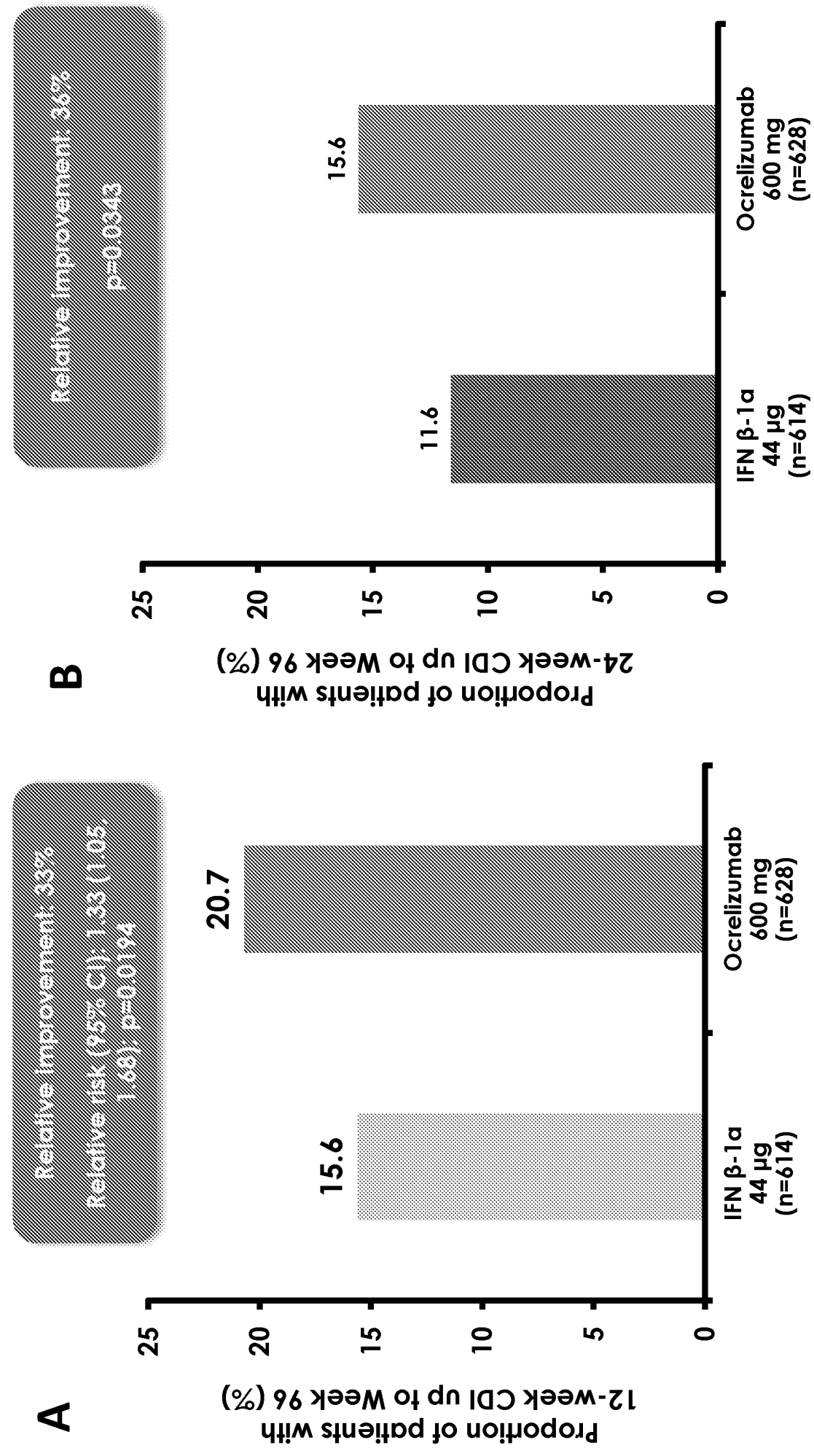
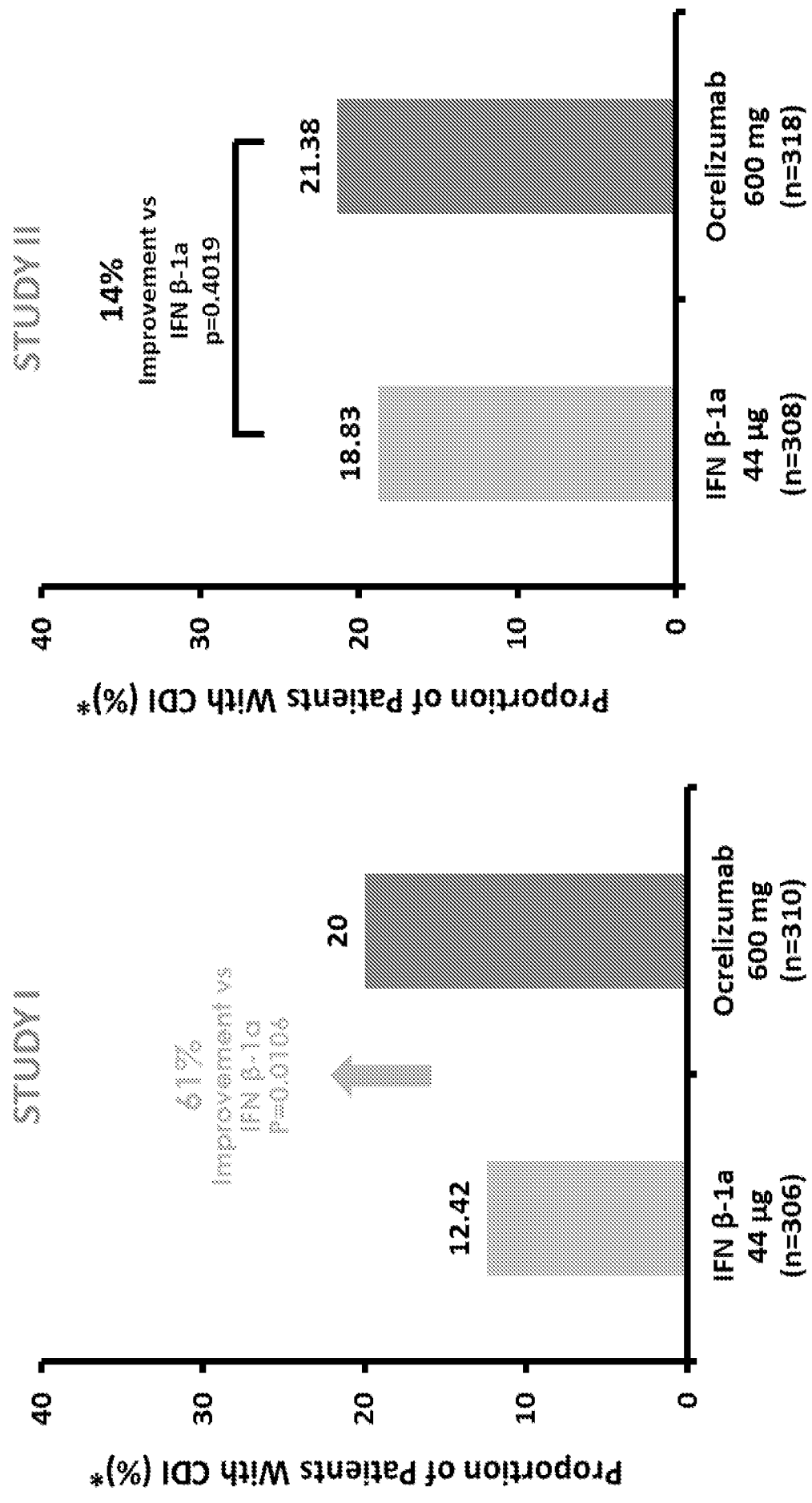

A
Relative improvement: 33%
Relative risk (95% CI): 1.33 (1.05, 1.68); p=0.0194
Proportion of patients with 12-week CDI up to Week 96 (%)
25
20
15
10
5
0
15.6
20.7
IFN β-1a 44 µg (n=614)
Ocrelizumab 600 mg (n=628)
B
Relative improvement: 36%
p=0.0343
Proportion of patients with 24-week CDI up to Week 96 (%)
25
20
15
10
5
0
11.6
15.6
IFN β-1a 44 µg (n=614)
Ocrelizumab 600 mg (n=628)

FIG. 16



*Compared using the Cochran–Mantel–Haenszel X2 test stratified by geographic region (United States vs ROW) and baseline EDSS score (≤4.0 vs ≥4.0).
t_cdi_cmh_CDII2_IT_092_0.3, t_cdi_cmh_CDII2_IT_093_0.2

FIG. 16C

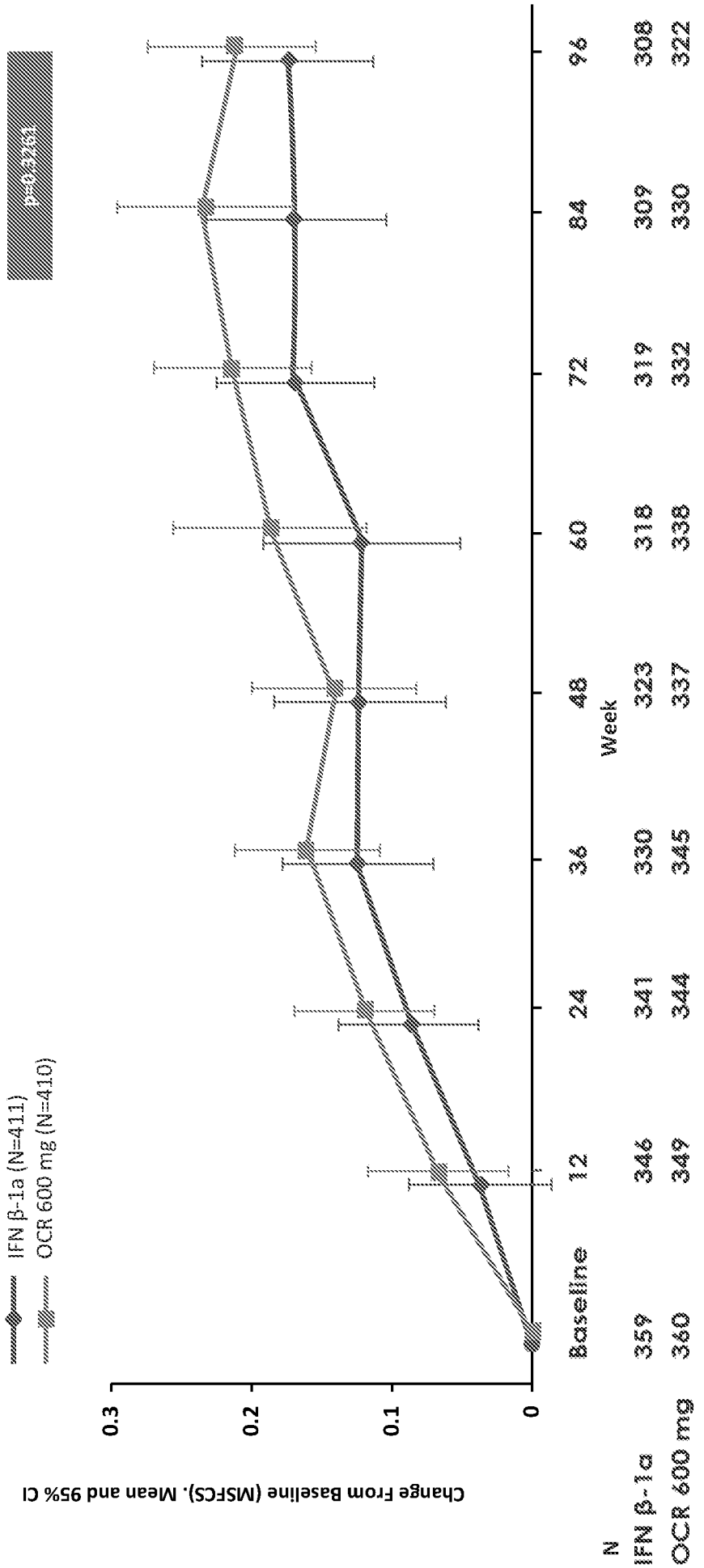

IFN β-1a (N=411)
OCR 600 mg (N=410)
p=0.3261
Change From Baseline (MSFCS). Mean and 95% CI
0.3
0.2
0.1
0
Week
Baseline 12 24 36 48 60 72 84 96
N
IFN β-1a 359 346 341 330 323 318 319 309 308
OCR 600 mg 360 349 344 345 337 338 332 330 322

FIG. 16D

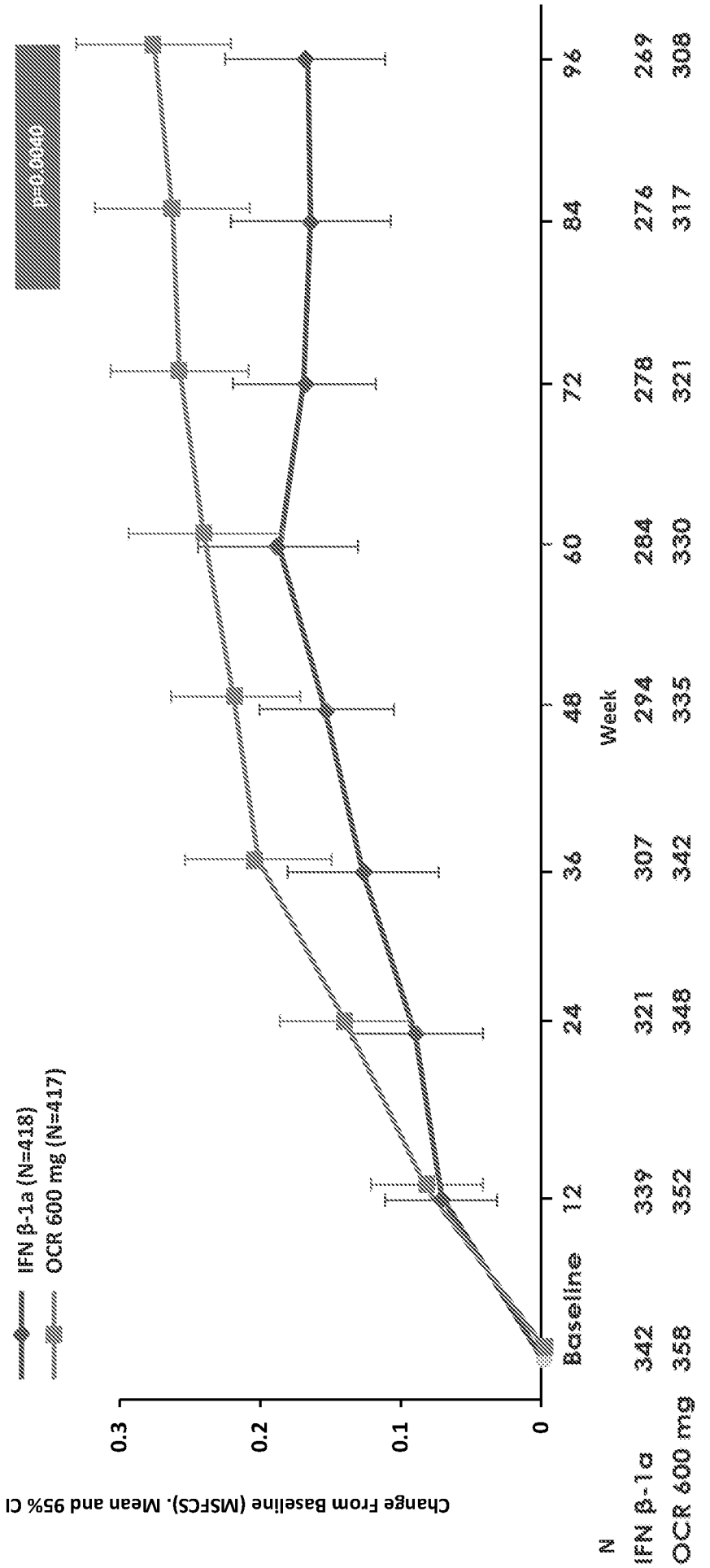
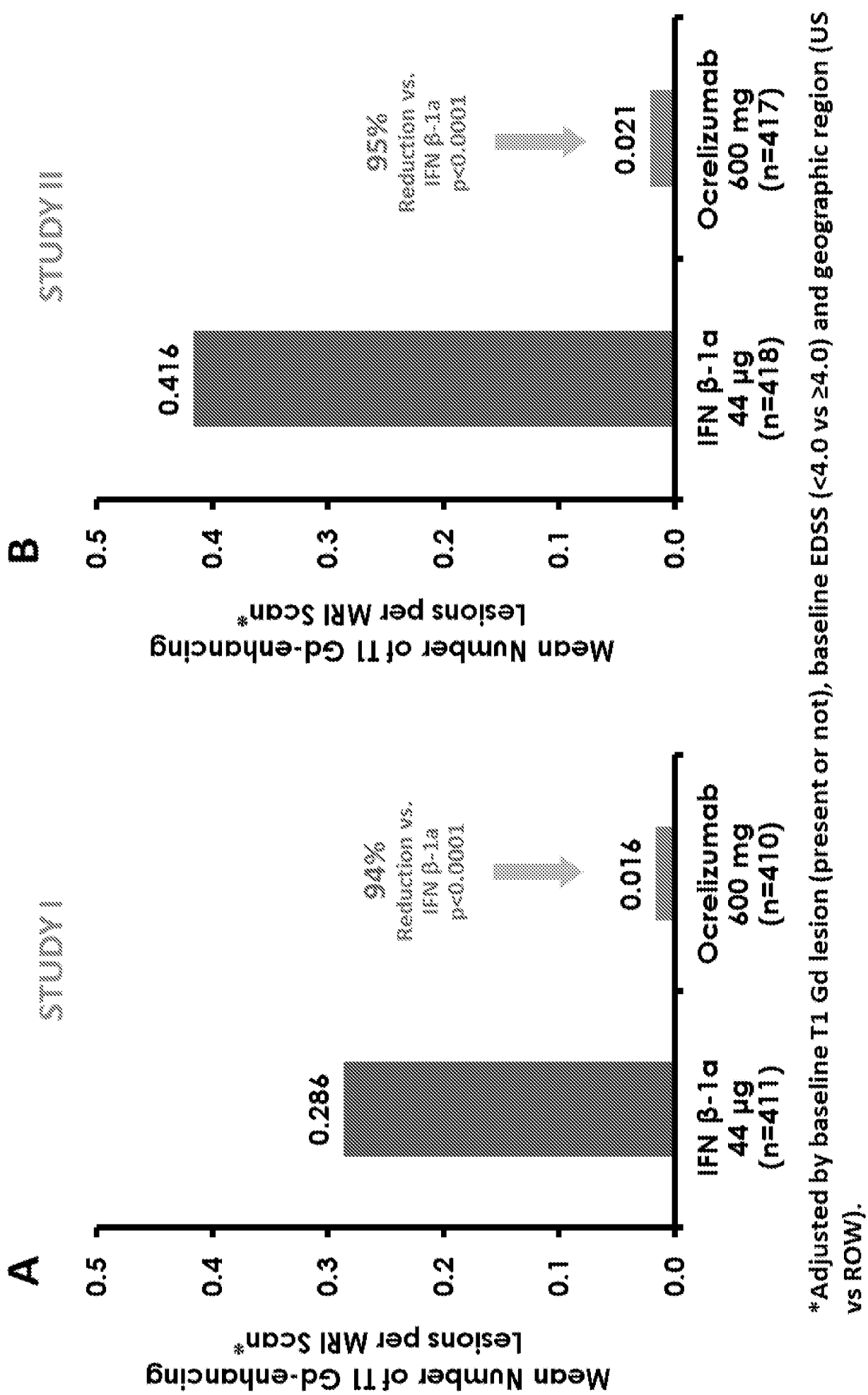
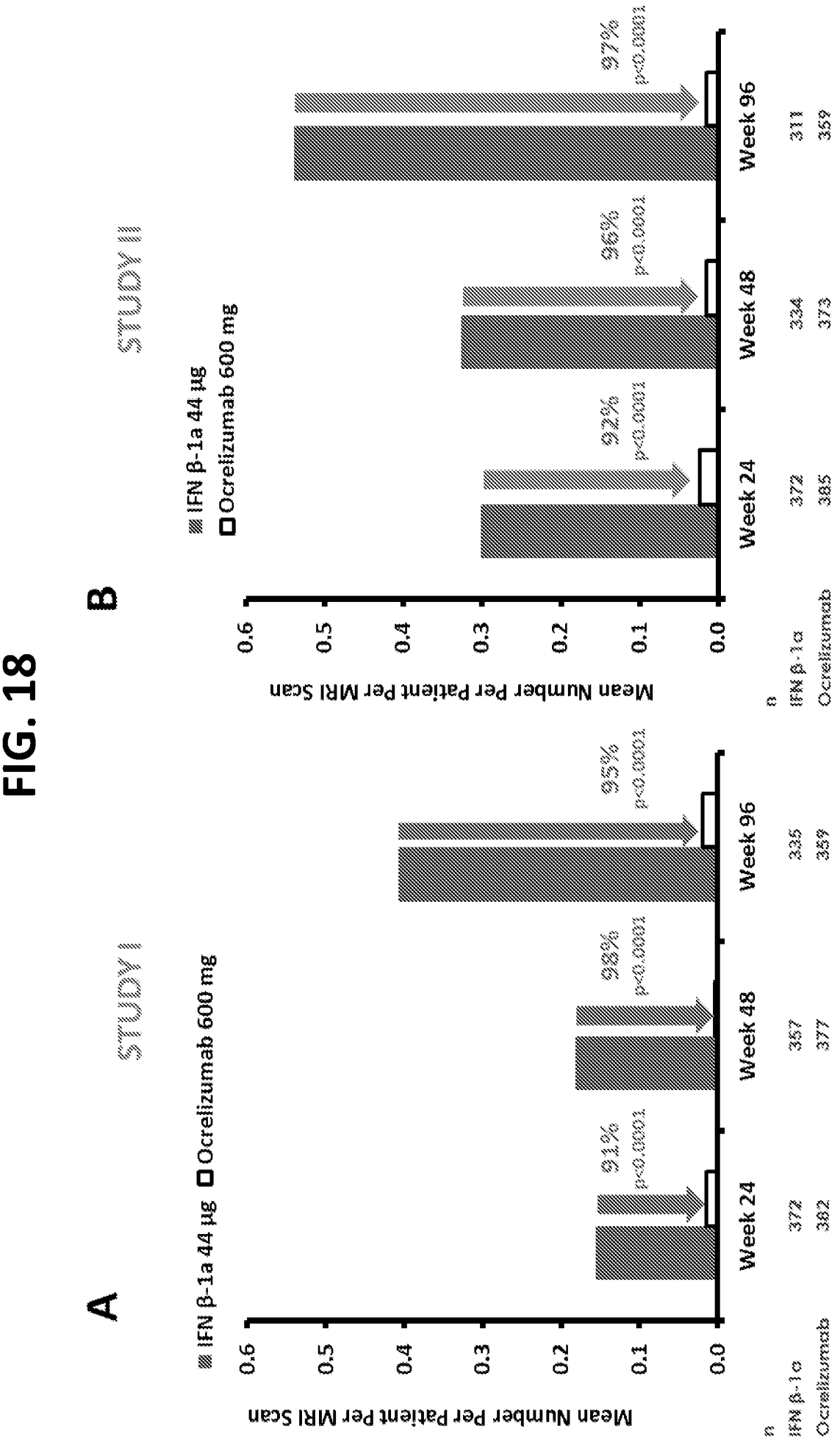

IFN β-1a (N=418)
OCR 600 mg (N=417)
p=0.0019
Change From Baseline (MSFCS). Mean and 95% CI
0.3
0.2
0.1
0
Baseline
12
24
36
48
60
72
84
96
Week
N
IFN β-1a 342 339 321 307 294 284 278 276 269
OCR 600 mg 358 352 348 342 335 330 321 317 308

FIG. 17



*Adjusted by baseline T1 Gd lesion (present or not), baseline EDSS (<4.0 vs ≥4.0) and geographic region (US vs ROW).

FIG. 18



*Adjusted by baseline T1 Gd lesion (present or not), baseline EDSS (<4.0 vs ≥4.0) and geographic region (US vs ROW).

FIG. 19

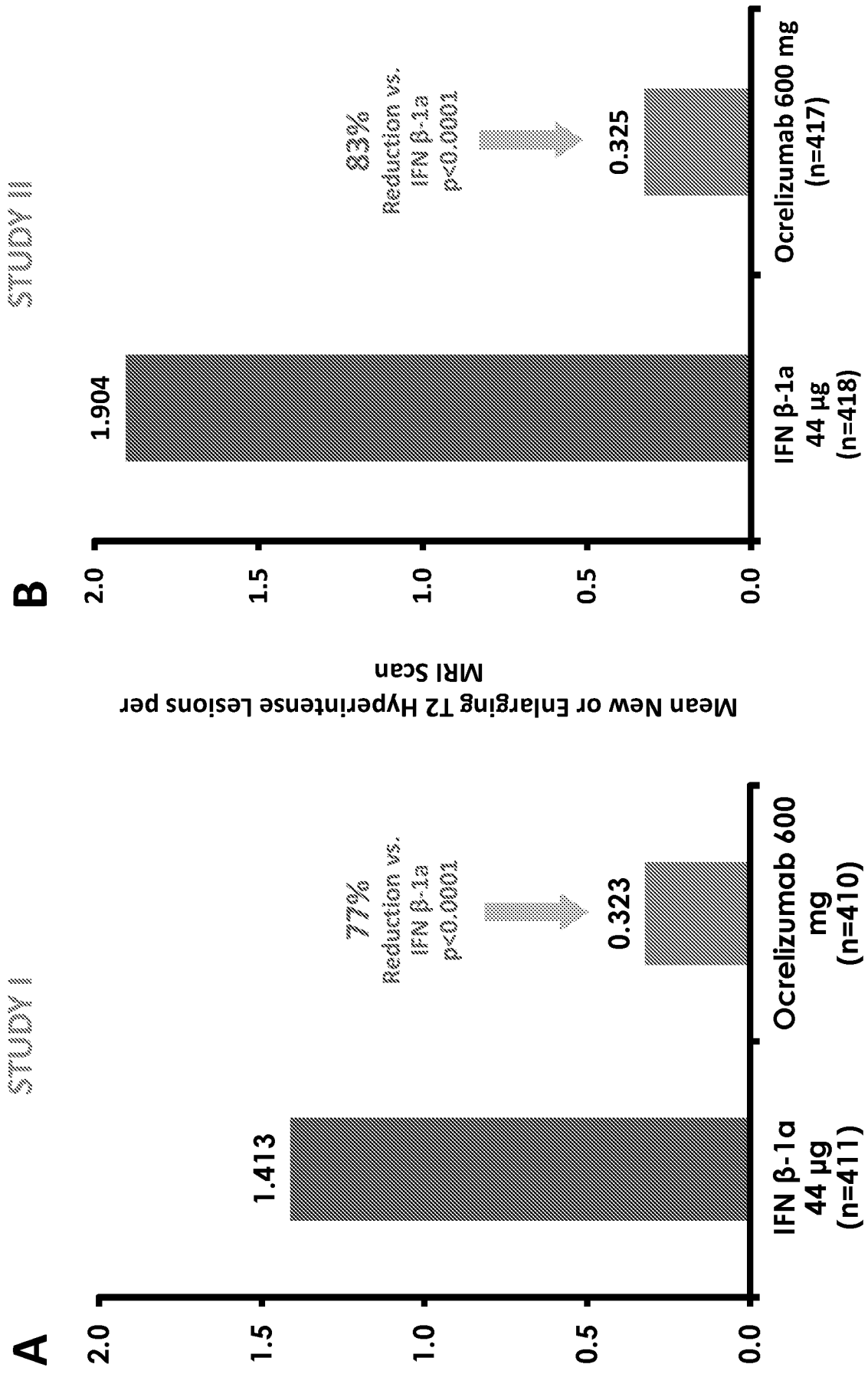

A
STUDY I
Mean New or Enlarging T2 Hyperintense Lesions per MRI Scan
2.0
1.5
1.0
0.5
0.0
1.413
0.323
77% Reduction vs. IFN β-1a p<0.0001
IFN β-1a 44 µg (n=411)
Ocrelizumab 600 mg (n=410)
B
STUDY II
Mean New or Enlarging T2 Hyperintense Lesions per MRI Scan
2.0
1.5
1.0
0.5
0.0
1.904
0.325
83% Reduction vs. IFN β-1a p<0.0001
IFN β-1a 44 µg (n=418)
Ocrelizumab 600 mg (n=417)

FIG. 19 (cont'd)

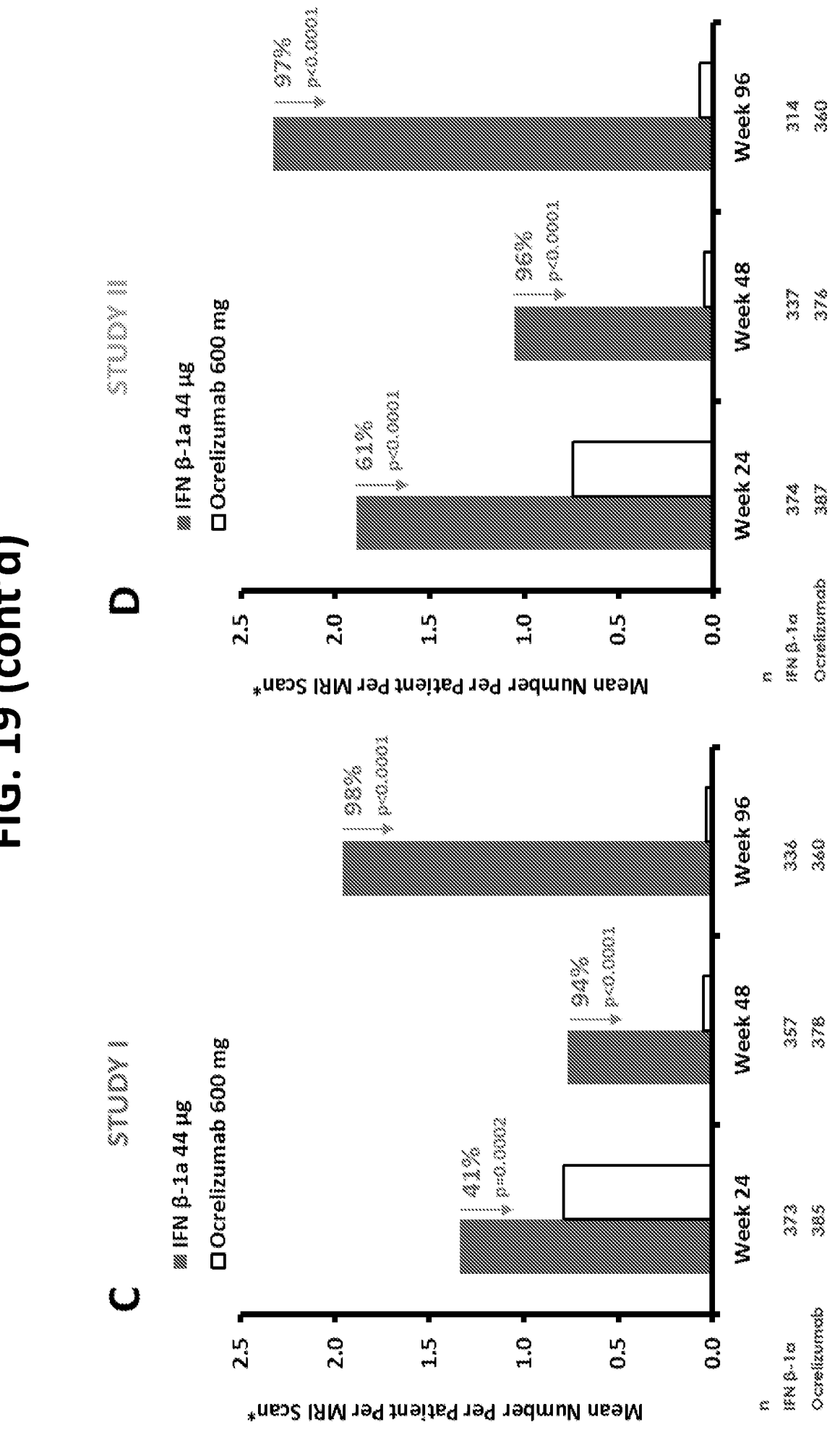

C
STUDY I
IFN β-1a 44 µg
Ocrelizumab 600 mg
Mean Number Per Patient Per MRI Scan*
2.5
2.0
1.5
1.0
0.5
0.0
41%
p=0.0002
94%
p<0.0001
98%
p<0.0001
Week 24
Week 48
Week 96
n
IFN β-1a 373 357 336
Ocrelizumab 385 378 360
D
STUDY II
IFN β-1a 44 µg
Ocrelizumab 600 mg
Mean Number Per Patient Per MRI Scan*
2.5
2.0
1.5
1.0
0.5
0.0
61%
p<0.0001
96%
p<0.0001
97%
p<0.0001
Week 24
Week 48
Week 96
n
IFN β-1a 374 337 314
Ocrelizumab 387 376 360

FIG. 20

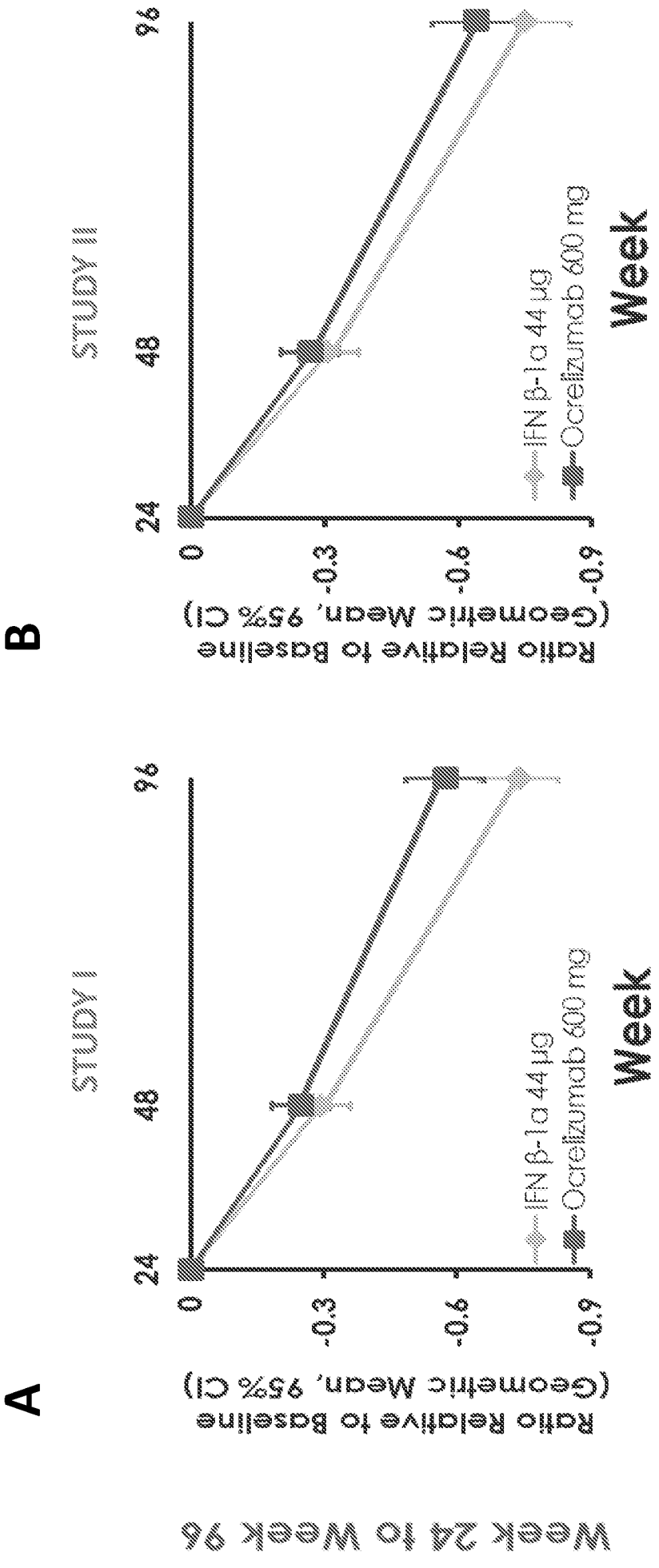

A
STUDY I
Week 24 to Week 96
Ratio Relative to Baseline
(Geometric Mean, 95% CI)
0
-0.3
-0.6
-0.9
24
48
96
Week
IFN β-1a 44 µg
Ocrelizumab 600 mg
B
STUDY II
Ratio Relative to Baseline
(Geometric Mean, 95% CI)
0
-0.3
-0.6
-0.9
24
48
96
Week
IFN β-1a 44 µg
Ocrelizumab 600 mg

FIG. 21

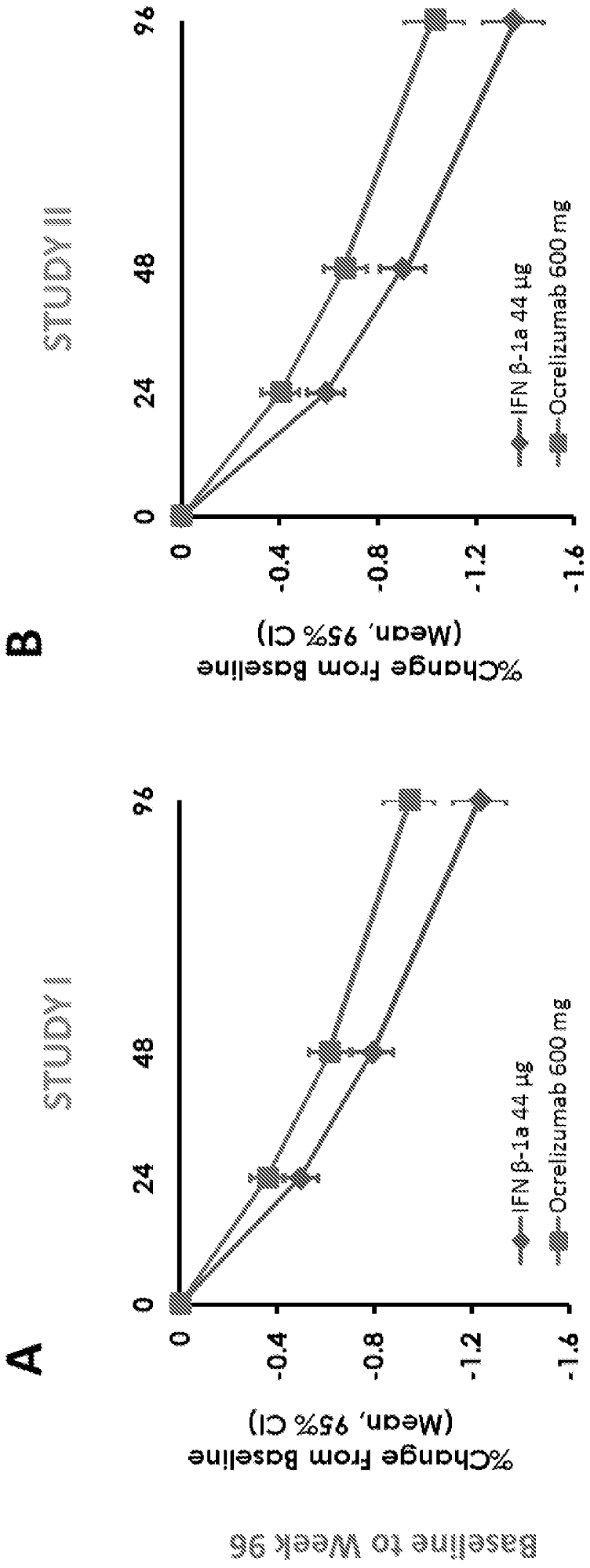
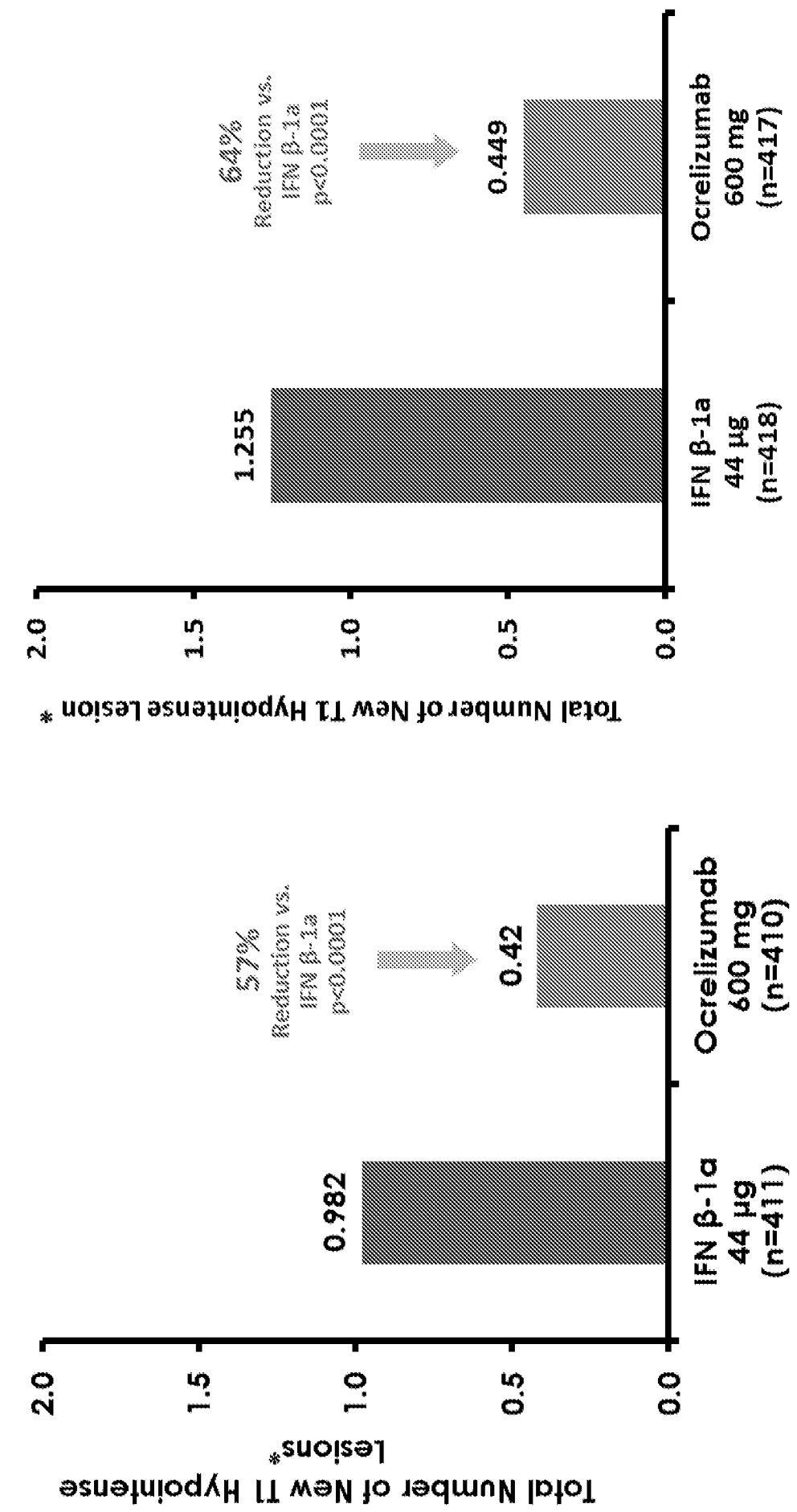

A
STUDY I
%Change from Baseline
(Mean, 95% CI)
0
-0.4
-0.8
-1.2
-1.6
0
24
48
96
Baseline to Week 96
IFN β-1a 44 µg
Ocrelizumab 600 mg
B
STUDY II
%Change from Baseline
(Mean, 95% CI)
0
-0.4
-0.8
-1.2
-1.6
0
24
48
96
IFN β-1a 44 µg
Ocrelizumab 600 mg

FIG. 22



*Adjusted by baseline T1 hypointense lesion count, baseline EDSS (<4.0 vs ≥4.0) and geographic region (US vs ROW).

FIG. 23

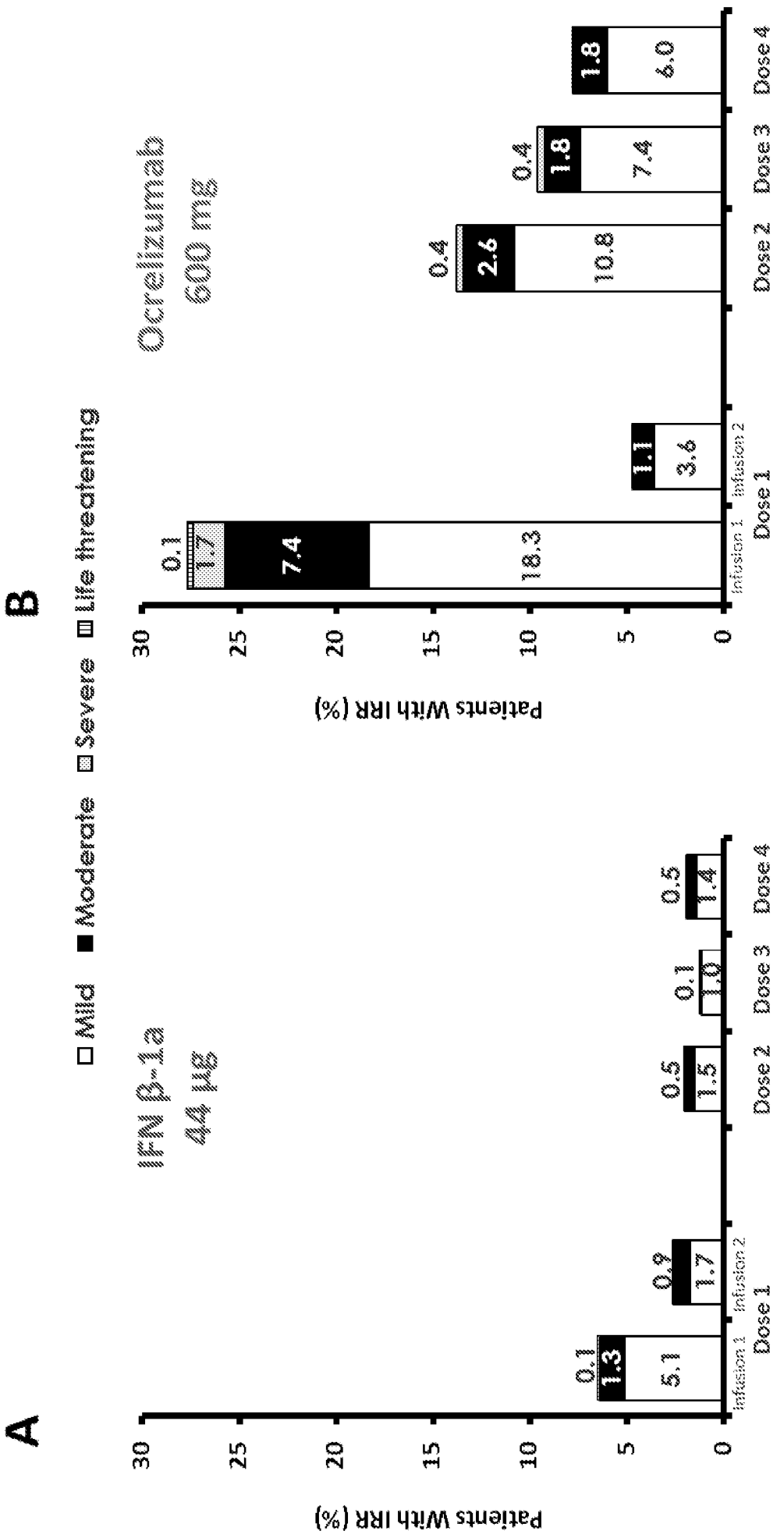
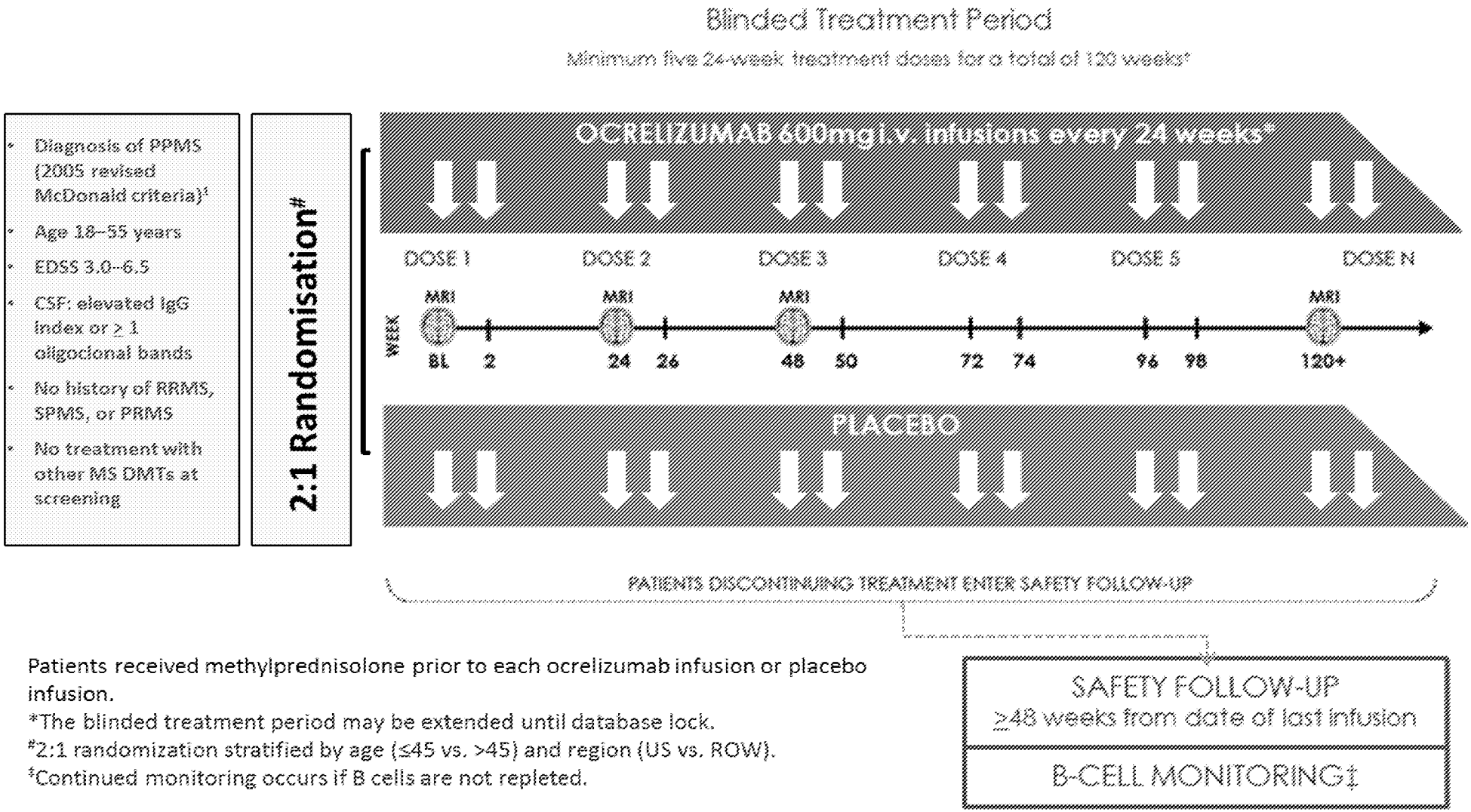

A
B
Mild
Moderate
Severe
Life threatening
IFN β-1a
44 µg
Ocrelizumab
600 mg
Patients With IRR (%)
Infusion 1
Infusion 2
Dose 1
Dose 2
Dose 3
Dose 4
0.1
1.3
5.1
0.9
1.7
0.5
1.5
0.1
1.0
0.5
1.4
0.1
1.7
7.4
18.3
1.1
3.6
0.4
2.6
10.8
0.4
1.8
7.4
1.8
4.0

FIG. 24



Patients received methylprednisolone prior to each ocrelizumab infusion or placebo infusion.
*The blinded treatment period may be extended until database lock.
#2:1 randomization stratified by age (≤45 vs. >45) and region (US vs. ROW).
‡Continued monitoring occurs if B cells are not repleted.

CSF, cerebrospinal fluid; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; i.v., intravenous; MRI, magnetic resonance imaging.

FIG. 25

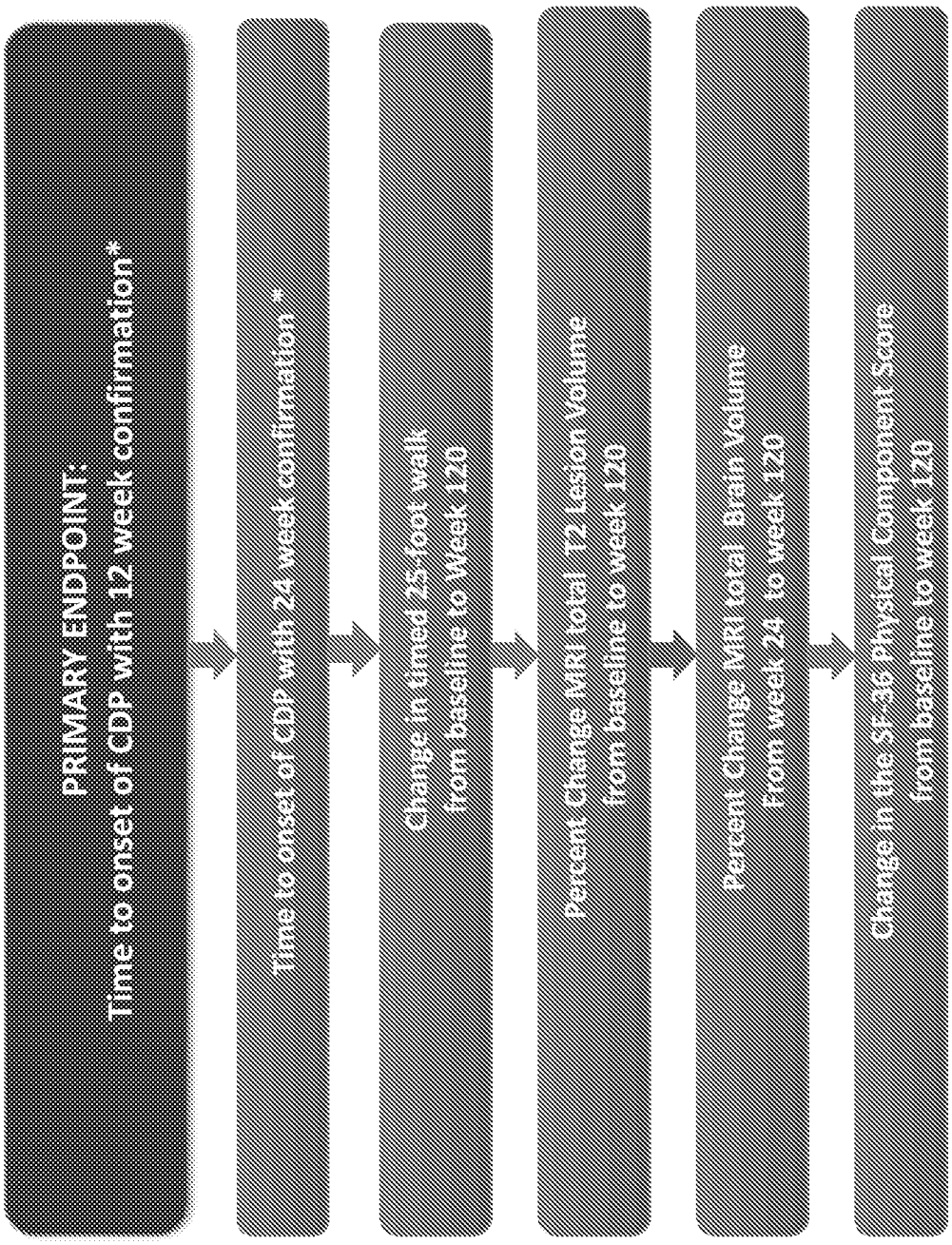

PRIMARY ENDPOINT:
Time to onset of CDP with 12 week confirmation*
Time to onset of CDP with 24 week confirmation *
Change in Timed 25-foot walk
from baseline to Week 120
Percent Change MRI total T2 Lesion Volume
from baseline to week 120
Percent Change MRI total Brain Volume
from week 24 to week 120
Change in the SF-36 Physical Component Score
from baseline to week 120

FIG. 26

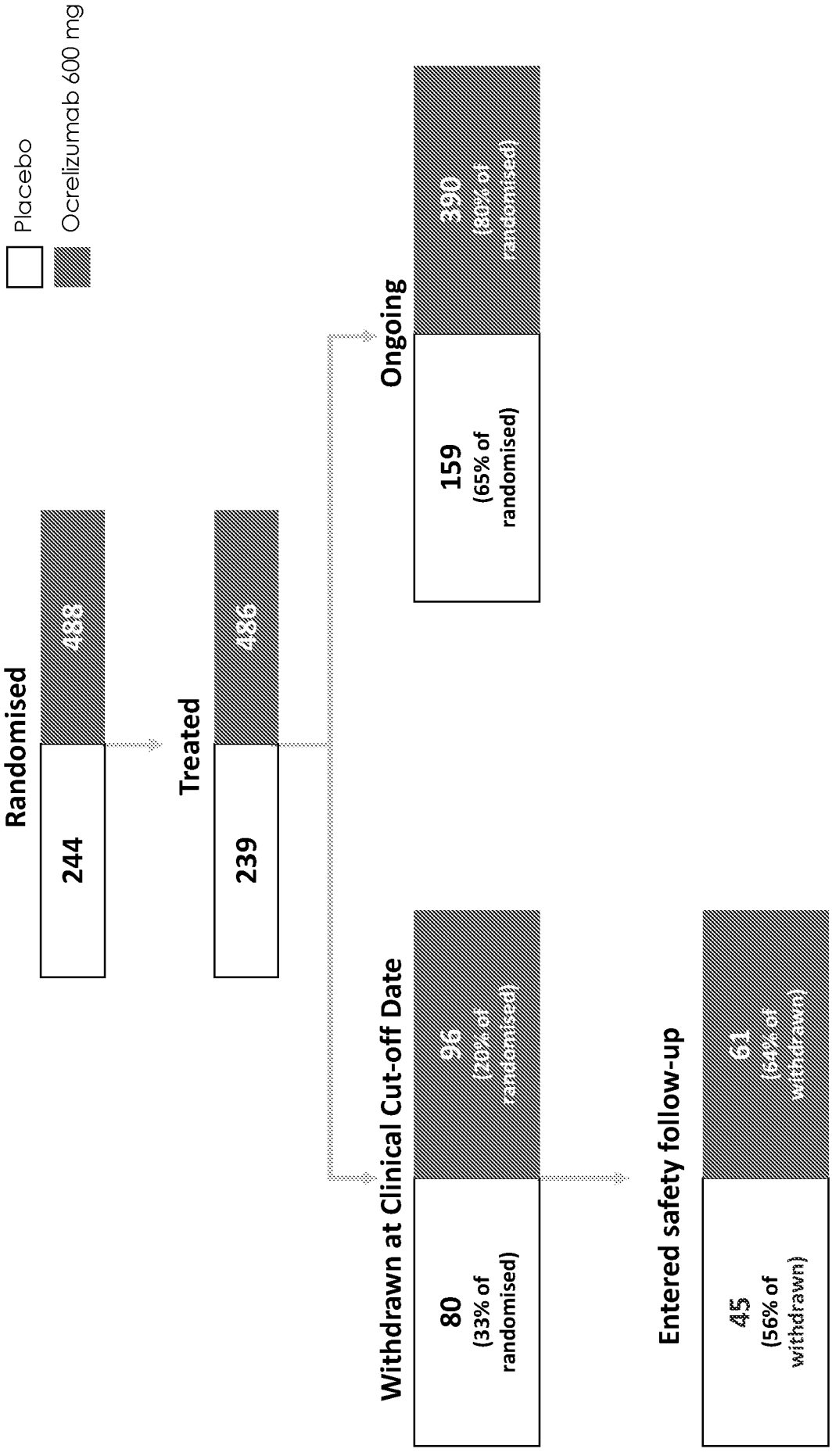

Placebo
Ocrelizumab 600 mg
Randomised
244
488
Treated
239
486
Ongoing
159
(65% of randomised)
390
(80% of randomised)
Withdrawn at Clinical Cut-off Date
80
(33% of randomised)
96
(20% of randomised)
Entered safety follow-up
45
(56% of withdrawn)
61
(64% of withdrawn)

FIG. 27

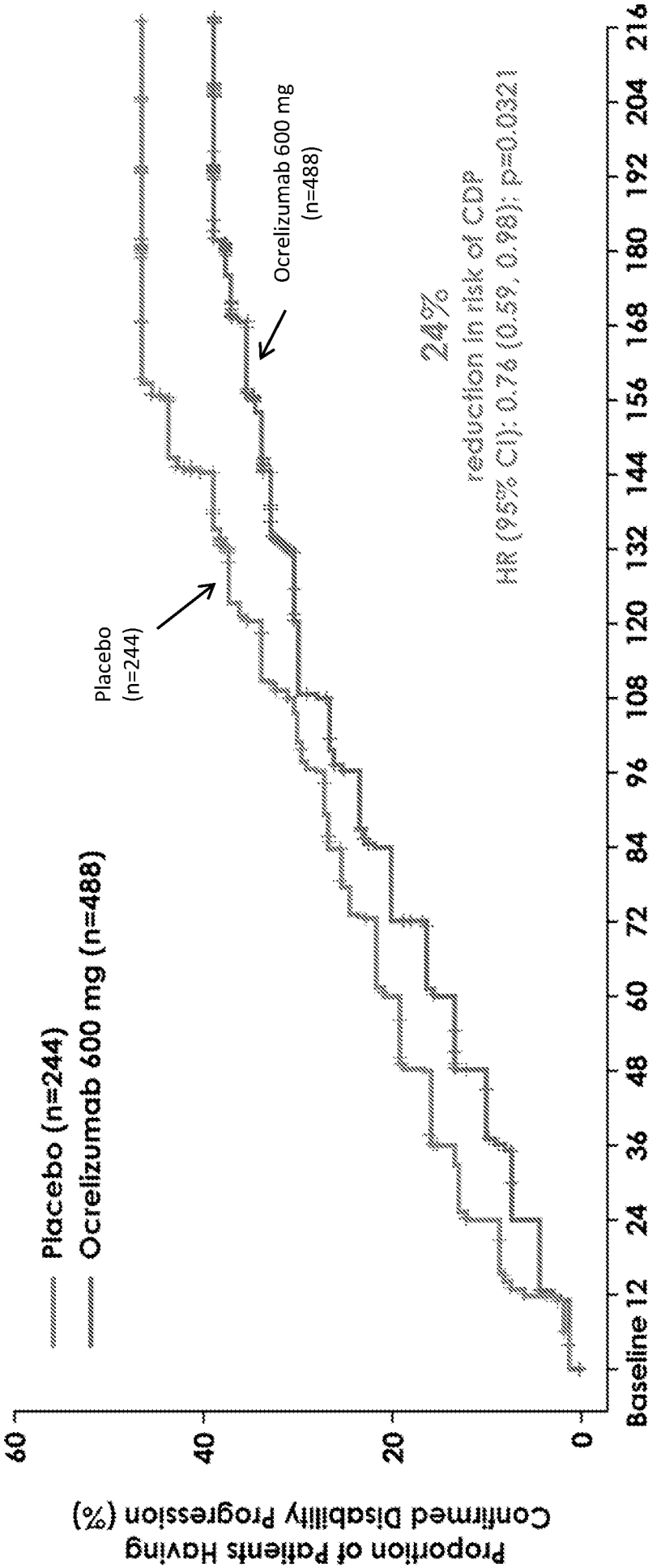

Proportion of Patients Having Confirmed Disability Progression (%)
60
40
20
0
Placebo (n=244)
Ocrelizumab 600 mg (n=488)
Placebo (n=244)
Ocrelizumab 600 mg (n=488)
24%
reduction in risk of CDP
HR (95% CI): 0.76 (0.59, 0.98); p=0.0321
Baseline 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
Time to Confirmed Disability Progression (Weeks)

FIG. 28

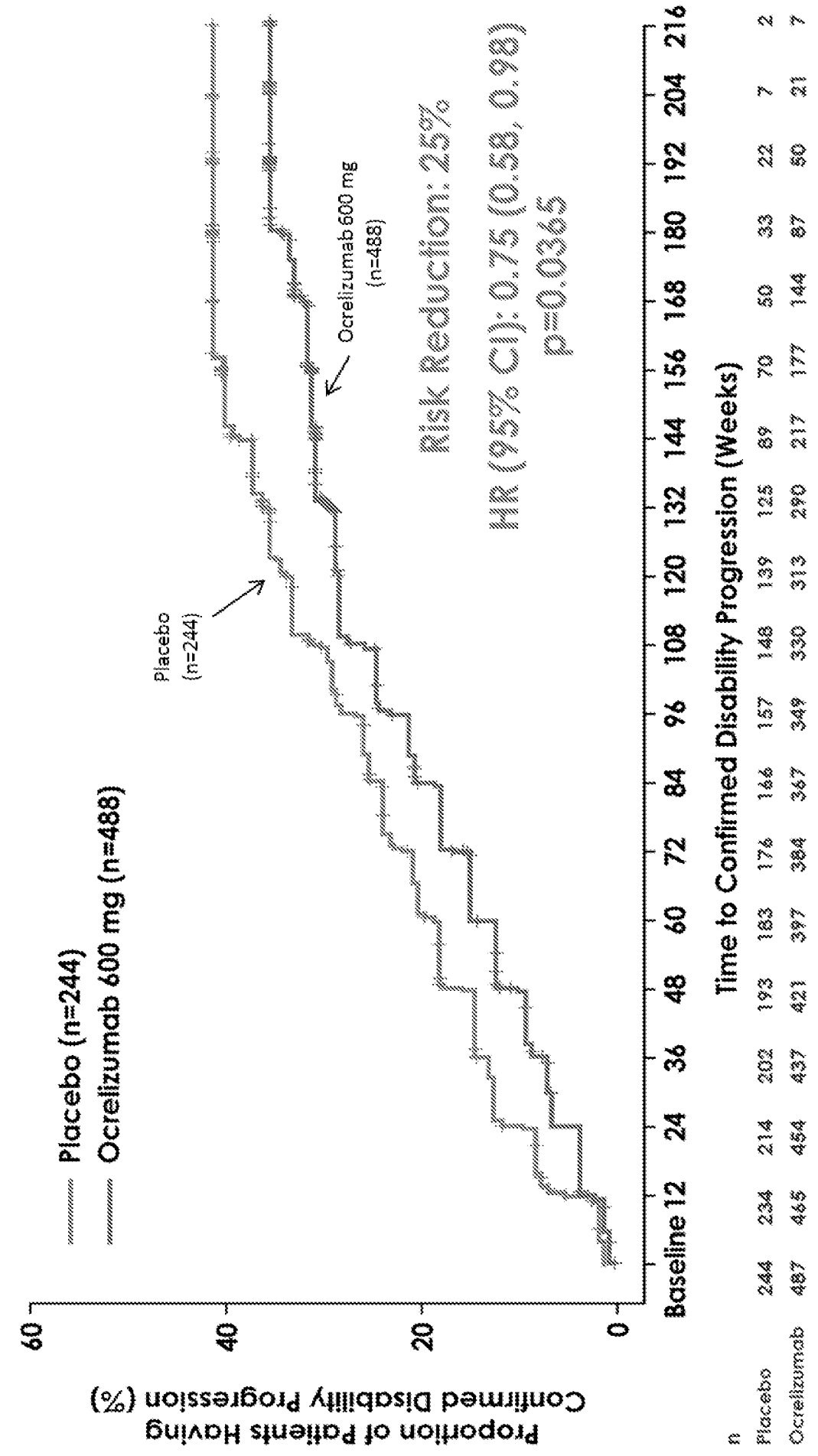

Placebo (n=244)
Ocrelizumab 600 mg (n=488)
Proportion of Patients Having Confirmed Disability Progression (%)
60
40
20
0
Placebo (n=244)
Ocrelizumab 600 mg (n=488)
Risk Reduction: 25%
HR (95% CI): 0.75 (0.58, 0.98)
p=0.0365
Baseline 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
Time to Confirmed Disability Progression (Weeks)
n
Placebo 244 234 214 202 193 183 176 166 157 148 139 125 89 70 50 33 22 7 2
Ocrelizumab 487 465 454 437 421 397 384 367 349 330 313 290 217 177 144 87 50 21 7

FIG. 29

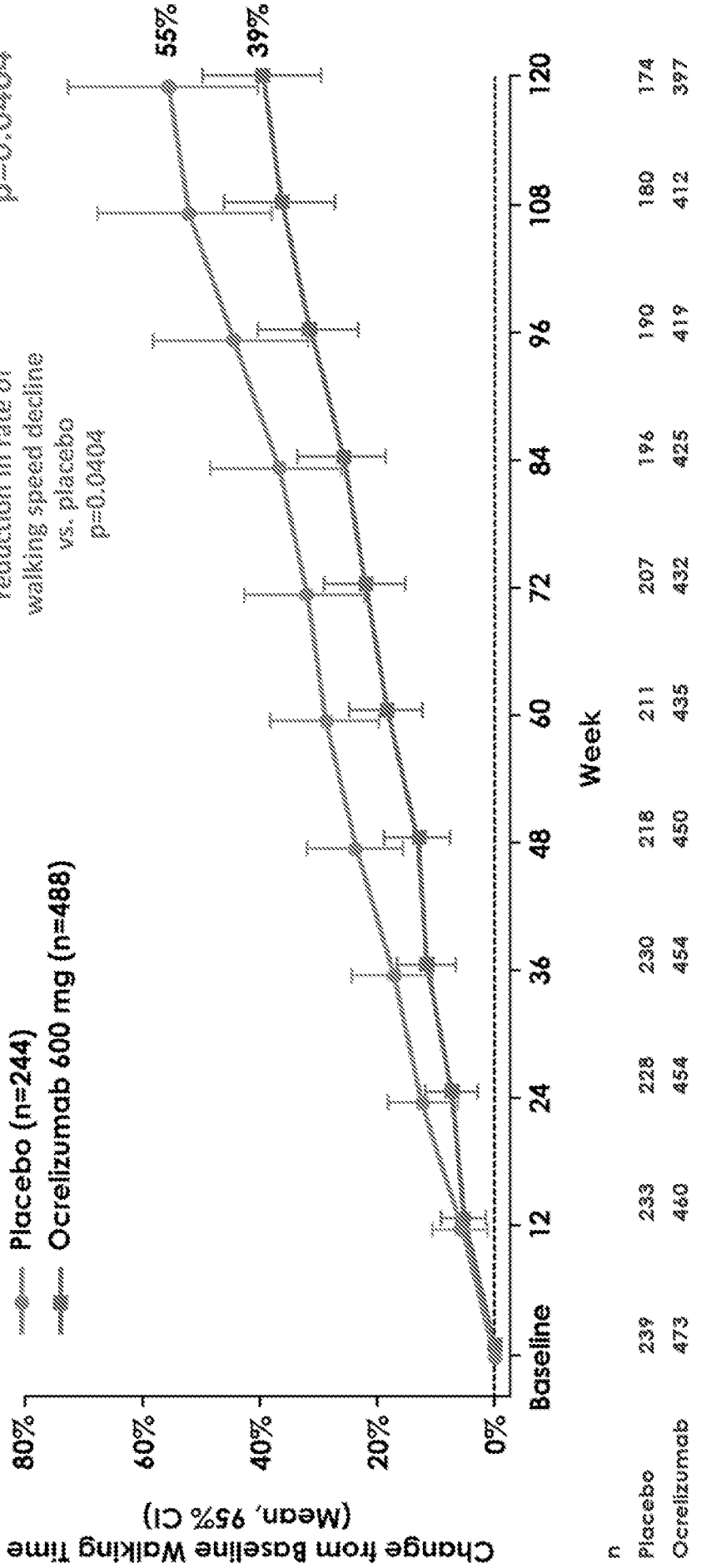
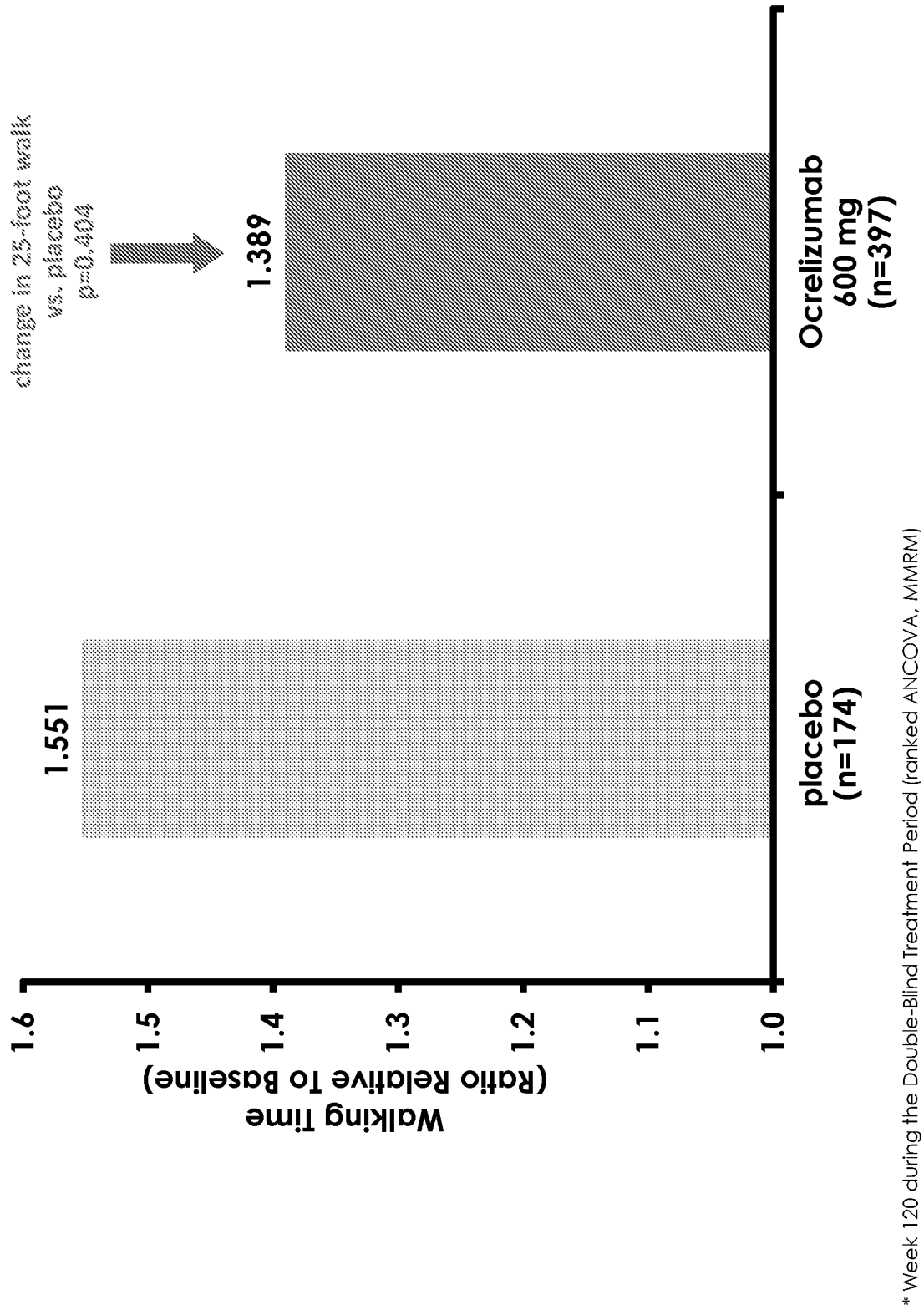

Placebo (n=244)
Ocrelizumab 600 mg (n=488)
29%
reduction in rate of
walking speed decline
vs. placebo
p=0.0404
p=0.0404*
55%
39%
Change from Baseline Walking Time
(Mean, 95% CI)
80%
60%
40%
20%
0%
Baseline
12
24
36
48
60
72
84
96
108
120
Week
n
Placebo 239 233 228 230 218 211 207 196 190 180 174
Ocrelizumab 473 460 454 454 450 435 432 425 419 412 397

FIG. 30



* Week 120 during the Double-Blind Treatment Period (ranked ANCOVA, MMRM)

FIG. 31

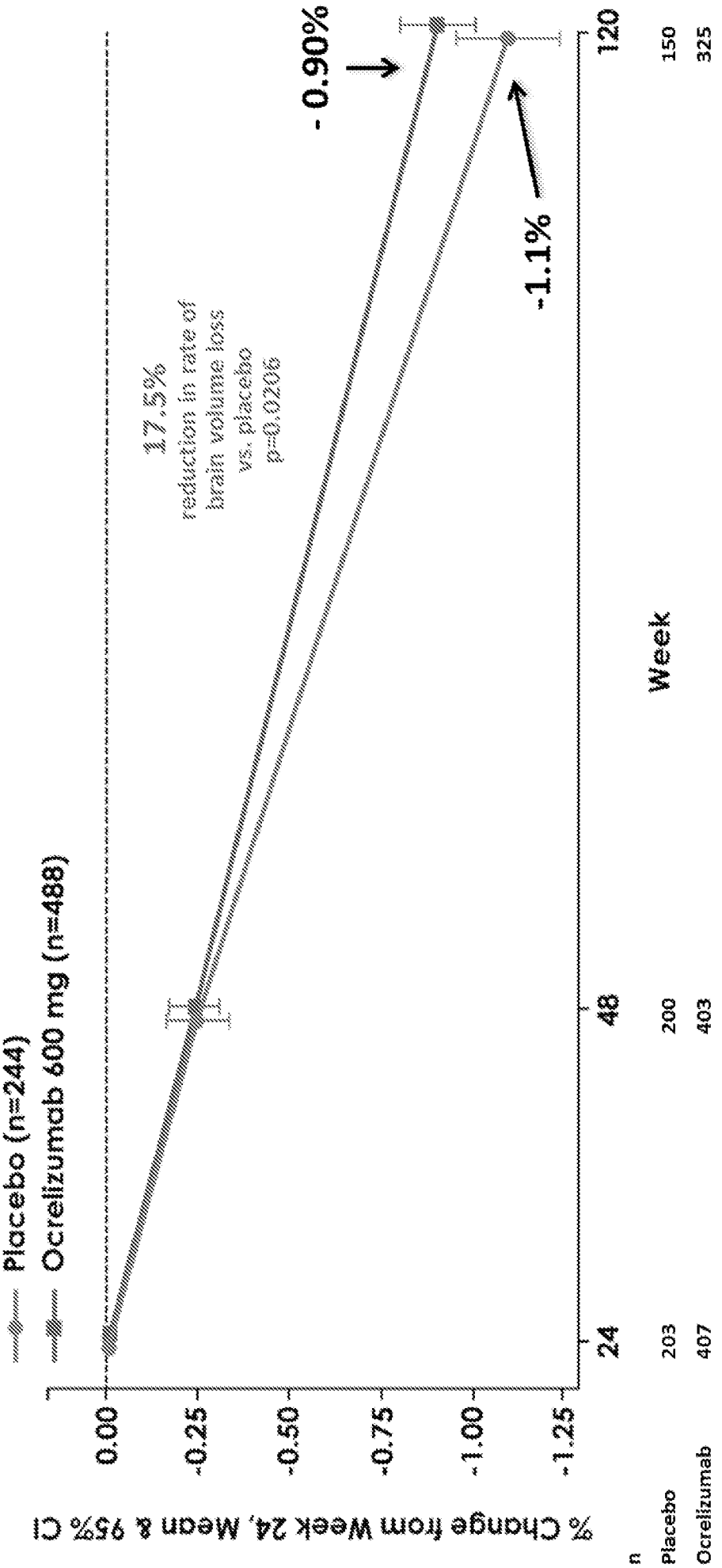

Placebo (n=244)
Ocrelizumab 600 mg (n=488)
17.5%
reduction in rate of
brain volume loss
vs. placebo
p=0.0206
- 0.90%
-1.1%
% Change from Week 24, Mean & 95% CI
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
24
48
120
Week
n
Placebo 203 200 150
Ocrelizumab 407 403 325

FIG. 32

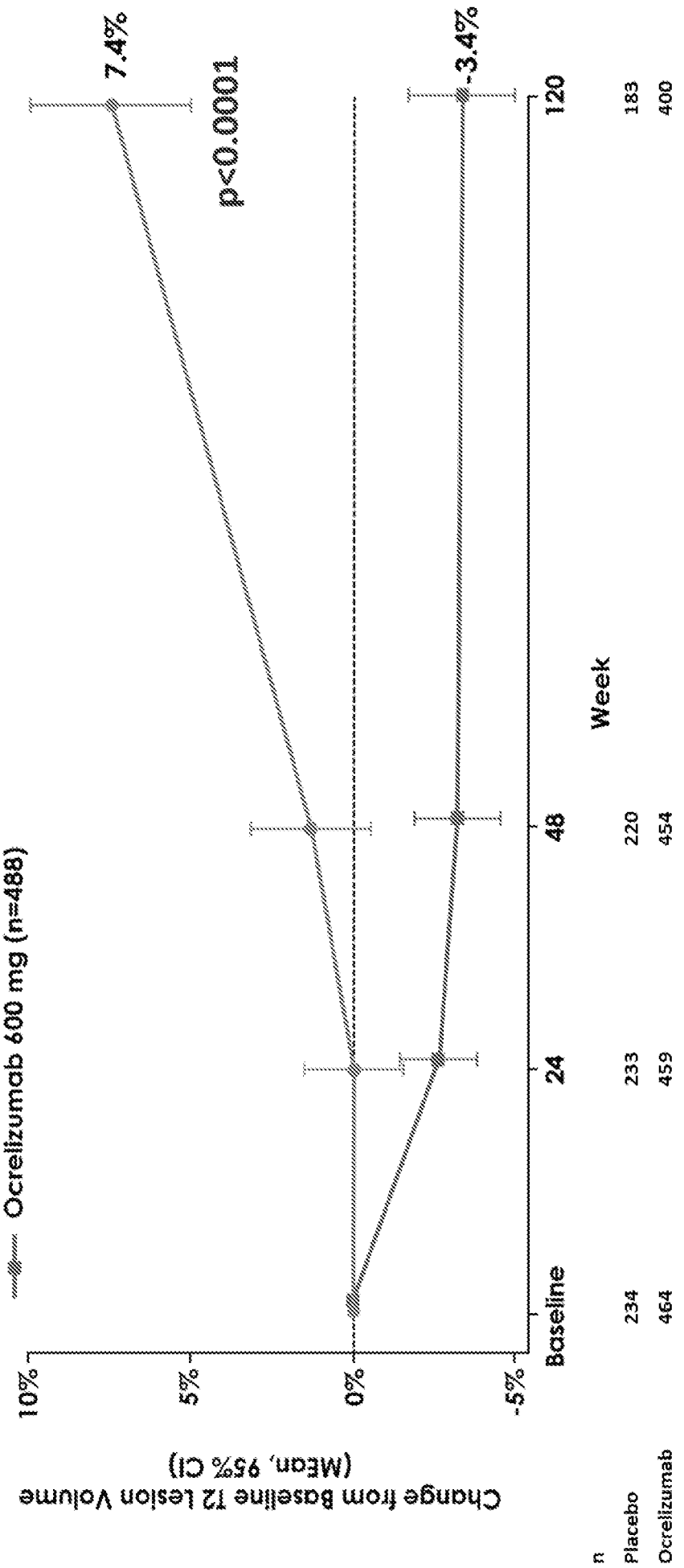

Placebo (n=244)
Ocrelizumab 600 mg (n=488)
7.4%
-3.4%
p<0.0001
10%
5%
0%
-5%
Change from Baseline T2 Lesion Volume
(Mean, 95% CI)
Baseline
24
48
120
Week
n
Placebo 234 233 220 183
Ocrelizumab 464 459 454 400

FIG. 33

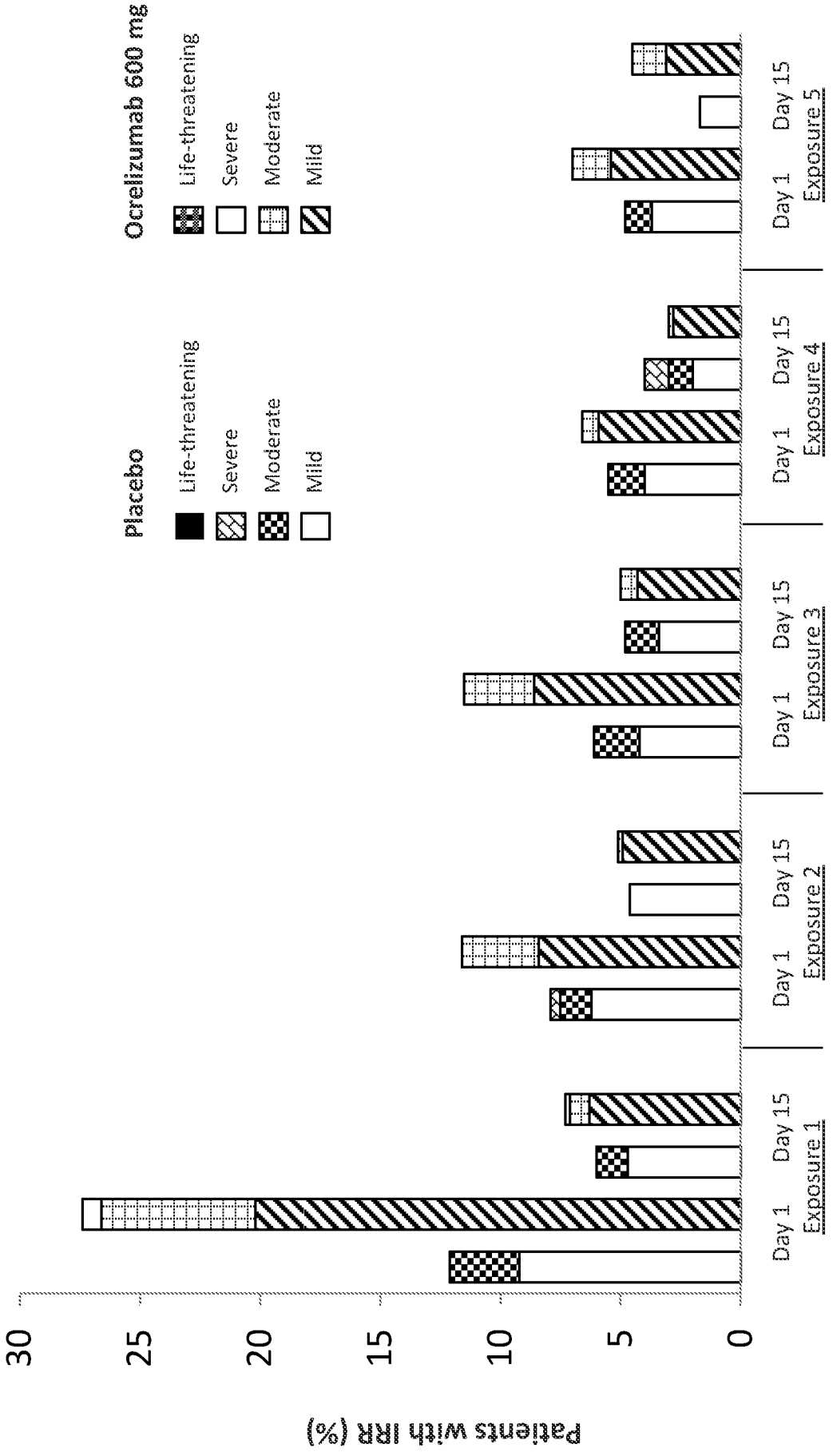
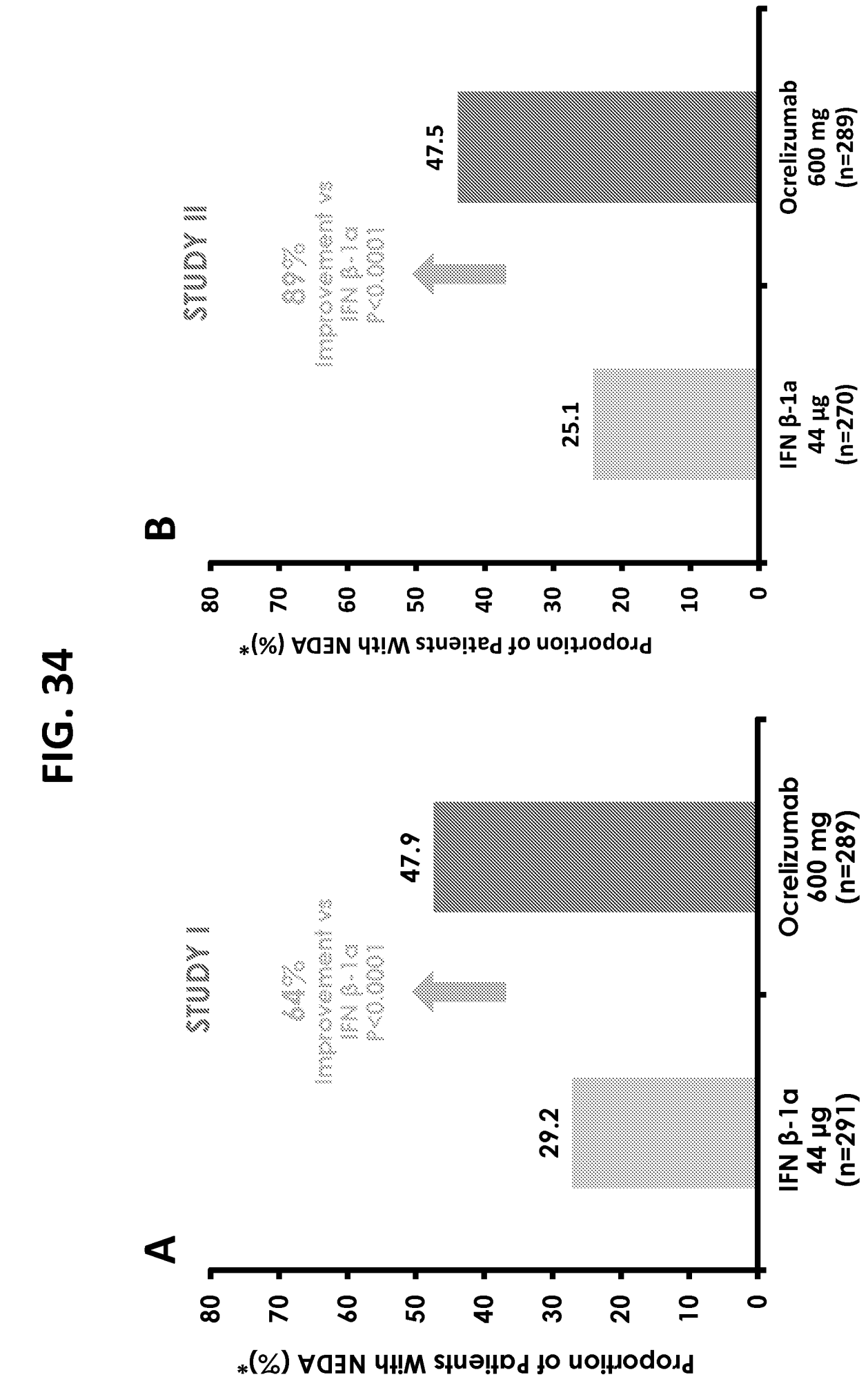

Placebo
Life-threatening
Severe
Moderate
Mild
Ocrelizumab 600 mg
Life-threatening
Severe
Moderate
Mild
Patients with IRR (%)
0
5
10
15
20
25
30
Day 1
Day 15
Exposure 1
Day 1
Day 15
Exposure 2
Day 1
Day 15
Exposure 3
Day 1
Day 15
Exposure 4
Day 1
Day 15
Exposure 5

FIG. 34



*Compared using the Cochran–Mantel–Haenszel X2 test stratified by geographic region (United States vs ROW) and baseline EDSS score (<4.0 vs. ≥4.0).
t_neda_cmh_IT_092_0.2, t_neda_cmh_IT_093_0.1

FIG. 35

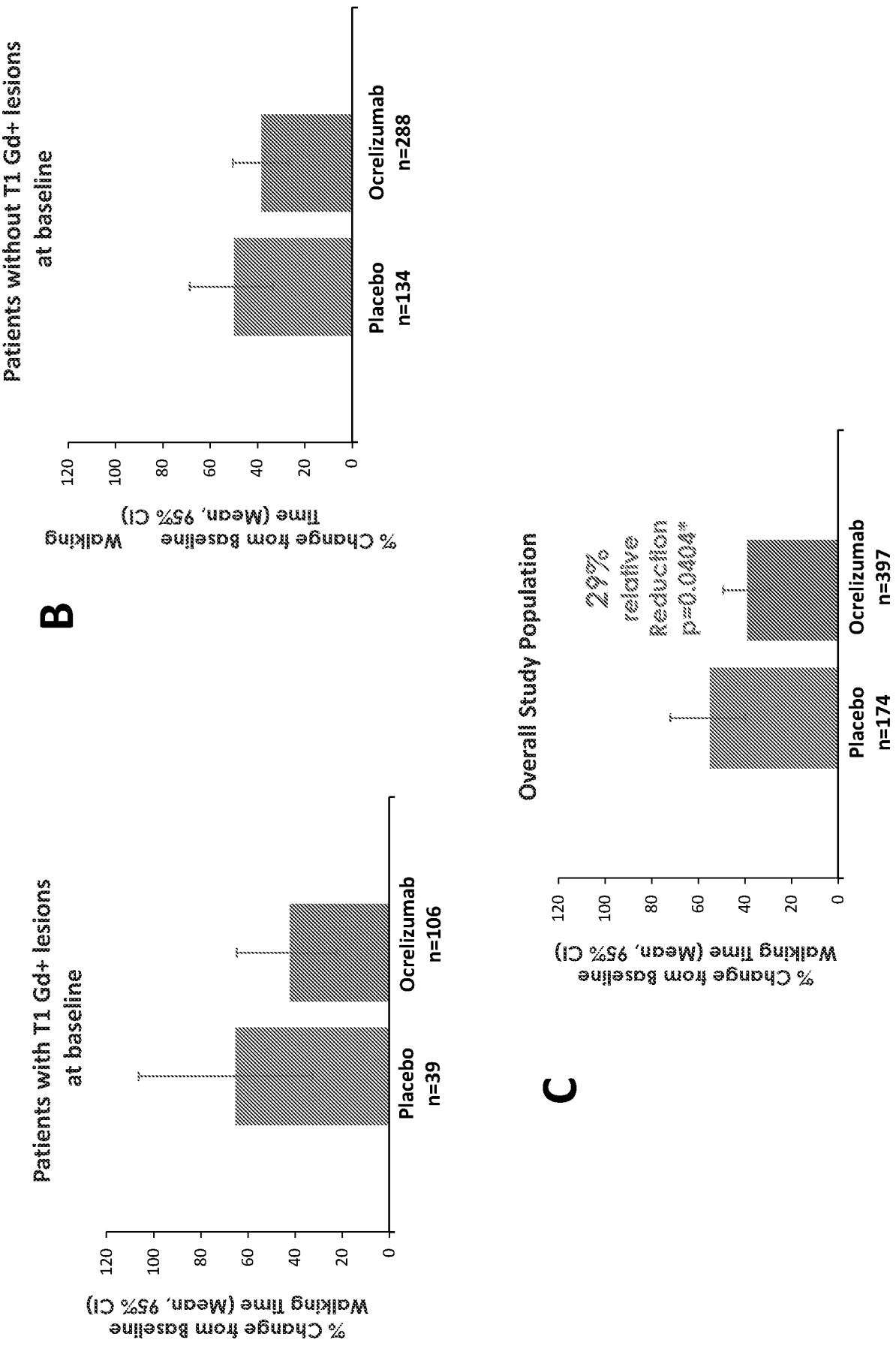

A
Patients with T1 Gd+ lesions at baseline
% Change from baseline Walking Time (Mean, 95% CI)
120
100
80
60
40
20
0
Placebo n=39
Ocrelizumab n=106
B
Patients without T1 Gd+ lesions at baseline
% Change from baseline Walking Time (Mean, 95% CI)
120
100
80
60
40
20
0
Placebo n=134
Ocrelizumab n=288
C
Overall Study Population
29% relative Reduction p=0.0404*
% Change from baseline Walking Time (Mean, 95% CI)
120
100
80
60
40
20
0
Placebo n=174
Ocrelizumab n=397

FIG. 36

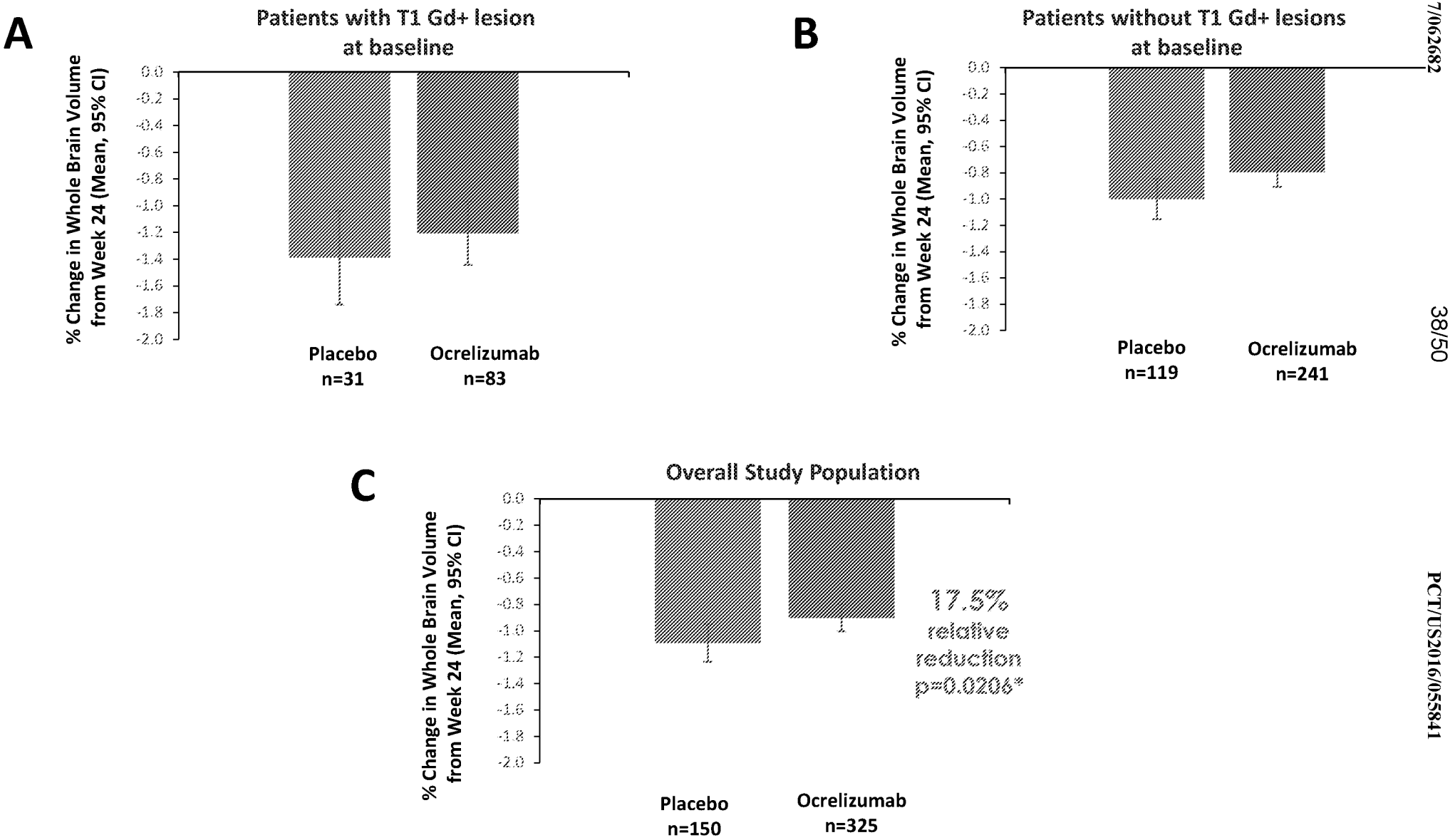

A
Patients with T1 Gd+ lesion at baseline
% Change in Whole Brain Volume from Week 24 (Mean, 95% CI)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
Placebo n=31
Ocrelizumab n=83
B
Patients without T1 Gd+ lesions at baseline
% Change in Whole Brain Volume from Week 24 (Mean, 95% CI)
Placebo n=119
Ocrelizumab n=241
C
Overall Study Population
% Change in Whole Brain Volume from Week 24 (Mean, 95% CI)
17.5% relative reduction p=0.0206*
Placebo n=150
Ocrelizumab n=325

FIG. 37

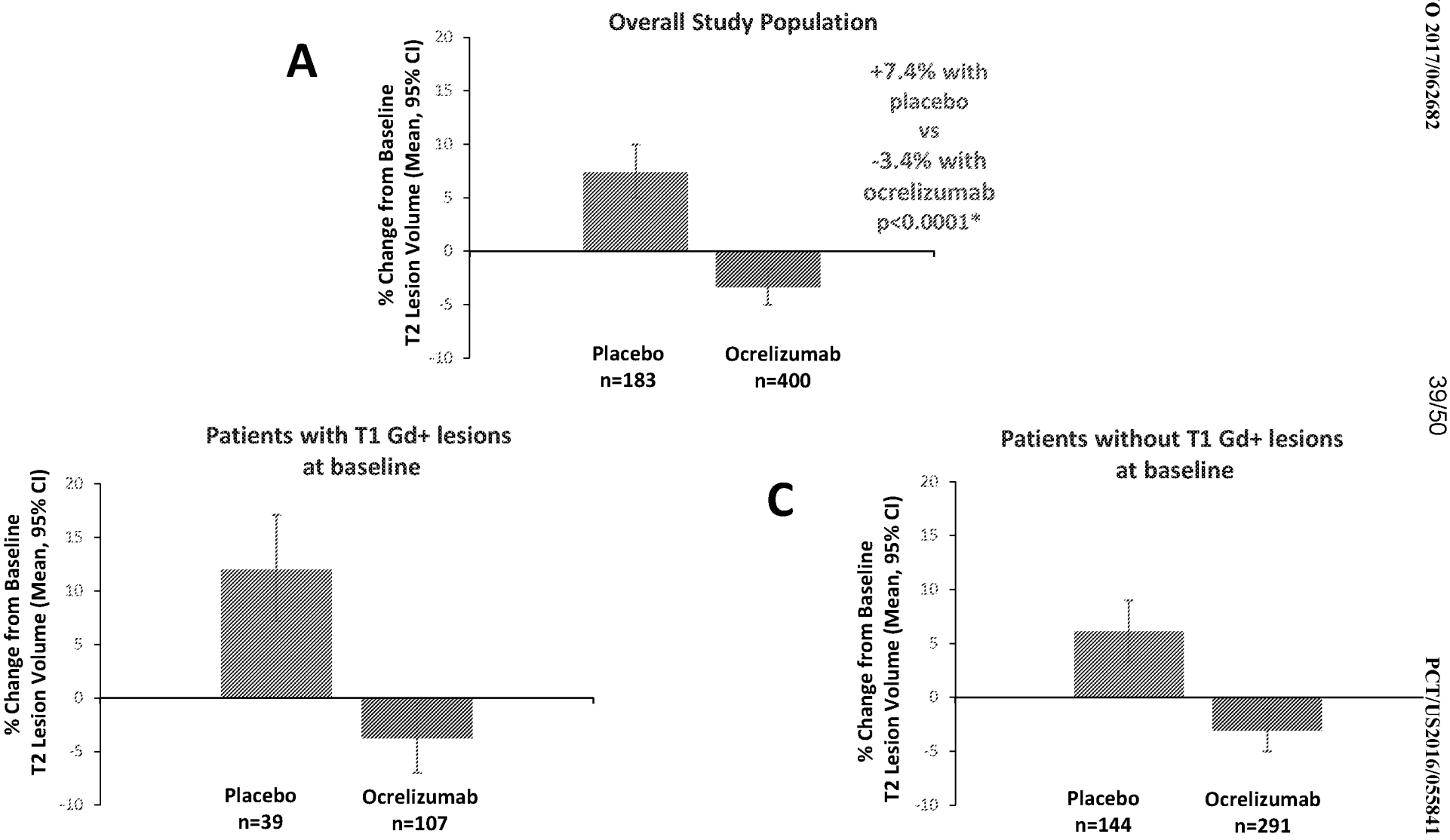
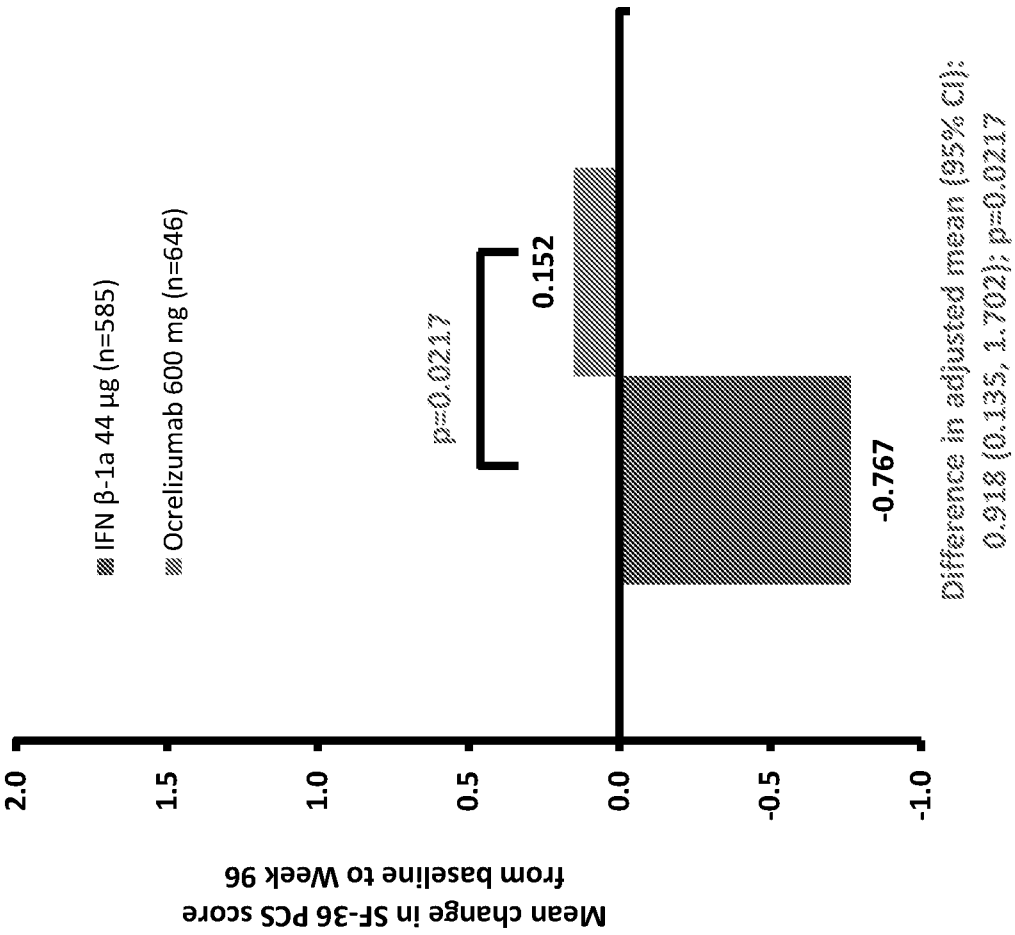

A
Overall Study Population
% Change from Baseline
T2 Lesion Volume (Mean, 95% CI)
20
15
10
5
0
-5
-10
+7.4% with placebo vs -3.4% with ocrelizumab p<0.0001*
Placebo n=183
Ocrelizumab n=400
B
Patients with T1 Gd+ lesions at baseline
% Change from Baseline
T2 Lesion Volume (Mean, 95% CI)
Placebo n=39
Ocrelizumab n=107
C
Patients without T1 Gd+ lesions at baseline
% Change from Baseline
T2 Lesion Volume (Mean, 95% CI)
Placebo n=144
Ocrelizumab n=291

FIG. 38

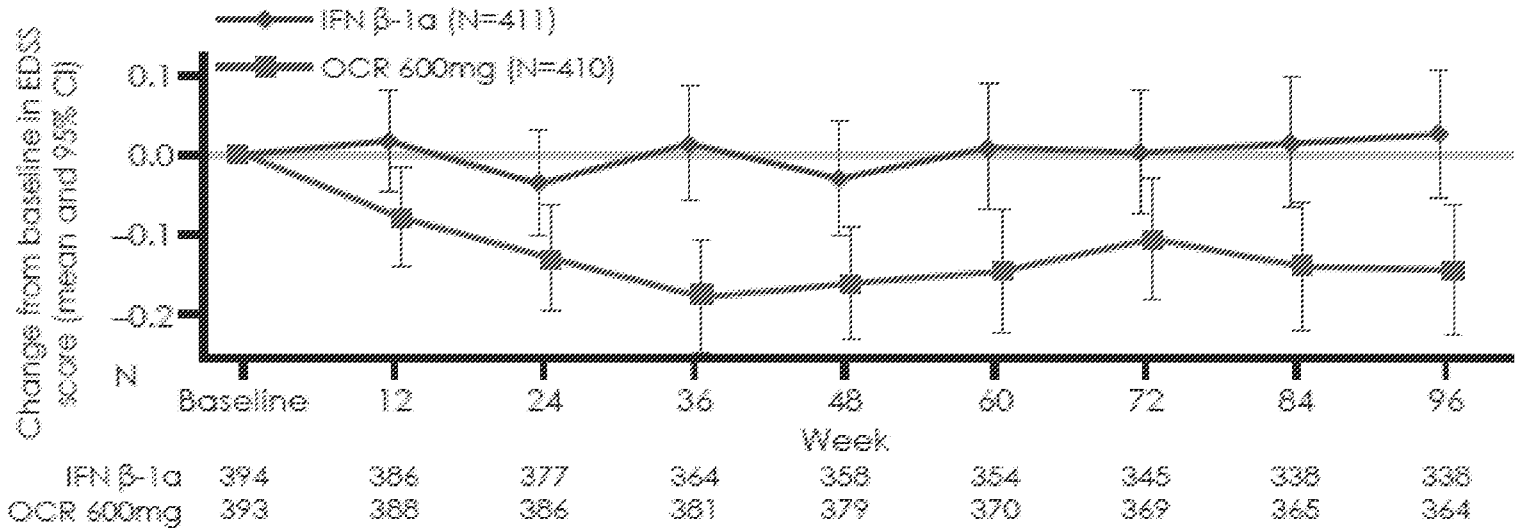


ITT. Post-hoc analysis, pooled OPERA I and II.
p value for difference in adjusted means is based on mixed-effect model of repeated measures using unstructured covariance matrix, adjusted by study, baseline PCS Score, baseline EDSS score (<4.0 vs ≥4.0), and geographic region (US vs ROW).
EDSS, Expanded Disability Status Scale; IFN, interferon; PCS, Physical Component Summary; ROW, rest of the world; SF-36, Short Form (36) Health Survey.

FIG. 39

A

STUDY 1



Difference in change from baseline to Week 96, adjusted mean (95% CI): -0.171 (-0.282, -0.059) p=0.0028

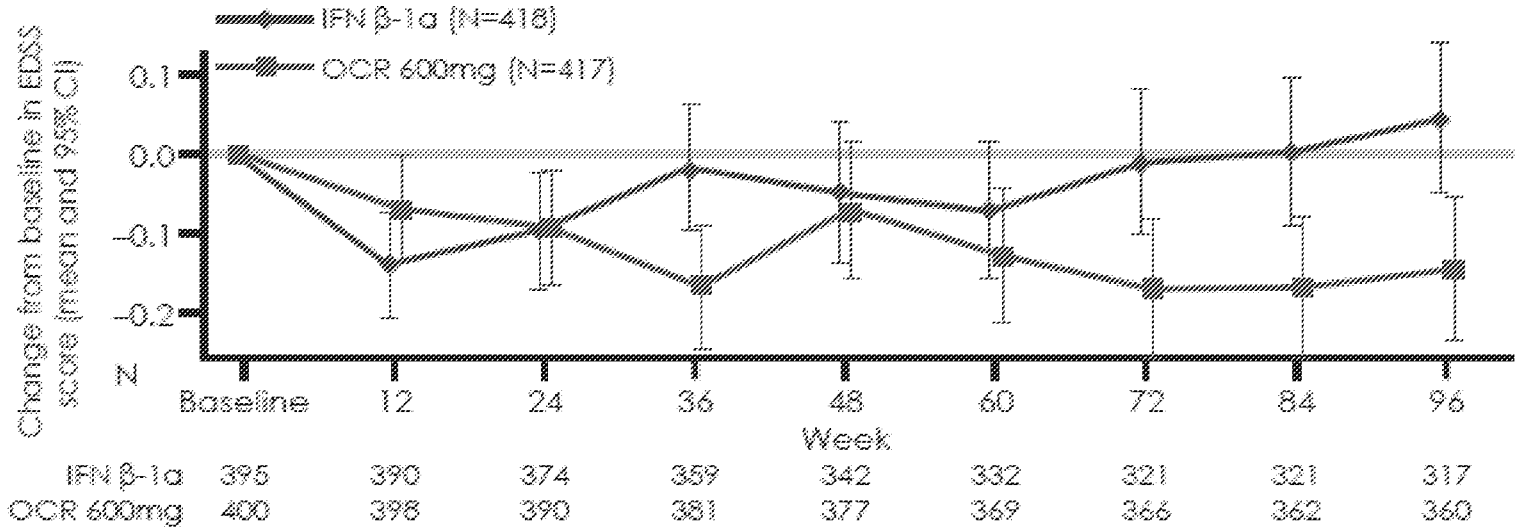
ITT. Estimates are from analysis based on mixed-effect model of repeated measures using unstructured covariance matrix adjusted for baseline EDSS score (continuous without rounding) and geographic region (US vs ROW).

EDSS, Expanded Disability Status Scale; IFN, interferon; ROW, rest of the world.

FIG. 39

B

STUDY II

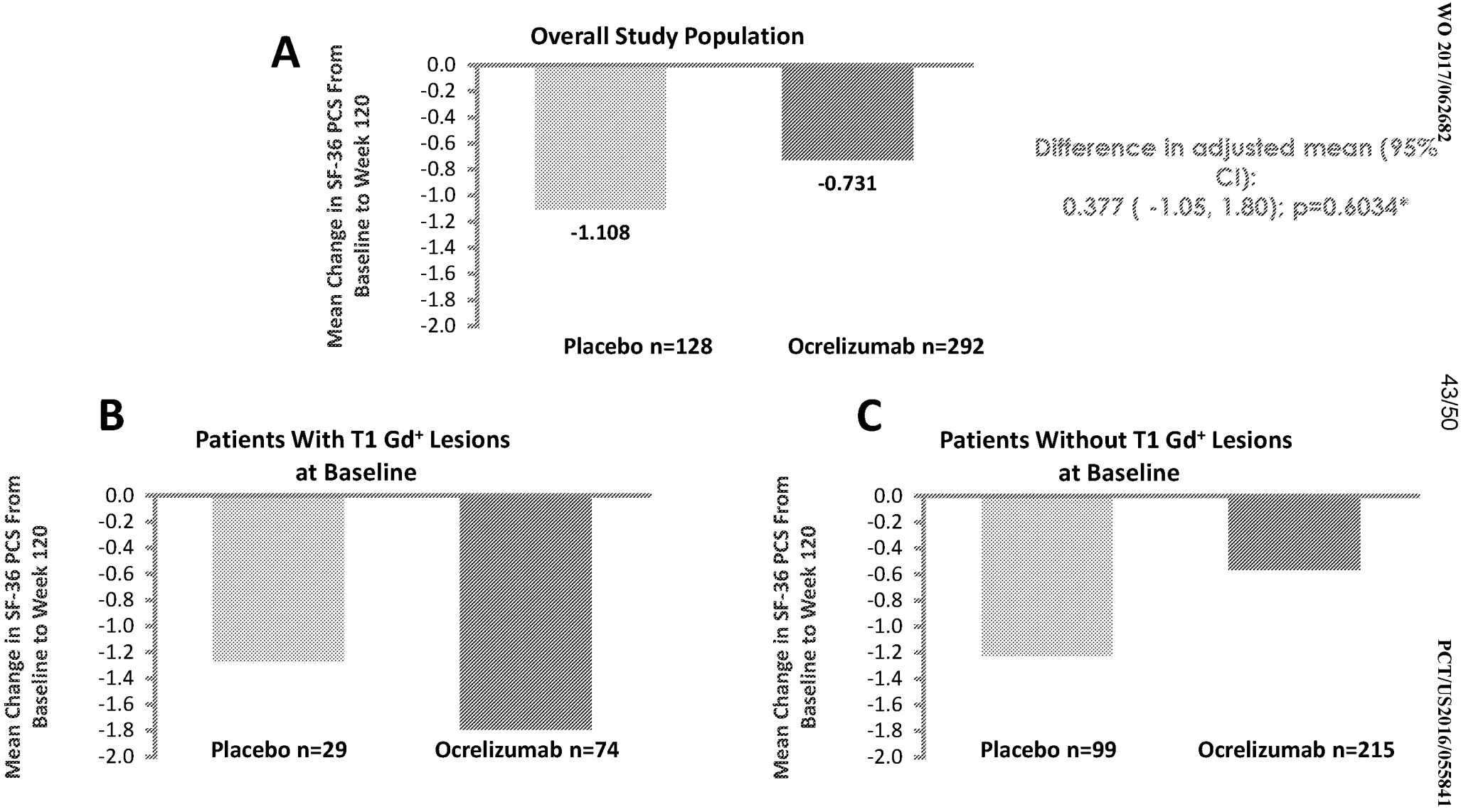


Difference in change from baseline to Week 96, adjusted mean (95% CI): -0.192 (-0.320, -0.064) p=0.0033

ITT. Estimates are from analysis based on mixed-effect model of repeated measures using unstructured covariance matrix adjusted for baseline EDSS score (continuous without rounding) and geographic region (US vs ROW).

EDSS, Expanded Disability Status Scale; IFN, interferon; ROW, rest of the world.

FIG. 40



*Analysis based on ITT population with assessment at baseline and at least one post-baseline value; p-value based on MMRM using unstructured covariance matrix. Non-confirmatory testing per hierarchical testing procedure.
CI, confidence interval; Gd+, gadolinium-enhancing; MMRM, mixed-effect model of repeated measures;
PCS, physical component summary; SF-36, short form (36).

FIG. 41

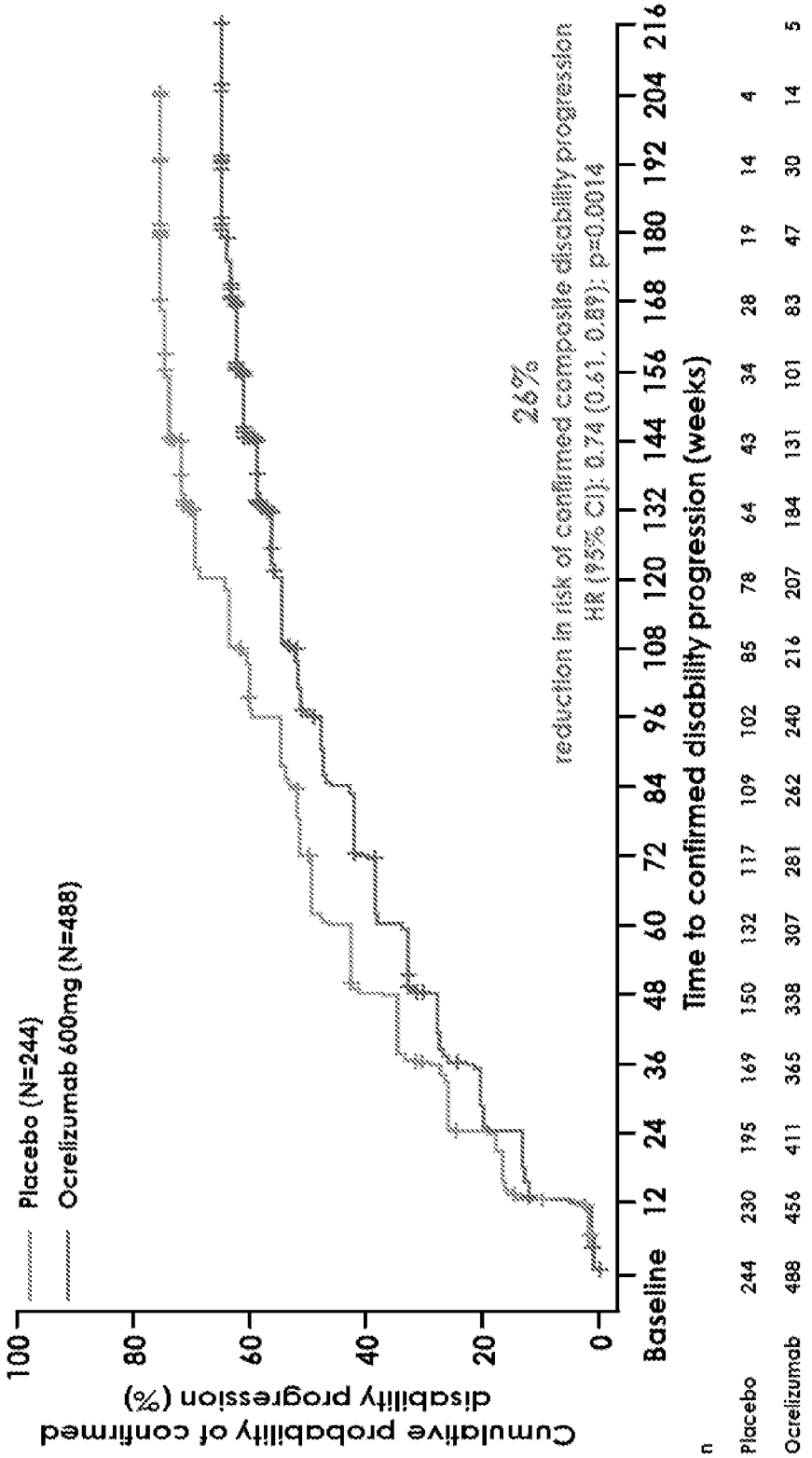

Placebo (N=244)
Ocrelizumab 600mg (N=488)
Cumulative probability of confirmed disability progression (%)
100
80
60
40
20
0
26%
reduction in risk of confirmed composite disability progression
HR (95% CI): 0.74 (0.61, 0.89); p=0.0014
Baseline 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
Time to confirmed disability progression (weeks)
n
Placebo 244 230 195 169 150 132 117 109 102 85 78 64 43 34 28 19 14 4
Ocrelizumab 488 456 411 365 338 307 281 262 240 216 207 184 131 101 83 47 30 14 5

FIG. 42

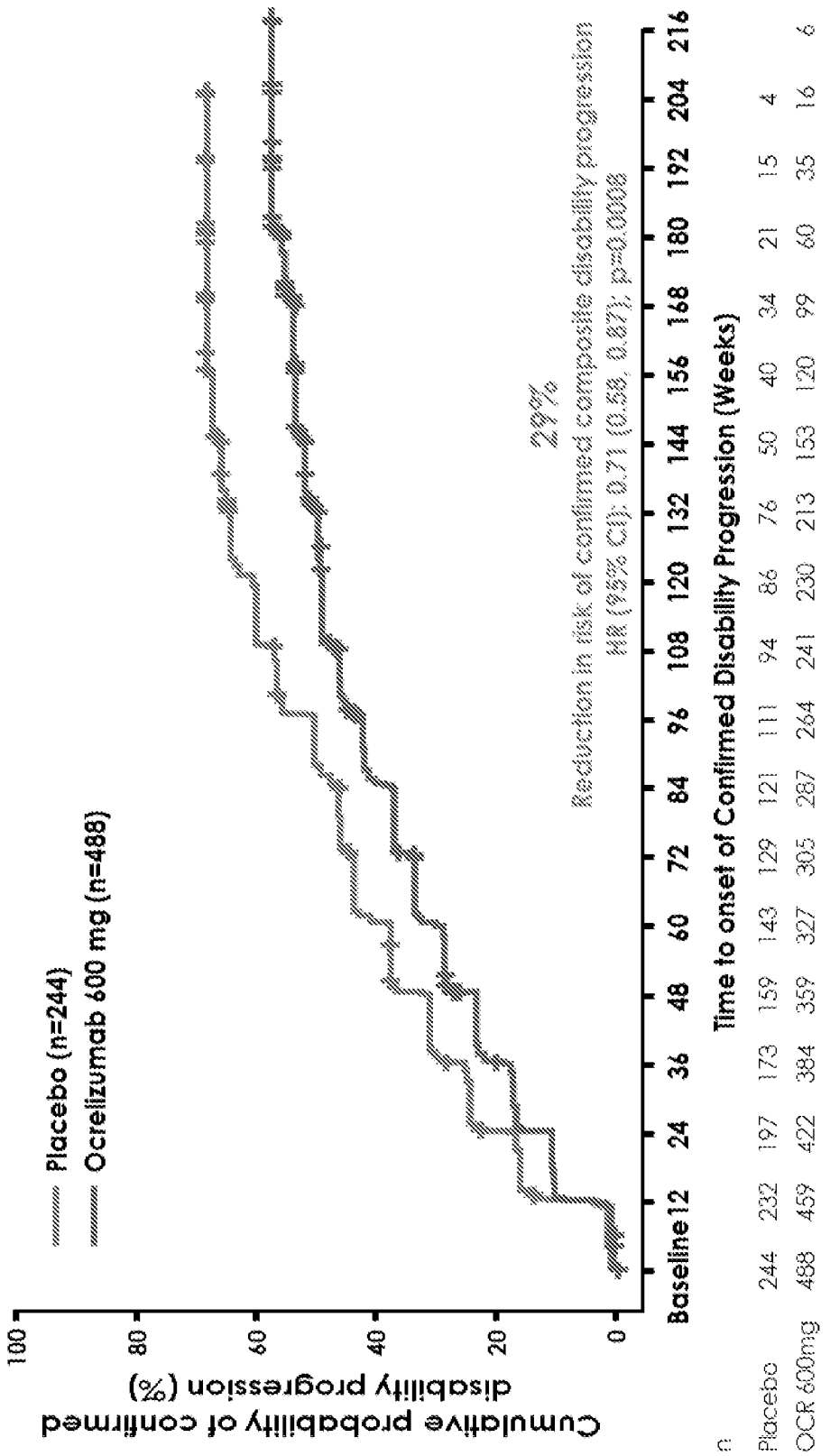
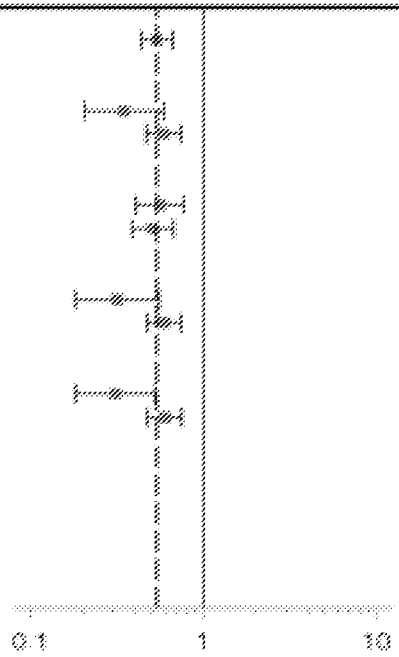

Placebo (n=244)
Ocrelizumab 600 mg (n=488)
Cumulative probability of confirmed disability progression (%)
100
80
60
40
20
0
29%
Reduction in risk of confirmed composite disability progression
HR (95% CI): 0.71 (0.58, 0.87); p=0.0008
Time to onset of Confirmed Disability Progression (Weeks)
Baseline 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
n
Placebo 244 232 197 173 159 143 129 121 111 94 86 76 50 40 34 21 15 4
OCR 600mg 488 459 422 384 359 327 305 287 264 241 230 213 153 120 99 60 35 16 6

FIG. 43

Annualized Protocol Defined Relapse Rate by Week 96 (Negative Binomial Model) by Subgroups - Inadequate Responder and Treatment-Naive,
Intent-to-Treat Population
Pooled: WA21092 and WA21093

Baseline Risk Factors	IFN beta-1a (N=629)			OCR 600mg (N=627)			Rate Ratio	95% CI	p-value
	No. in group	No. of Relapses	Patient years	No. in group	No. of Relapses	Patient years			
All Patients	629	334	1339.16	627	194	1415.72	0.535	(0.435,0.659)	<.0001
Active inadequate responder									
Yes	148	66	235.25	153	26	261.21	0.345	(0.204,0.585)	<.0001
No	681	268	1103.91	674	168	1154.51	0.584	(0.466,0.733)	<.0001
Active treatment naive									
Yes	311	137	510.85	313	69	557.40	0.536	(0.405,0.764)	0.0003
No	518	197	828.32	504	105	858.33	0.506	(0.385,0.668)	<.0001
Highly active inadequate responder									
Yes	140	64	233.56	143	23	243.07	0.317	(0.181,0.556)	<.0001
No	689	270	1115.60	684	171	1172.65	0.569	(0.471,0.736)	<.0001
Highly active treatment naive									
Yes	107	65	173.89	112	33	194.50	0.306	(0.180,0.518)	<.0001
No	722	269	1165.31	715	171	1221.22	0.583	(0.473,0.743)	<.0001

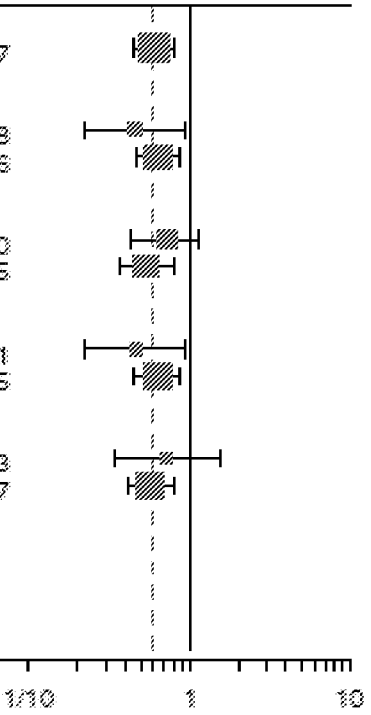


Adjusted by Study, Geographical Region (US vs. ROW) and Baseline EDSS (<=5.0 vs. >=5.5).
Log-transformed exposure time is included as an offset variable.
p-values have not been adjusted to account for multiple treatment comparisons.

FIG. 44

Time to Onset of CDP for at least 12 weeks during the Double-Blind Treatment Period by Subgroups - Inadequate Responder and Treatment-Naive, Intent-to-Treat Population
Pooled: WA21092 and WA21093

		IFN beta-1a (N=829)		OCR 600mg (N=827)					
Baseline Risk Factors	Total n	n	Events	n	Events	Hazard Ratio	95% CI	p-value (Wald)	
All Patients	1655	828	113	827	75	0.60	(0.45, 0.81)	0.0007	
Active inadequate responder									
Yes	301	148	22	153	12	0.46	(0.23, 0.93)	0.0318	
No	1354	680	91	674	63	0.64	(0.46, 0.88)	0.0066	
Active treatment naive									
Yes	634	311	39	323	31	0.72	(0.44, 1.16)	0.1750	
No	1021	517	74	504	44	0.54	(0.37, 0.79)	0.0015	
Highly active inadequate responder									
Yes	283	140	22	143	12	0.47	(0.23, 0.96)	0.0351	
No	1372	688	91	684	63	0.63	(0.46, 0.87)	0.0055	
Highly active treatment naive									
Yes	219	107	15	112	13	0.72	(0.34, 1.52)	0.3893	
No	1436	721	98	715	62	0.57	(0.42, 0.79)	0.0007	

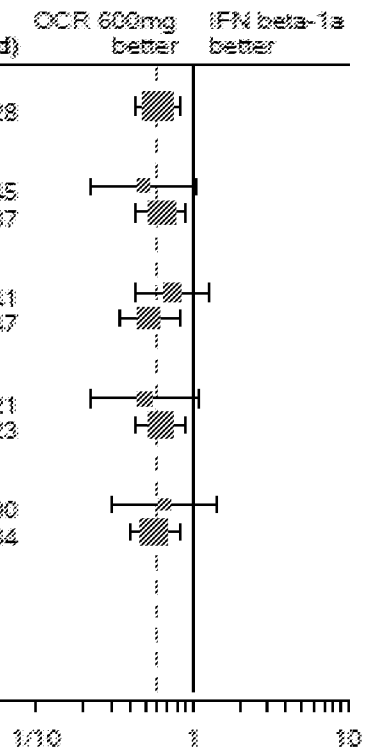


Stratified by Study, Geographical Region (US vs. ROW) and Baseline EDSS (<4.0 vs. >=4.0).
p-values have not been adjusted to account for multiple treatment comparisons.

FIG. 45

Time to Onset of CDP for at least 24 weeks during the Double-Blind Treatment Period by Subgroups - Inadequate Responder and Treatment-Naive, Intent-to-Treat Population
Pooled: WA21092 and WA21093

Baseline Risk Factors	Total n	IFN beta-1a (N=829) n	IFN beta-1a (N=829) Events	OCR 600mg (N=827) n	OCR 600mg (N=827) Events	Hazard Ratio	95% CI	p-value (Wald)
All Patients	1655	828	87	827	57	0.60	(0.43, 0.84)	0.0028
Active inadequate responder								
Yes	301	148	17	153	10	0.49	(0.22, 1.07)	0.0745
No	1354	680	70	674	47	0.63	(0.43, 0.91)	0.0137
Active treatment naive								
Yes	634	311	33	323	26	0.74	(0.44, 1.25)	0.2641
No	1021	517	54	504	31	0.53	(0.34, 0.82)	0.0047
Highly active inadequate responder								
Yes	283	140	17	143	10	0.50	(0.23, 1.09)	0.0821
No	1372	688	70	684	47	0.62	(0.43, 0.90)	0.0123
Highly active treatment naive								
Yes	219	107	15	112	12	0.67	(0.31, 1.43)	0.3000
No	1436	721	72	715	45	0.57	(0.39, 0.83)	0.0034

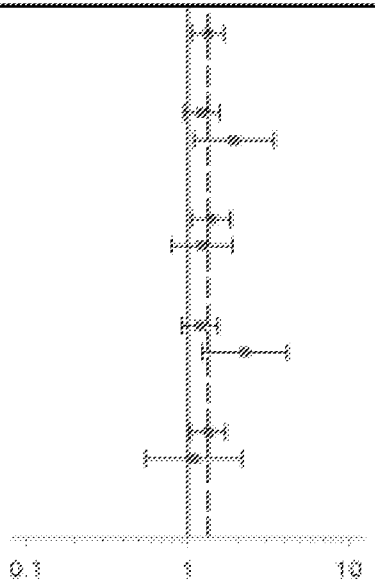


Stratified by Study, Geographical Region (US vs. ROW) and Baseline EDSS (<4.0 vs. >=4.0).
p-values have not been adjusted to account for multiple treatment comparisons.

FIG. 46

Proportion of patients who have CDI for at least 12 weeks during the Double-Blind Treatment Period Patients with Baseline EDSS >= 2.0 by Subgroups - Inadequate Responder and Treatment-Naive, Intent-to-Treat Population
Pooled: WA21092 and WA21093

Baseline Risk Factors	IFN beta-1a (N=614)			OCR 600mg (N=628)			Relative Risk	95% CI of RR	p-value (CMH)	Forest plot IFN Beta-1a better / OCR 600mg better
	No. in group	No. of Pat. with CDI	Proportion %	No. in group	No. of Pat. with CDI	Proportion %				
All Patients	614	96	15.64	628	130	20.70	1.33	(1.05, 1.68)	0.0194	
Active inadequate responder										
No	501	82	16.37	501	99	19.76	1.21	(0.93, 1.58)	0.1565	
Yes	113	14	12.39	127	31	24.41	1.97	(1.06, 3.40)	0.0207	
Active treatment naive										
No	279	64	16.89	290	91	23.33	1.38	(1.04, 1.84)	0.0246	
Yes	235	32	13.62	238	39	16.39	1.21	(0.79, 1.87)	0.3853	
Highly active inadequate responder										
No	506	84	16.60	509	108	19.65	1.19	(0.91, 1.54)	0.2031	
Yes	108	12	11.11	119	30	25.21	2.33	(1.22, 4.15)	0.0083	
Highly active treatment naive										
No	535	84	15.70	547	133	21.02	1.34	(1.04, 1.72)	0.0244	
Yes	79	12	15.19	81	15	18.52	1.68	(0.84, 3.16)	0.8313	

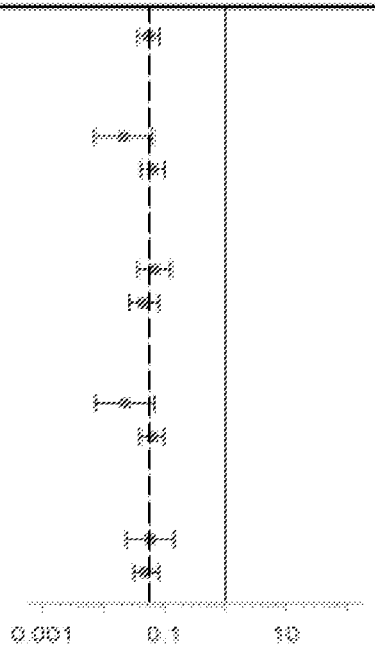


CMH: Cochran-Mantel-Haenszel Chi-Squared test.
95% CI of proportion were constructed using Pearson-Clopper method.
Stratified by Study, Geographical Region (US vs. ROW) and Baseline EDSS (<4.5 vs. >=4.5).
Patients with missing EDSS or no confirmation after onset of disability improvement are counted as not having Confirmed Disability Improvement.
p-values have not been adjusted to account for multiple treatment comparisons.

FIG. 47

Total Number of T1 Gadolinium-Enhancing Lesions as Detected by Brain MRI at scheduled visits during the Double-Blind Treatment Period by Subgroups - Inadequate Responder and Treatment-Naive, Intent-to-Treat Population
Pooled: WA21092 and WA21093

Baseline Risk Factors	IFN beta-1a (N=829)			OCR 600mg (N=827)			Rate Ratio	95% CI	p-value	Forest plot
	No. in group	No. of Lesions	No. of MRIs	No. in group	No. of Lesions	No. of MRIs				OCR 600mg better / IFN beta-1a better
All Patients	829	802	2661	827	42	2235	0.053	(0.037,0.082)	<.0001	
Active inadequate responder										
Yes	148	178	358	153	4	416	0.021	(0.007,0.063)	<.0001	
No	681	624	1713	674	38	1819	0.064	(0.041,0.100)	<.0001	
Active treatment naive										
Yes	311	324	797	323	20	888	0.058	(0.037,0.135)	<.0001	
No	518	478	1264	504	22	1347	0.046	(0.026,0.079)	<.0001	
Highly active inadequate responder										
Yes	149	163	350	143	4	387	0.022	(0.007,0.068)	<.0001	
No	680	639	1731	684	38	1848	0.063	(0.040,0.098)	<.0001	
Highly active treatment naive										
Yes	197	222	371	192	13	313	0.057	(0.023,0.140)	<.0001	
No	722	580	1810	715	29	1922	0.048	(0.031,0.075)	<.0001	



Negative Binomial Model, adjusted by Study, Baseline T1 Gd Lesion (present or not), Baseline EDSS (<4.0 vs. >=4.0) and Geographical Region (US vs. ROW).
Log-transformed number of brain MRI scans is included as an offset variable.
p-values have not been adjusted to account for multiple treatment comparisons.

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

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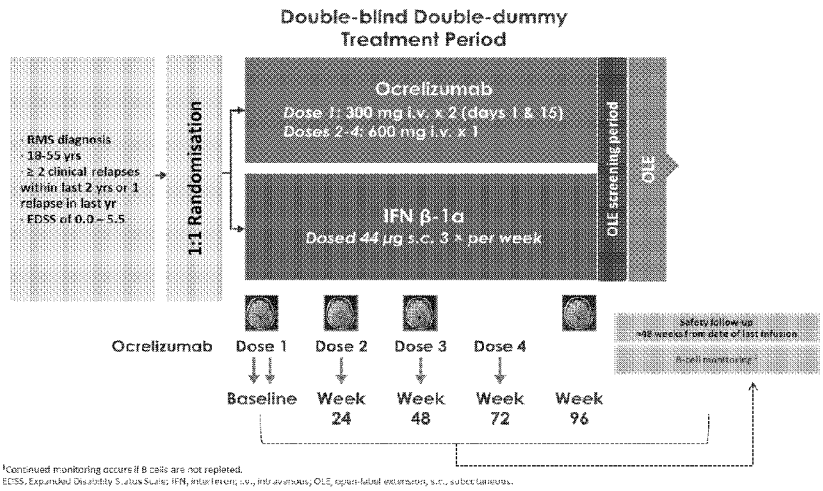
(74) Agents: **ONUFRYK, Christina** et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).

(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, JP, KE, KG, KN, KP, 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,

[Continued on next page]

(54) Title: METHOD FOR TREATING MULTIPLE SCLEROSIS

FIG. 7



[1]Continued monitoring occurs if B cells are not repleted.
EDSS, Expanded Disability Status Scale; IFN, interferon; i.v., intravenous; OLE, open-label extension; s.c., subcutaneous.

(57) Abstract: The present invention concerns methods for treating multiple sclerosis (MS) in a patient, and an article of manufacture with instructions for such use.

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TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
26 May 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/055841

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/055841

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15(completely); 126-133, 137-145(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055841

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P25/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Genentech ET AL: "Genentech: Press Releases ¦ Phase II Study Showed Ocrelizumab Maintained Significant Reduction in Disease Activity for Multiple Sclerosis Patients for Almost Two Years", , 19 October 2011 (2011-10-19), XP055203108, Retrieved from the Internet: URL:http://www.gene.com/media/press-releases/13627/2011-10-19/phase-ii-study-showed-ocrelizumab-mainta [retrieved on 2015-07-17]	1-15, 126-130, 137-145
A	page 1, paragraph 3 page 3, paragraph 7 page 3, paragraph 12 page 2, paragraph 3 - page 3, paragraph 1	131-133

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2017	31/03/2017

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. Kappos et al.: "Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial", Lancet, 19 November 2011 (2011-11-19), pages 1779-87, XP055333854, DOI: 10.1016/S0140- Retrieved from the Internet: URL:http://www.sciencedirect.com/science/article/pii/S0140673611616498/pdfft?md5=158b2ed9ca46655cb0f78883fa5517c2&pid=1-s2.0-S0140673611616498-main.pdf [retrieved on 2017-01-10] the whole document	1-15, 126-133, 137-145
A	J. L. JONES ET AL: "Improvement in disability after alemtuzumab treatment of multiple sclerosis is associated with neuroprotective autoimmunity", BRAIN., vol. 133, no. 8, 21 July 2010 (2010-07-21), pages 2232-2247, XP055334857, GB ISSN: 0006-8950, DOI: 10.1093/brain/awq176 abstract; figure 1	1-15, 126-133, 137-145
A	SIDDHARAMA PAWATE ET AL: "Newer Agents in the Treatment of Multiple Sclerosis", NEUROLOGIST, vol. 19, no. 4, 1 April 2015 (2015-04-01), pages 104-117, XP055333727, US ISSN: 1074-7931, DOI: 10.1097/NRL.0000000000000020 page 107, right-hand column, last paragraph - page 108, left-hand column, paragraph 1	1-15, 126-133, 137-145
X,P	F Hoffmann-La Roche: "Media Release: Roche's ocrelizumab first investigational medicine to show positive pivotal study results in both relapsing and primary progressive forms of multiple sclerosis", , 8 October 2015 (2015-10-08), XP055334454, Retrieved from the Internet: URL:http://www.roche.com/dam/jcr:8e278040-1e67-4e30-9ef7-972833f56c51/en/med-cor-2015-10-08-e.pdf [retrieved on 2017-01-11]	1-15, 126-130, 137-145
A,P	the whole document	131-133

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(completely); 126-133, 137-145(partially)

Subject-matter of claims 1 to 15 and subject-matter of claims 126 to 133 and 137 to 145 as far as it refers to claims 1 to 15.

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2. claims: 16-27(completely); 126-145(partially)

Subject-matter of claims 16 to 27 and subject-matter of claims 126 to 145 as far as it refers to claims 16 to 27.

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3. claims: 28-39(completely); 126-145(partially)

Subject-matter of claims 28 to 39 and subject-matter of claims 126 to 145 as far as it refers to claims 28 to 39.

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4. claims: 40-55(completely); 126-145(partially)

Subject-matter of claims 40 to 55 and subject-matter of claims 126 to 145 as far as it refers to claims 40 to 55.

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5. claims: 56-65, 99-107(completely); 126-145(partially)

Subject-matter of claims 56 to 65 and 99 to 107 and subject-matter of claims 126 to 145 as far as it refers to claims 56 to 65 and 99 to 107.

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6. claims: 66-75(completely); 126-145(partially)

Subject-matter of claims 66 to 75 and subject-matter of claims 126 to 145 as far as it refers to claims 66 to 75.

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7. claims: 76-89(completely); 126-145(partially)

Subject-matter of claims 76 to 89 and subject-matter of claims 126 to 145 as far as it refers to claims 76 to 89.

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8. claims: 90-98(completely); 126-145(partially)

Subject-matter of claims 90 to 98 and subject-matter of claims 126 to 145 as far as it refers to claims 90 to 98.

9-19. claims: 108-116(completely); 126-145(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Subject-matter of claims 108 to 116 and subject-matter of claims 126 to 145 as far as it refers to claims 108 to 116. The inventions 9 to 19 correspond to the alternatives a) to k) of claim 108, respectively.

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20. claims: 117-125(completely); 126-145(partially)

Subject-matter of claims 117 to 125 and subject-matter of claims 126 to 145 as far as it refers to claims 117 to 125

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(19)中华人民共和国国家知识产权局

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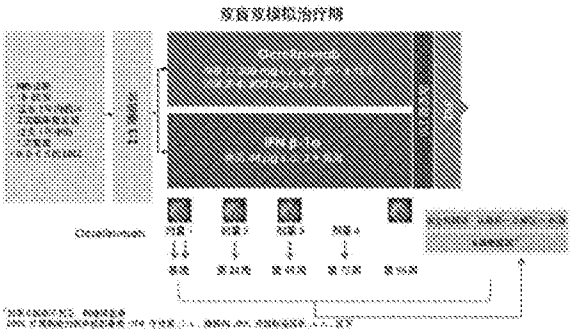
权利要求书11页 说明书109页
序列表15页 附图50页

(54)发明名称

治疗多发性硬化的方法

(57)摘要

本发明涉及治疗患者中的多发性硬化(MS)的方法,以及用于这种用途的具有说明书的制品。



CN 108431038 A

1.一种改善患有多发性硬化的人类患者中的功能能力的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述患者在治疗后具有功能能力的改善；其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区；和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

2.根据权利要求1所述的方法，其中所述患者在治疗后具有12周确认的能力丧失改善。

3.根据权利要求1所述的方法，其中所述患者在治疗后具有24周确认的能力丧失改善。

4.根据权利要求1-3中任一项所述的方法，其中所述患者中功能能力的改善持续至少12周。

5.根据权利要求1-3中任一项所述的方法，其中所述患者中功能能力的改善持续至少24周。

6.根据权利要求1-5中任一项所述的方法，其中通过计时25英尺行走(T-25FW)测试或EDSS评分来测量功能能力的改善。

7.根据权利要求1-5中任一项所述的方法，其中通过计时25英尺行走(T-25FW)测试和EDSS评分来测量功能能力的改善。

8.根据权利要求1-7中任一项所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

9.根据权利要求8所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

10.根据权利要求9所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

11.根据权利要求8-10中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

12.根据权利要求11所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

13.根据权利要求8-10中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

14.根据权利要求8-13中任一项所述的方法，其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，所述患者具有改善的功能能力。

15.根据权利要求1-14中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

16.一种抑制患有多发性硬化的人类患者中的复合能力丧失进展的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述施用导致确认的能力丧失进展事件减少，并且其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区；和b)

包含SEQ ID NO:2的氨基酸序列的轻链可变区。

17.根据权利要求16所述的方法,其中所述施用导致12周确认的复合能力丧失进展减少。

18.根据权利要求16所述的方法,其中所述施用导致24周确认的复合能力丧失进展减少。

19.根据权利要求16-17中任一项所述的方法,其中通过扩展的能力丧失状态量表(EDSS)评分、计时25英尺行走(T25-FW)和9孔钉测试(9-HPT)来确定所述确认的复合能力丧失进展。

20.根据权利要求16-19中任一项所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露,随后是第二次抗-CD20抗体暴露,其中第一次和第二次暴露各自为约600mg的抗体,并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

21.根据权利要求20所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露,其中第三次暴露为约600mg的抗体,并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

22.根据权利要求21所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露,其中第四次暴露为约600mg的抗体,并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

23.根据权利要求20-22中任一项所述的方法,其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且所述第一剂量和第二剂量隔开约两周或约14天。

24.根据权利要求23所述的方法,其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

25.根据权利要求20-22中任一项所述的方法,其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且所述第一剂量和第二剂量隔开约两周或约14天。

26.根据权利要求20-25中任一项所述的方法,其中在所述抗-CD20抗体的一次或者两次、三次或四次暴露后,所述患者中的复合能力丧失进展得到抑制。

27.根据权利要求16-26中任一项所述的方法,其中所述抗-CD20抗体包含:包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

28.一种抑制患有多发性硬化的人类患者中的能力丧失进展的方法,所述方法包括向所述患者施用有效量的抗-CD20抗体,其中所述施用导致确认的能力丧失进展事件减少,并且其中所述抗-CD20抗体包含:a)包含SEQ ID NO:8的氨基酸序列的重链可变区;和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

29.根据权利要求28所述的方法,其中所述施用导致12周确认的能力丧失进展减少。

30.根据权利要求28所述的方法,其中所述施用导致24周确认的能力丧失进展减少。

31.根据权利要求28-30中任一项所述的方法,其中通过扩展的能力丧失状态量表(EDSS)评分来确定所述确认的能力丧失进展。

32.根据权利要求28-31中任一项所述的方法,其中将所述抗-CD20抗体施用至所述患

者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

33.根据权利要求32所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

34.根据权利要求33所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

35.根据权利要求32-34中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

36.根据权利要求35所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

37.根据权利要求32-34中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

38.根据权利要求32-37中任一项所述的方法，其中在所述抗-CD20抗体的一次或者两次、三次或四次暴露后，所述患者中的复合能力丧失进展得到抑制。

39.根据权利要求28-38中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

40.一种使患有多发性硬化的人类患者中确认的能力丧失进展的发病延迟或确认的能力丧失进展的风险降低的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区；和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

41.根据权利要求40所述的方法，其中所述施用导致12周确认的能力丧失进展的发病延迟或风险降低。

42.根据权利要求40所述的方法，其中所述施用导致24周确认的能力丧失进展的发病延迟或风险降低。

43.根据权利要求40-42中任一项所述的方法，其中所述患者在基线处具有T1钆染色损伤。

44.根据权利要求40-42中任一项所述的方法，其中所述患者在基线处不具有T1钆染色损伤。

45.根据权利要求40-44中任一项所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

46.根据权利要求45所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴

露之间的间隔为约20-24周或约5-6个月。

47.根据权利要求46所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

48.根据权利要求45-47中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

49.根据权利要求48所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

50.根据权利要求45-47中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

51.根据权利要求40-50中任一项所述的方法，其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，所述患者中确认的能力丧失进展的发病延迟或确认的能力丧失进展的风险降低。

52.根据权利要求51所述的方法，其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，所述患者中12周确认的能力丧失进展的发病延迟或风险降低。

53.根据权利要求51所述的方法，其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，所述患者中24周确认的能力丧失进展的发病延迟或风险降低。

54.根据权利要求51-53中任一项所述的方法，其中通过扩展的能力丧失状态量表(EDSS)评分来确定所述确认的能力丧失进展。

55.根据权利要求40-54中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

56.一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致无疾病活动迹象(NEDA)达至少12周，其中所述抗-CD20抗体包含：其中所述抗-CD20抗体包含：包含SEQ ID NO:8的氨基酸序列的重链可变区和包含SEQ ID NO:2的氨基酸序列的轻链可变区。

57.根据权利要求56所述的方法，其中治疗导致无疾病活动迹象(NEDA)达至少24周。

58.根据权利要求56-57中任一项所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

59.根据权利要求58所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

60.根据权利要求59所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

61.根据权利要求58-59中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20

抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

62.根据权利要求61所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

63.根据权利要求58-59中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

64.根据权利要求58-63中任一项所述的方法，其中治疗导致在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，NEDA达至少12周。

65.根据权利要求56-64中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

66.一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致所述患者在96周时不具有确认的能力丧失进展事件，其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区；和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

67.根据权利要求66所述的方法，其中治疗还导致以下中的一个或多个：

a)所述患者在96周时无复发；

b)所述患者在96周时不具有T1钆增强性损伤；

c)所述患者在96周时不具有新的和/或扩大的T2损伤。

68.根据权利要求66或67所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

69.根据权利要求68所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

70.根据权利要求69所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

71.根据权利要求68-70中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

72.根据权利要求71所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

73.根据权利要求68-70中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

74.根据权利要求68-73中任一项所述的方法，其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后，所述患者在96周时不具有确认的能力丧失进展事件。

75.根据权利要求66-74中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID

NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

76.一种治疗患有高度活跃的多发性硬化的人类患者的方法,所述方法包括向所述患者施用有效量的抗-CD20抗体,其中所述抗-CD20抗体包含:a)包含SEQ ID NO:8的氨基酸序列的重链可变区;和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

77.根据权利要求76所述的方法,其中所述患有高度活跃的多发性硬化的患者是对其它多发性硬化疗法的不充分应答者。

78.根据权利要求77所述的方法,其中所述其它多发性硬化疗法是干扰素或醋酸格拉默。

79.根据权利要求76所述的方法,其中所述患有高度活跃的多发性硬化的患者先前未使用其它多发性硬化疗法进行治疗。

80.根据权利要求76-79中任一项所述的方法,其中所述抗-CD20抗体的施用在以下一个或多个方面有效:(1)减少所述患者脑损伤的数量;(2)降低年复发率;(3)降低能力丧失进展;和(4)改善功能能力。

81.根据权利要求76-80中任一项所述的方法,所述方法还包括进行MRI扫描,并且在将所述抗-CD20抗体施用至所述患者之前确定所述患者是否患有高度活跃的多发性硬化。

82.根据权利要求76-81中任一项所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露,随后是第二次抗-CD20抗体暴露,其中第一次和第二次暴露各自为约600mg的抗体,并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

83.根据权利要求82所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露,其中第三次暴露为约600mg的抗体,并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

84.根据权利要求83所述的方法,其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露,其中第四次暴露为约600mg的抗体,并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

85.根据权利要求82-84中任一项所述的方法,其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且所述第一剂量和第二剂量隔开约两周或约14天。

86.根据权利要求85所述的方法,其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

87.根据权利要求82-84中任一项所述的方法,其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且所述第一剂量和第二剂量隔开约两周或约14天。

88.根据权利要求82-87中任一项所述的方法,其中在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后,所述患者具有:(1)患者脑损伤的数量减少;(2)年复发率降低;(3)能力丧失进展降低;和/或(4)功能能力改善。

89.根据权利要求76-88中任一项所述的方法,其中所述抗-CD20抗体包含:包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

90.一种治疗患有早期多发性硬化的人类患者的方法,所述方法包括向所述患者施用

有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区；和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

91.根据权利要求90所述的方法，所述方法还包括在将所述抗-CD20抗体施用至所述患者之前诊断患有早期多发性硬化的患者。

92.根据权利要求90-91中任一项所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

93.根据权利要求92所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

94.根据权利要求93所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

95.根据权利要求92-94中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

96.根据权利要求95所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

97.根据权利要求92-94中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约14天。

98.根据权利要求90-97中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

99.一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述治疗导致所述患者中无疾病活动迹象(NEDA)，并且其中所述抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

100.根据权利要求99所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

101.根据权利要求100所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

102.根据权利要求101所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

103.根据权利要求100-101中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量

隔开约两周或约14天。

104.根据权利要求103所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

105.根据权利要求100-101中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

106.根据权利要求100-105中任一项所述的方法，其中治疗导致在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后的NEDA。

107.根据权利要求99-106中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

108.一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致以下中的一个或多个：

a)年复发率降低至少约30％；

b)确认的能力丧失进展达至少12周的风险降低至少约30％；

c)确认的能力丧失进展达至少24周的风险降低至少约30％；

d)T1钆$^{+}$损伤的数量减少至少约90％；

e)第24周、第48周和/或第96周的T1钆$^{+}$损伤的平均数减少至少约90％；

f)新的和/或扩大的T2高强度损伤的数量减少至少约70％；

g)第24周、第48周和/或第96周的新的和/或扩大的T2高强度损伤的平均数减少至少约40％；

h)全脑体积损失的速率降低至少约10％；

i)持续至少12周的确认的能力丧失改善提高至少约10％；

j)新的T1低强度损伤的数量减少至少约50％；和

k)NEDA(无疾病活动迹象)提高至少约55％；

其中a)-k)中的降低或提高是与接受干扰素β-1a治疗的患者相比的，并且其中所述抗-CD20抗体包含：1)包含SEQ ID NO:8的氨基酸序列的重链可变区和2)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

109.根据权利要求108所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

110.根据权利要求109所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

111.根据权利要求110所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

112.根据权利要求109-111中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

113.根据权利要求112所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

114.根据权利要求109-111中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

115.根据权利要求109-114中任一项所述的方法，其中治疗导致在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后存在a)至k)中的一个或多个。

116.根据权利要求108-115中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

117.一种治疗患有原发进行性多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致以下中的一个或多个：

a)与不接受治疗的患者相比，确认的能力丧失进展达至少12周的风险降低至少约15％；

b)与不接受治疗的患者相比，确认的能力丧失进展达至少24周的风险降低至少约15％；

c)与不接受治疗的患者相比，通过计时25英尺行走所测量的行走时间的进展率降低至少约15％；

d)与不接受治疗的患者相比，T2损伤体积减小至少约10％；

e)从基线至第24周的T2损伤体积减小至少约3％；和

f)与不接受治疗的患者相比，全脑体积损失的速率降低至少约12％；

其中抗-CD20抗体包含：1)包含SEQ ID NO:8的氨基酸序列的重链可变区和2)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

118.根据权利要求117所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。

119.根据权利要求118所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。

120.根据权利要求119所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

121.根据权利要求118-120中任一项所述的方法，其中所述第一次暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

122.根据权利要求121所述的方法，其中所述第二次、第三次和/或第四次暴露包括约600mg的单一剂量。

123.根据权利要求118-120中任一项所述的方法，其中初次暴露以及第二次、第三次和/或第四次另外的暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

124.根据权利要求118-123中任一项所述的方法，其中治疗导致在所述抗-CD20抗体的一次、两次、三次和/或四次暴露后存在a)至f)中的一个或多个。

125.根据权利要求117-124中任一项所述的方法，其中所述抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:26的氨基酸序列的重链和包含SEQ ID NO:13的氨基酸序列的轻链。

126.根据权利要求1-125中任一项所述的方法，其中所述患者在治疗期间保持产生针对抗原的体液免疫应答的能力。

127.根据权利要求126所述的方法，其中所述抗原是腮腺炎抗原、风疹抗原、水痘抗原、肺炎链球菌(S.pneumonia)抗原、破伤风类毒素抗原、肺炎球菌抗原或流感抗原。

128.根据权利要求1-127中任一项所述的方法，其中与初次暴露或后面的暴露一起施用第二药物，其中所述抗-CD20抗体是第一药物。

129.根据权利要求1-116和126-128中任一项所述的方法，其中所述多发性硬化是复发型多发性硬化。

130.根据权利要求129所述的方法，其中所述复发型多发性硬化是复发缓和型多发性硬化(RRMS)。

131.根据权利要求129所述的方法，其中所述复发型多发性硬化是具有重叠复发的继发进行性多发性硬化(rSPMS)。

132.根据权利要求1-65和76-128中任一项所述的方法，其中所述多发性硬化是进行性多发性硬化。

133.根据权利要求132所述的方法，其中所述多发性硬化是原发进行性多发性硬化(PPMS)。

134.根据权利要求10、22、34、47、60、70、84、94、102、111和120中任一项所述的方法，其中将所述抗-CD20抗体施用至所述患者以提供第四次暴露后的一次或多次另外的抗-CD20抗体暴露，其中第四次暴露后的一次或多次另外的暴露为约600mg的抗体，并且其中每次暴露之间的间隔为约20-24周或约5-6个月。

135.根据权利要求134所述的方法，其中第四次暴露后的一次或多次另外的抗-CD20抗体暴露包括所述抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且所述第一剂量和第二剂量隔开约两周或约14天。

136.根据权利要求134所述的方法，其中第四次暴露后的一次或多次另外的抗-CD20抗体暴露包含约600mg的单一剂量。

137.根据前述权利要求中任一项所述的方法，其中所述抗-CD20抗体是ocrelizumab。

138.根据前述权利要求中任一项所述的方法，其中所述抗-CD20抗体是其抗原结合片段。

139.根据前述权利要求中任一项所述的方法，其中所述抗-CD20抗体在药用组合物中。

140.根据前述权利要求中任一项所述的方法，其中静脉内施用所述抗-CD20抗体。

141.根据权利要求140所述的方法，其中对于每次抗体暴露，静脉内施用所述抗-CD20抗体。

142.根据权利要求1-139中任一项所述的方法，其中皮下施用所述抗体。

143.根据权利要求142所述的方法，其中对于每次抗体暴露，皮下施用所述抗-CD20抗体。

144.一种用于根据前述权利要求中任一项的方法的抗-CD20抗体，其中所述抗-CD20抗体包含：其中所述抗-CD20抗体包含：1)包含SEQ ID NO:8的氨基酸序列的重链可变区和2)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

145.一种制品，其包含：(a)包含ocrelizumab的容器；和(b)具有用于根据前述权利要求中任一项治疗患者中的多发性硬化的说明的包装插页。

治疗多发性硬化的方法

[0001] 对相关申请的交叉引用

[0002] 本申请要求2015年10月6日提交的美国临时专利申请号62/238,103、2015年10月7日提交的美国临时专利申请号62/238,674、2015年12月28日提交的美国临时专利申请号62/271,985、2016年2月16日提交的美国临时专利申请号62/296,049、2016年4月14日提交的美国临时专利申请号62/322,734、2016年5月27日提交的美国临时专利申请号62/342,633、和2016年6月27日提交的美国临时专利申请号62/355,299的权益，上述公开内容通过引用整体结合于此用于全部目的。

[0003] ASCII文本文件中的序列表的提交

[0004] ASCII文本文件中的以下提交的内容通过引用整体并入本文：序列表的计算机可读形式(CRF)(文件名：146392035245SEQLISTING.txt，记录日期：2016年10月4日，大小：41KB)。

发明领域

[0005] 本发明涉及用于治疗患者中多发性硬化的方法，以及用于这种用途的具有说明书的制品。

[0006] 发明背景

[0007] 多发性硬化(multiple sclerosis,MS)是人中枢神经系统(CNS)的炎性脱髓鞘退行性疾病。它是一种世界范围的疾病，在美国影响约300,000名患者；它是一种年轻成人的疾病，70％-80％在20至40岁之间发病(Anderson等人Ann Neurology 31(3)：333-6(1992)；Noonan等人Neurology 58：136-8(2002))。MS是基于临床病程、磁共振成像(MRI)扫描评价以及活检和尸检材料的病理分析的异质性病变(Lucchinetti等人Ann Neurol 47：707-17(2000))。该疾病自身表现为缺陷的许多可能组合，包括脊髓、脑干、颅神经、小脑、大脑和认知综合征。进行性伤残是大多数MS患者的命运，尤其是在包括25年前景时。一半患者在发病后的15年内行走需要拐杖。MS是年轻和中年成人中神经性伤残的主要原因，直至过去十年，仍未有已知的有效治疗方法。由于非特异性临床表现，所以MS难以诊断，这导致开发了包含数种技术进步在内的高度结构化诊断标准，其由MRI扫描、诱发电位和脑脊液(CSF)研究组成。所有诊断标准依赖于在不同时间出现的中心白质内的分散性损伤的一般原理，而不是从诸如感染、血管病症或自身免疫性病症等其它病因方面来解释(McDonald等人Ann Neurol 50：121-7(2001))。MS有四种疾病样式：复发缓和型MS(RRMS；发病案例的80％-85％)、原发进行性MS(PPMS；发病案例的10％-15％)、进行性复发型MS(PRMS；发病案例的5％)；以及继发进行性MS(SPMS)(Kremenchutzky等人Brain 122(Pt 10)：1941-50(1999)；Confavreux等人N Engl J Med 343(20)：1430-8(2000))。估计50％的RRMS患者将在10年内发展成SPMS，而高达90％的RRMS患者最终将发展成SPMS(Weinshenker等人Brain 112(Pt 1)：133-46(1989))。

[0008] 在美国批准了五个种类中的几种减轻疾病的药物用于治疗RRMS，而没有药物已被批准用于PPMS。RRMS治疗包括以下：干扰素类，IFN-β-1a((REBIF®、Extavia、AVONEX®

和PLEGRIDY™)和IFN-β-1b(BETASERON®);醋酸格拉默(glatiramer acetate)(COPAXONE®),即一种多肽;那他珠单抗(natalizumab)(TYSABRI®),阿仑单抗(alemtuzumab)(LEMTRADA®),即两种单克隆抗体;富马酸二甲酯(TECFIDERA®)和芬戈莫德(fingolimod)(GILENYA®),即两种小分子,以及米托蒽醌(mitoxantrone)(NOVANTRONE®),即一种细胞毒剂;特立氟胺(teriflunomide)(AUBAGIO®)。已经使用了目的为减轻疾病的其它药物并且获得了不同程度的成功,包括甲氨蝶呤、环磷酰胺,硫唑嘌呤和静脉内(IV)免疫球蛋白。目前的疗法缺乏足够的效力以消除早期疾病,或者由于相关副作用而不能用于早期MS(Hartung等人(2011)Expert Rev Neurother.11,351-62;Freedman等人,Mult.Scler.Relat.Disord.3(2):147-55,2014)。因此,仍需要具有可接受的收益风险比的高效疗法,其可以在病程早期给予以减少累积伤残的长期后果并改善患者的生活质量。

[0009] 本文引用的所有参考文献通过引用整体结合于此。

[0010] 发明概述

[0011] 本文提供一种用于治疗人类患者中多发性硬化的方法,所述方法包括施用有效量的抗-CD20抗体。可以施用本文描述的抗-CD20抗体中的任一种。在一些实施方案中,抗-CD20抗体包含:a)重链可变区,所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3,以及b)轻链可变区,所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。

[0012] 在一个方面,本文提供一种改善患有多发性硬化的人类患者中功能能力的方法,所述方法包括向所述患者施用有效量的抗-CD20抗体,其中在治疗后所述患者的功能性能力得到改善;其中所述抗-CD20抗体包含:a)重链可变区,所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3,以及b)轻链可变区,所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中,在治疗后,患者中的功能能力得到改善。在一些实施方案中,在治疗后,患者具有12周确认的能力丧失改善(confirmed disability improvement)。在一些实施方案中,在治疗后,患者具有24周确认的能力丧失改善。在一些实施方案中,患者中功能能力的改善持续至少12周。在一些实施方案中,患者中功能能力的改善持续至少24周。在一些实施方案中,通过计时25英尺行走(Timed 25-Foot Walk,T-25FW)测试或EDSS评分来测量功能能力的改善。在一些实施方案中,通过计时25英尺行走(T-25FW)测试和EDSS评分来测量功能能力的改善。

[0013] 在一些实施方案中,患者在基线处具有T1钆染色损伤。在一些实施方案中,患者在基线处不具有T1钆染色损伤。

[0014] 在某些实施方案中,抗-CD20抗体包含:a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中,抗-CD20抗体包含:包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链,和包含SEQ ID NO:13的氨基酸序列的轻链。

[0015] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0016] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0017] 在一些实施方案中，在抗-CD20抗体的一次、两次、三次和/或四次暴露后，患者具有改善的功能能力。

[0018] 在另一个方面，提供一种抑制患有多发性硬化的人类患者中复合能力丧失进展(composite disability progression)的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中该施用导致确认的能力丧失进展事件减少，并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，施用导致12周确认的复合能力丧失进展减少。在一些实施方案中，施用导致24周确认的复合能力丧失进展减少。在一些实施方案中，通过扩展的能力丧失状态量表(Expanded Disability Status Scale，EDSS)评分或计时25英尺行走(T25-FW)或9孔钉测试(9-Hole Peg Test，9-HPT)来确定确认的复合能力丧失进展。在一些实施方案中，通过扩展的能力丧失状态量表(Expanded Disability Status Scale，EDSS)评分或计时25英尺行走(Timed 25-Foot Walk，T25-FW)和9孔钉测试(9-Hole Peg Test，9-HPT)来确定确认的复合能力丧失进展。

[0019] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0020] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg

的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0021] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0022] 在一些实施方案中，在抗-CD20抗体的一次或者两次、三次和/或四次暴露后，患者中的复合能力丧失进展得到抑制。在一些实施方案中，在抗-CD20抗体的一次、两次、三次和/或四次暴露后，患者中12周确认的能力丧失进展的发病延迟或风险降低。在一些实施方案中，在抗-CD20抗体的一次或者两次、三次和/或四次暴露后，患者中的24周确认的能力丧失进展的发病得到延迟。在一些实施方案中，在抗-CD20抗体的一次或者两次、三次和/或四次暴露后，患者中的24周确认的能力丧失进展的风险降低。在一些实施方案中，通过扩展的能力丧失状态量表(EDSS)评分来确定确认的能力丧失进展。

[0023] 在另一个方面，本文提供一种抑制患有多发性硬化的人类患者中能力丧失进展的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中该施用导致确认的能力丧失进展事件减少，并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，施用导致12周确认的能力丧失进展减少。在一些实施方案中，施用导致24周确认的能力丧失进展减少。在一些实施方案中，通过扩展的能力丧失状态量表(EDSS)评分来确定确认的能力丧失进展。在一些实施方案中，通过EDSS的增加来确定确认的能力丧失进展。

[0024] 在一些实施方案中，通过计时25英尺行走(T25-FW)的变化来确定确认的能力丧失进展。在一些实施方案中，通过MRI总T2损伤体积的百分比变化来确定确认的能力丧失进展。在一些实施方案中，通过MRI总脑体积的百分比变化来确定确认的能力丧失进展。在一些实施方案中，通过简式-36(SF-36)健康要素评分(Short Form-36(SF-36)physical component score)的变化来确定确认的能力丧失进展。

[0025] 在一些实施方案中，患者在基线处具有T1钆染色损伤。在一些实施方案中，患者在基线处不具有T1钆染色损伤。

[0026] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0027] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，

并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0028] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0029] 在一些实施方案中，在抗-CD20抗体的一次或者两次、三次或四次暴露后，患者具有抑制的复合能力丧失进展。

[0030] 在另一个方面，本文提供一种使患有多发性硬化的人类患者中确认的能力丧失进展的发病延迟或确认的能力丧失进展的风险降低的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，施用导致12周确认的能力丧失进展的发病延迟或风险降低。在一些实施方案中，施用导致24周确认的能力丧失进展的发病延迟或风险降低。

[0031] 在一些实施方案中，患者在基线处具有T1钆染色损伤。在一些实施方案中，患者在基线处不具有T1钆染色损伤。

[0032] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0033] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0034] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0035] 在一些实施方案中，在抗-CD20抗体的一次、两次、三次和/或四次暴露后，患者中

确认的能力丧失进展的发病延迟或确认的能力丧失进展的风险降低。

[0036] 本文提供一种减小患有多发性硬化的人类患者中T2损伤体积的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，患者在基线处具有T1钆染色损伤。在某些实施方案中，患者在基线处不具有T1钆染色损伤。

[0037] 在另一个方面，本文提供一种减缓或阻止患有多发性硬化的人类患者中脑体积减小的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中减缓或阻止了患者中的脑体积减小；并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，减缓或阻止了已经经历脑体积损失的患者中脑体积的进一步减少。

[0038] 本文还提供一种减小患有多发性硬化的人类患者中的脑萎缩的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中减缓或阻止了脑萎缩，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，减缓或阻止了已经经历脑萎缩的患者中的进一步脑萎缩。

[0039] 本文提供一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致无疾病活动迹象(no evidence of disease activity,NEDA)达至少12周，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，治疗导致无疾病活动迹象(NEDA)达至少24周。

[0040] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0041] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，

并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0042] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0043] 在一些实施方案中，治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后，NEDA达至少12周。

[0044] 本文还提供一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致患者在治疗24、48或96周后达到无损伤状态，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，治疗导致患者在治疗48周后达到无损伤状态。在某些实施方案中，治疗导致患者在治疗24周后达到无损伤状态。在某些实施方案中，治疗导致患者在治疗48周后达到无损伤状态。在某些实施方案中，治疗导致患者在治疗96周后达到无损伤状态。在某些实施方案中，治疗导致患者不具有钆染色损伤。在某些实施方案中，治疗导致患者不具有T2损伤。

[0045] 本文提供一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致以下中的一个或多个：

[0046] a)患者在96周时无复发；

[0047] b)患者在96周时不具有确认的能力丧失进展事件；

[0048] c)患者在96周时不具有T1钆增强性损伤；

[0049] d)患者在96周时不具有新的和/或扩大的T2损伤；

[0050] 其中抗-CD20抗体包含：1)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有氨基酸SEQ ID NO:11的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及2)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。

[0051] 本文提供一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致患者在96周时不具有确认的能力丧失进展事件，其中抗-CD20抗体包含：1)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有氨基酸SEQ ID NO:11的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及2)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。

[0052] 在某些实施方案中，治疗导致以下中的一个或多个：

[0053] a)患者在96周时无复发；

[0054] b)患者在96周时不具有T1钆增强性损伤；和

[0055] c)患者在96周时不具有新的和/或扩大的T2损伤。

[0056] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0057] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0058] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0059] 在一些实施方案中，治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后，患者在96周时不具有确认的能力丧失进展事件。在某些实施方案中，治疗还导致以下中的一个或多个：

[0060] a)患者在96周时无复发；

[0061] b)患者在96周时不具有T1钆增强性损伤；和/或

[0062] c)在抗-CD20抗体的一次、两次、三次和/或四次暴露后，患者在96周时不具有新的和/或扩大的T2损伤。

[0063] 本文提供一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致以下中的一个或多个：

[0064] a)患者无复发；

[0065] b)患者不具有确认的能力丧失进展事件；

[0066] c)患者不具有T1钆增强性损伤；

[0067] d)患者不具有新的和/或扩大的T2损伤；

[0068] 其中抗-CD20抗体包含：1)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有氨基酸SEQ ID NO:11的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及2)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3，并且其中治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后存在a)-d)中的任一个或多个。

[0069] 在另一个方面，本文提供一种治疗患有高度活跃的多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，患有高度活跃的多发性硬化的患者是对其它多发性硬化疗法的不充分应答者。在一些实施方案中，患有高度活跃的多发性硬化的患者先前未使用多发性硬化的其它疗法进行治疗。在一些实施方案中，多发性硬化症的其它疗法是干扰素或醋酸格拉默(glatiramer acetate)。在一些实施方案中，抗-CD20抗体的施用在以下一个或多个方面有效：(1)减少患者脑损伤的数量；(2)降低年复发率；(3)降低能力丧失进展；和(4)改善功能能力。在一些实施方案中，所述方法还包括进行MRI扫描，并且在将抗-CD20抗体施用至患者之前确定患者是否患有高度活跃的多发性硬化。

[0070] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0071] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0072] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0073] 在一些实施方案中，在抗-CD20抗体的一次、两次、三次和/或四次暴露后，对于患有高度活跃的多发性硬化的患者：(1)患者脑损伤的数量减少；(2)年复发率降低；(3)能力丧失进展降低；和/或(4)功能能力改善。

[0074] 在另一个方面，本文提供一种治疗患有早期多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，所述方法还包括在将

抗-CD20抗体施用至患者之前诊断患有早期多发性硬化的患者。在一些实施方案中，患者已被诊断为患有多发性硬化，但是在施用抗-CD20抗体之前至少两年未接受治疗。在某些实施方案中，早期多发性硬化症是患者中多发性硬化的第一次临床表现。在某些实施方案中，多发性硬化症的第一次临床表现是第一次临床脱髓鞘事件(FCDE)(也称为临床孤立综合征(clinical isolated syndrome,CIS))，即持续至少24小时并由中枢神经系统的炎症或脱髓鞘引起的神经症状的第一次发病。在某些实施方案中，FDCE影响视神经、脑干、皮质下白质或脊髓。在某些实施方案中，早期多发性硬化症是指临床上确定的多发性硬化症(CDMS)的诊断，其中患者经历FCDE，随后是第二次临床发病。

[0075] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0076] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0077] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0078] 在另一个方面，本文提供一种治疗患有多发性硬化的人类患者中的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致患者中无疾病活动迹象(NEDA)，并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，NEDA被定义为：没有方案定义的复发，没有CDP事件，没有新的或扩大的T2损伤，以及没有Gd增强性T1损伤。

[0079] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0080] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中

第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0081] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0082] 在一些实施方案中，治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后达到NEDA。

[0083] 在某些实施方案中，抗-CD20抗体向患有复发型多发性硬化的人类患者的治疗或施用导致以下中的一个或多个：

[0084] a)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，随时间(例如，至少约1年、1.5年或2年的时间段内)的复发率降低约30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％或50％中的任一个(包括介于这些值之间的任何范围)；

[0085] b)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，确认的能力丧失进展达至少12周的风险降低约30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％或50％中的任一个(包括介于这些值之间的任何范围)；

[0086] c)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，确认的能力丧失进展达至少24周的风险降低约30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％或50％中的任一个(包括介于这些值之间的任何范围)；

[0087] d)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，T1钆+损伤的总数减少约90％、91％、92％、93％、94％、95％、96％、97％、98％或99％中的任一个(包括介于这些值之间的任何范围)；

[0088] e)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，第24周、第48周和/或第96周的$T1钆^{+}$损伤的平均数减少约90％、91％、92％、93％、94％、95％、96％、97％、98％或99％中的任一个(包括介于这些值之间的任何范围)；

[0089] f)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，新的和/或扩大的T2高强度损伤的数量减少约70％、71％、72％73％74％、75％、76％、77％、78％、79％、80％、81％、82％、83％、84％或85％中的任一个(包括介于这些值之间的任何范围)；

[0090] g)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，第24周、第48周和/或第96周的新的和/或扩大的T2高强度损伤的平均数减少约40％、41％、42％、43％、44％、45％、46％、47％、48％、49％、50％、51％、52％、53％、54％、55％、56％、57％、58％、59％、60％、

61%、62%、63%、64%、65%、66%、67%、68%、69%、70%、70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%中的任一个(包括介于这些值之间的任何范围)；

[0091] h)与不接受治疗的患者相比，全脑体积损失的速率降低约10%、10.5%、11%、11.5%、12%、12.5%、13%、13.5%、14%、14.5%、15%、15,5%、16%、16.5%、17%、17.5%、18%、18.5%、19%、19.5%或20%中的任一个(包括介于这些值之间的任何范围)；

[0092] i)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，持续至少12周的确认的能力丧失改善提高约10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、21%、22%、23%、24%、25%、26%、27%、28%、29%、30%、31%、32%、33%、34%、35%、36%、37%、38%、39%、40%、41%、42%、43%、44%、45%、46%、47%、48%、49%、50%、51%、52%、53%、54%、55%、56%、57%、58%、59%、60%、61%、62%、63%、64%或65%中的任一个(包括介于这些值之间的任何范围)；

[0093] j)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，新的T1低强度损伤的数量减少约50%、51%、52%、53%、54%、55%、56%、57%、58%、59%、60%、61%、62%、63%、64%、65%、66%、67%、68%、69%、70%、71%、72%、73%、74%或75%中的任一个(包括介于这些值之间的任何范围)；和

[0094] k)与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，达到NEDA(无疾病活动迹象)的几率增加约55%、56%、57%、58%、59%、60%、61%、62%、63%、64%、65%、66%、67%、68%、69%，70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%或85%中的任一个(包括介于这些值之间的任何范围)，

[0095] 其中NEDA被定义为没有方案定义的复发，没有CDP事件，没有新的或扩大的T2损伤，以及没有Gd增强性T1损伤。

[0096] 在某些实施方案中，治疗可以另外地或备选地导致，与接受干扰素β-1a(诸如REBIF®)治疗的患者相比，脑萎缩减少约10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、21%、22%、23%、24%或25%中的任一个(包括介于这些值之间的任何范围)。

[0097] 在某些实施方案中，抗-CD20抗体包含:a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含:包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0098] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0099] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每

个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0100] 在一些实施方案中，治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后存在a)-k)中的一个或多个。

[0101] 在某些实施方案中，抗-CD20抗体向患有原发进行性多发性硬化的患者的治疗或施用导致以下中的一个或多个：

[0102] a)与不接受治疗的患者相比，确认的能力丧失进展达至少12周的风险降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％或30％中的任一个(包括介于这些值之间的任何范围)；

[0103] b)与不接受治疗的患者相比，确认的能力丧失进展达至少24周的风险降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％或30％中的任一个(包括介于这些值之间的任何范围)；

[0104] c)与不接受治疗的患者相比，行走时间的进展率(如通过计时25英尺行走所测量的)降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％、30％、31％、32％、33％、34％或35％中的任一个(包括介于这些值之间的任何范围)；

[0105] d)与不接受治疗的患者相比，T2损伤体积减小约1％、2％、3％、4％、5％、6％、7％、8％、9％、10％、11％、12％、13％、14％或15％中的任一个(包括介于这些值之间的任何范围)；

[0106] e)在约第20周、第24周、第28周、第32周、第36周、第40周、第44周、第48周、第52周、第56周、第60周、第64周、第68周、第72周、第76周、第80周、第84周、第88周、第92周、第96周、第100周、第104周、第108周、第112周、第116周和第120周中的任一个，从基线的高强度T2损伤体积减小约1％、1.5％、2％、2.5％、3％、3.4％、3.5％、4％、4.5％或5％；和

[0107] f)与不接受治疗的患者相比，全脑体积损失的速率降低约12％、12.5％、13％、13.5％、14％、14.5％、15％、15.5％、16％、16.5％、17％、17.5％、18％、18.5％、19％、19.5％或20％中的任一个(包括介于这些值之间的任何范围)；

[0108] 在某些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0109] 在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg

的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0110] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0111] 在一些实施方案中，治疗导致在抗-CD20抗体的一次、两次、三次和/或四次暴露后存在a)-f)中的一个或多个。

[0112] 在某些实施方案中，提供一种降低患有原发进行性多发性硬化的患者中确认的能力丧失进展达至少12周的风险的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致患者中确认的能力丧失进展达至少12周的风险降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％或30％中的任一个(包括介于这些值之间的任何范围)，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

[0113] 在某些实施方案中，提供一种降低患有原发进行性多发性硬化的患者中确认的能力丧失进展达至少24周的风险的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致患者中确认的能力丧失进展达至少24周的风险降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％或30％中的任一个(包括介于这些值之间的任何范围)，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

[0114] 在某些实施方案中，提供一种降低患有原发进行性多发性硬化的患者中如通过计时25英尺行走所测量的行走时间进展率的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致患者中行走时间的进展率降低约15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％、30％、31％、32％、33％、34％或35％中的任一个(包括介于这些值之间的任何范围)，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

[0115] 在某些实施方案中，提供一种减小患有原发进行性多发性硬化的患者中T2损伤体积的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致患者中T2损伤体积减小约1％、2％、3％、4％、5％、6％、7％、8％、9％、10％、11％、12％、13％、14％或15％中的任一个(包括介于这些值之间的任何范围)，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

[0116] 在某些实施方案中，提供一种减小患有原发进行性多发性硬化的患者中高强度T2损伤体积的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致在约第20周、第24周、第28周、第32周、第36周、第40周、

第44周、第48周、第52周、第56周、第60周、第64周、第68周、第72周、第76周、第80周、第84周、第88周、第92周、第96周、第100周、第104周、第108周、第112周、第116周和第120周中的任一个，患者中从基线的高强度T2损伤体积减小约1％、1.5％、2％、2.5％、3％、3.4％、3.5％、4％、4.5％或5％中的任一个，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。

[0117] 在某些实施方案中，提供一种降低患有原发进行性多发性硬化的患者中全脑体积损失的速率的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中与不接受治疗的患者相比，施用抗-CD20抗体导致患者中全脑体积损失的速率降低约12％、12.5％、13％、13.5％、14％、14.5％、15％、15.5％、16％、16.5％、17％、17.5％、18％、18.5％、19％、19.5％或20％中的任一个(包括介于这些值之间的任何范围)，并且其中所述抗体包含：a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻链可变区。在一些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0118] 在一些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0119] 在某些实施方案中，在抗-CD20抗体的一次、两次、三次和/或四次暴露后，a)确认的能力丧失进展达至少12周的风险、b)确认的能力丧失进展达至少24周的风险、c)如通过计时25英尺行走所测量的行走时间的进展率、d)T2损伤体积、e)高强度T2损伤体积和/或f)全脑体积损失的速率得以降低。在某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0120] 在任一种方法的某些实施方案中，患者在治疗期间保持产生针对抗原的体液应答的能力。在某些实施方案中，抗原是腮腺炎抗原、风疹抗原、水痘抗原、肺炎链球菌(S.pneumonia)抗原、破伤风类毒素抗原、肺炎球菌抗原或流感抗原。

[0121] 在某些实施方案中，患者在用抗-CD20抗体输注之前进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30分钟用甲泼尼龙(methylprednisolone)(或等效物)进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30分钟用100mg IV甲泼尼龙(methylprednisolone)(或等效物)进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30-60分钟另外地(或备选地)用抗组胺药(例如苯海拉明)进行前驱用药。在某些实施方案中，患者另外地(或备选地)用解热药(例

如,对乙酰氨基酚/扑热息痛)进行前驱用药。

[0122] 在以上和本文所述方法的一些实施方案中,与初次暴露或后面的暴露一起施用第二药物,其中抗-CD20抗体是第一药物。在一些实施方案中,第二药物选自由以下组成的组:干扰素、醋酸格拉默、细胞毒性剂、化学治疗剂、米托蒽醌、甲氨喋呤、环磷酰胺、苯丁酸氮芥、硫唑嘌呤、γ球蛋白、Campath、抗-CD4、克拉屈滨(cladribine)、皮质类固醇、麦考酚酸吗乙酯(mycophenolate mofetil,MMF)、环孢菌素、他汀(statin)类降胆固醇药物、雌二醇、睾酮、激素替代药物、TNF抑制剂、减轻疾病的抗风湿药(DMARD)、非甾体抗炎药(NSAID)、左甲状腺素、环孢菌素A、促生长素抑制素(somatastatin)类似物、细胞因子或细胞因子受体拮抗剂、抗代谢物、免疫抑制剂、整联蛋白拮抗剂或抗体、LFA-1抗体、依法珠单抗(efalizumab)、α4整联蛋白抗体、那他珠单抗(natalizumab)、和其它B细胞表面标志抗体。

[0123] 在以上和本文所述方法的一些实施方案中,多发性硬化是复发型多发性硬化。在一些实施方案中,多发性硬化是复发缓和型多发性硬化(RRMS)。在一些实施方案中,复发型多发性硬化是具有重叠复发的继发进行性多发性硬化(rSPMS)。在一些实施方案中,多发性硬化是进行性多发性硬化。在一些实施方案中,多发性硬化是原发进行性多发性硬化(PPMS)。

[0124] 在根据(或如应用于)任一个上述实施方案的某些实施方案中,基于患有复发型多发性硬化(诸如RRMS或rSPMS)来选择患者用于治疗。在根据(或如应用于)任一个上述实施方案的某些实施方案中,治疗以患有复发型多发性硬化(诸如RRMS或rSPMS)的患者为基础。在根据(或如应用于)任一个上述实施方案的某些实施方案中,在治疗前,患者诊断为患有复发型多发性硬化(诸如RRMS或rSPMS)。

[0125] 在根据(或如应用于)任一个上述实施方案的某些实施方案中,基于患有进行型多发性硬化(诸如PPMS)来选择患者用于治疗。在根据(或如应用于)任一个上述实施方案的某些实施方案中,治疗以患有进行型多发性硬化(诸如PPMS)的患者为基础。在根据(或如应用于)任一个上述实施方案的某些实施方案中,在治疗前,患者诊断为患有进行型多发性硬化(诸如PPMS)。

[0126] 在以上和本文所述方法的一些实施方案中,将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露,随后是一次或多次另外的抗-CD20抗体暴露,其中每次暴露为约600mg的抗体,并且将每次暴露作为抗-CD20抗体的一次或两次剂量提供至患者,并且其中每次暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中,“约20-24周”是指20周至24周之间的时间点。在一些实施方案中,“约20-24周”是指第24周之前或之后1周或7天的变动。在一些实施方案中,“约5-6个月”是指5至6个月之间的时间点。

[0127] 在一些实施方案中,第一次暴露包括抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中,“约14天”是指第14天之前或之后1天的变动。在一些实施方案中,第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在一些实施方案中,初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。

[0128] 在根据(或如应用于)任一个上述实施方案的某些实施方案中,抗-CD20抗体包含:a)包含SEQ ID NO:8的氨基酸序列的重链可变区和b)包含SEQ ID NO:2的氨基酸序列的轻

链可变区。在根据(或如应用于)任一个上述实施方案的某些实施方案中，抗-CD20抗体包含：包含SEQ ID NO:14或SEQ ID NO:15或SEQ ID NO:26或SEQ ID NO:27的氨基酸序列的重链，和包含SEQ ID NO:13的氨基酸序列的轻链。

[0129] 在根据(或如应用于)任一个上述实施方案的某些实施方案中，将抗-CD20抗体施用至患者以提供初次抗-CD20抗体暴露，随后是第二次抗-CD20抗体暴露，其中第一次和第二次暴露各自为约600mg的抗体，并且其中第一次暴露和第二次暴露之间的间隔为约20-24周或约5-6个月。在根据(或如应用于)任一个上述实施方案的某些实施方案中，将抗-CD20抗体施用至患者以提供第三次抗-CD20抗体暴露，其中第三次暴露为约600mg的抗体，并且其中第二次暴露和第三次暴露之间的间隔为约20-24周或约5-6个月。在根据(或如应用于)任一个上述实施方案的某些实施方案中，将抗-CD20抗体施用至患者以提供第四次抗-CD20抗体暴露，其中第四次暴露为约600mg的抗体，并且其中第三次暴露和第四次暴露之间的间隔为约20-24周或约5-6个月。

[0130] 在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一次暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在根据(或如应用于)任一个上述实施方案的某些实施方案中，静脉内施用第一和第二剂量。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自包含浓度为约1.2mg/ml的250ml抗-CD20抗体。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自以30ml/小时的速率进行输注。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量的输注速率可以以30ml/小时的增量增加至180ml/小时的最大速率。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自在大约2.5小时内给予。

[0131] 在一些实施方案中，第二次、第三次和/或第四次暴露包括约600mg的单一剂量。在根据(或如应用于)任一个上述实施方案的某些实施方案中，静脉内施用第二次、第三次和/或第四次暴露。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第二次、第三次和/或第四次暴露各自包含浓度为约1.2mg/ml的500ml抗-CD20抗体。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第二次、第三次和/或第四次暴露各自以40ml/小时的速率进行输注。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第二次、第三次和/或第四次暴露的输注速率可以以40ml/小时的增量增加至200ml/小时的最大速率。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自在大约3.5小时内给予。

[0132] 在一些实施方案中，初次暴露以及第二次、第三次和/或第四次另外的暴露包括抗-CD20抗体的第一剂量和第二剂量，其中每个剂量为约300mg，并且第一剂量和第二剂量隔开约两周或约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在根据(或如应用于)任一个上述实施方案的某些实施方案中，静脉内施用第一和第二剂量。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自包含浓度为约1.2mg/ml的250ml抗-CD20抗体。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量各自以30ml/小时的速率进行输注。在根据(或如应用于)任一个上述实施方案的某些实施方案中，第一和第二剂量的输注速率可以以

30ml/小时的增量增加至180ml/小时的最大速率。在根据(或如应用于)任一个上述实施方案的某些实施方案中,第一和第二剂量各自在大约2.5小时内给予。

[0133] 在某些实施方案中,患者接受至少2次、3次、4次或多于4次抗-CD20抗体暴露。

[0134] 在一些实施方案中,将抗-CD20抗体施用至患者以提供第四次暴露后的一次或多次另外的抗-CD20抗体暴露,其中一次或多次另外的暴露为约600mg的抗体,并且其中第四次暴露和另外的暴露之间的间隔为约20-24周或约5-6个月。在一些实施方案中,"约20-24周"是指20周至24周之间的时间点。在一些实施方案中,"约20-24周"是指第24周之前或之后1周或7天的变动。在一些实施方案中,"约5-6个月"是指5至6个月之间的时间点。在一些实施方案中,第四次暴露后每次另外的暴露之间的间隔为20-24周或约5-6个月。在一些实施方案中,一次或多个暴露包括抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且第一剂量和第二剂量隔开约14天(诸如13天或15天)。在一些实施方案中,"约14天"是指第14天之前或之后1天的变动。在一些实施方案中,一次或多个暴露包括抗-CD20抗体的第一剂量和第二剂量,其中每个剂量为约300mg,并且第一剂量和第二剂量隔开约14天(诸如13天或15天)。

[0135] 在以上和本文所述方法的一些实施方案中,抗-CD20抗体包含:包含SEQ ID NO:8的氨基酸序列的重链可变区和包含SEQ ID NO:2的氨基酸序列的轻链可变区。在一些实施方案中,抗-CD20抗体包含:包含SEQ ID NO:14或SEQ ID NO:26或SEQ ID NO:15或SEQ ID NO:27的氨基酸序列的重链,和包含SEQ ID NO:13的氨基酸序列的轻链。在一些情况中,抗-CD20抗体是ocrelizumab。在一些实施方案中,抗-CD20抗体是药用组合物。在一些实施方案中,抗-CD20抗体在包含30mg/mL抗体、20mM乙酸钠、106mM海藻糖、0.02%聚山梨醇酯20、pH 5.3的制剂中。在一些实施方案中,制剂中的抗体以300mg/小瓶储存于约2-8℃。在一些实施方案中,将抗体在IV袋中稀释于盐水(0.9%氯化钠)中,用于通过输注施用。在一些实施方案中,抗-CD20抗体是其抗原结合片段。

[0136] 在以上和本文所述方法的一些实施方案中,静脉内施用抗-CD20抗体。在一些实施方案中,对于每次抗体暴露,静脉内施用抗-CD20抗体。在以上和本文所述方法的一些实施方案中,皮下施用所述抗体。在一些实施方案中,对于每次抗体暴露,皮下施用抗-CD20抗体。

[0137] 在一些实施方案中,提供包含抗-CD20抗体的组合物,所述抗-CD20抗体包含:a)重链可变区,所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3,以及b)轻链可变区,所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3,其用于根据任一种上述方法治疗患者中的多发性硬化。

[0138] 在一些实施方案中,提供一种抗-CD20抗体,所述抗体包含:a)重链可变区,所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3,以及b)轻链可变区,所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3,其用于制备用于根据任一种上述方法治疗患者中的多发性硬化的药物。

[0139] 在以上和/或本文所述任一种方法的某些实施方案中，施用抗-CD20抗体后的降低或减少或改善可以与基线水平比较、与未治疗的患者中的水平比较、和/或与患者中的水平(例如诸如接受不同治疗(诸如干扰素β-1a或REBIF®)的一组患者的均值、平均数或中位数水平)比较。

[0140] 在另一个方面，本文提供一种制品，所述制品包含：(a)包含抗-CD20抗体(例如ocrelizumab)的容器；和(b)具有用于根据以上和本文所述的任一种方法治疗患者中的多发性硬化的说明的包装插页。

[0141] 应该理解，本文所述的各种实施方案中的一个、一些或全部特征可以组合以形成本发明的其它实施方案。

[0142] 附图简述

[0143] 图1A是比较了鼠2H7(SEQ ID NO:1)、人源化2H7.v16变体(SEQ ID NO:2)和人κ轻链亚组I(SEQ ID NO:3)中每一个的轻链可变结构域(V_L)的氨基酸序列的序列比对。2H7和hu2H7.v16的V_L的CDR如下：CDR1(SEQ ID NO:4)、CDR2(SEQ ID NO:5)和CDR3(SEQ ID NO:6)。

[0144] 图1B是比较了鼠2H7(SEQ ID NO:7)、人源化2H7.v16变体(SEQ ID NO:8)和重链亚组III的人共有序列(SEQ ID NO:9)中每一个的重链可变结构域(V_H)的氨基酸序列的序列比对。2H7和hu2H7.v16的V_H的CDR如下：CDR1(SEQ ID NO:10)、CDR2(SEQ ID NO:11)和CDR3(SEQ ID NO:12)。

[0145] 在图1A和图1B中，如所指明的，每条链中的CDR1、CDR2和CDR3都包含在括号中，两侧是框架区FR1-FR4。2H7是指鼠2H7抗体。两行序列之间的星号表示两个序列之间不同的位置。残渣编号根据Kabat等人，Sequences of Immunological Interest，第5版，Public Health Service，National Institutes of Health，Bethesda，Md.(1991)进行，插入显示为a、b、c、d和e。

[0146] 图2显示了成熟2H7.v16轻链的氨基酸序列(SEQ ID NO:13)。

[0147] 图3显示了成熟2H7.v16重链的氨基酸序列(SEQ ID NO:14)。

[0148] 图4显示了成熟2H7.v31重链的氨基酸序列(SEQ ID NO:15)。2H7.v31的轻链与2H7.v16相同。

[0149] 图5显示了成熟2H7.v16和2H7.v511轻链(分别为SEQ ID NOS.13和16)的比对，其具有Kabat可变结构域残基编号和Eu恒定结构域残基编号。

[0150] 图6显示了成熟2H7.v16和2H7.v511重链(分别为SEQ ID NOS.14和17)的比对，其具有Kabat可变结构域残基编号和Eu恒定结构域残基编号。

[0151] 图7显示了与患有复发型多发性硬化的患者中使用干扰素β-1a(REBIF®)相比，ocrelizumab的两个相同的III期研究(即，研究I和研究II)的研究设计的概述。

[0152] 图8显示了研究I和研究II的层级结构统计学分析计划。

[0153] 图9显示了研究I和研究II中的患者分布。

[0154] 图10A显示了在研究I中，与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周时的年复发率(ARR)。

[0155] 图10B显示了在研究II中，与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周时的年复发率(ARR)。

[0156] 图11显示了与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展(CDP)达至少12周的发病时间(来自研究I和研究II的合并数据)。

[0157] 图12A显示了在研究I中，与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展达至少12周的发病时间。

[0158] 图12B显示了在研究II中，与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展达至少12周的发病时间。

[0159] 图13显示了与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展达至少24周的发病时间(来自研究I和研究II的合并数据)。

[0160] 图14A显示了在研究I中，与用于扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展达至少24周的发病时间。

[0161] 图14B显示了在研究II中，与用干扰素β-1a治疗的患者相比，用ocrelizumab治疗的患者在96周内确认的能力丧失进展达至少24周的发病时间。

[0162] 图15A显示了与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中具有确认的能力丧失改善(即CDI)达至少12周的患者(具有2.0以上的基线EDSS评分)的比例(来自研究I和研究II的合并数据)。

[0163] 图15B显示了与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中具有确认的能力丧失改善(即CDI)达至少24周的患者(具有2.0以上的基线EDSS评分)的比例(来自研究I和研究II的合并数据)。

[0164] 图16A显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中具有确认的能力丧失改善(即CDI)达至少12周的患者(具有2.0以上的基线EDSS评分)的比例。

[0165] 图16B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中具有确认的能力丧失改善(即CDI)达至少12周的患者(具有2.0以上的基线EDSS评分)的比例。

[0166] 图16C显示了在研究I中，与接受干扰素β-1a的患者相比，从基线至接受ocrelizumab的第96周患者的多发性硬化症功能综合评分的变化。

[0167] 图16D显示了在研究II中，与接受干扰素β-1a的患者相比，从基线至接受ocrelizumab的第96周患者的多发性硬化症功能综合评分的变化。

[0168] 图17A显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的T1钆增强性损伤的总数。

[0169] 图17B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的T1钆增强性损伤的总数。

[0170] 图18A显示了在研究I中，与接受IFN β-1a的患者相比，在第24周、第48周和第96周时在接受ocrelizumab的患者中的T1钆增强性损伤的平均总数。

[0171] 图18B显示了在研究II中，与接受IFN β-1a的患者相比，在第24周、第48周和第96周时在接受ocrelizumab的患者中的T1钆增强性损伤的平均总数。

[0172] 图19A显示了在研究I和研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的新的和/或扩大的高强度T2损伤的总数。

[0173] 图19B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的新的和/或扩大的高强度T2损伤的总数。

[0174] 图19C显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的新的和/或扩大的高强度T2损伤的平均数。

[0175] 图19D显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周检测到的新的和/或扩大的高强度T2损伤的平均数。

[0176] 图20A显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中从第24周至第96周的脑体积损失速率。

[0177] 图20B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中从第24周至第96周的脑体积损失速率。

[0178] 图21A显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中从基线至第96周的脑体积损失速率。

[0179] 图21B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中从基线至第96周的脑体积损失速率。

[0180] 图22A显示了在研究I中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周的T1低强度损伤/MRI扫描的总数。

[0181] 图22B显示了在研究II中，与接受干扰素β-1a的患者相比，在接受ocrelizumab的患者中第24周、第48周和第96周的T1低强度损伤/MRI扫描的总数。

[0182] 图23A显示了在研究I和研究II中(合并的)，接受IFN β-1a的患者的输注相关反应。

[0183] 图23B显示了在研究I和研究II中(合并的)，接受ocrelizumab的患者的输注相关反应。

[0184] 图24显示了患有原发进行性多发性硬化(PPMS)的患者中Ocrelizumab的III期研究的研究设计。

[0185] 图25提供了PPMS患者中Ocrelizumab的III期研究的统计层次结构。

[0186] 图26显示了III期PPMS研究的临床截止日期的患者分布。

[0187] 图27显示了对于III期PPMS研究，与接受安慰剂的患者相比，在用ocrelizumab治疗的患者中确认的能力丧失进展达至少12周的发病时间。

[0188] 图28显示了对于III期PPMS研究，与接受安慰剂的患者相比，在用ocrelizumab治疗的患者中确认的能力丧失进展达至少24周的发病时间。

[0189] 图29显示了对于III期PPMS研究，与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中从基线至第120周的行走速度的下降速率(如通过计时25英尺行走所测量的)。

[0190] 图30显示了对于III期PPMS研究，与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中第120周行走速度相对于基线的下降速率。

[0191] 图31显示了对于III期PPMS研究，与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中从第24周至第96周的全脑体积的百分比变化。

[0192] 图32显示了对于III期PPMS研究，与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中T2损伤体积的变化。

[0193] 图33显示了对于III期PPMS研究，与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中通过暴露和直至临床截止日期的严重程度表示的输注相关反应(IRR)。

[0194] 图34A显示了在研究I中，与服用ocrelizumab的NEDA患者的比率相比，服用IFN β-1a的无疾病活动迹象(NEDA)的患者的比例。

[0195] 图34B显示了在研究II中，与服用ocrelizumab的NEDA患者的比率相比，服用IFN β-1a的无疾病活动迹象(NEDA)的患者的比例。

[0196] 图35A显示了接受ocrelizumab的在基线处具有Gd^+损伤的患者比上接受安慰剂的在基线处具有Gd+损伤的患者中，计时25英尺行走测试中从基线行走时间至第120周的百分比变化的降低。

[0197] 图35B显示了接受ocrelizumab的在基线处不具有Gd^+损伤的患者比上接受安慰剂的在基线处不具有Gd^+损伤的患者中，计时25英尺行走测试中从基线行走时间至第120周的百分比变化的降低。

[0198] 图35C显示了接受ocrelizumab的总研究群体中的患者比上接受安慰剂的总研究群体中的患者中，计时25英尺行走测试中从基线行走时间至第120周的百分比变化的降低。

[0199] 图36A显示了与接受安慰剂的在基线处具有T1 Gd^+损伤的患者相比，在基线处具有T1 Gd^+损伤的用ocrelizumab治疗的患者中从第24周至第120周的全脑体积损失的差异。

[0200] 图36B显示了与接受安慰剂的在基线处不具有T1 Gd^+损伤的患者相比，在基线处不具有T1 Gd^+损伤的用ocrelizumab治疗的患者中从第24周至第120周的全脑体积损失的差异。

[0201] 图36C显示了与接受安慰剂的总研究群体中的患者相比，总研究群体中用ocrelizumab治疗的患者中从第24周至第120周的全脑体积损失速率。

[0202] 图37A显示了接受ocrelizumab的患者比上接受安慰剂的患者中，从基线至第120周的T2损伤体积的差异。

[0203] 图37B显示了接受ocrelizumab的在基线处具有T1 Gd^+损伤的患者比上接受安慰剂的在基线处具有T1 Gd^+损伤的患者中，从基线至第120周的T2损伤体积的差异。

[0204] 图37C显示了接受ocrelizumab的在基线处不具有T1 Gd^+损伤的患者比上接受安慰剂的在基线处不具有T1 Gd^+损伤的患者中，从基线至第120周的T2损伤体积的差异。

[0205] 图38显示了如通过简式-36(SF-36)健康要素总结(Physical Component Summary，PCS)所测量的，接受ocrelizumab的患者和接受IFN β-1a的患者之间报告的生活质量变化的改善的差异。

[0206] 图39A显示了在研究I中，接受ocrelizumab的患者比上接受IFN β-1a的患者中，从基线至第96周的EDSS评分变化之间的差异。

[0207] 图39B显示了在研究II中，接受ocrelizumab的患者比上接受IFN β-1a的患者中，从基线至第96周的EDSS评分变化之间的差异。

[0208] 图40A显示了与接受安慰剂的总研究群体中的患者相比，接受600mg ocrelizumab的总研究群体中的患者中从基线至第120周的SF-36健康要素总结评分的变化。

[0209] 图40B显示了与接受安慰剂的在基线处具有T1钆增强型损伤的患者相比，接受600mg ocrelizumab的在基线处具有T1钆增强型损伤的患者中从基线至第120周的SF-36健

康要素总结评分的变化。

[0210] 图40C显示了与接受安慰剂的在基线处不具有T1钆增强型损伤的患者相比，接受600mg ocrelizumab的在基线处不具有T1钆增强型损伤的患者中从基线至第120周的SF-36健康要素总结评分的变化。

[0211] 图41显示了对于III期PPMS研究，与接受安慰剂的患者相比，在用ocrelizumab治疗的患者中确认的复合能力丧失进展达至少12周的发病时间。

[0212] 图42显示了对于III期PPMS研究，与接受安慰剂的患者相比，在用ocrelizumab治疗的患者中确认的复合能力丧失进展达至少24周的发病时间。

[0213] 图43显示了以下亚组中到第96周的方案定义的年复发率：活跃的不充分应答者、活跃的初治治疗患者、高度活跃的不充分应答者和高度活跃的初治治疗患者。

[0214] 图44显示了以下亚组中CDP达至少12周的发病时间：活跃的不充分应答者、活跃的初治治疗患者、高度活跃的不充分应答者和高度活跃的初治治疗患者。

[0215] 图45显示了以下亚组中CDP达至少24周的发病时间：活跃的不充分应答者、活跃的初治治疗患者、高度活跃的不充分应答者和高度活跃的初治治疗患者。

[0216] 图46显示了以下亚组中CDI达至少12周的患者的比例：活跃的不充分应答者、活跃的初治治疗患者、高度活跃的不充分应答者和高度活跃的初治治疗患者。

[0217] 图47显示了以下亚组中如通过脑MRI检测的T1钆增强性损伤的总数：活跃的不充分应答者、活跃的初治治疗患者、高度活跃的不充分应答者和高度活跃的初治治疗患者。

[0218] 发明详述

[0219] I.定义

[0220] “B细胞”是在骨髓中成熟的淋巴细胞，并且包括幼稚B细胞、记忆B细胞或效应B细胞(浆细胞)。本文中的B细胞可以是正常的或非恶性的B细胞。

[0221] 本文中的“B细胞表面标志”或“B细胞表面抗原”是可以被与其结合的抗体靶向的B细胞表面上表达的抗原。示例性B细胞表面标志包括CD10、CD19、CD20、CD21、CD22、CD23、CD24、CD37、CD40、CD53、CD72、CD73、CD74、CDw75、CDw76、CD77、CDw78、CD79a、CD79b、CD80、CD81、CD82、CD83、CDw84、CD85和CD86白细胞表面标志(描述参见The Leukocyte Antigen Facts Book，第2版.1997，ed.Barclay等人，Academic Press，Harcourt Brace&Co.，New York)。其它B细胞表面标志包括RP105、FcRH2、B细胞CR2、CCR6、P2X5、HLA-DOB、CXCR5、FCER2、BR3、Btig、NAG14、SLGC16270、FcRH1、IRTA2、ATWD578、FcRH3、IRTA1、FcRH6、BCMA和239287。相较于哺乳动物的其它非B细胞组织，本文中特别感兴趣的B细胞表面标志优先在B细胞上表达，并且可以在前体B细胞和成熟B细胞两者上表达。本文中优选的B细胞表面标志是CD20。

[0222] “CD20”抗原或“CD20”是一种约35-kDa非糖基化磷蛋白，其存在于来自外周血或淋巴器官的超过90％的B细胞的表面上。CD20存在于正常B细胞以及恶性B细胞二者上，但是在干细胞上不表达。文献中CD20的其它名称包括“B淋巴细胞限制性抗原”和“Bp35”。例如，CD20抗原描述于Clark等人，Proc.Natl.Acad.Sci.(USA)82:1766(1985)中。

[0223] 本文中的“抗体拮抗剂”是在结合B细胞上的B细胞表面标志后破坏或消耗哺乳动物中的B细胞和/或干扰一种或多种B细胞功能(例如通过降低或阻止由B细胞引发的体液应答)的抗体。优选地，抗体拮抗剂能够在用其处理的哺乳动物中消耗B细胞(即降低循环B细

胞水平)。这种消耗可以通过各种机制来实现,诸如抗体依赖性细胞介导的细胞毒性(ADCC)和/或补体依赖性细胞毒性(CDC)、抑制B细胞增殖、和/或诱导B细胞死亡(例如通过细胞凋亡)。

[0224] "抗体依赖性细胞介导的细胞毒性"和"ADCC"是指细胞介导的反应,其中表达Fc受体(FcR)的非特异性细胞毒性细胞(例如,天然杀伤(NK)细胞、嗜中性粒细胞和巨噬细胞)识别靶细胞上的结合抗体,随后引起靶细胞溶解。介导ADCC的原代细胞,即NK细胞,仅表达FcγRIII,而单核细胞表达FcγRI、FcγRII和FcγRIII。造血细胞上的FcR表达汇总于Ravetch和Kinet,Annu.Rev.Immunol.9:457-92(1991)的第464页上的表3中。为了评价目的分子的ADCC活性,可以进行体外ADCC测定,诸如美国专利号5,500,362或5,821,337中描述的体外ADCC测定。用于这种测定的效应细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。备选地或额外地,可以在体内,例如,在动物模型(诸如在Clynes等人,PNAS(USA)95:652-656(1998)中公开的)中评价目的分子的ADCC活性。

[0225] "人效应细胞"是表达一种或多种FcR并且行使效应子功能的白细胞。在一些实施方案中,该细胞至少表达FcγRIII并且执行ADCC效应子功能。介导ADCC的人白细胞的实例包括外周血单核细胞(PBMC)、天然杀伤(NK)细胞、单核细胞、细胞毒性T细胞和嗜中性粒细胞;优选PBMC和NK细胞。

[0226] 术语"Fc受体"和"FcR"用于描述与抗体Fc区结合的受体。在一些实施方案中,FcR是天然序列人FcR。此外,优选的FcR是与IgG抗体结合的FcR(γ受体),并且包括FcγRI、FcγRII和FcγRIII亚类的受体,包括这些受体的等位基因变体和可变剪接形式。FcγRII受体包括FcγRIIA("激活受体")和FcγRIIB("抑制受体"),它们具有相似的氨基酸序列,所述氨基酸序列的区别主要在于其胞质结构域。激活受体FcγRIIA在其胞质结构域中含有基于免疫受体酪氨酸的激活基序(ITAM)。抑制受体FcγRIIB在其胞质结构域中含有基于免疫受体酪氨酸的抑制基序(ITIM)。(参见Daëron,Annu.Rev.Immunol.15:203-234(1997))。FcR综述于Ravetch和Kinet,Annu.Rev.Immunol 9:457-92(1991);Capel等人,Immunomethods 4:25-34(1994);和de Haas等人,J.Lab.Clin.Med.126:330-41(1995)中。本文中的术语"FcR"涵盖其它FcR,包括未来将会鉴定的FcR。该术语还包括新生儿受体,FcRn,其负责将母体IgG转移至胎儿(Guyer等人,J.Immunol,117:587(1976)和Kim等人,J.Immunol.24:249(1994))。

[0227] "补体依赖性细胞毒性"或"CDC"是指在补体的存在下分子溶解靶标的能力。补体激活途径起始于补体系统的第一组分(C1q)与复合于相关抗原的分子(例如抗体)的结合。为了评价补体激活,可以进行CDC测定,例如如Gazzano-Santoro等人,J.Immunol.Methods 202:163(1996)中所描述的。

[0228] "生长抑制性"抗体是阻止或降低表达抗体所结合的抗原的细胞增殖的那些抗体。例如,抗体可以在体外和/或在体内阻止或降低B细胞增殖。

[0229] "诱导凋亡"的抗体是如通过标准凋亡测定(诸如,结合膜联蛋白V、DNA片段化、细胞收缩、内质网膨胀、细胞破碎和/或形成膜囊泡(称作凋亡小体))所确定的,诱导例如B细胞的程序性细胞死亡的那些抗体。

[0230] 本文中的术语"抗体"以最广意义使用,并且特别涵盖单克隆抗体、多克隆抗体、由至少两种完整抗体形成的多特异性抗体(例如双特异性抗体)、和抗体片段,只要它们展现

所需生物活性即可。

[0231] “抗体片段”包含完整抗体的一部分，优选包含其抗原结合区。抗体片段的实例包括Fab、Fab'、F(ab')$_2$和Fv片段；双抗体(diabody)；线性抗体；单链抗体分子；和由抗体片段形成的多特异性抗体。

[0232] 为了本文的目的，“完整抗体”是包含重链和轻链可变结构域以及Fc区的抗体。

[0233] “天然抗体”通常是约150,000道尔顿的异源四聚体糖蛋白，由两条相同的轻(L)链和两条相同的重(H)链组成。每条轻链通过一个共价二硫键与重链连接，而二硫键的数量在不同免疫球蛋白同种型的重链之间有所不同。每条重链和轻链还具有规律地间隔的链内二硫桥。每条重链在一端具有可变结构域(V_H)，之后是若干个恒定结构域。每条轻链在一端具有可变结构域(V_L)并且在其另一端具有恒定结构域；轻链的恒定结构域与重链的第一恒定结构域对齐，并且轻链的可变结构域与重链的可变结构域对齐。据信，特定的氨基酸残基在轻链和重链可变结构域之间形成界面。

[0234] 术语“可变”是指这样的事实，即在抗体之间可变结构域的某些部分在序列上差异很大并且被用于各特定抗体对其特定抗原的结合和特异性。然而，可变性在抗体的可变结构域上并不是均匀分布的。其集中于在轻链和重链可变结构域中的三个被称为高变区的区段。可变结构域的更高度保守的部分被称为框架区(FR)。天然重链和轻链的可变结构域各自包含四个FR，其大多采取β折叠构型，通过三个高变区连接，所述三个高变区形成环连接，并且在一些情况下形成β折叠结构的部分。每条链中的高变区通过FR非常接近地保持在一起，并与另一条链的高变区一起促成抗体的抗原结合位点的形成(参见Kabat等人，Sequences of Proteins of Immunological Interest，第5版，Public Health Service，National Institutes of Health，Bethesda，MD.(1991))。恒定结构域不直接参与抗体与抗原的结合，但是展现出多种效应子功能，诸如在抗体依赖性细胞毒性(ADCC)中抗体的参与。

[0235] 用木瓜蛋白酶消化抗体产生称作“Fab”片段的两个相同的抗原结合片段(各自具有单个抗原结合位点)和一个残余“Fc”片段，其名称反映了它易于结晶的能力。木瓜蛋白酶处理产生具有两个抗原结合位点并且仍然能够交联抗原的F(ab')$_2$片段。

[0236] “Fv”是含有完整抗原识别和抗原结合位点的最小抗体片段。该区由一个重链可变结构域和一个轻链可变结构域以紧密的、非共价缔合的二聚体组成。在这种构型中，每个可变结构域的三个高变区相互作用从而限定在V_H-V_L二聚体的表面上的抗原结合位点。总而言之，六个高变区将抗原结合特异性赋予抗体。然而，即使单个可变结构域(或只包含对抗原具有特异性的三个高变区的Fv的一半)也具有识别和结合抗原的能力，虽然亲和性低于完整结合位点。

[0237] Fab片段还含有轻链的恒定结构域和重链的第一恒定结构域(CH1)。Fab'片段与Fab片段的不同之处在于在重链CH1结构域的羧基末端添加了几个残基，包括来自抗体铰链区的一个或多个半胱氨酸。Fab'-SH是本文对Fab'的命名，其中恒定结构域的一个或多个半胱氨酸残基带有至少一个游离硫醇基团。F(ab')$_2$抗体片段最初是作为在它们之间具有铰链半胱氨酸的Fab'片段对而产生的。抗体片段的其它化学偶联也是已知的。

[0238] 来自任何脊椎动物物种的抗体(免疫球蛋白)的“轻链”可以基于其恒定结构域的氨基酸序列而分配为两种截然不同类型(称作κ和λ)之一。

[0239] 根据其重链的恒定结构域的氨基酸序列，可以将抗体分配为不同的类别。存在完整抗体的五种主要类别：IgA、IgD、IgE、IgG和IgM，并且这些类别中的几种可以进一步划分成亚类(同种型)，例如IgG1、IgG2、IgG3、IgG4、IgA和IgA2。对应不同类别的抗体的重链恒定结构域分别称作α、δ、ε、γ和μ。不同类别的免疫球蛋白的亚基结构和三维构型是公知的。

[0240] “单链Fv”或“scFv”抗体片段包含抗体的V_H和V_L结构域，其中这些结构域存在于一条多肽链上。在一些实施方案中，Fv多肽还包含V_H和V_L结构域之间的多肽接头，其使得sFv能够形成抗原结合所需的结构。关于scFv的综述，Plückthun，在The Pharmacology of Monoclonal Antibodies，卷113，Rosenburg和Moore编著，Springer-Verlag，New York，pp.269-315(1994)。

[0241] 术语“双抗体”是指具有两个抗原结合位点的小型抗体片段，该片段在同一条多肽链(V_H-V_L)中包含与轻链可变结构域(V_L)相连的重链可变结构域(V_H)。通过使用过短的接头使得同一条链上的两个结构域之间不能配对，迫使这些结构域与另一条链的互补结构域配对，并且产生两个抗原结合位点。双抗体更完整地描述于例如EP 404,097；WO 93/11161；和Hollinger等人，Proc.Natl.Acad.Sci.USA，90:6444-6448(1993)中。

[0242] 如本文所用，术语“单克隆抗体”是指从基本上同质的抗体的群体中获得的抗体，即，构成该群体的各个抗体是相同的和/或结合相同的表位，除了产生单克隆抗体期间可以出现的可能变体以外，这种变体通常以微量存在。与通常包括针对不同决定簇(表位)的不同抗体的多克隆抗体制备物相反，每种单克隆抗体针对抗原上的单一决定簇。除了它们的特异性之外，单克隆抗体的优点在于它们不被其它免疫球蛋白污染。修饰语“单克隆”表示如从基本上同质的抗体群体获得的抗体的特征，并且不解释为需要通过任何特殊方法产生该抗体。例如，根据本发明待使用的单克隆抗体可以通过Kohler等人，Nature，256:495(1975)首先描述的杂交瘤方法制备，或者可以通过重组DNA方法(参见例如美国专利号4,816,567)制备。“单克隆抗体”也可以使用例如Clackson等人，Nature，352:624-628(1991)和Marks等人，J.Mol.Biol.，222:581-597(1991)中描述的技术从噬菌体抗体文库中分离。

[0243] 本文中的单克隆抗体具体包括“嵌合”抗体(免疫球蛋白)，其中重链和/或轻链的一部分与来源于特定物种或属于特定抗体类别或亚类的抗体中的相应序列相同或同源，而链的其余部分与来源于另一种物种或属于另一种抗体类别或亚类的抗体中的相应序列相同或同源，以及这些抗体的片段，只要它们呈现所需生物活性即可(美国专利号4,816,567；Morrison等人，Proc.Natl.Acad.Sci.USA，81:6851-6855(1984))。本文中的目的嵌合抗体包括“灵长源化的(primatized)”抗体，其包含来源于非人灵长类(例如，旧大陆猴(Old World Monkey)，诸如狒狒、恒河猴(rhesus)或食蟹猴(cynomolgus monkey))的可变结构域抗原结合序列以及人类恒定区序列(美国专利号5,693,780)。

[0244] “人源化”形式的非人(例如鼠)抗体是含有来源于非人免疫球蛋白的最小序列的嵌合抗体。对于最大的部分，人源化抗体是人免疫球蛋白(接受体抗体)，其中来自接受体高变区的残基被来自非人物种(诸如小鼠、大鼠、兔或非人灵长动物)的具有需要的特异性、亲和力和能力的高变区(供体抗体)的残基替代。在一些情形中，人免疫球蛋白的框架区(FR)残基被相应的非人残基替代。此外，人源化抗体可以包含在接受体抗体中或在供体抗体中不存在的残基。进行这些修饰以进一步优化抗体性能。通常，人源化抗体将包含基本上全部的至少1个、并且典型2个可变结构域，其中全部或基本上全部的高变环与非人免疫球蛋白

的那些对应，并且全部或基本上全部的FR与人免疫球蛋白序列的那些对应，除了如上所述的FR替代以外。人源化抗体任选地还将包含免疫球蛋白恒定区的至少一部分，典型地包含人免疫球蛋白恒定区的至少一部分。关于进一步的细节，参见Jones等人，Nature 321:522-525(1986)；Riechmann等人，Nature 332:323-329(1988)；和Presta，Curr.Op.Struct.Biol.2:593-596(1992)。

[0245] 当在本文中使用时，术语“高变区”是指负责抗原结合的抗体的氨基酸残基。高变区包含来自“互补决定区”或“CDR”的氨基酸残基(例如，轻链可变结构域中的残基24-34(L1)、50-56(L2)和89-97(L3)以及重链可变结构域中的残基31-35(H1)、50-65(H2)和95-102(H3)；Kabat等人，Sequences of Proteins of Immunological Interest，第5版，Public Health Service，National Institutes of Health，Bethesda，MD.(1991))，和/或来自“高变环”的那些残基(例如，轻链可变结构域中的残基26-32(L1)、50-52(L2)和91-96(L3)以及重链可变结构域中的残基26-32(H1)、53-55(H2)和96-101(H3)；Chothia和Lesk J.Mol.Biol.196:901-917(1987))。“框架”或“FR”残基是不同于如本文所限定的高变区残基的那些可变结构域残基。

[0246] “裸抗体”是不与异源分子(诸如细胞毒性部分或放射性标记物)缀合的抗体(如本文所定义的)。

[0247] 纯粹为了本文的目的并且除非另有说明，“人源化2H7”是指与人CD20结合的人源化抗体或其抗原结合片段，其中所述抗体在体内有效消耗灵长类动物B细胞，所述抗体在其重链可变区(V_H)中至少包含来自抗-人CD20抗体的CDR H3序列SEQ ID NO:12(图1B)和基本上人重链亚组III(V_HIII)的人共有构架(FR)残基。在一些实施方案中，该抗体还包含重链CDR H1序列SEQ ID NO:10和CDR H2序列SEQ ID NO:11，并且在一些实施方案中，还包含轻链CDR L1序列SEQ ID NO:4、CDR L2序列SEQ ID NO:5、CDR L3序列SEQ ID NO:6和基本上人轻链κ亚组I($V_κI$)的人共有构架(FR)残基，其中V_H区可以连接至人IgG链恒定区，其中所述区可以是例如IgG1或IgG3。在一些实施方案中，这种抗体包含SEQ ID NO:8的V_H序列(v16，如图1B所示)，任选地还包含SEQ ID NO:2的V_I序列(v16，如图1A所示)，其可以在H链中具有氨基酸置换D56A和N100A以及在L链中具有S92A(v96)。在一些实施方案中，抗体是分别包含SEQ ID NOS:13和14(如图2和3所示)的轻链和重链氨基酸序列的完整抗体。在一些实施方案中，抗体是分别包含SEQ ID NOS:13和15(如图2和4所示)的轻链和重链氨基酸序列的2H7.v31。本文中的抗体还可以在Fc区中包含至少一个改善ADCC和/或CDC活性的氨基酸置换，诸如其中氨基酸置换是S298A/E333A/K334A的抗体，并且在一些实施方案中是具有SEQ ID NO:15的重链氨基酸序列(如图4所示)的2H7.v31。任何这些抗体还可以在Fc区中包含至少一个降低CDC活性的氨基酸置换，例如至少包含K322A置换。参见美国专利号6,528,624B1(Idusogie等人)。

[0248] 本文中的术语“ocrelizumab”(CAS登记号637334-45-3)是指针对CD20抗原的基因工程改造的人源化单克隆抗体，并且其包含(a)包含SEQ ID NO:13的氨基酸序列的轻链，和(b)包含SEQ ID NO:14的氨基酸序列的重链，包括保留结合CD20能力的其片段。Ocrelizumab可从Genentech获得。

[0249] “分离的”抗体是已经经过鉴定并从它的天然环境的组分分离和/或回收的抗体。它的天然环境的污染物组分是将干扰抗体的诊断或治疗用途的物质，且可以包括酶、激素

和其它蛋白性的或非蛋白性的溶质。在一些实施方案中，抗体将被纯化(1)至大于95重量％的抗体，如通过Lowry方法所确定的，并且在一些实施方案中超过99重量％，(2)纯化至足以获得N-末端或内部氨基酸序列的至少15个残基的程度，如通过使用旋杯式序列分析仪，或(3)纯化至同质性，通过在还原或非还原条件下使用考马斯蓝或在一些实施方案中使用银染料的SDS-PAGE。分离的抗体包括在重组细胞内的原位抗体，因为抗体的天然环境的至少一种组分将是不存在的。然而，通常将通过至少一个纯化步骤制备分离的抗体。

[0250] 本文中的“受试者”或“患者”是人受试者或患者。通常，受试者或患者符合接受多发性硬化治疗的条件。为了本文的目的，这种符合条件的受试者或患者是这样的受试者或患者：其正在经历、已经经历、或可能经历多发性硬化的一种或多种征候、症状或其它指标；已经诊断有多发性硬化，无论是例如最近诊断的(具有“新发病”的MS)、先前诊断具有新复发或恶化的、先前诊断并处于缓和的等；和/或有风险发展多发性硬化。患有或有风险患有多发性硬化的受试者或患者可以任选地被鉴定为这样的受试者或患者，其已经针对血清、脑脊液(CSF)和/或MS损伤中CD20阳性B细胞的升高的水平进行了筛选和/或使用检测自身抗体的测定进行筛选，定性且优选定量地进行评价。与多发性硬化有关的示例性的这种自身抗体包括抗髓鞘碱性蛋白(MBP)、抗髓鞘少突胶质细胞糖蛋白(MOG)、抗神经节苷脂和/或抗神经丝抗体。可以在受试者的血清、脑脊液(CSF)和/或MS损伤中检测这种自身抗体。本文中“升高的”自身抗体或B细胞水平意指所述自身抗体或B细胞的水平显著超过未患有MS的个体中的水平。

[0251] 如本文中使用的，“治疗”是获得有益的或期望的结果(包括临床结果)的方案。对于本发明的目的，有益或所需的临床结果包括但不限于以下中的一种或多种：减少由疾病产生的一种或多种症状、减轻疾病程度、稳定疾病(例如阻止或延迟疾病的恶化)、延迟或减缓疾病进展、改善疾病状态、减少一种或多种治疗疾病所需的其它药物的剂量、和/或增加生活质量。

[0252] 如本文所使用的，“延迟”或“减缓”多发性硬化的进展意指阻止、延缓、阻碍、减缓、放缓、稳定和/或推迟疾病的发展。这种延迟可以具有不同长度的时间，这取决于疾病的历史和/或被治疗的个体。

[0253] 如本文所使用的，“开始治疗时”是指在第一次暴露于多发性硬化药物(诸如抗-CD20抗体)之时或之前的时期。在一些实施方案中，“开始治疗时”是在多发性硬化药物(诸如抗-CD20抗体)之前约1年、9个月、6个月、3个月、2个月或1个月中的任一时期。在一些实施方案中，“开始治疗时”是在第一次暴露于多发性硬化药物(诸如抗-CD20抗体)之前立即发生或与之同时发生。

[0254] 如本文所使用的，“基于”包括(1)评价、确定或测定如本文描述的患者特征(并且优选地选择适于接受治疗的患者)；和(2)施用本文描述的治疗。

[0255] MS的“症状”是受试者所经历的且指示MS的结构、功能或感觉方面的任何病态现象或与正常的偏差。

[0256] “多发性硬化”是指以髓鞘的进行性破坏为特征的中枢神经系统的慢性且通常为致残性的疾病。国际上公认有四种形式的MS，即原发进行性多发性硬化(primary progressive multiple sclerosis，PPMS)、复发缓和型多发性硬化(relapsing-remitting multiple sclerosis，RRMS)、继发进行性多发性硬化(secondary progressive multiple

sclerosis,SPMS)和进行性复发型多发性硬化(progressive relapsing multiple sclerosis,PRMS)。

[0257] 如本文所使用的“进行性多发性硬化”是指原发进行性多发性硬化(PPMS)、继发进行性多发性硬化(SPMS)和进行性复发型多发性硬化(PRMS)。在一些实施方案中,进行性多发性硬化的特征在于持续≥6个月的神经功能的有记录的、不可逆的丧失,其中该丧失不能归因于临床复发。

[0258] “原发进行性多发性硬化”或“PPMS”的特征在于,疾病从发病开始渐进性的发展,而几乎没有重叠的复发和缓和。可能存在疾病活动的平稳时期,并且可能存在好的和坏的数天或数周。PPMS与RRMS和SPMS的区别在于其发病典型地在三十多岁后期或四十多岁早期,男性与女性对于罹患此病具有相同可能性,并且最初的疾病活动常常在脊髓中而不是在脑中。还可以在脑中观察到(或发现)PPMS疾病活动。PPMS是MRI扫描上显示炎性(钆增强性)损伤的可能性最小的MS亚型。原发进行性形式的疾病影响所有多发性硬化患者的10-15%。可以根据Polman等人,Ann Neurol 69:292-392(2010)中的标准来定义PPMS。本文中所治疗的PPMS受试者通常是可能或确定诊断为患有PPMS的受试者。

[0259] “复发缓和型多发性硬化”或“RRMS”的特征在于复发(也称为恶化),在此期间可能出现新症状且老症状重新出现或恶化。复发之后是缓和期,在此期间患者完全或部分地从复发期间所造成的缺陷中恢复。复发可以持续数天、数周或数月,并且恢复可以是缓慢和逐步的,或者几乎是瞬间的。绝大部分表现出MS的患者首先诊断为RRMS。这通常是在他们二十多岁或三十多岁的时候,但是已知早得多或晚得多的诊断。表现出此MS亚型的女性患者是男性患者的两倍。在复发期间,髓鞘(即中枢神经系统(CNS)白质区中神经纤维(神经元)周围的保护性隔离鞘)可能被身体自身免疫系统的炎性反应损伤。这引起各种各样的神经症状,该神经症状根据损伤的CNS区域而变化甚大。在复发后,炎性反应立即逐渐消失,并且CNS中一种特殊类型的神经胶质细胞(称为少突胶质细胞)发起髓鞘再生-通过其可以修复轴突周围髓鞘的过程。可能是这种髓鞘再生造成了病情缓和。大约50%的RRMS患者在发病10年内转变成SPMS。30年后,该数字上升至90%。在任一时间,复发缓和型疾病占所有MS患者的55%。

[0260] “继发进行性多发性硬化”或“SPMS”的特征在于临床神经损伤的稳步发展,具有或不具有重叠的复发及小的缓和和平稳状态。发展为SPMS的患者先前将经历过RRMS时期,该时期可能已经持续从2年至40年或更长时间的任何时长。存在的任何重叠的复发和缓和都倾向于随时间逐渐变少。从疾病的继发进行阶段开始,能力丧失开始发展得比其在RRMS期间快得多,尽管在一些个体中发展仍然可以是相当缓慢的。10年后,50%的RRMS患者将发展成SPMS。到25至30年,该数字将上升至90%。与RRMS相比,SPMS倾向于与较低水平的炎性损伤形成相关,但是总体病情负担持续进展。在任一时间,SPMS都占所有多发性硬化患者的30%左右。

[0261] “进行性复发型多发性硬化”或“PRMS”的特征在于临床神经损伤的稳定发展,其具有重叠的复发和缓和。复发后会立即有显著的恢复,但是在复发之间症状逐渐恶化。PRMS占所有多发性硬化患者的5%左右。一些神经学家认为PRMS是PPMS的一种变体。表述“有效量”是指有效改善或治疗多发性硬化的抗体(或其它药物)的量。这种有效量通常将导致MS的征候、症状或其它指标的改善,诸如降低复发率、防止能力丧失、减少脑MRI损伤的数量和/或

体积、改善计时25英尺行走、减缓或延迟疾病的进展(诸如延长疾病进展的时间(例如使用扩展的能力丧失状态量表(EDSS))等。

[0262] “抗体暴露”是指接触或暴露于在约1-20天的一段时间内施用的一个或多个剂量的本文抗体。所述剂量可以一次性给予,或者在该暴露时间段内以固定的或无规律的间隔给予。如本文所详细描述的,初次和稍后(例如第二次或第三次)的抗体暴露彼此在时间上隔开。

[0263] 如本文所使用的,抗体暴露之间的“间隔”是指较早抗体暴露于较晚抗体暴露之间的时间段。本发明的抗体暴露可以包括一个或两个剂量。在抗体暴露含有一个剂量的情况中,两次抗体暴露之间的间隔是指一次抗体暴露的剂量(例如第1天)和下一次抗体暴露的剂量之间经过的时间量。如果一次抗体暴露包括两个剂量并且下一次抗体暴露包括一个剂量,则两次抗体暴露之间的间隔是指第一次抗体暴露的两个剂量中的第一个(例如第1天)和下一次抗体暴露的剂量之间经过的时间量。在两次抗体暴露中每一次都含有两个剂量的情况中,抗体暴露之间的间隔是指第一次抗体暴露的两个剂量中的第一个(例如第1天)和第二次抗体暴露的两个剂量中的第一剂量之间经过的时间量。例如,如果本发明的方法包括具有两个剂量的第一次抗体暴露和具有两个剂量的第二次抗体暴露,并且直到第一次抗体暴露后约24周或6个月才提供第二次抗体暴露,则第一次抗体暴露的第一剂量和第二次抗体暴露的第一剂量之间的间隔是约24周或6个月。在某些实施方案中,直到第一次抗体暴露后约20-24周或约5-6个月才提供第二次抗体暴露。在一些实施方案中,第一次抗体暴露的第一剂量和第二次抗体暴露的第一剂量之间的间隔是约20-24周或约5-6个月。在一些实施方案中,“约20-24周”是指20周至24周之间的时间点。在一些实施方案中,“约20-24周”是指第24周之前或之后1周或7天的变动。在一些实施方案中,“约5-6个月”是指5至6个月之间的时间点。

[0264] 术语“免疫抑制剂”在本文中用于辅助疗法时是指用于抑制或掩蔽本文所治疗的哺乳动物的免疫系统的物质。这将包括抑制细胞因子生成、下调或抑制自体抗原表达、或掩蔽MHC抗原的物质。这种药剂的实例包括2-氨基-6-芳基-5-取代的嘧啶(参见美国专利号4,665,077);非甾体抗炎药(NSAID);更昔洛韦(ganciclovir)、他克莫司(tacrolimus)、糖皮质激素(诸如皮质醇或醛固酮)、抗炎药(诸如环加氧酶抑制剂、5-脂氧合酶抑制剂或白三烯受体拮抗剂);嘌呤拮抗剂,诸如硫唑嘌呤或麦考酚酸吗乙酯(MMF);烷化剂,诸如环磷酸胺;溴隐亭(bromocryptine);达那唑(danazol);氨苯砜(dapsone);戊二醛(如美国专利号4,120,649中所描述的,其掩蔽MHC抗原);针对MHC抗原和MHC片段的抗独特型抗体;环孢菌素A;类固醇,诸如皮质类固醇或糖皮质类固醇或糖皮质激素类似物,例如泼尼松(prednisone)、甲泼尼龙和地塞米松(dexamethasone);二氢叶酸还原酶抑制剂,诸如甲氨喋呤(口服或皮下);羟氯喹;柳氮磺吡啶;来氟米特(leflunomide);细胞因子或细胞因子受体拮抗剂,包括抗干扰素-α、-β或-γ抗体、抗肿瘤坏死因子-α抗体(英夫利昔单抗(infliximab)或阿达木单抗(adalimumab))、抗TNF-α免疫粘附素(依那西普(etanercept))、抗肿瘤坏死因子-β抗体、抗白细胞介素-2抗体和抗IL-2受体抗体;抗LFA-1抗体,包括抗CD11a和抗CD18抗体;抗L3T4抗体;异源性抗淋巴细胞球蛋白;泛T(pan-T)抗体,优选抗CD3或抗CD4/CD4a抗体;含有LFA-3结合结构域的可溶性肽(90年7月26日公布的WO 90/08187);链激酶;TGF-β;链道酶(streptodornase);来自宿主的RNA或DNA;FK506;RS-

61443;脱氧精胍菌素(deoxysperguali n);雷帕霉素(rapamycin);T细胞受体(Cohen等人,美国专利号5,114,721);T细胞受体片段(Offner等人,Science,251:430-432(1991);WO 90/11294;Janeway,Nature,341:482(1989);和WO 91/01133);以及T细胞受体抗体(EP 340,109),诸如T10B9。

[0265] 如本文所使用的术语"细胞毒性剂"是指抑制或阻止细胞功能和/或引起细胞破坏的物质。该术语旨在包括放射性同位素(例如,At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}和Lu的放射性同位素)、化疗剂、和毒素(诸如小分子毒素或细菌、真菌、植物或动物源的酶促活性毒素或其片段)。

[0266] "化疗剂"是可用于治疗癌症的化学化合物。化疗剂的实例包括:烷化剂类,诸如噻替派(thiotepa)和CYTOXAN®环磷酰胺;烷基磺酸酯类,诸如白消安(busulfan)、英丙舒凡(improsulfan)和哌泊舒凡(piposulfan);氮丙啶类,诸如苯佐替哌(benzodopa)、卡波醌(carboquone)、美妥替派(meturedopa)和乌瑞替派(uredopa);乙烯亚胺类和甲基蜜胺类(methylamelamines),包括六甲蜜胺(altretamine)、三乙撑蜜胺(triethylenemelamine)、三乙撑磷酰胺(triethylenephosphoramide)、三乙撑硫代磷酰胺(triethiylenethiophosphoramide)和三羟甲基蜜胺(trimethylolomelamine);多聚乙酰类(acetogenin)(尤其是布拉他辛(bullatacin)和布拉他辛酮(bullatacinone));喜树碱(camptothecin)(包括合成类似物拓扑替康(topotecan));苔藓抑素(bryostatin);callystatin;CC-1065(包括其阿多来新(adozelesin)、卡折来新(carzelesin)和比折来新(bizelesin)合成类似物);念珠藻素(cryptophycins)(特别是念珠藻素1和念珠藻素8);多拉司他汀(dolastatin);多卡米星(duocarmycin)(包括合成类似物KW-2189和CB 1-TM1);eleutherobin;pancratistatin;sarcodictyin;海绵抑素(spongistatin);氮芥类,诸如苯丁酸氮芥(chlorambucil)、萘氮芥(chlornaphazine)、胆磷酰胺(cholophosphamide)、雌氮芥(estramustine)、异磷酰胺(ifosfamide)、双氯乙基甲胺(mechlorethamine)、盐酸氧氮芥(mechlorethamine oxide hydrochloride)、美法仑(melphalan)、新氮芥(novembichin)、苯芥胆甾醇(phenesterine)、松龙苯芥(prednimustine)、曲磷胺(trofosfamide)、尿嘧啶氮芥(uracil mustard);亚硝基脲类,诸如卡莫司汀(carmustine)、氯脲菌素(chlorozotocin)、福莫司汀(fotemustine)、洛莫司汀(lomustine)、尼莫司汀(nimustine)和雷莫司汀(ranimnustine);抗生素类,抗生素类,诸如烯二炔类(enediyne)抗生素(例如加利车霉素(calicheamicin),尤其是加利车霉素γ1I和加利车霉素ωI1(参见例如Agnew,Chem Intl.Ed.Engl.,33:183-186(1994));dynemicin,包括dynemicin A;双膦酸盐类(bisphosphonates),诸如氯膦酸盐(clodronate);埃斯培拉霉素(esperamicin);以及新制癌菌素(neocarzinostatin)发色团和相关色蛋白烯二炔类抗生素发色团)、阿克拉霉素(aclacinomysins)、放线菌素(actinomycin)、氨茴霉素(authramycin)、氮丝氨酸(azaserine)、博来霉素(bleomycins)、放线菌素C(cactinomycin)、carabicin、洋红霉素(carminomycin)、嗜癌霉素(carzinophilin)、色霉素(chromomycinis)、放线菌素D(dactinomycin)、柔红霉素(daunorubicin)、地托比星(detorubicin)、6-二氮-5-氧-L-正亮氨酸、ADRIAMYCIN®多柔比星(doxorubicin)(包括吗琳代-多柔比星、氰基吗琳代-多柔比星、2-吡咯啉并-多柔比星和脱氧多柔比星)、表柔比星(epirubicin)、依索比星(esorubicin)、伊达比星

(idarubicin)、麻西罗霉素(marcellomycin)、丝裂霉素(mitomycins)(睹如丝裂霉素C)、霉酚酸(mycophenolic acid)、诺加霉素(nogalamycin)、橄榄霉素(olivomycins)、培洛霉素(peplomycin)、泊非美素(potfimromycin)、嘌呤霉素(puromycin)、三铁阿霉素(quelamycin)、罗多比星(rodorubicin)、链黑菌素(streptigrin)、链脲霉素(streptozocin)、杀结核菌素(tubercidin)、乌苯美司(ubenimex)、净司他丁(zinostatin);抗代谢物类,诸如甲氨蝶呤和5-氟尿嘧啶(5-FU);叶酸类似物,诸如二甲叶酸(denopterin)、甲氨蝶呤(methotrexate)、蝶酰三谷氨酸蝶呤(pteropterin)、三甲曲沙(trimetrexate);嘌呤类似物,诸如氟达拉滨(fludarabine)、6-巯基嘌呤(6-mercaptopurine)、硫咪嘌呤(thiamiprine)、硫鸟嘌呤(thioguanine);嘧啶类似物,诸如安西他滨(ancitabine)、阿扎胞苷(azacitidine)、6-氮尿苷(6-azauridine)、卡莫氟(carmofur)、阿糖胞苷(cytarabine)、双脱氧尿苷(dideoxyuridine)、多西氟尿啶(doxifluridine)、依诺他滨(enocitabine)、氟尿苷(floxuridine);雄性激素,诸如卡普睾酮(calusterone)、丙酸屈他雄酮(dromostanolone propionate)、环硫雄醇(epitiostanol)、美雄烷(mepitiostane)、睾内酯(testolactone);抗肾上腺类,诸如氨鲁米特(aminoglutethimide)、米托坦(mitotane)、曲洛司坦(trilostane);叶酸补充剂,诸如亚叶酸;醋葡内酯(aceglatone);醛磷酰胺糖苷(aldophosphamide glycoside);氨基酮戊酸(aminolevulinic acid);安吖啶(amsacrine);胺苯吖啶(amsacrine);bestrabucil;比生群(bisantrene);依达曲沙(edatraxate);defofamine;地美可辛(demecolcine);地吖醌(diaziquone);依洛尼塞(elfornithine);醋酸羟吡咔唑(elliptinium acetate);埃博霉素(epothilone);依托格鲁(etoglucid);硝酸镓;羟脲;香菇多糖(lentinan);氯尼达明(lonidainine);美登木素生物碱(maytansinoid),诸如美登素和安丝菌素(ansamitocin);米托胍腙(mitoguazone);米托蒽醌(mitoxantrone);莫哌达醇(mopidanmol);二胺硝吖啶(nitraerine);喷司他丁(pentostatin);蛋氨氮芥(phenamet);吡柔比星(pirarubicin);洛索蒽醌(losoxantrone);鬼臼酸;2-乙基酰肼;丙卡巴肼(procarbazine);PSK®多糖复合物(JHS Natural Products,Eugene,OR);雷佐生(razoxane);根霉菌素(rhizoxin);西索菲兰(sizofiran);锗螺胺(spirogermanium);细交链孢菌酮酸(tenuazonic acid);三亚胺醌(triaziquone);2,2′,2″-三氯三乙胺;单端孢菌毒素(trichothecenes)(尤其是T-2毒素、verracurin A、杆孢菌素(roridin)A和蛇形菌素(anguidine));乌拉坦(urethan);长春地辛(vindesine);达卡巴嗪(dacarbazine);甘露醇氮芥(mannomustine);二溴甘露醇(mitobronitol);二溴卫矛醇(mitolactol);哌泊溴烷(pipobroman);gacytosine;阿糖胞苷(arabinoside)(“Ara-C”);环磷酰胺;噻替派;紫杉烷类,例如TAXOL®紫杉醇(Bristol-Myers Squibb Oncology,Princeton,N.J.)、ABRAXANE™紫杉醇的不含克列莫佛(Cremophor)的、白蛋白工程改造的纳米颗粒制剂(American Pharmaceutical Partners,Schaumberg,Illinois)和TAXOTERE®多西他赛(doxetaxel)(Rhône-Poulenc Rorer,Antony,France);苯丁酸氮芥(chlorambucil);GEMZAR®吉西他滨(gemcitabine);6-硫鸟嘌呤;巯基嘌呤;甲氨蝶呤;铂类似物,诸如顺铂和卡铂;长春花碱(vinblastine);铂;依托泊苷(etoposide)(VP-16);异磷酰胺(ifosfamide);米托蒽醌(mitoxantrone);长春新碱(vincristine);NAVELBINE®长春瑞滨(vinorelbine);诺安托(novantrone);替尼泊苷(teniposide);依达曲沙(edatrexate);柔红霉素(daunomycin);氨基蝶呤

(aminopterin)；希罗达(xeloda)；伊班膦酸盐(ibandronate)；CPT-11；拓扑异构酶抑制剂RFS 2000；二氟甲基鸟氨酸(DMFO)；类视黄醇，诸如视黄酸；卡培他滨(capecitabine)；和上述任何物质的药用盐、酸或衍生物。

[0267] 该定义还包括：对于调节或抑制激素对肿瘤的作用起作用的抗激素剂，诸如抗雌激素类和选择性雌激素受体调节剂(SERM)，包括例如他莫昔芬(tamoxifen)(包括NOLVADEX®他莫昔芬)、雷洛昔芬(raloxifene)、屈洛昔芬(droloxifene)、4-羟基他莫昔芬、曲沃昔芬(trioxifene)、keoxifene、LY117018、奥那司酮(onapristone)和FARESTON托瑞米芬(toremifene)；抑制调节肾上腺中雌激素生成的芳香酶的芳香酶抑制剂，诸如例如4(5)-咪唑、氨鲁米特(aminoglutethimide)、MEGASE®醋酸甲地孕酮(megestrol acetate)、AROMASIN®依西美坦(exemestane)、福美司坦(formestanie)、法倔唑(fadrozole)、RIVISOR®伏氯唑(vorozole)、FEMARA®来曲唑(letrozole)和ARIMIDEX®阿那曲唑(anastrozole)；以及抗雄激素类，诸如氟他胺(flutamide)、尼鲁米特(nilutamide)、比卡鲁胺(bicalutamide)、亮丙瑞林(leuprolide)和戈舍瑞林(goserelin)；以及曲沙他滨(troxacitabine)(1,3-二氧戊环核苷胞嘧啶类似物)；反义寡核苷酸，特别是抑制涉及异常细胞增殖的信号传导途经中的基因表达的那些反义寡核苷酸，所述基因诸如例如PKC-α、Ralf和H-Ras；疫苗，诸如基因疗法疫苗，例如ALLOVECTIN®疫苗、LEUVECTIN®疫苗和VAXID®疫苗；PROLEUKIN® rIL-2；LURTOTECAN®拓扑异构酶1抑制剂；ABARELIX® rmRH；和上述任何物质的药用盐、酸或衍生物。

[0268] 术语“细胞因子”是由一个细胞群释放的、作为细胞间介质作用于另一细胞的蛋白质的通称。这种细胞因子的实例是淋巴因子、单核因子；白细胞介素(IL)，诸如IL-1、IL-1α、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8、IL-9、IL-11、IL-12、IL-15；肿瘤坏死因子，诸如TNF-α或TNF-β；及其它多肽因子，包括LIF和kit配体(KL)。如本文所使用的，术语细胞因子包括来自天然来源或来自重组细胞培养物的蛋白质和天然序列细胞因子的生物活性等效物，包括合成产生的小分子实体及其药用衍生物和盐。

[0269] 术语“激素”是指多肽激素，其通常由具有导管的腺器官分泌。激素包括例如生长激素，诸如人生长激素、N-甲硫氨酰人生长激素和牛生长激素；甲状旁腺激素；甲状腺素；胰岛素；胰岛素原；松弛素(relaxin)；松弛素原(prorelaxin)；糖蛋白激素，诸如促卵泡激素(FSH)、促甲状腺激素(TSH)和促黄体生成素(LH)；催乳素、胎盘催乳激素、小鼠促性腺激素相关肽、抑制素；激活素(activin)；缪勒抑制物质(mullerian-inhibiting substance)；和血小板生成素。如本文所使用的，术语激素包括来自天然来源或来自重组细胞培养物的蛋白质和天然序列激素的生物活性等效物，包括合成产生的小分子实体及其药用衍生物和盐。

[0270] 术语“生长因子”是指促进生长的蛋白质，并且包括例如肝生长因子；成纤维细胞生长因子；血管内皮生长因子；神经生长因子，诸如NGF-β；血小板衍生生长因子；转化生长因子(TGF)，诸如TGF-α和TGF-β；胰岛素样生长因子-I和-II；促红细胞生成素(EPO)；骨诱导因子；干扰素，诸如干扰素-α、-β和-γ；以及集落刺激因子(CSF)，诸如巨噬细胞-CSF(M-CSF)；粒细胞-巨噬细胞-CSF(GM-CSF)；和粒细胞-CSF(G-CSF)。如本文所使用的，术语生长因子包括来自天然来源或来自重组细胞培养物的蛋白质和天然序列生长因子的生物活性

等效物，包括合成产生的小分子实体及其药用衍生物和盐。

[0271] 术语“整联蛋白”是指允许细胞结合和应答于胞外基质并且涉及多种细胞功能(诸如伤口愈合、细胞分化、肿瘤细胞归巢以及凋亡)的受体蛋白质。它们是参与细胞-胞外基质和细胞-细胞相互作用的细胞黏附受体大家族的一部分。功能性整联蛋白由非共价结合的两个跨膜糖蛋白亚基(称为α和β)组成。α亚基彼此都享有一定同源性，β亚基也是如此。受体总是含有一条α链和一条β链。实例包括α6β1、α3β1、α7β1、LFA-1、α4整联蛋白等。如本文所使用的，术语整联蛋白包括来自天然来源或来自重组细胞培养物的蛋白质和天然序列整联蛋白的生物活性等效物，包括合成产生的小分子实体及其药用衍生物和盐。

[0272] 本文中“整联蛋白拮抗剂或抗体”的实例包括LFA-1抗体；α4整联蛋白抗体，诸如可从Biogen Idec/Elan Pharmaceuticals，Inc.获得的那他珠单抗(TYSABRI®)；二氮杂环苯丙氨酸衍生物(WO 2003/89410)；苯丙氨酸衍生物(WO 2003/70709、WO 2002/28830、WO 2002/16329和WO 2003/53926)；苯基丙酸衍生物(WO 2003/10135)；烯胺衍生物(WO 2001/79173)；丙酸衍生物(WO 2000/37444)；链烷酸衍生物(WO 2000/32575)；取代苯基衍生物(美国专利号6,677,339和6,348,463)；芳香胺衍生物(美国专利号6,369,229)；以及ADAM去整联蛋白(disintegrin)结构域多肽(US2002/0042368)；针对αvβ3整联蛋白的抗体(EP 633945)；氮杂桥连二环氨基酸衍生物(WO 2002/02556)等。

[0273] 为了本文的目的，“肿瘤坏死因子α(TNF-α)”是指包含如Pennica等人，Nature，312:721(1984)或Aggarwal等人，JBC，260:2345(1985)中所描述的氨基酸序列的人TNF-α分子。

[0274] 本文中的“TNF-α抑制剂”是指通常通过与TNF-α结合和中和其活性而在一定程度上抑制TNF-α的生物功能的药剂。本文明确涵盖的TNF抑制剂的实例是依那西普(ENBREL®)、英夫利昔单抗(REMICADE®)和阿达木单抗(HUMIRA™)。

[0275] “减轻疾病的抗风湿药”或“DMARD”的实例包括羟氯喹、柳氮磺吡啶、甲氨喋呤、来氟米特、依那西普、英夫利昔单抗(加上口服和皮下甲氨喋呤)、硫唑嘌呤、D-青霉胺、金(口服)、金(肌内)、米诺环素(minocycline)、环孢菌素、葡萄球菌蛋白A免疫吸附，包括其盐和衍生物等。

[0276] “非甾体抗炎药”或“NSAID”的实例是乙酰水杨酸、布洛芬(ibuprofen)、萘普生(naproxen)、吲哚美辛(indomethacin)、舒林酸(sulindac)、托美丁(tolmetin)，包括其盐和衍生物等。

[0277] “皮质类固醇”是指具有类固醇的一般化学结构、模拟或提高天然存在的皮质类固醇的效果的数种合成的或天然存在的物质中的任一种。合成的皮质类固醇的实例包括泼尼松、泼尼松龙(包括甲泼尼龙)、地塞米松、糖皮质激素和倍他米松(betamethasone)。

[0278] 术语“包装插页”用来指通常包含在治疗产品的商品包装中的使用说明，其含有关于涉及此类治疗产品的使用的适应症、用法、剂量、施用、禁忌症、与包装产品组合的其它治疗性产品和/或警告的信息等。

[0279] “标签”在本文中用来指通常附随着药物制剂的商用包装(包括容器诸如小瓶和包装插页)以及其它类型的包装所包含的信息。

[0280] 本文中对“约”某个值或参数的提及包括(并且描述)涉及该值或参数本身的变体。例如，提及“约X”的描述包括“X”的描述。

[0281] 如本文中所用，除非上下文另有明确指明，否则单数形式“一个(a)”、“或”和“所述(the)”包括复数指代。可以理解的是，本文所述的发明的方面和变体包括“由.....组成”和/或“基本上由.....组成”的方面和变体。

[0282] 应该理解，本文所述的各种实施方案中的一个、一些或全部特征可以组合以形成本发明的其它实施方案。本发明的这些方面和其他方面将对于本领域技术人员变得显而易见。

[0283] 本文中引用的全部参考文献，包括专利申请和出版物，通过引用整体结合。

[0284] II.治疗方法

[0285] 在某些实施方案中，提供一种提供患有多发性硬化的患者中功能能力改善的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述患者在治疗后具有功能能力的改善。在一些实施方案中，所述方法还包括在抗-CD20抗体的1次、2次、3次、4次或多于4次暴露后测量患者功能能力的步骤(例如，施用本文其它地方描述的方法，诸如测量EDSS评分和/或计时25英尺行走(T25-FW))。在某些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。

[0286] 在某些实施方案中，在治疗后，患者具有至少约12周确认的能力丧失改善。在某些实施方案中，在治疗后，患者具有至少约24周确认的能力丧失改善。在某些实施方案中，通过扩展的能力丧失状态量表(EDSS)评分来确定确认的能力丧失改善。在某些实施方案中，患者的EDSS评分减少至少约0.1、约0.2、约0.3、约0.4、约0.5、约0.6、约0.7、约0.8、约0.9、约1.0或多于1.0分(诸如约1.1、约1.2、约1.3、约1.4或约1.5分)。

[0287] 在某些实施方案中，功能能力的改善维持至少约1周、至少约2周、至少约3周、至少约4周、至少约5周、至少约6周、至少约7周、至少约8周、至少约9周、至少约10周、至少约11周、至少约12周、至少约13周、至少约14周、至少约15周、至少约16周、至少约17周、至少约18周、至少约19周、至少约20周、至少约21周、至少约22周、至少约23周(包括介于这些值之间的任何范围)。在某些实施方案中，功能能力的改善维持至少约24周、至少约25周、至少约26周、至少约27周、至少约28周、至少约29周、至少约30周、至少约35周、至少约40周、至少约45周、至少约50周、至少约55周、至少约60周、至少约65周、至少约70周、至少约75周或多于约75周(包括介于这些值之间的任何范围)。

[0288] 在某些实施方案中，通过计时25英尺行走(T25-FW)测试来测量功能能力的改善。在某些实施方案中，相对于立即开始治疗前行走25英尺的时间，开始治疗后行走25英尺的时间减少约5秒、约10秒、约30秒、约60秒、约90秒、约2分钟、约2.5分钟、约3分钟、约3.5分钟、约4分钟、约4.5分钟、约5分钟、约5.5分钟、约6分钟、约6.5分钟、约7分钟、约7.5分钟、约8分钟、约8.5分钟、约9分钟、约9.5分钟或约10分钟。

[0289] 在某些实施方案中，通过无疾病活性迹象(NEDA)来证明功能能力的改善。在某些实施方案中，通过磁共振成像上不存在新的或扩大的T2损伤或T1钆增强性损伤来证明NEDA。在某些实施方案中，通过不存在复发来证明NEDA。在某些实施方案中，通过不存在进展来证明NEDA。在某些实施方案中，通过不存在EDSS恶化来证明NEDA。在某些实施方案中，NEDA被定义为:没有方案定义的复发，没有CDP事件，没有新的或扩大的T2损伤，以及没有钆增强性T1损伤。在某些实施方案中，NEDA维持至少约1周、至少约2周、至少约3周、至少约4周、至少约5周、至少约6周、至少约7周、至少约8周、至少约9周、至少约10周、至少约11周、至少约12周、至少约13周、至少约14周、至少约15周、至少约16周、至少约17周、至少约18周、至少约19周、至少约20周、至少约21周、至少约22周、至少约23周(包括介于这些值之间的任何范围)。在某些实施方案中，功能能力的改善维持至少约24周、至少约25周、至少约26周、至少约27周、至少约28周、至少约29周、至少约30周、至少约35周、至少约40周、至少约45周、至少约50周、至少约55周、至少约60周、至少约65周、至少约70周、至少约75周或多于约75周(包括介于这些值之间的任何范围)。

[0290] 在某些实施方案中，患者在基线处具有T1钆染色损伤(即，开始治疗前)。在某些实施方案中，患者在基线处不具有T1钆染色损伤(即，开始治疗前)。

[0291] 在某些实施方案中，提供一种抑制患有多发性硬化的人类患者中复合能力丧失进展的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中该施用导致确认的能力丧失进展事件减少，并且其中所述抗-CD20抗体包含:a)重链可变区，所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含:a)重链可变区，所述重链可变区包含:包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含:包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，施用导致12周确认的复合能力丧失进展减少。在某些实施方案中，施用导致24周确认的复合能力丧失进展减少。

[0292] 在某些实施方案中，提供一种延迟患有多发性硬化的人类患者中复合能力丧失进展的发病的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中该施用导致确认的能力丧失进展事件减少，并且其中所述抗-CD20抗体包含:a)重链可变区，所述重链可变区包含:具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含:具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含:a)重链可变区，所述重链可变区包含:包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含:包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，施用导致12周确认的复合能力丧失进展减少。在某些实施方案中，施用导致24周确认的复合能力丧失进展减少。

[0293] 在某些实施方案中，通过扩展的能力丧失状态量表(EDSS)评分来确定确认的复合能力丧失进展。在某些实施方案中，确认的复合能力丧失进展定义为EDSS进展(即，EDSS评分增加)。在某些实施方案中，通过计时25英尺行走(T25-FW)来确定确认的复合能力丧失进展。在某些实施方案中，确认的复合能力丧失进展定义为T25-FW中的至少20％。在某些实施方案中，通过9孔钉测试(9-HPT)来确定确认的复合能力丧失进展。在某些实施方案中，确认的复合能力丧失进展定义为9孔钉测试(9-HPT)时间增加至少20％。在某些实施方案中，通过EDSS进展、计时25英尺行走和9孔钉测试来确定确认的复合能力丧失进展。

[0294] 在某些实施方案中，提供一种抑制患有多发性硬化的人类患者中的能力丧失进展的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中施用导致确认的能力丧失进展事件减少。在某些实施方案中，在抗-CD20抗体的1次、2次、3次、4次或多于4次暴露后观察到确认的能力丧失进展事件的减少。在某些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，施用导致12周确认的能力丧失进展减少。在某些实施方案中，施用导致24周确认的能力丧失进展减少。在某些实施方案中，施用导致12周确认的能力丧失进展的风险降低。在某些实施方案中，施用导致24周确认的能力丧失进展的风险降低。

[0295] 在某些实施方案中，患者在基线处具有T1钆染色损伤(即，开始治疗前)。在某些实施方案中，患者在基线处不具有T1钆染色损伤(即，开始治疗前)。

[0296] 在某些实施方案中，提供一种延迟患有多发性硬化的人类患者中确认的能力丧失进展的发病的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。

[0297] 在某些实施方案中，通过扩展的能力丧失状态量表(EDSS)评分来确定确认的能力丧失进展。在某些实施方案中，患者的EDSS评分从约5.5以下的基线EDSS评分增加至少约1分。在某些实施方案中，患者的EDSS评分从高于5.5的基线EDSS评分增加约0.5分。在某些实施方案中，在初次神经学恶化后至少12周时确认EDSS的增加。

[0298] 在某些实施方案中，患者在基线处具有T1钆染色损伤(即，开始治疗前)。在某些实

施方案中，患者在基线处不具有T1钆染色损伤(即，开始治疗前)。

[0299] 在某些实施方案中，提供一种减小患有多发性硬化的人类患者中T2损伤体积的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，患者在基线处具有T1钆染色损伤。在某些实施方案中，患者在基线处不具有T1钆染色损伤。

[0300] 在某些实施方案中，提供一种减缓或阻止患有多发性硬化的患者中脑体积减小的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中减缓或阻止了患者中的脑体积减小；并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，减缓了脑体积减小。在某些实施方案中，在未经历脑体积损失的患者中减缓或阻止了脑体积减小。在某些实施方案中，在已经经历脑体积损失的患者中减缓或阻止了脑体积进一步减小。

[0301] 在某些实施方案中，提供一种减缓或阻止患有多发性硬化的患者中脑萎缩的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中减缓或阻止了患者中的脑萎缩；并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，减缓了脑萎缩。在某些实施方案中，在未经历脑萎缩的患者中减缓或阻止了脑萎缩。在某些实施方案中，在已经经历脑萎缩的患者中减缓或阻止了进一步脑萎缩。

[0302] 在某些实施方案中，提供一种治疗患有多发性硬化的人类患者的方法，所述方法

包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致无疾病活动迹象(no evidence of disease activity，NEDA)达至少12周，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，治疗导致无疾病活动迹象(NEDA)达至少24周。

[0303] 本文还提供一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致患者在治疗96周后达到无损伤状态，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，治疗导致患者在治疗48周后达到无损伤状态。在某些实施方案中，治疗导致患者在治疗24周后达到无损伤状态。在某些实施方案中，治疗导致患者不具有钆染色损伤。在某些实施方案中，治疗导致患者不具有T2损伤。

[0304] 在某些实施方案中，提供一种治疗患有复发型多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致以下中的一个或多个：a)患者在96周时无复发；b)患者在96周时不具有确认的能力丧失进展事件；c)患者在96周时不具有T1钆增强性损伤；d)患者在96周时不具有新的和/或扩大的T2损伤；其中抗-CD20抗体包含：1)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有氨基酸SEQ ID NO:11的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及2)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。

[0305] 在某些实施方案中，患者先前未使用其它多发性硬化疗法进行治疗(即，“初治(naïve)患者”)。在某些实施方案中，初治患者在开始治疗之前2年中经历至少2次复发。在某些实施方案中，初治患者在开始治疗之前最后一年中经历至少1次复发。

[0306] 在某些实施方案中，患者是对其它多发性硬化疗法的不充分应答者。在某些实施方案，作为不充分应答者的患者先前使用干扰素β-1a或醋酸格拉默治疗至少1年。在某些实施方案中，作为不充分应答者的患者在用另一种多发性硬化疗法治疗时经历至少一次复发或者经历至少1次基线钆增强性损伤。

[0307] 在某些实施方案中，提供一种治疗患有高度活跃的多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。

[0308] 在某些实施方案中，患有高度活跃的多发性硬化的患者先前未使用多发性硬化的其它疗法进行治疗(即，“初治患者”)。在某些实施方案中，患有高度活跃的多发性硬化的初治患者在随机化之前的最后一年中经历至少2次复发，并且与先前的MRI相比，经历了(a)至少1次基线钆损伤或(b)基线访问时T2损伤计数的增加(绝对变化从0-5至6-9个损伤或从6-9个损伤至＞9个损伤)。

[0309] 在某些实施方案中，患有高度活跃的多发性硬化的患者是对其它多发性硬化疗法的不充分应答者。在某些实施方案，患有高度活跃的多发性硬化的患者(其为不充分应答者)先前使用干扰素β-1a或醋酸格拉默治疗至少1年。在某些实施方案中，患有高度活跃的多发性硬化的患者(其为不充分应答者)已经经历至少一次复发，并且在用多发性硬化的另一种疗法治疗时在基线处(a)具有至少九个T2损伤或(b)具有至少一个钆损伤。

[0310] 在某些实施方案中，向患有高度活跃的多发性硬化的患者施用抗-CD20抗体在以下一个或多个方面有效：(1)减少患者脑损伤的数量；(2)降低年复发率；(3)降低能力丧失进展；和(4)改善功能能力。在某些实施方案中，治疗患有高度活跃的多发性硬化的方法还包括进行MRI扫描，并且在将抗-CD20抗体施用至患者之前确定患者是否患有高度活跃的多发性硬化。

[0311] 在某些实施方案中，提供一种治疗患有早期多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。在某些实施方案中，所述方法还

包括在将抗-CD20抗体施用至患者之前诊断患有早期多发性硬化的患者。

[0312] 在某些实施方案中，提供一种治疗患有多发性硬化的人类患者的方法，所述方法包括向所述患者施用有效量的抗-CD20抗体，其中治疗导致患者中无疾病活动迹象，并且其中所述抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：具有SEQ ID NO:10的氨基酸序列的CDR1、具有SEQ ID NO:11的氨基酸序列的CDR2、和具有SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：具有SEQ ID NO:4的氨基酸序列的CDR1、具有SEQ ID NO:5的氨基酸序列的CDR2、和具有SEQ ID NO:6的氨基酸序列的CDR3。在一些实施方案中，抗-CD20抗体包含：a)重链可变区，所述重链可变区包含：包含SEQ ID NO:10的氨基酸序列的CDR1、包含SEQ ID NO:11的氨基酸序列的CDR2、和包含SEQ ID NO:12的氨基酸序列的CDR3，以及b)轻链可变区，所述轻链可变区包含：包含SEQ ID NO:4的氨基酸序列的CDR1、包含SEQ ID NO:5的氨基酸序列的CDR2、和包含SEQ ID NO:6的氨基酸序列的CDR3。

[0313] 在某些实施方案中，患者患有复发型多发性硬化。在一些实施方案中，复发型MS(RMS)是指通常由RRMS和具有重叠的复发的SPMS(通常也称为“复发型SPMS”)两者构成的患者群。在某些实施方案中，多发性硬化是复发缓和型多发性硬化(RRMS)。在某些实施方案中，复发型多发性硬化是具有重叠复发的继发进行性多发性硬化(rSPMS)。在某些实施方案中，患者小于18岁。在某些实施方案中，患者为18至55岁。在某些实施方案中，患者超过55岁。在某些实施方案中，根据2010年修订的McDonald标准(Polman等人，(2011)“Diagnostic criteria for multiple sclerosis:2010revisions to the McDonald criteria.”Ann Neurol 69,292-302)，患者诊断为患有多发性硬化。另外地或备选地，在某些实施方案中，在筛选时患者的扩展的能力丧失状态量表(EDSS，参见world-wide-web.neurostatus.org)评分为0至5.5。另外地或备选地，在某些实施方案中，患者在先前2年内具有至少两次有记录的临床发作，或者在1年内出现一次临床发作。另外地或备选地，在某些实施方案中，患者具有存在与多发性硬化一致的异常的有记录的脑MRI。

[0314] 在某些实施方案中，患有复发型多发性硬化的患者未诊断为患有原发进行性多发性硬化。另外地或备选地，在某些实施方案中，患者先前未使用任何靶向B细胞的疗法、系统性皮质类固醇和/或免疫抑制剂进行治疗。另外地或备选地，在某些实施方案中，患者不具有原发性或继发性免疫缺陷的历史、活动性感染或存在复发性或慢性感染(例如，乙型肝炎或丙型肝炎、HIV、梅毒、结核病)，或者进行性多灶性脑白质病的历史。

[0315] 在某些实施方案中，患者患有进行性多发性硬化。在某些实施方案中，进行性多发性硬化是原发进行性多发性硬化。在某些实施方案中，患者小于18岁。在某些实施方案中，患者为18至55岁。在某些实施方案中，患者超过55岁。在某些实施方案中，根据2005年修订的McDonald标准(Polman等人，(2011)“Diagnostic criteria for multiple sclerosis:2005 revisions to the McDonald criteria.”Ann Neurol 58,840-846)，患者诊断为患有原发进行性多发性硬化。另外地或备选地，在某些实施方案中，患者的扩展的能力丧失状态量表(EDSS)评分为3至6.5分。另外地或备选地，在某些实施方案中，患者在功能系统量表(FSS)的金字塔形功能要素上具有至少2.0的评分。另外地或备选地，在某些实施方案中，患者具有以下的有记录的历史或在筛选时存在以下：脑脊液(CSF)样本中升高的IgG指数和/或通过脑脊液(CSF)样本中的等电聚焦检测的一个或多个IgG寡克隆条带。另外地或备选

地，在某些实施方案中，患者不具有复发缓和型多发性硬化(RRMS)的历史。另外地或备选地，在某些实施方案中，患者不具有继发进行性多发性硬化(SPMS)的历史。另外地或备选地，在某些实施方案中，患者不具有进行性复发型多发性硬化(PRMS)的历史。

[0316] 在某些实施方案中，患者先前已使用靶向B细胞的疗法(例如，利妥昔单抗(rituximab)、ocrelizumab、阿塞西普(atacicept)、贝利木单抗(belimumab)或奥法木单抗(ofatumumab))进行治疗。在某些实施方案中，患者先前未使用靶向B细胞的疗法(例如，利妥昔单抗、ocrelizumab、阿塞西普、贝利木单抗或奥法木单抗)进行治疗。

[0317] 在一些实施方案中，“确认的能力丧失进展(Confirmed Disability Progression)”或“CDP”是指在96周期间，基线评分为5.5以下的患者中从基线EDSS评分增加至少1.0分，或者基线评分高于5.5的患者中增加0.5分，其中在最初的神经恶化后至少12周以有规律的定期访问方式确认EDSS的增加。

[0318] 在一些实施方案中，“确认的能力丧失改善(Confirmed Disability Improvement)”或“CDI”是指与基线相比，基线EDSS评分为5.5以下的患者中EDSS评分降低至少1.0，或者基线EDSS评分高于5.5的患者中EDSS评分降低0.5分。

[0319] 在一些实施方案中，脑萎缩是指以下中的一项或多项：脑中的轴突损失、灰质损伤或白质损伤内的组织损失、与损伤相关的途径中的沃勒变性(Wallerian degeneration)、或损伤负荷。在某些实施方案中，脑萎缩是指全脑体积减小。在某些实施方案中，脑萎缩是指一种或多种脑结构(包括但不限于大脑、小脑、丘脑，额颞新皮质、脑干、海马、顶叶和/或下丘脑)的体积减小。在某些实施方案中，脑萎缩是指在中央前回、额上回、丘脑和/或壳核中的皮质变薄。在某些实施方案中，脑萎缩是指每年脑体积损失为至少约0.4％、至少约0.5％、至少约0.6％或至少0.7％。关于脑萎缩的另外的细节在例如Riley等人，(2012)Expert Rev Neurother 12(3)，323-333中详细描述。

[0320] 在一些实施方案中，患者或受试者患有高度活跃的多发性硬化。在一些实施方案中，初治治疗患者中的“高度活跃的多发性硬化”是指先前未使用其它多发性硬化疗法治疗并且在随机化之前的最后一年中经历至少2次复发的患者，并且与先前的MRI相比，所述患者具有(a)至少1次基线钆损伤或(b)基线访问时T2损伤计数的增加(绝对变化从0-5至6-9个损伤或从6-9个损伤至＞9个损伤)。在一些实施方案中，“高度活跃的多发性硬化”处于这样的患者中：所述患者先前已使用其它多发性硬化疗法治疗并且在先前一年具有至少一次复发，并且在基线处(a)具有至少九个T2损伤或(b)具有至少一个钆损伤。

[0321] 在某些实施方案中，患者中的基线水平是指在将抗-CD20抗体施用至患者或使用抗-CD20抗体治疗患者之前的水平，例如将抗-CD20抗体施用至患者或使用抗-CD20抗体治疗患者之前约2个月、约1.5个月、约1个月、约30天、约25天、约21天、约14天、约7天、约6天、约5天、约4天、约3天、约2天、约1天时的水平。

[0322] 在某些实施方案中，患者在治疗期间保持产生针对抗原的体液免疫应答的能力。在某些实施方案中，抗原是腮腺炎抗原、风疹抗原、水痘抗原、肺炎链球菌(S.pneumonia)抗原、破伤风类毒素抗原、肺炎球菌抗原或流感抗原。

[0323] 本文描述的方法可以涵盖本文描述的实施方案的任何组合。

[0324] III.剂量

[0325] 根据本文描述的任一个方法或制品的一些实施方案，所述方法或说明书包括将有

效量的抗-CD20抗体施用至多发性硬化患者以提供约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的初次抗体暴露,随后是约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的第二次抗体暴露,其中直到距初次抗体暴露约16周至约60周时才提供第二次抗体暴露。为了本发明的目的,第二次抗体暴露是在初次抗体暴露之后用抗-CD20抗体治疗患者的下一时间,其中在初次暴露与第二次暴露之间没有居间的抗-CD20抗体治疗或暴露。在一些实施方案中,初次抗体暴露和/或第二次抗体暴露是约0.3克、0.4克、0.5克、0.6克、0.7克、0.8克、0.9克或1.0克中的任一个。

[0326] 初次与第二次或随后的抗体暴露之间的间隔可以从初次抗体暴露的第一剂量算起。

[0327] 在一些实施方案中,抗体暴露间隔大约24周或6个月;或者间隔大约48周或12个月。在一些实施方案中,抗体暴露间隔约20-24周或约5-6个月。在一些实施方案中,"约20-24周"是指20周至24周之间的时间点。在一些实施方案中,"约20-24周"是指第24周之前或之后1周或7天的变动。在一些实施方案中,"约5-6个月"是指5至6个月之间的时间点。

[0328] 在一些实施方案中,直至距初次暴露约20周至约30周时才提供第二次抗体暴露,任选地随后提供约0.3克至约4克(优选约0.3克至约1.5克)的第三次抗体暴露,直至距初次暴露约46至60周(优选约46至54周)时才施用第三次暴露,然后在一些实施方案中,直至距初次暴露至少约70-75周时才提供另外的抗体暴露。在一些实施方案中,第三次抗体暴露是约0.3克、0.4克、0.5克、0.6克、0.7克、0.8克、0.9克或1.0克中的任一个。

[0329] 在备选实施方案中,直到距初次暴露约46至60周时才提供第二次抗体暴露,并且直到距前次抗体暴露约46至60周时才提供随后的抗体暴露(如果有的话)。

[0330] 根据本文描述的任一个方法或制品的一些实施方案,所述方法或说明书包括将有效量的抗-CD20抗体施用至多发性硬化患者以提供约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的初次抗体暴露,随后是约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的第二次抗体暴露,其中直到距初次抗体暴露约20周至约30周时才提供第二次抗体暴露,随后是约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的第三次抗体暴露,其中直到距初次暴露约46周至约54周时才提供第三次抗体暴露,随后是约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的第四次抗体暴露,其中直到距初次暴露约70周至约75周时才提供第四次抗体暴露。

[0331] 在某些实施方案中,第四次抗体暴露之后是一次或多次约0.3克至约4克(优选约0.3克至约1.5克,诸如约0.6克或约1.0克)的抗体暴露。在某些实施方案中,每次随后的抗体暴露距前次暴露约20周至约30周。

[0332] 为了本发明的目的,每次随后的抗体暴露是在初次抗体暴露之后用抗-CD20抗体治疗患者的下一时间,其中在例如初次暴露与第二次暴露之间、第二次与第三次暴露之间或第三次与第四次暴露之间等没有居间的抗-CD20抗体治疗或暴露。在一些实施方案中,初次、第二次、第三次、第四次和/或随后的抗体暴露是约0.3克、0.4克、0.5克、0.6克、0.7克、0.8克、0.9克或1.0克中的任一个。

[0333] 本文中的抗体暴露中的任何一次或多次暴露可以作为单一剂量的抗体或者作为两个分开剂量的抗体(即,由第一剂量和第二剂量构成)来提供给患者。每次抗体暴露所采用的具体剂量数(无论是一个或两个)可以取决于例如所治疗的MS类型、所采用的抗体类

型、是否采用第二药物或采用哪种类型的第二药物、以及施用的方法和频率。在施用两个分开剂量的情况中，第二剂量优选在距施用第一剂量的时间约3-17天、更优选约6-16天、并且最优选约13-16天时施用。在一些实施方案中，在施用两个分开剂量的情况中，第二剂量为约14天(诸如13天或15天)。在一些实施方案中，“约14天”是指第14天之前或之后1天的变动。在施用两个分开剂量的情况中，抗体的第一和第二剂量优选为约0.3克至1.5克，更优选为约0.3克至约1.0克。在一些实施方案中，在施用两个分开剂量的情况中，抗体的第一剂量和第二剂量为约0.3克、0.4克、0.5克或0.6克中的任一个。在一些实施方案中，初次ocrelizumab暴露包括第一剂量和第二剂量的ocrelizumab，其中ocrelizumab的第一剂量和第二剂量为约0.3克。在一些实施方案中，第二次ocrelizumab暴露包括单一剂量的ocrelizumab，其中ocrelizumab的单一剂量为0.6克。

[0334] 在一个实施方案中，向患者提供至少约三次、至少约四次或至少约五次抗体暴露，例如约3-60次暴露，并且更特别的是约3-40次暴露，最特别的是约3-20次暴露。在任何方法的一些实施方案中，所述方法还包括提供约一次至约三次后续的ocrelizumab暴露。在一些实施方案中，该暴露以各自为约24周或6个月或者48周或12个月的间隔施用。在某些实施方案中，间隔缩短约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天。在某些实施方案中，多于1个间隔缩短约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天。在某些实施方案中，间隔延长约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天.在某些实施方案中，多于1个间隔延长约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天。在某些实施方案中，多于1个间隔缩短约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天，或者延长约4周、约3.5周、约3周、约2.5周、约2周、约1.5周、约1周、约6天、约5天、约4天、约3天、约2天或约1天。

[0335] 在一个实施方案中，每次抗体暴露作为单一剂量的抗体提供。在备选实施方案中，每次抗体暴露作为两个分开剂量的抗体提供。在一些实施方案中，一些暴露作为单一剂量或作为两个分开剂量提供。

[0336] 抗体可以是裸抗体，或者可以缀合至另一分子，诸如细胞毒性剂，诸如放射性化合物。在一些实施方案中，抗体是利妥昔单抗、人源化2H7(例如包含SEQ ID NOS.2和8中的可变结构域序列)或包含SEQ ID NOS.23和24中的可变结构域序列的人源化2H7、或huMax-CD20(Genmab)。在一些实施方案中，抗体是ocrelizumab(例如包含(a)包含SEQ ID NO:13的氨基酸序列的轻链和(b)包含SEQ ID NO:14的氨基酸序列的重链)。

[0337] 在一个实施方案中，患者先前从未用药物(诸如免疫抑制剂)治疗以治疗多发性硬化和/或先前从未用针对B细胞表面标志的抗体治疗(例如先前从未用CD20抗体治疗)。

[0338] 抗体可以以任何合适的方式施用，包括肠胃外、局部、皮下、腹膜内、肺内、鼻内和/或病灶内施用。肠胃外输注包括肌肉内、静脉内、动脉内、腹膜内或皮下施用。还考虑鞘内施用(参见例如美国专利申请号2002/0009444，Grillo-Lopez，A concerning intrathecal delivery of a CD20 antibody)。另外，可以通过脉冲输注来合适地施用抗体，例如使用剂量逐渐减少的抗体。在一些实施方案中，静脉内、皮下或鞘内给药。在一些实施方案中，通过静脉内输注进行给药。

[0339] 在某些实施方案中，患者在用抗-CD20抗体输注之前进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30分钟用甲泼尼龙(methylprednisolone)(或等效物)进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30分钟用100mg IV甲泼尼龙(methylprednisolone)(或等效物)进行前驱用药。在某些实施方案中，患者在抗-CD20抗体的每次输注之前大约30-60分钟另外地(或备选地)用抗组胺药(例如苯海拉明)进行前驱用药。在某些实施方案中，患者另外地(或备选地)用解热药(例如，对乙酰氨基酚/扑热息痛)进行前驱用药。

[0340] 虽然CD20抗体可以是为了治疗多发性硬化而施用于患者的唯一药物，但是可以任选地与结合B细胞表面标志的抗体一起(例如与CD20抗体一起)施用第二药物，诸如细胞毒性剂、化疗剂、免疫抑制剂、细胞因子、细胞因子拮抗剂或抗体、生长因子、激素、整联蛋白、整联蛋白拮抗剂或抗体(例如LFA-1抗体，或α4整联蛋白抗体，诸如可从Biogen Idec/Elan Pharmaceuticals，Inc获得的那他珠单抗(TYSABRI®))等。

[0341] 在联合疗法的一些实施方案中，抗体可以与以下药物组合：干扰素类药物，诸如IFN-β-1a(REBIF®和AVONEX®)或IFN-β-1b(BETASERON®)；寡肽，诸如醋酸格拉默(COPAXONE®)；细胞毒性剂，诸如米托蒽醌(NOVANTRONE®)、甲氨喋呤、环磷酰胺、苯丁酸氮芥、硫唑嘌呤；静脉内免疫球蛋白(γ球蛋白)；淋巴细胞消耗性疗法(例如，米托蒽醌、环磷酰胺、阿仑单抗(alemtuzumab)(Campath®、LEMTRADA™)、抗-CD4、克拉屈滨、全身照射、骨髓移植)；皮质类固醇(例如，甲泼尼龙、泼尼松、地塞米松或糖皮质激素)，包括系统性皮质类固醇疗法；非淋巴细胞消耗性免疫抑制疗法(例如，麦考酚酸吗乙酯(MMF)或环孢菌素)；“他汀”类降胆固醇药物，其包括西立伐他汀(cerivastatin)(BAYCOL®)、氟伐他汀(luvastatin)(LESCOL®)、阿托伐他汀(atorvastatin)(LIPITOR®)、洛伐他汀(lovastatin)(MEVACOR®)、普伐他汀(pravastatin)(PRAVACHOL®)、辛伐他汀(Simvastatin)(ZOCOR®)；雌二醇；睾酮(任选以提高的剂量；Stuve等人，Neurology 8：290-301(2002))；激素替代疗法；用于MS继发的或相关的症状(例如，痉挛、失禁、疼痛、疲劳)的治疗；TNF抑制剂；减轻疾病的抗风湿药(DMARD)；非甾体抗炎药(NSAID)；血浆去除术(plasmapheresis)；左甲状腺素；环孢菌素A；促生长素抑制素类似物；细胞因子或细胞因子受体拮抗剂；抗代谢物；免疫抑制剂；康复性手术(rehabilitative surgery)；放射性碘；甲状腺切除术；其它B细胞表面拮抗剂/抗体等。

[0342] 第二药物与CD20抗体的初次暴露和/或随后暴露一起施用，这种组合施用包括使用分开的制剂或单个药物制剂的共同施用(同时施用)，以及以任何顺序的连续施用，其中优选地两种(或全部)活性剂同时发挥其生物学活性需要一段时间。

[0343] 除对患者施用抗体外，本申请考虑通过基因疗法来施用抗体。表述施用“有效量”的抗体涵盖编码抗体的核酸的施用。参见例如1996年3月14日公布的WO96/07321，其涉及使用基因疗法来生成胞内抗体。

[0344] 存在两种主要方法使核酸(任选地包含在载体中)进入患者的细胞；即体内和离体。对于体内递送，通常在需要抗体的部位将核酸直接注射到患者中。对于离体治疗，移出患者的细胞，将核酸引入这些分离的细胞，并且将修饰的细胞直接施用至患者，或者例如包

封在植入患者中的多孔膜内而施用至患者(参见例如美国专利号4,892,538和5,283,187)。存在多种技术可用于将核酸引入活细胞。这些技术根据是将核酸转移至体外培养细胞还是预期宿主的体内细胞而有所变化。适于在体外将核酸转移至哺乳动物细胞中的技术包括使用脂质体、电穿孔、显微注射、细胞融合、DEAE-葡聚糖、磷酸钙沉淀法等。常用于离体递送基因的载体是逆转录病毒。

[0345] 在一些实施方案中,体内核酸转移技术包括用病毒载体(诸如腺病毒、I型单纯疱疹病毒或腺伴随病毒)和基于脂质的系统(可用于脂质介导的基因转移的脂质为例如DOTMA、DOPE和DC-Chol)进行的转染。在一些情况中,需要与靶向靶细胞的药剂一起提供核酸源,所述药剂诸如对细胞表面膜蛋白或靶细胞具有特异性的抗体、靶细胞上受体的配体等。在采用脂质体的情况中,与胞吞作用相关的细胞表面膜蛋白结合的蛋白质可以用于靶向和/或促进摄入,例如对特定细胞类型具有向性的衣壳蛋白或其片段、在循环中进行内在化的蛋白质的抗体、和靶向细胞内定位和增加细胞内半衰期的蛋白质。受体介导的胞吞技术描述于例如Wu等人,J.Biol.Chem.262:4429-4432(1987);和Wagner等人,Proc.Natl.Acad.Sci.USA 87:3410-3414(1990)中。关于目前已知的基因标记和基因疗法方案的综述参见Anderson等人,Science 256:808-813(1992)。还参见WO 93/25673及其中引用的文献。

[0346] IV.抗体及其生产

[0347] 本发明的方法和制品使用或并入与B细胞表面标志结合的抗体,尤其是与CD20结合的抗体。因此,本文将描述用于生成这种抗体的方法。

[0348] 在一些实施方案中,本文描述的方法中使用的抗-CD20抗体通过包括以下步骤的方法产生:在宿主细胞中表达编码分别包含SEQ ID NO:14或13的重链和轻链氨基酸序列的人源化抗体的氨基酸,和回收宿主细胞中表达的人源化抗体或其抗原结合片段。在一些实施方案中,宿主细胞是哺乳动物细胞(例如CHO细胞)、昆虫细胞或植物细胞。在一些实施方案中,宿主细胞是细菌细胞。生产抗-CD20的方法还详细描述于例如美国专利号7,799,900中。

[0349] 可用于生成或筛选抗体的B细胞表面标志可以是例如包含所需表位的标志的可溶形式或其部分。备选地或另外的,在其细胞表面表达所述标志的细胞可以用于生成或筛选抗体。可用于生成抗体的其它形式的B细胞表面标志对本领域技术人员将是显而易见的。

[0350] 下面的描述例示了用于生产依照本发明使用的抗体的技术。

[0351] (i)多克隆抗体

[0352] 多克隆抗体优选通过多次皮下(sc)或腹膜内(ip)注射相关抗原和佐剂而在动物中生成。可能有用的是,使用双官能或衍生化试剂,例如马来酰亚胺苯甲酰磺基琥珀酸亚胺酯(通过半胱氨酸残基缀合)、N-羟基琥珀酰亚胺(通过赖氨酸残基)、戊二醛、琥珀酸酐、$SOCl_2$或$R^1N=C=NR$(其中R和R^1是不同的烷基),将相关抗原缀合至在待免疫的物种中具有免疫原性的蛋白质,例如匙孔戽螺血蓝蛋白(keyhole limpet hemocyanin)、血清白蛋白、牛甲状腺球蛋白或大豆胰蛋白酶抑制剂。

[0353] 通过如下方式将动物针对抗原、免疫原性缀合物或衍生物进行免疫:将例如100μg或5μg蛋白质或缀合物(分别用于兔或小鼠)与3倍体积的弗氏完全佐剂混和,并且将溶液皮内注射于多个部位。一个月后,通过多个部位的皮下注射,用弗氏完全佐剂中的1/5-1/10初

始量的肽或缀合物对动物进行加强免疫。7-14天后，采集动物的血液，并且测定血清的抗体滴度。对动物进行加强免疫直到滴度达到平稳状态。在一些实施方案中，将动物用与不同蛋白质缀合的和/或通过不同交联剂缀合的相同抗原的缀合物进行加强免疫。缀合物还可以在重组细胞培养物中作为蛋白质融合物来制备。此外，聚集剂(诸如明矾)也适合用来增强免疫应答。

[0354] (ii)单克隆抗体

[0355] 单克隆抗体是从基本上同质的抗体的群体中获得的，即，构成该群体的各个抗体是相同的和/或结合相同的表位，除了产生单克隆抗体期间出现的可能变体以外，这种变体通常以微量存在。因此，修饰语“单克隆”表示抗体的特征为其不是分立的或多克隆的抗体的混合物。

[0356] 例如，单克隆抗体可以使用Kohler等人，Nature，256:495(1975)首先描述的杂交瘤方法制备，或者可以通过重组DNA方法(美国专利号4,816,567)制备。

[0357] 在杂交瘤方法中，如本文所描述的免疫小鼠或其它合适的宿主动物(诸如仓鼠)以引发产生或能够产生将特异性结合用于免疫的蛋白质的抗体的淋巴细胞。备选地，可以在体外免疫淋巴细胞。然后使用合适的融合剂(诸如聚乙二醇)将淋巴细胞与骨髓瘤细胞融合以形成杂交瘤细胞(Goding，Monoclonal Antibodies:Principles and Practice，PP.59-103(Academic Press，1986))。

[0358] 由此制备的杂交瘤细胞可以在合适的培养基中接种和生长，所述培养基优选含有一种或多种抑制未融合的亲本骨髓瘤细胞的生长或存活的物质。例如，如果亲本骨髓瘤细胞缺乏次黄嘌呤鸟嘌呤磷酸核糖转移酶(HGPRT或HPRT)，则用于杂交瘤的培养基通常将包括次黄嘌呤、氨基喋呤和胸苷(HAT培养基)，这些物质阻止HGPRT缺陷型细胞的生长。

[0359] 在一些实施方案中，骨髓瘤细胞是高效融合、支持所选抗体生产细胞稳定高水平生产抗体、并对诸如HAT培养基的培养基敏感的那些骨髓瘤细胞。其中，在一些实施方案中，骨髓瘤细胞系是鼠骨髓瘤系，诸如可从alk Institute Cell Distribution Center，San Diego，California USA获得的来源于MOPC-21和MPC-11小鼠肿瘤的那些，以及可从美国模式培养物保藏所(American Type Culture Collection)，Rockville，Maryland USA获得的SP-2或X63-Ag8-653细胞。还已经描述了用于生产人单克隆抗体的人骨髓瘤和小鼠-人杂交骨髓瘤细胞系(Kozbor，J.Immunol.，133:3001(1984)；Brodeur等人，Monoclonal Antibody Production Techniques and Applications，pp.51-63(Marcel Dekker，Inc.，New York，1987))。

[0360] 测定其中生长杂交瘤细胞的培养基，用于针对抗原的单克隆抗体的生产。在一些实施方案中，通过免疫沉淀或通过体外结合测定(诸如放射性免疫测定法(RIA)或酶联免疫吸附测定(ELISA))，确定由杂交瘤细胞产生的单克隆抗体的结合特异性。

[0361] 单克隆抗体的结合亲和力可以通过例如Munson等人，Anal.Biochem.，107:220(1980)的Scatchard分析来确定。

[0362] 在鉴定产生具有所需特异性、亲和力和/或活性的抗体的杂交瘤细胞后，所述克隆可以通过有限稀释方法进行亚克隆，并且通过标准方法进行培养(Goding，Monoclonal Antibodies:Principles and Practice，PP.59-103(Academic Press，1986))。适用于该目的的培养基包括例如D-MEM或RPMI-1640培养基。另外，杂交瘤细胞可以在动物中作为腹水

瘤体内生长。

[0363] 合适地，通过常规免疫球蛋白纯化方法，诸如蛋白A-Sepharose，羟基磷灰石层析、凝胶电泳、透析或亲和层析，将亚克隆分泌的单克隆抗体与培养基、腹水或血清分开。

[0364] 可以使用常规方法(例如，通过使用能够与编码鼠抗体重链和轻链的基因特异性结合的寡核苷酸探针)，轻易地分离编码单克隆抗体的DNA并将其测序。在一些实施方案中，杂交瘤细胞作为这种DNA的来源。一旦分离，可以将DNA置于表达载体中，然后将该表达载体转染到宿主细胞(诸如，不以其它方式产生免疫球蛋白蛋白质的大肠杆菌(E.coli)细胞、猿猴COS细胞、中国仓鼠卵巢(CHO)细胞或骨髓瘤细胞)，以在重组宿主细胞中获得单克隆抗体的合成。关于编码抗体的DNA在细菌中的重组表达的综述性论文包括Skerra等人，Curr.Opinion in Immunol.，5:256-262(1993)和Plückthun，Immunol.Revs.，130:151-188(1992)。

[0365] 在另外的实施方案中，可以从使用McCafferty等人，Nature，348:552-554(1990)描述的技术产生的抗体噬菌体文库中分离抗体或抗体片段。Clackson等人，Nature，352:624-628(1991)和Marks等人，J.Mol.Biol.，222:581-597(1991)分别描述了使用噬菌体文库分离鼠和人抗体。后续出版物描述了通过链改组(Marks等人，Bio/Technology，10:779-783(1992))，以及组合感染和体内重组作为构建非常大的噬菌体文库的策略(Waterhouse等人，Nuc.Acids.Res.，21:2265-2266(1993))，产生高亲和力(nM范围)的人抗体。因此，这些技术是用于分离单克隆抗体的传统单克隆抗体杂交瘤技术的可行替代方法。

[0366] 还可以修饰DNA，例如通过用人重链和轻链恒定结构域的编码序列替代同源鼠序列(美国专利号4,816,567；Morrison等人，Proc.Natl Acad.Sci.USA，81:6851(1984))，或通过共价连接至免疫球蛋白编码序列和非免疫球蛋白多肽的整个或部分编码序列。

[0367] 通常，用这种非免疫球蛋白多肽替代抗体的恒定结构域，或者用它们替代抗体的一个抗原结合位点的可变结构域，以产生嵌合二价抗体，该抗体包含对一种抗原具有特异性的一个抗原结合位点以及对不同抗原具有特异性的另一个抗原结合位点。

[0368] (iii)人源化抗体

[0369] 本领域中已经描述了用于将非人抗体人源化的方法。在一些实施方案中，人源化抗体具有从非人来源引入其中的一个或多个氨基酸残基。这些非人氨基酸残基通常被称为“进口”残基，其典型地取自“进口”可变结构域。人源化可以基本上遵循Winter及同事(Jones等人，Nature，321:522-525(1986)；Riechmann等人，Nature，332:323-327(1988)；Verhoeyen等人，Science，239:1534-1536(1988))的方法，通过用高变区序列置换人抗体的相应序列进行。因此，这种”人源化”抗体是嵌合抗体(美国专利号4,816,567)，其中基本上少于完整人可变结构域被来自非人物种的相应序列置换。实际上，人源化抗体典型地是这样的人抗体，其中一些高变区残基和可能的一些FR残基被来自啮齿动物抗体中的类似位点的残基置换。

[0370] 选择要用于制备人源化抗体的人可变结构域(轻链和重链二者)对于降低抗原性是十分重要的。根据所谓的“最佳-配合(best-fit)”方法，针对已知的人可变结构域序列的整个文库来筛选啮齿动物抗体的可变结构域的序列。然后接受与啮齿动物序列最接近的人序列作为人框架区(FR)，用于人源化抗体(Sims等人，J.Immunol.，151:2296(1993)；Chothia等人，J.Mol.Biol.，196:901(1987))。另一种方法使用来源于所有特定轻链或重链

可变区亚组的人抗体的共有序列的特定框架区。相同的框架可以用于若干不同的人源化抗体(Carter等人,Proc.Natl.Acad.Sci.USA,89:4285(1992);Presta等人,J.Immunol.,151:2623(1993))。

[0371] 此外重要的是,抗体在人源化后保持对抗原的高亲和力以及其它有利的生物特性。为了实现这一目的,在这些方法的一些实施方案中,使用亲本和人源化序列的三维模型,通过分析亲本序列和各种概念性人源化产物的方法,制备人源化抗体。三维免疫球蛋白分子模型是通常可获得的,并且为本领域技术人员所熟悉。可获得用于图解和显示所选择的候选免疫球蛋白序列的可能三维构象结构的计算机程序。通过检查这些显示结果,允许分析残基在候选免疫球蛋白序列的功能中行使的可能作用,即分析影响候选免疫球蛋白结合其抗原的能力的残基。这样,可以从接受者和输入序列中选出FR残基并进行组合,从而获得所需抗体特征,诸如对靶抗原的亲和力提高。通常,高变区残基直接且最实质地参与影响抗原结合。

[0372] 在一些实施方案中,抗-CD20抗体是人源化2H7抗体。在一些实施方案中,人源化2H7抗体优选包含图1A和1B所示CDR序列中的一种、两种、三种、四种、五种或六种。在一些实施方案中,人源化2H7抗体优选包含以下CDR序列中的一种、两种、三种、四种、五种或六种:

[0373] CDR L1序列RASSSVSYXH,其中X是M或L(SEQ ID NO:18),例如RASSSVSYMH(SEQ ID NO:4)(图1A),

[0374] CDR L2序列APSNLAS(SEQ ID NO:5)(图1A),

[0375] CDR L3序列QQWXFNPPT,其中X是S或A(SEQ ID NO:19),例如QQWSFNPPT(SEQ ID NO:6)(图1A),

[0376] CDR H1序列GYTFTSYNMH(SEQ ID NO:10)(图1B),

[0377] CDR H2序列AIYPGNGXTSYNQKFKG,其中X是D或A(SEQ ID NO:20),例如AIYPGNGDTSYNQKFKG(SEQ ID NO:11)(图1B),和

[0378] CDR H3序列VVYYSXXYWYFDV,其中位置6处的X是N、A、Y、W或D,并且位置7处的X是S或R(SEQ ID NO:21),例如VVYYSNSYWYFDV(SEQ ID NO:12)(图1B)。

[0379] 以上CDR序列通常存在于人可变轻链框架序列和可变重链框架序列中,诸如人轻链κ亚组I(V_L6I)的基本上人共有FR残基,和人重链亚组III(V_HIII)的基本上人共有FR残基。还参见WO 2004/056312(Lowman等人)。

[0380] 在一些实施方案中,可变重链区可以与人IgG链恒定区连接,其中该区域可以是例如IgG1或IgG3,包括天然序列和变体恒定区。

[0381] 在一些实施方案中,这种抗体包含SEQ ID NO:8的可变重链结构域序列(v16,如图1B所示),任选地还包含SEQ ID NO:2的可变轻链结构域序列(v16,如图1A所示),其任选地包含可变重链结构域中位置56、100和/或100a处的一个或多个氨基酸置换(例如,D56A、N100A或N100Y和/或S100aR)以及可变轻链结构域中位置32和/或92处的一个或多个氨基酸置换(例如,M32L和/或S92A)。在一些实施方案中,抗体是包含SEQ ID NOs.13或16的轻链氨基酸序列和SEQ ID NO.14、15、17、22或25的重链氨基酸序列的完整抗体。在一些实施方案中,人源化2H7抗体是ocrelizumab(Genentech)。

[0382] 在一些实施方案中,人源化2H7是完整抗体或抗体片段,其包含可变轻链序列:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKP

[0383] LIYAPSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSF

NPPTFGQGTKVEIKR (SEQ ID NO:2)；

[0384] 和可变重链序列：

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG

[0385] LEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE

DTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID NO:8)。

[0386] 在一些实施方案中，人源化2H7抗体是完整抗体，在一些实施方案中，其包含轻链氨基酸序列：

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKP

LIYAPSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSF

[0387] NPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY

PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD

YEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)；

[0388] 和重链氨基酸序列：

[0389]

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG

LEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE

DTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPL

APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL

QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS

HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG

SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO:14)

[0390] 或重链氨基酸序列：

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG

[0391] LEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE

DTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPL

APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
[0392] DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
(SEQ ID NO:26)

[0393] 或重链氨基酸序列：

[0394]

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG
LEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE
DTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:15)

[0395] 或重链氨基酸序列：

[0396]

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG
LEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE
DTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG

[0397]

SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:27)。

[0398] 在一些实施方案中，移除重链C末端处的氨基酸K。

[0399] 在一些实施方案中，人源化2H7抗体包含2H7.v511可变轻链结构域序列：

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPL

[0400] IYAPSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFN PPTFGQGTKVEIKR (SEQ ID NO:23)

[0401] 和2H7.v511可变重链结构域序列：

[0402]

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG LEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE DTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS (SEQ ID NO:24)。

[0403] 在一些实施方案中，人源化2H7.v511抗体是完整抗体，其可以包含轻链氨基酸序列：

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPL IYAPSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFN

[0404] PPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:16)

[0405] 以及SEQ ID NO:17或以下的重链氨基酸序列：

[0406]

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKG LEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAE DTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQ DWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG

[0407]

SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:25)。

[0408] 在一些实施方案中，本文中的抗体还可以在Fc区中包含至少一个改善ADCC活性的氨基酸置换，诸如其中使用重链残基的Eu编号，氨基酸置换位于位置298、333和334(优选S298A、E333A和K334A)的抗体。还参见美国专利号6,737,056B1,Presta。这些抗体中的任一种可以在Fc区中包含至少一个改善FcRn结合或血清半衰期的置换，例如重链位置434处的置换，诸如N434W。还参见美国专利号6,737,056B1,Presta。这些抗体中的任一种还可以在Fc区中包含至少一个增加CDC活性的氨基酸置换，例如包含位置326处的至少一个置换，优选K326A或K326W。还参见美国专利号6,528,624B1(Idusogie等人)。

[0409] 在一些实施方案中，人源化2H7变体是包含SEQ ID NO:2的可变轻链结构域和SEQ ID NO:8的可变重链结构域的那些，包括Fc区(如果存在)中存在置换或不存在置换的那些，以及包含在SEQ ID NO:8中具有变化N100A;或D56A和N100A;或D56A、N100Y和S100aR的可变重链结构域以及在SEQ ID NO:2中具有变化M32L;或S92A;或M32L和S92A的可变轻链结构域的那些。2H7.v16的重链可变结构域中的M34已经被鉴定为抗体稳定性的潜在源，并且是用于置换的另一潜在候选者。

[0410] 在本发明的一些实施方案中，基于2H7.v16的变体的可变区包含v16的氨基酸序列，除了具有下表1所示的位置处的氨基酸置换。除非另有说明，否则2H7变体将具有与v16相同的轻链。

[0411] 表1:示例性人源化2H7抗体变体

[0412]

2H7 版本	重链 (V_H)变化	轻链 (V_L)变化	Fc 变化
16 用于参照			-
31	-	-	S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N100A	M32L, S92A	S298A, E333A, K334A

[0413]

115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K334A, K322A
138	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
477	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
375	-	-	K334L
588	-	-	S298A, E333A, K334A, K326A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, E333A, K334A, K326A

[0414] (iv)人抗体

[0415] 作为人源化的替代方法,可以生成人抗体。例如,目前可以产生在缺乏内源性免疫球蛋白生成的情况下能够在免疫后产生全人抗体库的转基因动物(例如小鼠)。例如,已经描述了嵌合和种系突变小鼠中抗体重链连接区(J_H)基因的纯合缺失导致内源性抗体生成的完全抑制。在该种系突变小鼠中转移人种系免疫球蛋白基因阵列将导致在抗原攻击后产生人抗体。参见,例如,Jakobovits等人,Proc.Natl.Acad.Sci.USA,90:2551(1993);Jakobovits等人,Nature,362:255-258(1993);Bruggermann等人,Year in Immuno.,7:33(1993);和美国专利号5,591,669、5,589,369和5,545,807。

[0416] 备选地,噬菌体展示技术(McCafferty等人,Nature 348:552-553(1990))可以用于在体外从来自未免疫供体的免疫球蛋白可变(V)结构域基因库生成人抗体和抗体片段。根据这种技术,将抗体V结构域基因以符合读框的方式(in-frame)克隆到丝状噬菌体(诸如M13或fd)的主要或次要外壳蛋白基因中,并且在噬菌体颗粒表面上展示为功能性抗体片段。因为丝状颗粒含有噬菌体基因组的单链DNA拷贝,所以以抗体的功能特性为基础进行的选择也导致编码展示那些特性的抗体的基因的选择。因此,噬菌体模拟B细胞的一些特性。噬菌体展示可以以多种形式进行;其综述参见例如Johnson,Kevin S.和Chiswell,DavidJ.,Current Opinion in Structural Biology 3:564-571(1993)。若干来源的V基因区段可以用于噬菌体展示。Clackson等人,Nature,352:624-628(1991)从来源于经免疫小鼠的脾的小型V基因随机组合文库中分离出一系列的不同抗[illegible]唑酮抗体。可以基本上依照Marks等人,J.Mol.Biol.222:581-597(1991)或Griffith等人,EMBO J.12:725-734(1993)描述的技术,构建来自未免疫的人供体的V基因库,并且分离针对一系列不同抗原(包括自体抗原)的抗体。还参见美国专利号5,565,332和5,573,905。

[0417] 还可以通过体外激活的B细胞来生成人抗体(参见美国专利号5,567,610和5,229,

275)。

[0418] (v)抗体片段

[0419] 已经开发了用于生成抗体片段的多种技术。传统上,通过蛋白水解消化完整抗体来衍生这些片段(参见例如Morimoto等人,Journal of Biochemical and Biophysical Methods 24:107-117(1992)和Brennan等人,Science,229:81(1985))。然而,目前可以直接由重组宿主细胞生成这些片段。例如,可以从上文讨论的噬菌体抗体文库中分离抗体片段。备选地,可以直接从大肠杆菌回收Fab'-SH片段,并且将其进行化学偶联以形成$F(ab')_2$片段(Carter等人,Bio/Technology 10:163-167(1992))。根据另一种方法,可以直接从重组宿主细胞培养物分离$F(ab')_2$片段。用于产生抗体片段的其它技术对于本领域技术人员将是显而易见的。在其它实施方案中,选择的抗体是单链Fv片段(scFv)。参见WO 93/16185;美国专利号5,571,894;和美国专利号5,587,458。例如,抗体片段也可以是"线性抗体",例如如美国专利号5,641,870中描述的。这种线性抗体片段可以是单特异性或双特异性的。

[0420] (vi)双特异性抗体

[0421] 双特异性抗体是对至少两个不同表位具有结合特异性的抗体。示例性的双特异性抗体可以与B细胞表面标志的两种不同表位结合。其它这种抗体可以与B细胞表面标志结合,并且还与第二种不同的B细胞表面标志结合。备选地,抗-B细胞表面标志结合臂可以与结合白细胞上的触发分子(诸如T细胞受体分子(例如CD2或CD3),或IgG的Fc受体(FcγR)(诸如FcγRI(CD64)、FcγRII(CD32)和FcγRIII(CD16))的臂组合,使得细胞防御机制聚焦于该B细胞。双特异性抗体还可以用于将细胞毒性剂定位于B细胞。这些抗体拥有B细胞表面标志结合臂以及与细胞毒性剂(例如皂草毒蛋白(saporin)、抗-干扰素-α、长春花生物碱、蓖麻毒蛋白A链、甲氨喋呤或放射性同位素半抗原)结合的臂。可以将双特异性抗体制备为全长抗体或抗体片段(例如,$F(ab')_2$双特异性抗体)。

[0422] 用于产生双特异性抗体的方法是本领域已知的。传统上,全长双特异性抗体的产生基于共表达两条免疫球蛋白重链-轻链对,其中两条链具有不同的特异性(Millstein等人,Nature,305:537-539(1983))。由于免疫球蛋白重链和轻链的随机分配,这些杂交瘤(四体瘤(quadroma))产生10种不同抗体分子的可能混合物,其中仅一种分子具有正确的双特异性结构。正确分子的纯化(通常通过亲和层析步骤进行)是相当繁琐的,并且产物产率低。类似的方法在WO 93/08829中以及在Traunecker等人,EMBO J.,10:3655-3659(1991)中公开。

[0423] 根据不同的方案,具有所需结合特异性的抗体可变结构域(抗体-抗原结合位点)与免疫球蛋白恒定结构域序列融合。在一些实施方案中,融合物具有免疫球蛋白重链恒定结构域,包括至少部分的铰链区、CH2区和CH3区。在一些实施方案中,含有轻链结合必需的位点的第一重链恒定区(CH1)在至少一种融合物中存在。将编码免疫球蛋白重链融合物和(如有需要)免疫球蛋白轻链的DNA插入单独的表达载体中,并且共转染至合适的宿主生物中。在构建中所用的比率不等的3种多肽链提供最佳产率时的实施方案中,这在调节3种多肽片段的相互比例方面提供了良好的灵活性。然而,当至少两条多肽链以相等比率的表达导致高产率时或当该比率没有特定意义时,可能将2条或全部3条多肽链的编码序列在一个表达载体中插入。

[0424] 在这种方案的一些实施方案中,双特异性抗体由一条臂中具有第一结合特异性的

杂合免疫球蛋白重链和另一条臂中的杂合免疫球蛋白重链-轻链对(提供第二结合特异性)组成。发现这种非对称结构促进了所需双特异性化合物与不需要的免疫球蛋白链组合的分离,因为免疫球蛋白轻链在仅一半的所述双特异性分子中的存在提供了便利的分离方式。这种方案在WO 94/04690中公开。关于产生双特异性抗体的进一步细节,参见,例如Suresh等人,Methods in Enzymology,121:210(1986)。

[0425] 根据美国专利号5,731,168中描述的另一个方案,可以将一对抗体分子之间的界面进行工程改造以最大化从重组细胞培养物回收的异二聚体的百分比。在一些实施方案中,该界面包含抗体恒定结构域的C_H3结构域的至少一部分。在这种方法中,来自第一抗体分子界面的一个或多个小氨基酸侧链由较大侧链(例如,酪氨酸或色氨酸)替换。通过将大氨基酸侧链替换为较小侧链(例如,丙氨酸或苏氨酸),在第二抗体分子的界面上产生针对大体积侧链的相同或相似尺寸的补偿"腔"。这提供了相对于其它不需要的产物(诸如同型二聚体)增加异二聚体产率的机制。

[0426] 双特异性抗体包括交联抗体或"异质缀合(heteroconjugate)"抗体。例如,异质缀合中抗体的一种可以偶联于抗生物素蛋白(avidin),另一种偶联于生物素。例如,已经提出这种抗体将免疫系统细胞靶向至不需要的细胞(美国专利号4,676,980),并用于治疗HIV感染(WO 91/00360、WO 92/200373和EP 03089)。可以使用任何便利的交联方法产生异质缀合抗体。合适的交联剂是本领域中熟知的并且连同许多交联技术一起在美国专利号4,676,980中公开。

[0427] 也已经在文献中描述从抗体片段产生双特异性抗体的技术。例如,可以利用化学键制备双特异性抗体。Brennan等人,Science 229:81(1985)描述了其中完整抗体以蛋白酶解方式被切割以产生$F(ab')_2$片段的方法。这些片段在二硫酚(dithiol)络合剂亚砷酸钠存在下被还原以稳定邻位二硫酚并防止分子间二硫化物形成。产生的Fab'片段随后转化成硫代硝基苯甲酸酯(TNB)衍生物。随后将Fab'-TNB衍生物中的一者通过用巯基乙胺还原来转化成Fab'-巯基并且与等摩尔量的其它Fab'-TNB衍生物混合以形成双特异性抗体。产生的双特异性抗体可以用作用于酶的选择性固定的药剂。

[0428] 还已经描述了从重组细胞培养物直接产生并分离双特异性抗体片段的多种技术。例如,已经使用亮氨酸拉链产生双特异性抗体。Kostelny等人,J.Immunol.,148(5):1547-1553(1992)。来自Fos和Jun蛋白的亮氨酸拉链肽通过基因融合与两个不同抗体的Fab'部分连接。抗体同型二聚体在铰链区被还原以形成单体,随后被再氧化以形成抗体异二聚体。也可以将这种方法用于产生抗体同型二聚体。Hollinger等人,Proc.Natl.Acad.Sci.USA,90:6444-6448(1993)描述的"双抗体"技术已经为产生双特异性抗体片段提供了替代的机制。片段包含通过接头与轻链可变结构域(V_L)连接的重链可变结构域(V_H),其中所述接头太短以至于不允许相同链上的这两个结构域之间配对。因此,迫使一个片段的V_H和V_L结构域与另一个片段的互补V_L和V_H结构域配对,从而形成两个抗原结合位点。还已经报道了通过利用单链Fv(sFv)二聚体产生双特异性抗体片段的另一种策略。参见Gruber等人,J.Immunol.,152:5368(1994)。

[0429] 构思了大于二价的抗体。例如,可以制备三特异性抗体。Tutt等人J.Immunol.147:60(1991)。

[0430] V.抗体的缀合物和其它修饰

[0431] 将本文方法中所使用的或制品中所包含的抗体任选地与细胞毒性剂缀合。例如，可以将抗体与WO2004/032828中描述的药物缀合。

[0432] 上文已经描述了可用于生成这种抗体-细胞毒性剂缀合物的化疗剂。

[0433] 本文还考虑抗体与一种或多种小分子毒素的缀合物，所述小分子毒素诸如加利车霉素、美登素(美国专利号5,208,020)、单端孢菌毒素(trichothene)和CC1065。在本发明的一个实施方案中，将抗体与一个和多个美登素分子缀合(例如约1个至约10个美登素分子/抗体分子)。例如可以将美登素转变为May-SS-Me，May-SS-Me可以还原为May-SH3并与修饰的抗体反应(Chari等人，Cancer Research 52:127-131(1992))，以产生美登木素生物碱-抗体缀合物。

[0434] 备选地，将抗体与一个或多个加利车霉素分子缀合。加利车霉素抗生素家族能够在亚皮摩尔浓度产生双链DNA断裂。可以使用的加利车霉素的结构类似物包括但不限于γ_1^I、α_2^I、α_3^I、N-乙酰基-γ_1^I、PSAG和θ^I_1(Hinman等人，Cancer Research 53:3336-3342(1993)和Lode等人，Cancer Research 58:2925-2928(1998))。

[0435] 可以使用的酶活性毒素及其片段包括白喉A链、白喉毒素的非结合活性片段、外毒素A链(来自铜绿假单胞菌(Pseudomonas aeruginosa))、蓖麻毒蛋白(ricin)A链、相思豆毒蛋白(abrin)A链、蒴莲根毒蛋白(modeccin)A链、α-帚曲霉素(alpha-sarcin)、油桐(Aleurites fordii)蛋白、香石竹毒蛋白(dianthin)蛋白、美洲商陆(Phytolaca americana)蛋白(PAPI、PAPII和PAP-S)、苦瓜(momordica charantia)抑制剂、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制剂、多花白树毒蛋白(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、依诺霉素(enomycin)和单端孢霉烯(tricothecenes)。参见，例如，1993年10月28日公开的WO 93/21232。

[0436] 本发明还构思了与具有核酸水解活性的化合物(例如，核糖核酸酶或DNA核酸内切酶(诸如脱氧核糖核酸酶;DNA酶))缀合的抗体。

[0437] 多种放射性同位素可用于生产放射性缀合的抗体。实例包括At^{211}、I^{131}、I^{125}、y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}以及Lu的放射性同位素。

[0438] 可以使用多种双功能的蛋白偶联剂来制备抗体与细胞毒性剂的缀合物，所述蛋白偶联剂诸如3-(2-吡啶基二硫醇)丙酸N-琥珀酰亚胺酯(SPDP)、4-(N-马来酰亚胺基甲基)环己烷-1-甲酸琥珀酰亚胺酯、亚氨基硫杂环戊烷(iminothiolane)(IT)、亚氨酸酯的双功能衍生物(诸如己二亚氨酸二甲酯HCl)、活性酯(诸如辛二酸二琥珀酰亚胺酯)、醛(诸如戊二醛)、双叠氮基化合物(诸如二(对-叠氮基苯甲酰基)己二胺)、双重氮鎓衍生物(诸如双-(对-重氮鎓苯甲酰基)-乙二胺)、二异氰酸酯(诸如2,6-二异氰酸亚甲苯酯)和双活性的氟化合物(诸如1,5-二氟-2,4-二硝基苯)。例如，如在Vitetta等人Science 238:1098(1987)中所描述的制备蓖麻毒蛋白免疫毒素。碳-14-标记的1-异硫氰酰基苄基-3-甲基二亚乙基三胺五乙酸(MX-DTPA)是用于将放射性核苷酸缀合至抗体的示例性螯合剂。参见WO94/11026。接头可以是便于在细胞中释放细胞毒性药物的“可切割接头”。例如，可以使用酸不稳定接头、肽酶敏感接头、二甲基接头或含二硫化物的接头(Chari等人，Cancer Research 52:127-131(1992))。

[0439] 备选地，可以例如通过重组技术或肽合成来制备包含抗体和细胞毒性剂的融合蛋

白。

[0440] 在又一个实施方案中，可以将抗体与“受体”(诸如链霉抗生物素蛋白(streptavidin))缀合，用于肿瘤预靶向，其中向患者施用抗体-受体缀合物，随后使用清除剂从循环中除去未结合的缀合物，然后施用与细胞毒性剂(例如放射性核苷)缀合的“配体”(例如抗生物素蛋白(avidin))。

[0441] 还可以将本发明的抗体与前体药物活化酶缀合，该活化酶将前体药物(例如肽基化疗剂，参见WO8I/01145)转变为活性抗癌药。参见，例如，WO 88/07378和美国专利号4,975,278。

[0442] 这种缀合物的酶成分包括能够作用于前体药物从而将其转变为更具活性的细胞毒性形式的任何酶。

[0443] 可以用于本发明方法的酶包括，但不限于，可用于将含磷酸酯的前体药物转变为游离药物的碱性磷酸酶；可用于将含硫酸酯的前体药物转变为游离药物的芳基硫酸酯酶；可用于将无毒5-氟胞嘧啶转变为抗癌药5-氟尿嘧啶的胞嘧啶脱氨酶；可用于将含肽的前体药物转变为游离药物的蛋白酶，诸如沙雷氏菌蛋白酶、嗜热菌蛋白酶、枯草杆菌蛋白酶、羧肽酶和组织蛋白酶(诸如组织蛋白酶B和L)；可用于转化含D-氨基酸置换的前体药物的D-丙氨酰羧肽酶；可用于将糖基化前体药物转变为游离药物的碳水化合物切割酶，诸如β-半乳糖苷酶和神经氨酸酶；可用于将β-内酰胺衍生的药物转变为游离药物的β-内酰胺酶；以及可用于将在其氨基氮处分别用苯氧乙酰基或苯乙酰基衍生的药物转变为游离药物的青霉素酰胺酶，诸如青霉素V酰胺酶或青霉素G酰胺酶。备选地，可以使用具有酶活性的抗体(本领域也称作“抗体酶(abzyme)”)，将本发明的前体药物转变为游离活性药物(参见，例如，Massey,Nature 328:457-458(1987))。可以如本文所描述的制备抗体-抗体酶缀合物，用于将抗体酶递送至肿瘤细胞群。

[0444] 可以通过本领域公知的技术将本发明的酶与抗体共价结合，例如使用上文讨论的异双官能交联剂。备选地，可以使用本领域公知的重组DNA技术，构建包含与本发明的酶的至少功能活性部分连接的本发明抗体的至少抗原结合区的融合蛋白(参见，例如，Neuberger等人,Nature,312:604-608(1984))。

[0445] 本文还考虑抗体的其它修饰。例如，可以将抗体与多种非蛋白性聚合物中的一种连接，所述聚合物诸如聚乙二醇(PEG)、聚丙二醇、聚氧化烯、或聚乙二醇和聚丙二醇的共聚物。在一些实施方案中，抗体片段(诸如Fab')与一个或多个PEG分子连接。

[0446] 还可以将本文中公开的抗体配制为脂质体。通过本领域已知的方法，诸如在Epstein等人，Proc.Natl.Acad.Sci.USA，82：3688(1985)；Hwang等人，Proc.Natl.Acad.Sci.USA,77:4030(1980)；美国专利号4,485,045和4,544,545;和1997年10月23日公开的WO97/38731中描述的方法，制备含有抗体的脂质体。在美国专利号5,013,556中公开了具有增加的循环时间的脂质体。

[0447] 通过反相蒸发方法用包含磷脂酰胆碱、胆固醇和PEG-衍生化的磷脂酰乙醇胺(PEG-PE)的脂质组合物可以产生特别有用的脂质体。将脂质体穿过确定孔径的过滤器挤出以产生具有所需直径的脂质体。本发明的抗体的Fab'片段可以经由二硫互换反应与脂质体(如Martin等人J.Biol.Chem.257:286-288(1982)中描述的)缀合。脂质体中任选地含有化学治疗剂。参见Gabizon等人J.National Cancer Inst.81(19)1484(1989)。

[0448] 构思了抗体的氨基酸序列修饰。例如，改善抗体的结合亲和力和/或其它生物学特性可能是所需的。通过将适合的核苷酸改变引入到抗体核酸或者通过肽合成来制备抗体的氨基酸序列变体。所述修饰包括，例如，从抗体的氨基酸序列内的残基缺失，和/或向抗体的氨基酸序列内的残基插入，和/或抗体的氨基酸序列内的残基置换。可以产生缺失、插入和置换的任意组合以实现最终构建体，条件是所述最终构建体拥有所需特征。氨基酸变化也可以改变抗体的翻译后加工，诸如改变糖基化位点的数量或位置。

[0449] 一种用于鉴定作为诱变的优选位置的某些抗体残基或区域的有用方法称作“丙氨酸扫描诱变”，如Cunningham和Wells Science，244:1081-1085(1989)所描述的。因此，将残基或一组靶残基(例如，带电荷残基诸如arg、asp、his、lys和glu)鉴定并且用中性或带负电荷的氨基酸(最优选是丙氨酸或多丙氨酸)替换以影响氨基酸与表位的相互作用。然后，对置换显示功能敏感性的那些氨基酸位置通过在置换位点处或者对于置换位点引入另外的或其它变体而完善。因此，虽然引入氨基酸序列变体的位点是预先确定的，但是突变本身的性质不需要预先确定。例如，为了分析给定位点处的突变的性能，丙氨酸扫描或随机诱变在目标密码子或区域进行，并且表达的抗体变体对所需活性进行筛选。

[0450] 氨基酸序列插入包括长度范围从1个残基至含有成百个或更多个残基的多肽的氨基端和/或羧基端融合，以及单个或多个氨基酸残基的序列内插入。末端插入的实例包括具有N-末端甲硫氨酰基残基的抗体或与细胞毒性多肽融合的抗体。抗体分子的其它插入性变体包括与酶或增加抗体血清半衰期的多肽的抗体N末端或C末端的融合。

[0451] 另一类型的变体是氨基酸置换变体。这些变体具有被不同残基替换的抗体分子中的至少一个氨基酸残基。对于一个或多个抗体的置换诱变最感兴趣的位点包括高变区，但也考虑FR改变。表2中在“优选的置换”的标题下显示了保守性置换。如果这种置换导致生物活性的改变，则可以引入表2中称为“示例性置换”的更实质性改变并筛选产物。

[0452] 表2

[0453]

原始残基	示例性置换	优选置换
Ala(A)	Val;Leu;Ile	Val
Arg(R)	Lys;Gin;Asn	Lvs
Asn(N)	Gin;His;Asp,Lys;Arg	Gin
Asp(D)	Glu;Asn	Glu
Cys(C)	Ser;Ala	Ser
Gin(Q)	Asn;Glu	Asn
Glu(E)	Asp;Gin	Asp
Gly(G)	Ala	Ala
His(H)	Asn;Gin;Lys;Arg	Arg
Ile(I)	Leu;Val;Met;Ala;Phe;正亮氨酸	Leu
Leu(L)	正亮氨酸;lie;Val;Met;Ala;Phe	Ile
Lys(K)	Arg;Gin;Asn	Arg
Met(M)	Leu;Phe;Ile	Leu
Phe(F)	Trp;Leu;Val;lie;Ala;Tyr	Tyr

Pro(P)	Ala	Ala
Ser(S)	Thr	Thr
Thr(T)	Vak;Ser	Ser
Trp(W)	Tyr;Phe	Tyr
Tyr(Y)	Trp;Phe;Thr;Ser	Phe
Val(V)	Ile;Leu;Met;Phe;Ala;正亮氨酸	Leu

[0454] 对抗体生物特性的实质性修饰可以通过选择对以下方面的保持具有显著不同的影响的置换来完成:(a)置换区域中的多肽主链的结构,例如作为折叠构象或螺旋构象,(b)靶位点处分子的电荷或疏水性,或(c)侧链的体积。根据其侧链特性的相似性,可以将氨基酸进行如下分组(A.L.Lehninger,在Biochemistry,第2版,pp.73-75,Worth Publishers,New York(1975)):

[0455] (1)非极性:Ala(A),Val(V),Leu(L),Ile(I),Pro(P),Phe(F),Trp(W),Met(M)

[0456] (2)非荷电极性:Gly(G),Ser(S),Thr(T),Cys(C),Tyr(Y),Asn(N),Gln(Q)

[0457] (3)酸性:Asp(D),Glu(E)

[0458] (4)碱性:Lys(K),Arg(R),His(H)

[0459] 备选地,天然存在的残基可以基于常见的侧链特性分组:

[0460] (1)疏水:正亮氨酸、Met、Ala、Val、Leu、Ile;

[0461] (2)中性亲水:Cys、Ser、Thr、Asn、Gln;

[0462] (3)酸性:Asp、Glu;

[0463] (4)碱性:His、Lys、Arg;

[0464] (5)影响链取向的残基:Gly、Pro;

[0465] (6)芳香性:Trp、Tyr、Phe。

[0466] 非保守性置换将需要将这些类别之一的成员交换为另一个类别的成员。

[0467] 也可以置换任何不涉及保持抗体正确构象的半胱氨酸残基(通常用丝氨酸置换),以改善分子的氧化稳定性和防止异常交联。相反地,可以向抗体中添加半胱氨酸键以改善其稳定性(特别是当抗体是抗体片段诸如Fv片段时)。

[0468] 特别优选的置换变体类型涉及置换亲本抗体的一个或多个高变区残基。通常,选择用于进一步开发的所得变体相对于产生它们的亲本抗体将具有改善的生物学特性。产生这种置换变体的一种便利方法是使用噬菌体展示进行的亲和力成熟。简言之,将若干高变区位点(例如6-7个位点)突变,以在各个位点产生所有可能的氨基酸置换。由此产生的抗体变体作为与各个颗粒内包装的M13基因III产物的融合物,以单价形式展示在丝状噬菌体颗粒上。然后,如本文所公开的对噬菌体展示的变体筛选其生物活性(例如结合亲和力)。为了鉴定用于修饰的候选高变区位点,可以进行丙氨酸扫描诱变以鉴定对抗原结合具有重要贡献的高变区残基。备选地或额外地,可能有益的是,分析抗原-抗体复合物的晶体结构以鉴定抗体和抗原之间的接触点。这种接触残基和邻近残基是用于依照本文详述的技术进行置换的候选物。一旦产生这种变体,如本文所描述的对该组变体进行筛选,并且可以选择在一种或多种相关测定中具有优良特性的抗体用于进一步开发。

[0469] 抗体的另一类氨基酸变体改变抗体的原始糖基化模式。这种改变包括:删除存在于抗体中的一个或多个碳水化合物部分,和/或添加抗体中不存在的一个或多个糖基化位

点。

[0470] 多肽的糖基化通常是N-连接的或O-连接的。N-连接是指碳水化合物部分连接于天冬酰胺残基的侧链。三肽序列天冬酰胺-X-丝氨酸和天冬酰胺-X-苏氨酸(其中X是除脯氨酸以外的任何氨基酸)是用于将碳水化合物部分酶促连接于天冬酰胺侧链的识别序列。因此,在多肽中存在这些三肽序列中的任一个产生了潜在的糖基化位点。O-连接的糖基化是指将糖N-乙酰半乳糖胺、半乳糖或木糖之一连接于羟基氨基酸,最常见的是丝氨酸或苏氨酸,但是也可以使用5-羟脯氨酸或5-羟赖氨酸。

[0471] 向抗体中添加糖基化位点可以通过改变氨基酸序列使得其含有一个或多个上述三肽序列而便利地完成(用于N-连接的糖基化位点)。还可以通过向原始抗体的序列中添加或置换一个或多个丝氨酸或苏氨酸残基来进行所述改变(用于O-连接的糖基化位点)。

[0472] 在抗体包含Fc区的情况下,可以改变与之连接的碳水化合物。例如,美国专利申请号US 2003/0157108 A1(Presta,L.)中描述了具有成熟碳水化合物结构的抗体,所述结构缺乏连接于抗体Fc区的岩藻糖;也参见US 2004/0093621 A1(Kyowa Hakko Kogyo Co.,Ltd),其涉及CD20抗体组合物的。WO03/011878(Jean-Mairet等人)和美国专利号6,602,684(Umana等人)中提到了在连接于抗体Fc区的碳水化合物中具有平分型N-乙酰葡糖胺(GlcNAc)的抗体。WO97/30087(Patel等人)中报道了在连接于抗体Fc区的寡糖中具有至少一个半乳糖残基的抗体;关于附着于其Fc区的碳水化合物更改了的抗体;关于具有连接于其Fc区的改变的碳水化合物的抗体,还参见WO98/58964(Raju,S.)和WO99/22764(Raju,S.)。

[0473] 在一些实施方案中,本文中的糖基化变体包含Fc区,其中连接于Fc区的碳水化合物结构缺乏岩藻糖。这种变体具有改善的ADCC功能。任选地,Fc区还包含其中进一步改善ADCC的一个或多个氨基酸置换,例如Fc区的位置298、333和/或334处的置换(残基的EU编号)。与“去岩藻糖基化”或“岩藻糖缺陷型”抗体相关的出版物的实例包括:美国专利申请号US 2003/0157108 A1(Presta,L);WO 00/61739A1;WO01/29246A1;US2003/0115614A1;US2002/0164328A1;US2004/0093621A1;US2004/0132140A1;US2004/0110704A1;US2004/0110282A1;US2004/0109865A1;WO03/085119A1;WO03/084570A1;WO2005/035778;WO2005/035586(描述岩藻糖基化的RNA抑制(RNAi));Okazaki等人J.Mol.Biol.336:1239-1249(2004);Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004)。产生去岩藻糖基化抗体的细胞系的实例包括在蛋白质岩藻糖基化方面缺陷的Lec13 CHO细胞(Ripka等人,Arch.Biochem.Biophys.249:533-545(1986);美国专利申请号US 2003/0157108 A1,Presta,L;和WO 2004/056312 A1,Adams等人,特别是在实施例11),和敲除细胞系,诸如α-1,6-岩藻糖基转移酶基因FUT8敲除CHO细胞(Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004))。

[0474] 通过本领域已知的多种方法来制备编码抗体的氨基酸序列变体的核酸分子。这些方法包括但不限于从天然来源分离(在天然存在的氨基酸序列变体的情况中),或者通过对早期制备的变体或非变体形式的抗体进行寡核苷酸介导的(或定点)诱变、PCR诱变和盒式诱变来制备。

[0475] 可能需要在效应子功能方面修饰本发明的抗体,例如以增强抗体的抗体依赖性细胞介导的细胞毒性(ADCC)和/或补体依赖性细胞毒性(CDC)。这可以通过在抗体Fc区中引入

一个或多个氨基酸置换来实现。备选地或另外地，可以在Fc区中引入半脱氨酸残基，从而使得在该区中形成链间二硫键。由此产生的同二聚体抗体可以具有改善的内在化能力和/或增加的补体介导的细胞杀伤和抗体依赖性细胞性细胞毒性(ADCC)。参见Caron等人，J.Exp Med.176:1191-1195(1992)和Shopes,B.J.Immunol.148:2918-2922(1992)。还可以使用如Wolff等人，Cancer Research 53:2560-2565(1993)中描述的异双官能交联剂来制备具有增强的抗肿瘤活性的同二聚体抗体。备选地，抗体可以工程改造为具有双重Fc区，并且因此可以具有增强的补体溶解和ADCC能力。参见Stevenson等人，Anti-Cancer Drug Design 3:219-230(1989)。

[0476] WO00/42072(Presta,L.)描述了在人效应细胞的存在下具有改善的ADCC功能的抗体，其中所述抗体在其Fc区中包含氨基酸置换。在一些实施方案中，具有改善的ADCC的抗体在Fc区的位置298、333和/或334处包含置换。在一些实施方案中，改变的Fc区是人IgG1 Fc区，该Fc区包含一个、两个或三个这些位置处的置换或由其组成。

[0477] WO99/51642、美国专利号6,194,551B1、美国专利号6,242,195B1、美国专利号6,528,624B1和美国专利号6,538,124(Idusogie等人)中描述了具有改变的C1q结合和/或补体依赖性细胞毒性(CDC)的抗体。所述抗体在其Fc区的氨基酸位置270、322、326、327、329、313、333和/或334中的一个或多个处包含氨基酸置换。

[0478] 为了提高抗体的血清半衰期，可以如例如美国专利5,739,277中所描述的，将救援受体(salvage receptor)结合表位并入抗体(尤其是抗体片段)中。如本文所使用的，术语“救援受体结合表位”是指IgG分子(例如IgG_1、IgG_2、IgG_3或IgG_4)的Fc区中负责提高IgG分子体内血清半衰期的表位。WO00/42072(Presta,L.)中也描述了在其Fc区中具有置换且血清半衰期得以提高的抗体。

[0479] 还考虑具有三个或更多个(优选四个)功能性抗原结合位点的工程改造抗体(美国专利申请号US2002/0004587A1,Miller等人)。

[0480] VI.药物制剂

[0481] 通过将具有所需纯度的抗体与任选的药用载体、赋形剂或稳定剂(Remington's Pharmaceutical Sciences,第16版,Osol,A.编著，(1980))混合，以冻干制剂或水溶液的形式制备依照本发明使用的抗体的治疗制剂用以储存。可接受的载体、赋形剂或稳定剂在采用的剂量和浓度下对接受者无毒，并且包括：缓冲剂，诸如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸和甲硫氨酸；防腐剂(诸如十八烷基二甲基苄基氯化铵(octadecyldimethylbenzyl ammonium chloride)；氯化六甲双胺(hexamethonium chloride)；苯扎氯铵(benzalkonium chloride)、苄索氯铵(benzethonium chloride)；苯酚、丁醇或苯甲醇；对羟基苯甲酸烷基酯，诸如对羟基苯甲酸甲酯或丙酯；儿茶酚；间苯二酚(resorcinol)；环己醇；3-戊醇；和间甲酚)；低分子量(少于约10个残基的)多肽；蛋白质，诸如血清白蛋白、明胶或免疫球蛋白；亲水聚合物，诸如聚乙烯吡咯烷酮；氨基酸，诸如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸；单糖类、二糖类和其它糖类包括葡萄糖、甘露糖或糊精；螯合剂，诸如EDTA；糖类，诸如蔗糖、甘露糖、海藻糖或山梨醇；形成盐的反离子，诸如钠；金属复合物(例如，Zn-蛋白质复合物)；和/或非离子表面活性剂，诸如TWEEN™、PLURONICS™或聚乙二醇(PEG)。

[0482] 示例性抗-CD20抗体制剂描述于WO 98/56418中。该出版物描述了pH5.0的液体多剂

量制剂，其包含40mg/mL利妥昔单抗、25mM乙酸盐、150mM海藻糖、0.9％苯甲醇、0.02％聚山梨醇酯20，该制剂在2-8BC具有最少2年的货架期。另一种感兴趣的抗-CD20制剂包含在9.0mg/mL氯化钠、7.35mg/mL柠檬酸钠二水合物、0.7mg/mL聚山梨醇酯80和注射用无菌水中的10mg/mL利妥昔单抗(pH 6.5)。

[0483] 适于皮下施用的冻干制剂描述于美国专利号6,267,958(Andya等人)。这种冻干制剂可以用合适的稀释剂重构至高蛋白质浓度，并且重构的制剂可以皮下施用至本文中待治疗的哺乳动物。

[0484] 还考虑结晶形式的抗体。参见，例如，US 2002/0136719A1(Shenoy等人)。

[0485] 本文中的制剂也可以根据所治疗的特定适应症的需要而含有多于一种活性化合物，在一些实施方案中是具有彼此不产生不利影响的互补活性的那些活性化合物。例如，可能还需要在制剂中提供细胞毒性剂；化疗剂；免疫抑制剂；细胞因子；细胞因子拮抗剂或抗体；生长因子；激素；整联蛋白；整联蛋白拮抗剂或抗体(例如LFA-1抗体，或α4整联蛋白抗体，诸如可从Biogen Idec/Elan Pharmaceuticals,Inc.获得的那他珠单抗/TYSABRI®)；干扰素类药物，诸如IFN-β-1a(REBIF®和AVONEX®)或IFN-β-1b(BETASERON®)；寡肽，诸如醋酸格拉默(COPAXONE®)；细胞毒性剂，诸如米托蒽醌(NOVANTRONE®)、甲氨喋呤、环磷酰胺、苯丁酸氮芥或硫唑嘌呤；静脉内免疫球蛋白(γ球蛋白)；淋巴细胞消耗性药物(例如，米托蒽醌、环磷酰胺、Campath、抗-CD4或克拉屈滨)；非淋巴细胞消耗性免疫抑制药物(例如，麦考酚酸吗乙酯(MMF)或环孢菌素)；“他汀”类降胆固醇药物；雌二醇；睾酮；激素替代疗法；治疗MS继发的或相关的症状(例如，痉挛、失禁、疼痛、疲劳)的药物；TNF抑制剂；减轻疾病的抗风湿药(DMARD)；非甾体抗炎药(NSAID)；皮质类固醇(例如，甲泼尼龙、泼尼松、地塞米松或糖皮质激素)；左甲状腺素；环孢菌素A；促生长素抑制素类似物；细胞因子拮抗剂；抗代谢物；免疫抑制剂；整联蛋白拮抗剂或抗体(例如，LFA-1抗体，诸如依法珠单抗，或α4整联蛋白抗体，诸如那他珠单抗)；或其它B细胞表面拮抗剂/抗体；等。所述其它药剂的类型和有效量取决于例如制剂中存在的抗体的量、所治疗的多发性硬化的类型、以及患者的临床参数。这些通常以如前文所使用的相同剂量以及施用途径使用，或以迄今为止采用的剂量的约1％至99％使用。

[0486] 活性成分还可以包埋于例如分别通过凝聚技术或通过界面聚合制备的微胶囊(例如，羟甲基纤维素微胶囊或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊)、胶质药物递送系统(例如，脂质体、白蛋白微球体、微乳液、纳米粒子和纳米胶囊)或巨乳液(macroemulsion)中。这种技术在Remington's Pharmaceutical Sciences第16版，Osol,A.编著(1980)中公开。

[0487] 可以制备持续释放制备物。持续释放制备物的合适实例包括含有抗体的固体疏水性聚合物的半透性基质，所述基质处于成形制品(例如，薄膜或微胶囊)形式。缓释基质的实例包括聚酯、水凝胶(例如，聚(2-羟乙基-甲基丙烯酸)或聚(乙烯醇))、聚交酯(美国专利号3,773,919)、L-谷氨酸和L-谷氨酸γ乙酯的共聚物、不可降解的乙烯-乙酸乙烯酯、可降解的乳酸-乙醇酸共聚物诸如LUPRON DEPOT™(可注射微球，其由乳酸-乙醇酸共聚物和乙酸亮丙瑞林组成)以及聚-D-(-)-3-羟基丁酸。

[0488] 用于体内施用的制剂必须是无菌的。这可通过借助无菌滤膜的过滤而轻易地实

现。

[0489] 在一些实施方案中，制剂包含由以下组成的组中的一种或多种：组氨酸缓冲液、海藻糖、蔗糖和聚山梨醇酯20。在一些实施方案中，组氨酸缓冲液是组氨酸-乙酸盐缓冲液，pH 6.0。适用于施用抗-CD20抗体的制剂的实例记载于Andya等人，US2006/0088523中，该专利申请中关于制剂的全部内容通过引用结合于此。

[0490] 示例性抗-CD20抗体制剂描述于Andya等人，US2006/0088523和WO98/56418中，其通过引用整体结合。在一些实施方案中，制剂是pH 5.0的液体多剂量制剂，其包含40mg/mL的抗-CD20抗体、25mM乙酸盐、150mM海藻糖、0.9％苯甲醇、0.02％聚山梨醇酯20，该制剂在2-8℃具有最少2年的货架期。在一些实施方案中，抗-CD20制剂包含在9.0mg/mL氯化钠、7.35mg/mL柠檬酸钠二水合物、0.7mg/mL聚山梨醇酯80和注射用无菌水中的10mg/mL抗体(pH 6.5)。在一些实施方案中，抗-CD20抗体在水性药物制剂中，所述水性药物制剂含有约pH 4.8至约pH 5.5(优选pH5.5)的10-30mM乙酸钠、约0.01-0.1％v/v的作为表面活性剂的聚山梨醇酯、量为约2-10％w/v的海藻糖、以及作为防腐剂的苯甲醇(U.S.6,171,586，其通过引用整体结合)。适用于皮下施用的冻干制剂描述于WO97/04801中，其通过引用整体结合。这种冻干制剂可以用合适的稀释剂重构至高蛋白质浓度，并且重构的制剂可以皮下施用至本文中待治疗的哺乳动物。

[0491] 在一些实施方案中，人源化2H7变体制剂是10mM组氨酸、6％蔗糖、0.02％聚山梨醇酯20、pH 5.8中的12-14mg/mL的抗体。在特定实施方案中，2H7变体(并且特别是2H7.v16)在10mM硫酸组氨酸、60mg/ml蔗糖、0.2mg/ml聚山梨醇酯20和注射用无菌水(pH5.8)中配制为20mg/mL抗体。在特定实施方案中，人源化2H7v16的一种IV制剂是：20mM乙酸钠、4％海藻糖二水合物、0.02％聚山梨醇酯20(Tween 20™)、pH 5.3中的30mg/ml抗体。在一些实施方案中，人源化2H7.v511变体制剂是10mM硫酸组氨酸、60mg/ml蔗糖(6％)、0.2mg/ml聚山梨醇酯20(0.02％)和注射用无菌水(pH5.8)中的15-30mg/ml抗体、优选20mg/mL抗体。在又一个实施方案中，2H7变体(并且特别是2H7.v511)的制剂是20mM乙酸钠、4％海藻糖二水合物、0.02％聚山梨醇酯20、pH 5.5中的用于静脉内施用的20mg/ml 2H7。在一些实施方案中，2H7.v114制剂是20mM乙酸钠、240mM(8％)海藻糖二水合物、0.02％聚山梨醇酯20、pH 5.3中的15-25mg/ml(优选20mg/ml)的抗体。在一些实施方案中，抗-CD20抗体(例如2H7.v16)在包含30mg/mL抗体、20mM乙酸钠、106mM海藻糖、0.02％聚山梨醇酯20和pH 5.3的制剂中。含有抗体的液体制剂可以是300mg/小瓶，并且可以在2-8℃避光储存。在一些实施方案中，在施用之前，将抗体制剂在IV袋中稀释于生理盐水(0.9％氯化钠)，用于通过输注施用。

[0492] VII.制品或试剂盒

[0493] 本发明还提供了含有可用于治疗本文描述的进行性多发性硬化的物质的制品或试剂盒(诸如多个部分的试剂盒)。在一些实施方案中，制品包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有多发性硬化的患者并且提供多发性硬化患者的功能能力的改善。

[0494] 在一些实施方案中，制品或试剂盒包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有多发性硬化的患者并且抑制多发性硬化患者的能力丧失进展。

[0495] 在一些实施方案中，制品或试剂盒包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有多发性硬化的患者并且延迟多发性硬化患者中确认的能力丧失进展的发病。在一些实施方案中，确认的疾病进展是持续12周的EDSS增加。在一些实施方案中，确认的疾病进展是持续24周的EDSS增加。

[0496] 在一些实施方案中，制品或试剂盒包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有多发性硬化的患者并且减缓或阻止多发性硬化患者中的脑体积损失。

[0497] 在一些实施方案中，制品或试剂盒包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有高度活跃的多发性硬化的患者。在一些实施方案中，标签还指示抗-CD20抗体或药物组合物适用于治疗患有高度活跃的多发性硬化的患者，所述患者先前未使用其它多发性硬化疗法进行治疗。在一些实施方案中，标签还指示抗-CD20抗体或药物组合物适用于治疗患有高度活跃的多发性硬化的患者，所述患者先前已使用其它多发性硬化疗法进行治疗。在一些实施方案中，标签还指示抗-CD20抗体或药物组合物适用于治疗患有高度活跃的多发性硬化的患者，所述患者为对其它多发性硬化疗法的不充分应答者。

[0498] 在一些实施方案中，制品或试剂盒包括包装在一起的药物组合物和标签，所述药物组合物包含抗-CD20抗体和药用载体，所述标签指示所述抗-CD20抗体或药物组合物适用于治疗患有多发性硬化的患者，并且所述抗-CD20抗体或药物组合物在以下一个或多个方面有效：(1)减少患者脑损伤的数量；(2)降低年复发率；(3)降低能力丧失进展；和(4)改善功能能力。

[0499] 在任一种制品或试剂盒的一些实施方案中，多发性硬化是进行性多发性硬化。在某些实施方案中，进行性多发性硬化是原发进行性多发性硬化。在一些实施方案中，多发性硬化是复发型多发性硬化。在某些实施方案中，多发性硬化是复发缓和型多发性硬化。在某些实施方案中，复发型多发性硬化是具有重叠复发的继发进行性多发性硬化(rSPMS)。

[0500] 在任一种制品或试剂盒的一些实施方案中，抗-CD20抗体包含：a)包含SEQ ID NO:10、SEQ ID NO:11和SEQ ID NO:12的重链可变区；和b)包含SEQ ID NO:4、SEQ ID NO:5和SEQ ID NO:6的轻链可变区。在一些情况中，抗-CD20抗体是ocrelizumab。

[0501] 在某些实施方案中，包装插页具有说明书，所述说明书指示(即显示)向所述患者施用有效量的ocrelizumab以提供约0.3克至约0.6克的初次ocrelizumab暴露，随后是约0.3克至约0.6克的第二次ocrelizumab暴露，其中直到距初次暴露约16至60周时才施用第二次暴露，并且每次ocrelizumab暴露作为一个或两个剂量的ocrelizumab提供给患者。在一些实施方案中，初次ocrelizumab暴露为约0.6克。在一些实施方案中，第二次ocrelizumab暴露为约0.6克。在一些实施方案中，从距初次暴露约20-24周来施用第二次暴露。在一些实施方案中，“约20-24周”是指20周至24周之间的时间点。在一些实施方案中，“约20-24周”是指第24周之前或之后1周或7天的变动。在一些实施方案中，一次或多次ocrelizumab暴露作为一个剂量的ocrelizumab提供给患者。在一些实施方案中，一次或多次ocrelizumab暴露作为两个剂量的ocrelizumab提供给患者。在一些实施方案中，两个剂量的ocrelizumab包含约0.3克的ocrelizumab。

[0502] 在某些实施方案中，制品或试剂盒包括容器和在所述容器上或与所述容器相结合的标签或包装插页。合适的容器包括，例如，瓶、小药瓶、注射器等。容器可以由多种材料诸如玻璃或塑料制成。所述容器容纳或含有对治疗多发性硬化有效的组合物，并且可以具有无菌接入口(例如，所述容器可以是静脉内输液袋或是具有可由皮下注射针头刺透的瓶塞的小药瓶)。组合物中的至少一种活性剂是抗体。在一些实施方案中，容器包含约0.3克至约4.0克的抗-CD20抗体。在一些实施方案中，容器包含约0.3克至约1.5克的抗-CD20抗体。

[0503] 所述标签或包装插页表明组合物用于治疗患有多发性硬化的患者中的多发性硬化，以及有关抗体和所提供的任何其它药物的给药量和间隔的具体指导。制品还可以含有第二容器，其包含药用稀释缓冲液，诸如注射用抑菌水(BWFI)、磷酸盐缓冲盐水、林格氏(Ringer)溶液和葡萄糖溶液。制品还可以包括从商业和用户观点来看所需的其它材料，包括其它缓冲液、稀释剂、滤器、针和注射器。

[0504] 任选地，本文提供的制品或试剂盒还包括含有除抗体以外的用于治疗的药剂的容器，并且还包括用所述药剂治疗患者的说明书，所述药剂优选地为化疗剂或免疫抑制剂，干扰素类药物，诸如IFN-β-1a(REBIF®和AVONEX®)或IFN-β-1b(BETASERON®)；寡肽，诸如醋酸格拉默(COPAXONE®)；细胞毒性剂，诸如米托蒽醌(NOVANTRONE®)、甲氨喋呤、环磷酰胺、苯丁酸氮芥或硫唑嘌呤；静脉内免疫球蛋白(γ球蛋白)；淋巴细胞消耗性药物(例如，米托蒽醌、环磷酰胺、Campath、抗-CD4或克拉屈滨)；非淋巴细胞-消耗性免疫抑制药物(例如，麦考酚酸吗乙酯(MMF)或环孢菌素)；“他汀”类降-胆固醇药物；雌二醇；激素替代疗法；治疗MS继发的或相关的症状(例如，痉挛、失禁、疼痛、疲劳)的药物；TNF抑制剂；减轻疾病的抗风湿药(DMARD)；非甾体抗炎药(NSAID)；皮质类固醇(例如，甲泼尼龙、泼尼松、地塞米松或糖皮质激素)；左甲状腺素；环孢菌素A；促生长素抑制素类似物；细胞因子或细胞因子受体拮抗剂；抗代谢物；免疫抑制剂；整联蛋白拮抗剂或抗体(例如，LFA-1抗体，诸如依法珠单抗，或α4整联蛋白抗体，诸如那他珠单抗)；和其它B细胞表面标记抗体；等。

实施例

[0505] 实施例1：在患有复发型多发性硬化的患者中Ocrelizumab相比于干扰素β-1α(Rebif)的III期研究

[0506] 多发性硬化是具有不可预期的病程且无法治愈的异质性疾病(Scalfari等人，(2013)JAMA Neurol.70,214-22；Tremlett等人，(2006)Neurology.66,172-7；Markowitz(2010)Am JManag Care.16,S211-8；Hauser等人，(2013)Ann Neurol.74,317-27)。尽管近年来对复发型多发性硬化症进行了各种治疗，但是许多患者继续累积神经能力丧失，因此仍然存在对更有效且耐受性良好的治疗的重要的、未满足的需求(Markowitz(2010)Am JManag Care.16,S211-8；Rotstein等人，(2015)JAMA Neurol.72,152-8；Sorensen(2007)J Neurol Sci.259,128-32)。另外，迄今为止，较高功效治疗的风险状况阻止了它们在病程早期的使用(Markowitz(2010)Am JManag Care.16,S211-8；Hartung等人，(2011)Expert Rev Neurother.11,351-62；Hauser SL.(2015)Mult Scler.21,8-21)。

[0507] B细胞是多发性硬化症发病的关键因素(Monson(2005)J Neuroimmunol.158,170-81；Hauser SL.(2015)Mult Scler.21,8-21)。在健康对照的脑脊液中很少观察到B细胞，但是其在多发性硬化患者的脑脊液中经常以低百分比发现(Cepok(2005)Brain.128,1667-

76;Cross等人,(2011)Biochim Biophys Acta.1812,231-8),脑脊液中的水平升高与复发缓和型多发性硬化和继发进行性多发性硬化中更快的疾病进展有关(Cepok(2005)Brain.128,1667-76)。B细胞通过许多功能影响多发性硬化的潜在发病机制:抗原呈递(Constant(1999)J Immunol.162,5695-703;Crawford等人,(2006)J Immunol.176,3498-506),autoantibody production(Bar-Or A(2010)Ann Neurol.67,452-61;Duddy(2007)J Immunol.178,6092-9),cytokine regulation(Genain等人(1999)Nat Med.5,170-5;Storch等人(1998)Ann Neurol.43,465-71),以及形成异位淋巴滤泡样聚集体(Magliozzi等人,(2010)Ann Neurol.68,477-93;Serafini等人,(2004)Brain Pathol.14:164-74。对B细胞的兴趣随着概念验证(proof-of-concept)和观察研究而增加,并且对其在多发性硬化中的利用的兴趣随着时间而发展(Hauser等人,(2008)N Engl J Med.358,676-88;Kappos等人,(2011)Lancet.378,1779-87;Lehmann-Horn等人,(2013)Ther Adv Neurol Disord.6,161-73)。

[0508] CD20是在前B细胞(pre-B cell)、成熟B细胞和记忆B细胞上发现的细胞表面抗原,但是其不在淋巴干细胞和浆细胞上表达(Stashenko等人,(1980)J Immunol.125,1678-85;Loken等人,(1987)Blood.70,1316-24;Tedder等人,(1994)Immunol Today.15,450-4)。在HERMES研究中,与安慰剂相比,抗-CD20嵌合单克隆抗体利妥昔单抗在患有复发缓和型多发性硬化的患者中显著减少了炎性脑损伤和临床复发;因此,这为选择性消耗$CD20^+$B细胞是多发性硬化中潜在有效的治疗方法提供了证据(Kappos等人,(2011)Lancet.378,1779-87)。

[0509] Ocrelizumab是重组人源化单克隆抗体,其选择性地消耗表达CD20的B细胞(Klein等人,(2013)MAbs.5,337-8;Genovese等人,(2008)Arthritis Rheum.58,2652-61),同时保留B细胞重建能力和预先存在的体液免疫(Martin等人,(2006)Annu Rev Immunol.24,467-96;DiLillo等人,(2008)J Immunol.180,361-71)。Ocrelizumab以高亲和力与CD20的大胞外环结合,通过几种机制选择性地消耗B细胞,所述机制包括抗体依赖性细胞介导的吞噬作用、抗体依赖性细胞介导的细胞毒性、补体依赖性细胞毒性和诱导细胞凋亡(Klein等人,(2013)MAbs.5,22-33)。

[0510] 进行两项完全相同的3期、多中心、随机、双盲、双模拟、平行组试验(研究I和研究II),以研究与干扰素(IFN)β-1a相比,ocrelizumab在患有复发型多发性硬化的患者中的功效和安全性。本文中报告了这两项研究的结果。

[0511] 方法

[0512] 合格和排除标准

[0513] 关键的合格标准包括:年龄为18至55岁;依照2010年修订的McDonald标准(Polman等人,(2011)"Diagnostic criteria for multiple sclerosis:2010 revisions to the McDonald criteria."Ann Neurol 69,292-302)诊断为多发性硬化;筛选时扩展的能力丧失状态量表(EDSS,参见world-wide-web.neurostatus.org)评分为0至5.5;在先前2年内具有至少两次有记录的临床发作,或者在筛选前1年内(但是不在筛选前30天内)出现一次临床发作;具有存在与多发性硬化一致的异常的有记录的脑MRI;在筛选和基线两者之前神经稳定性达至少30天。

[0514] 关键的排除标准包括:诊断为原发进行性多发性硬化;患者具有多于10年的病程

连同筛选时小于或等于2.0的EDSS评分;已知存在可以模仿多发性硬化的其它神经病症;妊娠或哺乳期;先前用靶向B细胞的疗法或其它禁忌药物进行治疗(即,研究过程中需要用系统性皮质类固醇或免疫抑制剂进行慢性治疗,具有原发性或继发性免疫缺陷的历史或目前活跃的原发性或继发性免疫缺陷,活动性感染,或者具有复发性或慢性感染(例如,乙型肝炎或丙型肝炎、HIV、梅毒、结核病)的历史或已知存在该复发性或慢性感染,具有进行性多灶性脑白质病的历史,禁忌或不耐受口服或静脉内皮质类固醇,或者禁忌Rebif或与Rebif使用不相容)。

[0515] 研究设计

[0516] 将患者随机化(1:1)以每24周通过静脉内输注接受ocrelizumab 600mg(对于第一剂量在第1天和第15天作为两次300-mg输注施用,并且以后对于每个24周治疗在第1天作为单次600mg输注施用),或者在整个96周治疗期内以44μg的剂量每周三次接受皮下IFN β-1a。参见图7。ocrelizumab组和IFN β-1a组的患者也分别接受皮下和静脉内安慰剂。所有患者在输注前均接受静脉内甲泼尼龙100mg(以及任选的镇痛药/解热药和抗组胺药)。随机化由独立供应商集中进行。患者按地区(美国/世界其它地区)和基线EDSS评分(小于4/大于或等于4)分层。

[0517] 为了维持研究组分配的隐蔽性,每个研究中心都具有独立的治疗研究者(在多发性硬化监护中有经验的神经病科医师)和检查研究者(神经病科医师或其它医疗从业者),他们在整个试验过程中全部是不知情的。治疗研究者可获得安全性和盲法功效数据,并且基于患者的临床反应和实验室检查作出治疗决定。检查研究者进行神经病学评价,包括:EDSS评分(Kurtze(1983)"Rating neurologic impairment in multiple sclerosis:an expanded disability status scale(EDSS)."Neurology.33,1444-52),功能系统评分(Kurtze(1983)"Rating neurologic impairment in multiple sclerosis:an expanded disability status scale(EDSS)."Neurology.33,1444-52;Haber和LaRocca编著Minimal Record of Disability for multiple sclerosis.New York:National Multiple Sclerosis Society;1985),多发性硬化功能综合(Multiple Sclerosis Functional Composite,MSFC)(Rudick等人,(2002)"The multiple sclerosis functional composite:anew clinical outcome measure for multiple sclerosis trials."Multiple sclerosis(Houndmills,Basingstoke,England)8,359-65),低对比度视敏度(Low Contrast Visual Acuity,LCVA)测试(Wieder等人,(2013)"Low contrast visual acuity testing is associated with cognitive performance in multiple sclerosis:apilot study."BMC Neurology.13,167),符号数字模式测试(Symbol Digit Modalities Test,SDMT)(Smith A.(1982).Symbol digit modalities test:Manual.Los Angeles:Western Psychological Services),和卡氏机能状态量表(Karnofsky Performance Status Scale)(Mor等人,"The Karnofsky Performance Status Scale.An examination of its reliability and validity in a research setting."Cancer.53.2002-2007)。MRI评价由对于治疗分配为盲法的中央MRI阅读器独立地进行分析。

[0518] 完成96周治疗期的患者有资格进入研究的开放标签(open-label)延长阶段。过早中断或不想参与开放标签延长阶段的患者进入48周安全性随访阶段,其中包括B细胞监测。

[0519] 研究过程

[0520] 在筛选、基线和每12周来确定EDSS评分;在基线和每12周来确定MSFC、LCVA和SDMT评分;并且在基线和每24周来确定卡氏机能状态量表。在基线以及第24周、第48周和第96周进行MRI。在整个研究中监测不良事件。

[0521] 主要终点是96周时方案定义的年复发率,其中复发被定义为仅由多发性硬化引起的持续超过24小时的新的或恶化的神经症状。新的或恶化的神经症状必然伴随着与以下一致的客观神经恶化:EDSS增加至少半级,一项EDSS功能系统评分增加2分,或两项以上EDSS功能系统评分每一个都增加1分。按照上述规则,通过自动算法将复发重新分类为方案定义的复发。该算法在数据库关闭和数据解盲之前编写。

[0522] 关键的次要终点包括:12周确认的能力丧失进展(即CDP)的发病时间,研究中将其定义为在96周期间,基线评分为5.5以下的患者中从基线EDSS评分增加至少1.0分,或者基线评分高于5.5的患者中增加0.5分,其中在最初的神经恶化后至少12周以有规律的定期访问方式确认EDSS的增加;第24周、第48周和第96周时T1钆增强性损伤的总数;第24周、第48周和第96周时新的和/或扩大的T2高强度损伤的总数;96周中具有12周确认的能力丧失改善(即CDI)的患者的比例(仅分析基线EDSS评分为至少2.0的患者的亚组);在96周中,24周CDP的发病时间,最初的神经恶化后24周确认的24周CDP的发病时间;ocrelizumab的药代动力学、免疫原性和药效学;以及ocrelizumab的安全性和耐受性。研究中的CDI被定义为与基线相比,基线EDSS评分为5.5以下的患者中EDSS评分降低至少1.0,或者基线EDSS评分高于5.5的患者中EDSS评分降低0.5分。

[0523] EDSS评分≥2.0的患者到第96周无疾病活动迹象(NEDA)的比例;所有患者中的NEDA分析为探索性终点。如从第24周到第96周通过脑MRI检测的脑体积的百分比变化;从基线到第96周的分析也作为探索性分析进行。

[0524] 统计学分析

[0525] 图8中提供了统计学层级结构。为了获得足够的统计效力来测试ocrelizumab对CDP和CDI的作用,预先指定这些终点将被合并用于这两项III期研究。对于每项研究将分别地分析所有其它终点。对意向性治疗人群进行主要和次要功效分析。

[0526] 对意向性治疗人群进行所有功效分析。使用负二项模型分析作为主要功效终点的年复发率(ARR),所述负二项模型对于每个患者包括统计学分析中随机化数据与早期治疗-停药/第96周的日期之间的发病以考虑暴露长度,并且治疗组、地区(美国/世界其它地区)和基线EDSS评分(小于4.0/大于或等于4.0)作为协变量。双侧$\alpha<0.05$的显著结果表明与IFN β-1a相比,ocrelizumab在降低ARR方面具有优异效果。

[0527] 每项研究的样本量均基于:ocrelizumab组估计的年复发率为0.165,并且IFN β-1a组为0.33。使用双侧t检验,计算出每组400名患者的样本将提供84%的统计效力以维持0.05的I型错误率,并且检测相比于IFNβ-1a,对于ocrelizumab的相对减少为50%(假设退出率(drop-out rate)为大约20%)。符合方案的(per-protocol)灵敏度分析评价了重大方案违规对主要终点的影响。

[0528] 以临床重要性降低的层级顺序(双侧α为0.05)测试了十个次要功效终点(参见图8;ARR=年复发率;CDI=确认的能力丧失改善;CDP=确认的能力丧失进展;Gd=钆;MSFC=多发性硬化功能综合;NEDA=无疾病活动迹象;SF-36PCS=简式-36健康要素总评)。NEDA被

定义为:没有方案定义的复发,没有CDP事件,没有新的或扩大的T2损伤,以及没有Gd增强性T1损伤。个体研究水平的次要功效终点的分析如下:

[0529] ●对于第一个次要功效终点(确认的能力丧失进展达至少12周的发病时间),研究水平p值将解释为非验证性的,因为研究水平的统计效力不足以检测相关治疗差异。

[0530] ●当且仅当在组合的两项研究的分析中第一个次要功效终点达到0.05的显著性水平(即,合并分析p≤0.05),第二个次要功效终点(第24周、第48周和第96周时T1 Gd增强性损伤的总数)才将以验证性方式测试。如果在组合研究的分析中,第一个次要功效终点合并分析p>0.05,则层级内的第二个和随后的次要功效终点p值将被解释为非验证性的。

[0531] ●当且仅当第二个次要功效终点(第24周、第48周和第96周时T1 Gd增强性损伤的总数)达到0.05的显著性水平(即,p≤0.05),第三个次要功效终点(第24周、第48周和第96周时新的和/或扩大的T2高强度损伤的总数)才将以验证性方式测试。如果第二个次要功效终点p>0.05,则层级内的第三个和随后的次要功效终点p值将被解释为非验证性的。

[0532] ●对于第四个(具有确认的能力丧失改善达至少12周的患者的比例)和第五个(确认的能力丧失进展达至少24周的发病时间)次要功效终点,研究水平p值将解释为非验证性的,因为研究水平的统计效力不足以检测相关治疗差异。

[0533] ●当且仅当在组合研究的分析中第五个次要功效终点(确认的能力丧失进展达至少12周的发病时间)达到0.05的显著性水平(即,合并分析p≤0.05),第六个次要功效终点(第24周、第48周和第96周时新T1低强度损伤(慢性黑洞)的总数)才将以验证性方式测试。如果在组合的两项研究的分析中,第五个次要功效终点合并分析p>0.05,则层级内的第六个和随后的次要功效终点p值将被解释为非验证性的。

[0534] ●当且仅当第六个(或紧前面的)次要功效终点达到0.05的显著性水平(即,p≤0.05),第七个(和随后的)次要功效终点才将以验证性方式测试。如果第六个(或紧前面的)次要功效终点p>0.05,则层级内的第七个(或当前的)和所有随后的次要功效终点p值将被解释为非验证性的。

[0535] 此外,对于其中组合两项研究数据的次要功效终点的分析(因此所有主要和次要疗效终点比较均具有足够的统计效力):

[0536] ●当且仅当主要功效终点(2年为止的方案定义的年复发率)达到0.05的显著性水平(即,合并分析p≤0.05),第一个次要功效终点(确认的能力丧失进展达至少12周的发病时间)才将以验证性方式测试。如果主要功效终点合并分析p>0.05,则层级内的所有次要功效终点合并分析p值将被解释为非验证性的。

[0537] ●当且仅当第一个(或紧前面的)次要功效终点达到0.05的显著性水平(即,合并分析p≤0.05),第二个(和随后的)次要功效终点才将以验证性方式测试。如果第一个(或紧前面的)次要功效终点合并分析p>0.05,则层级内的第二个(或当前的)和所有随后的次要功效终点合并分析p值将被解释为非验证性的。

[0538] 安全性人群用于所有安全性数据分析,并且包括已经接受任何研究治疗的所有患者。

[0539] 结果

[0540] 患者

[0541] 总体而言,1656名患者入选了第一项(N=821)和第二项(N=835)III期研究(意向

治疗人群)。基线人口统计学和疾病特征在每项研究和两项研究之间的人群中相似(参见表3)。

[0542]

表 3. 基线人口统计学和疾病特征

特征	研究 I		研究 II	
	IFN β-1a 44 µg (n=411)	Ocrelizumab 600 mg (n=410)	IFN β-1a 44 µg (n=418)	Ocrelizumab 600 mg (n=417)
年龄，岁，平均值(SD)	36.9 (9.3)	37.1 (9.3)	37.4 (9.0)	37.2 (9.1)
女性，n (%)	272 (66.2)	270 (65.9)	280 (67.0)	271 (65.0)
发病以来的时间，年，平均值(SD)	6.3 (6.0)	6.7 (6.4)	6.7 (6.1)	6.7 (6.1)
诊断以来的时间，年，平均值(SD)	3.7 (3.6)	3.8 (4.8)	4.1 (5.1)	4.2 (5.0)
在前 12 个月复发，平均值(SD)	1.3 (0.6)	1.3 (0.7)	1.3 (0.7)	1.3 (0.7)
先前未经治疗，数量(%)*	292 (71.4)	301 (73.8)	314 (75.3)	304 (72.9)
EDSS 平均分 Mean score on EDSS	2.8 (1.3)	2.9 (1.2)	2.8 (1.4)	2.8 (1.3)
T1 钆增强性损伤 - 数量(%)				
0	252 (61.9)	233 (57.5)	243 (58.6)	252 (61.0)
1	52 (12.8)	64 (15.8)	62 (14.9)	58 (14.0)
2	30 (7.4)	30 (7.4)	38 (9.2)	33 (8.0)
3	16 (3.9)	16 (3.9)	14 (3.4)	15 (3.6)
≥4	57 (14.0)	57 (14.0)	58 (14.0)	55 (13.3)

[0543]

T2 损伤 – 数量	51.06±39.91	51.04±39.00	51.01±35.69	49.26±38.59
T2 损伤的体积 - cm^3	9.74±11.28	10.84±13.90	10.61±12.30	10.73±14.28
标准化的脑体积 – cm^3	1499.18±87.68	1500.93±84.10	1501±90.98	1503±92.63
不具有 Gd^+ 损伤的患者，n (%)	252 (61.9)	233 (57.5)	243 (58.6)	252 (61.0)
具有 Gd^+ 损伤的患者，n (%)	155 (38.1)	172 (42.5)	172 (41.4)	161 (39.0)
Gd^+ T1 损伤数量，平均值(SD)	1.9 (5.2)	1.7 (4.2)	2.0 (4.9)	1.8 (5.0)
T2 损伤数量，平均值(SD)	51.1 (39.9)	51.0 (39.0)	51.0 (35.7)	49.3 (38.6)

† 在进入研究之前 2 年中未用减轻疾病的疗法治疗的患者

[0544] ocrelizumab组和IFN β-1a组中总计366名(89%)和340名(83%)患者分别完成了第一项研究(参见图9),并且ocrelizumab组和IFN β-1a组中360名(86%)和320名(77%)进

行了第二项研究(参见图9)。ocrelizumab组中超过85%的患者完成了研究I和研究II。所有随机患者均被纳入ITT人群。ITT(意向治疗)分析仍包括因任何原因过早退出研究的患者以及因任何原因未进行评价的患者。

[0545] 功效

[0546] 来自第一项和第二项研究的临床和MRI结果总结于表4中。在两项研究中ocrelizumab降低了多发性硬化的复发频率,调整的96周的年复发率(ARR)(主要终点)在研究I中对于ocrelizumab为0.156(相对于IFNβ-1a的0.292)并且在研究I中对于ocrelizumab为0.155(相对于IFNβ-1a的0.290)(参见表4和图10A和10B)。在研究I和研究II中,相对于IFN β-1,用ocrelizumab治疗的患者中年复发率(基于方案定义的复发)分别降低了46%和47%(对于两种比较,p<0.0001)。相对于IFN β-1,用ocrelizumab治疗的患者中年复发率(基于方案定义的复发)降低了46%(研究I和研究II,合并数据;p=0.0001)。通过二项回归计算调整的ARR,并且针对基线EDSS评分(<4.0比上≥4.0)和地理位置(美国比上世界其它地区)调整。在研究I和研究II中,相对于IFN β-1,用ocrelizumab治疗的患者中年复发率(基于所有临床复发)分别降低了42%(p=0.001)和47%(p<0.0001)。

[0547]

*表 4. 96 周研究期间临床和 MRI 终点(ITT 人群)**

终点	合并的研究 I 和研究 II		研究 I		研究 II	
	IFN β-1a 44 μg (n=829)	Ocrelizumab 600 mg (n=827)	IFN β-1a 44 μg (n=411)	Ocrelizumab 600 mg (n=410)	IFN β-1a 44 μg (n=418)	Ocrelizumab 600 mg (n=417)
临床终点						
96 周的年复发率			0.292	0.156	0.290	0.155
复发率(95% CI)			(0.235-0.361)	(0.122-0.200)	(0.234-0.361)	(0.121-0.198)
风险降低，相比于 IFN β-1a – % (95% CI；p 值)		46 (p<0.0001)		46 (p<0.0001)		47 (p<0.0001)
确认的能力度失进展达至少 12 周[†]						
具有事件的患者 – %	**13.6**	**9.1**				
危险比，相比于 IFN β-1a (95% CI)		**0.60 (0.45–0.81)**		**0.57 (0.37–0.90)**		**0.63 (0.42–0.92)**
风险降低，相比于 IFN β-1a – % (p 值)		**40 (p=0.0006)**		**43 (p=0.0139)**		**37 (p<0.0169)**

[0548]

确认的能力丧失进展达至少 24 周[1]						
具有事件的患者 – %	10.5	6.9				
危险比，相比于 IFN β-1a (95% CI)		0.60 (0.43–0.84)		0.57 (0.34–0.95)		0.63 (0.40–0.98)
风险降低，相比于 IFN β-1a – % (p 值)		40 (p=0.0025)		43 (p=0.0278)		37 (p=0.0370)
确认的能力丧失改善达至少 12 周[1,3]						
评价的患者的数量	614	628				
具有持续 12 周的改善的患者 – %	15.6	20.7				
相对改善，相比于 IFN β-1a – % (p 值)		33 (p=0.0194)		61 (p=0.0106)		14 (p=0.4019)
多发性硬化功能综合评分						
从基线到第 96 周的 MSFC 评分的平均变化			0.174±0.031	0.213±0.031)	0.169±0.029	0.276±0.028
调整平均值的差异(95% CI; p 值)				0.039±0.039 (-0.039-0.166; p = 0.3261)		0.107±0.037 (0.034-0.180; p=0040)
到第 96 周的 NEDA-3[3]						
评价的患者的数量			291	289	270	289

[0549]

达到 NEDA 的患者 – % 相对改善，相比于 IFN β-1a – % (p 值)			27.1	47.4 74 (p<0.0001)	24.1	43.9 81 (p<0.0001)
到第 96 周的 NEDA 要素[4,8] – %						
患者无复发			66.7	80.4	64.3	78.9
患者无确认的能力丧失进展			87.8	92.4	84.9	89.4
患者无 T1 钆增强性损伤			69.8	91.7	63.9	90.2
患者无新的和/或扩大的 T2 高强度损伤			38.7	61.7	38.0	60.9
MRI 终点						
第 24 周、第 48 周和第 96 周时 T1 钆增强性损伤[9]						
患者具有任何 T1 钆增强性损伤			30.2	8.3	36.1	9.8
损伤的平均数量(95% CI)			0.286 (0.200–0.409)	0.016 (0.0009–0.030)	0.416 (0.309–0.561)	0.021 (0.012–0.036)
风险降低，相比于 IFN β-1a – % (p 值)				94 (p<0.0001)		95 (p<0.0001)

[0550]

第 24 周、第 48 周和第 96 周时新的和/或扩大的 T2 高强度损伤[*]						
患者具有任何新的和/或扩大的 T2 高强度损伤 – %	61.3	38.3	62.0	39.1		
损伤的平均数量(95% CI)	1.413 (1.123–1.777)	0.323 (0.256–0.407)	1.904 (1.536–2.359)	0.325 (0.259–0.409)		
比率(95% CI)		0.229 (0.174–0.300)		0.171 (0.130–0.225)		
风险降低，相比于 IFN β-1a – % (p 值)		77 (P<0.0001)		83 (P<0.0001)		
第 24 周、第 48 周和第 96 周时 T1 低强度损伤[*]						
损伤的平均数量(95% CI)	0.982 (0.780–1.237)	0.420 (0.337–0.524)	1.255 (1.003–1.571)	0.449 (0.357–0.560)		
比率(95% CI)		0.428 (0.328–0.557)		0.357 (0.272–0.470)		
风险降低，相比于 IFN β-1a – % (p 值)		57 (P<0.0001)		64 (P<0.0001)		
来自第 24 周至第 96 周的脑体积						

[0551]

平均变化			-0.741±0.046	-0.572±0.044	-0.750±0.051	0.638±0.049
p 值				P=0.0042		P=0.0900
调整平均值的平均差异(95% CI)				0.168 (0.053–0.283)		0.112 (-0.018–0.241)
从基线到第 96 周的脑体积**						
平均百分比变化			-1.233±0.055	-0.943±0.054	-1.354±0.064	-1.032±0.062
p 值				p<0.0001		p<0.0001
调整平均值的平均差异(95% CI)				0.290 (0.148–0.432)		0.322 (0.156–0.488)
患者报告结果						
从基线到第 96 周的简式-36 健康要素总评						
平均值			45.399±0.529	45.065±0.507	44.552±0.544	44.307±0.541
p 值				P=0.2193		P=0.0404
调整平均值的差异				0.693 (-0.414–1.800)		1.159 (0.051–2.269)

*正负值是平均值±标准偏差

†粗体文本表示预先指定的合并终点

‡在基线 EDSS 评分至少 2.0 的患者中

[0552] §NEDA 被定义为：没有方案定义的复发，没有 CDP 事件，没有新的或扩大的 T2 损伤，以及没有轮增强性损伤通过基线损伤计数、EDSS(≤4.0/≥4.0)和地理区域(US/ROW)进行调整

[0553] 能力丧头

[0554] 与IFN β-1a相比，在96周研究期间，ocrelizumab使持续12周的确认的能力丧失进展(爱，CDP)的风险降低40％(研究I和研究II，合并数据；P＝0.0006；图11，CI＝置信区间；HR风险比)。图12A(研究I)和图12B(研究II)中显示了研究I和研究II的非合并数据，其表明与IFN β-1a相比，ocrelizumab降低了持续12周的确认的能力丧失进展(即，CDP)的风险。在96周研究期间，相对于IFN β-1a，ocrelizumab还使持续24周的CDP的风险降低40％(研究I

和研究II,合并数据;p=0.0025;图13)。图14A和图14B中显示了研究I和研究II的非合并数据,其表明与IFN β-1a相比,ocrelizumab降低了持续24周的确认的能力丧失进展(即,CDP)的风险。具有确认的能力丧失改善(即,CDI)达至少12周的患者(基线EDSS评分为2.0以上)在接受ocrelizumab的患者(n=628)中的比例为20.7%,而在接受IFN β-1a的患者(n=614)中的比例为15.6%,这表明使用ocrelizumab的风险改善为33%(研究I和研究II,合并数据;p=0.0194)。参见图15A。具有确认的能力丧失改善(即,CDI)达至少12周的患者(基线EDSS评分为2.0以上)在接受ocrelizumab的患者(n=628)中的比例为15.6%,而在接受IFN β-1a的患者(n=614)中的比例为11.6%,这表明使用ocrelizumab的风险改善为36%(研究I和研究II,合并数据;p=0.0343)。参见图15B。对于基线EDSS评分≥2.0且≤5.5的患者,能力丧失改善被定义为与基线EDSS评分相比,EDSS评分减少≥1.0分。对于基线EDSS评分>5.5的患者,能力丧失改善被定义为EDSS评分减少≥0.5分,相对改善的p值来自Cochran-Mantel-Haenszel卡方检验,按研究、基线EDSS评分(<4.0比上≥4.0)和地理区域(美国比上世界其它地区)分层,并且包括分层因子。缺少EDSS或在能力丧失改善发作后未确诊的患者被计为不具有CDI。

[0555] 图16A和图16B中提供了在研究I和研究II中具有确认的能力丧失改善达至少12周的患者(基线EDSS评分为2.0以上)的比例的非合并数据。图16C和图16D中提供了多发性硬化功能综合评分结果的非合并数据。图16C和图16D包括具有基线处的评估和至少一个基线后值的患者。估计值来自基于重复测量的混合效应模型(MMRM)的分析,使用非结构化协方差矩阵:变化=基线MSFCS评分+地理区域(美国比上世界其它地区)+基线EDSS(<4.0比上>=4.0)+周+治疗+治疗*周(随周的重复值)+基线MSFCS评分*周。15.6%的ocrelizumab治疗的患者在24周时达到CDI(而IFNβ-1a治疗的患者为11.6%),这表示36%的相对改善(相对风险1.36[p=0.0343])。

[0556] 在合并分析中,与用IFNβ-1a治疗的患者(15.0%[n=98])相比,用ocrelizumab治疗的患者具有提高的EDSS评分的比例更高(20.2%[n=146])(调整让步比(odds ratio)[aOR]1.288[0.964,1.72];p=0.0866])。在合并分析中,在ocrelizumab组中显著更少的患者具有恶化的EDSS评分(10.1%[n=73],与使用IFNβ-1a相比(16.6%[n=109])(aOR 0.575[0.414-0.797],p=0.0009)。参见图39。

[0557] 在研究I中,相比于接受IFN β-1a的患者((p=0.0242)),恶化的能力丧失(测量为与基线相比,第96周时EDSS评分增加>0.5)在ocrelizumab治疗的患者中降低44%,并且在研究II中,相比于接受IFN β-1a的患者(p=0.121),恶化的能力丧失在ocrelizumab治疗的患者中降低44%。(恶化相对减少的p值来自多项式对数回归(logistic regression),针对基线EDSS评分(<4.0比上≥4.0)以及地理区域(美国vs世界其它地区)调整。相比于接受IFN IFN β-1a的患者,恶化的能力丧失(测量为与基线相比,第96周时EDSS评分增加>0.5)在ocrelizumab治疗的患者中降低43%(研究I和研究II,合并数据;p=0.0009)。

[0558] MRI相关终点

[0559] 与IFN β-1a相比,ocrelizumab在第24周、第48周和第96周使T1钆增强性损伤的总数减少94%(在研究I中)以及95%(在研究II中)(对于两项研究,p<0.0001;参见表4和图17A和17B)。在研究I中,与IFN β-1a相比,ocrelizumab使T1钆增强性损伤的平均总数在第24周减少91%,在第48周减少98%,并且在第96周减少95%(对于所有时间点,p<0.0001,

参见图18A)。在研究II中,与IFN β-1a相比,ocrelizumab使T1钆增强性损伤的平均总数在第24周减少92%,在第48周减少96%,并且在第96周减少97%(对于所有时间点,p<0.0001,参见图18B)。图17A-B和18A-B的结果通过负二项回归计算平均值进行调整,并且针对基线T1 Gd损伤(存在与否)、基线EDSS(<4.0比上>4.0)和地理位置(美国比上世界其它地区)调整。相比于接受IFN β-1a的患者,接受ocrelizumab的患者中T1钆增强性损伤的累积数量减少94%(研究I和研究II,合并数据;p=0.0001)。

[0560] 在研究I中,与IFNβ-1a相比,ocrelizumab在第24周、第48周和第96周使新的/扩大的T2高强度损伤的总数减少77%,并且在研究II中与IFNβ-1a相比减少83%(对于两项研究,p<0.0001;参见表4和图19A和19B)。如图19C所示,在研究I中,与IFN β-1a相比,ocrelizumab使新的/扩大的T2高强度损伤的平均数在第24周减少41%;在第48周减少94%;并且在第96周减少98%。如图19D所示,在研究II中,与IFN β-1a相比,ocrelizumab使新的/扩大的T2高强度损伤的平均数在第24周减少61%;在第48周减少96%;并且在第97周减少97%。(通过负二项回归计算平均值进行调整,并且针对基线T2损伤计数、基线EDSS(<4.0比上>4.0)和地理区域(US比上ROW)调整。EDSS,扩展的能力丧失状态量表;IFN,干扰素;MRI,磁共振成像;世界其它地区。与IFN β-1a相比,ocrelizumab在24、48和96周时使MS相关脑损伤(T2高强度损伤)的更多慢性或生长区域的出现减少约80%(合并的研究I和研究II)。图19A-D的结果通过负二项回归计算的平均值进行调整,并且针对调整基线T2损伤计数、基线EDSS(<4.0比上≥4.0)和地理位置(US比上世界其它地区)调整。相比于接受IFN β-1a的患者,接受ocrelizumab的患者中新的和/或扩大的T2损伤的累积数量减少80%(研究I和研究II,合并数据;p=0.0001)。

[0561] 与IFN β-1a相比,ocrelizumab减缓了从第24周至第96周的脑体积损失(研究I,p=0.0042;研究II,p=0.0900;图20A和20B)。与IFN β-1a相比,ocrelizumab使脑体积损失速率降低18.8%(研究I和研究II,合并数据;p=0.0015;调整平均值的差异(0.140;95% CI:0.054,0.226)。分析基于第24周的ITT人群和至少一个第24周后的评价;p值基于96周访问的混合效应模型重复测量(MMRM),通过研究调整,针对第24周脑体积、地理区域和年龄)。

[0562] 与IFN β-1a相比,ocrelizumab还减缓了从基线至第96周的脑体积损失(研究I,p=<0.0001;研究II,p=0.0001;图2IA和21B)。使用按地理区域(US比上世界其他地区)和基线EDSS评分(<4.0比上)分层的Cochran-Mantel-Haenszel检验来比较图20A-B和21A-B中的终点。使用按地理区域(US比上世界其他地区)和基线EDSS评分(<4.0比上>4.0)分层的Cochran-Mantel-Haenszel检验来比较图20A-B和21A-B中的终点。与IFNβ-1a相比,ocrelizumab使第24周至第96周的脑体积损失速率降低22.8%(在研究I中)(p=0.0042)和14.9%(在研究II中)(p=0.0900)。参见图20A和B。在研究I中,相比于IFNβ-1a治疗的患者,ocrelizumab治疗的患者中脑体积损失(从基线至第96周)降低23.5%(P<0.0001)。在研究II中,相比于IFNβ-1a治疗的患者,ocrelizumab治疗的患者中脑体积损失(从基线至第96周)降低23.8%(P=0.0001)。参见图21A和B。

[0563] 与IFN β-1a治疗的患者相比,ocrelizumab治疗的患者显示出脑萎缩降低18.8%。

[0564] 在研究I中,与IFN β-1a相比,ocrelizumab使新T1低强度损伤的总数减少57%,并且在研究II中与IFN β-1a相比减少64%。参见图22A和22B。相比于接受IFN β-1a的患者,接受ocrelizumab的患者中新T1低强度损伤的累积数量减少62%(研究I和研究II,合并数据;

p＝0.0001)。在研究I中，与IFN β-1a相比，ocrelizumab使新T1低强度损伤的数量在第24周减少27％；在第48周减少95％；并且在第96周减少99％。在研究II中，与IFN β-1a相比，ocrelizumab使新T1低强度损伤的数量在第24周减少33％；在第48周减少95％；并且在第97周减少96％。(每个时间点(第24周、第48周或第96周)治疗组所有患者的新T1低强度损伤总数除以该时间点的脑MRI扫描总数。通过负二项回归计算平均值进行调整，并且针对基线T1低强度损伤计数、基线EDSS(＜4.0比上≥4.0)和地理区域(US比上世界其他地区)调整。

[0565] 研究I和研究II中的功效终点总结于表5中。

[0566]

表 5. 研究 I 和研究 II 的功效总结

	研究 I	研究 II	合并研究
终点	风险降低， p 值	风险降低， p 值	风险降低， p 值
ARR	46%， < 0.0001	47%， < 0.0001	46%， <0.0001
到 **CDP** 的时间 **12** 周	43%， 0.0139	37%， 0.0169	40%， 0.0006
总 **T1 Gd** 损伤 **(**第 **24** 周、第 **48** 周、第 **96** 周**)**	94%， <0.0001	95%， <0.0001	94%， <0.0001
总的新的/扩大的 **T2** 损伤 **(**第 **24** 周、第 **48** 周、第 **96** 周**)**	77%， <0.0001	83%， <0.0001	80%， <0.0001
CDI 12 周	提高 61%，0.0106	提高 14%，0.4019	提高 33%，0.0194
到 **CDP** 的时间 **24** 周	43%， 0.0278	37%， 0.0370	40%， 0.0025
总 **T1** 低强度损伤 **(**第 **24** 周、第 **48** 周、第 **96** 周**)**	57%， <0.0001	64%， <0.0001	62%， <0.0001

[0567]

平均 MSFC 的差异 (基线到第 96 周)	p=0.3261	p=0.0040	
平均脑体积的差异 (第 24 周到第 96 周)	p=0.0042	p=0.090	
SF-36 PCS 的变化 (基线到第 96 周)	p=0.2193	p=0.0404	
EDSS≥2.0 的患者中到第 96 周的 NEDA	提高 74%, <0.0001	提高 81%, <0.0001	提高 75%, <0.0001

[0568] 在研究I中,80.4%的ocrelizumab治疗的患者在96周时无复发,而IFNβ-1a治疗的患者为66.7%。在研究II中,78.9%的ocrelizumab治疗的患者在96周时无复发,而IFNβ-1a

治疗的患者为64.5%。

[0569] 在研究I中,92.4%的ocrelizumab治疗的患者在96周时无CDP,而IFNβ-1a治疗的患者为87.8%。在研究II中,89.4%的ocrelizumab治疗的患者在96周时无CDP,而IFNβ-1a治疗的患者为84.9%。

[0570] 在研究I中,92.3%的ocrelizumab治疗的患者具有改善的/稳定的能力丧失,而IFNβ-1a治疗的患者为86.1%。在研究II中,87.5%的ocrelizumab治疗的患者具有改善的/稳定的能力丧失,而IFNβ-1a治疗的患者为80.4%。

[0571] 在研究I中,7.7%的OCR治疗的患者具有恶化的能力丧失,而IFNβ-1a治疗的患者为13.9%(调整让步比0.559;p=0.0242)。在研究II中,12.5%的OCR治疗的患者具有恶化的能力丧失,而IFNβ-1a治疗的患者为19.6%(调整让步比0.577;p=0.0121)。

[0572] 在研究I中,91.7%的ocrelizumab治疗的患者在96周时无钆增强性T1损伤,而IFNβ-1a治疗的患者为69.8%。在研究II中,90.2%的ocrelizumab治疗的患者在96周时无钆增强性T1损伤,而IFNβ-1a治疗的患者为63.9%。

[0573] 在研究I中,61.7%的ocrelizumab治疗的患者在96周时无新的/扩大的T2损伤,而IFNβ-1a治疗的患者为38.7%。在研究II中,60.9%的ocrelizumab治疗的患者在96周时无新的/扩大的T2损伤,而IFNβ-1a治疗的患者为38.0%。

[0574] 在第24周后,≥96.0%的所有OCR治疗的患者无新的/扩大的T2损伤,与之相比,IFNβ1-a治疗的患者为60.8-70.9%。

[0575] 与接受IFNβ-1a的患者相比,更多的接受ocrelizumab的患者报告了生活质量变化中的改善,如通过简式-36(SF-36)健康要素总评(PCS)所测量的。参见图38。

[0576] 安全性

[0577] 不良事件

[0578] 在研究I和研究II(合并的)中,对于用IFN β-1a和ocrelizumab治疗的患者,不良事件的总发病率分别为83.3%和83.3%。(参见表6A)。

[0579] 表6A.不良事件(安全性人群)。

[0580]

n (%)	合并的研究 I 和研究 II	
	IFN β-1a 44 µg (n=826)	**Ocrelizumab 600 mg (n=825)**
具有≥1 个不良事件(AE)的患者总数	**688 (83.3)**	**687 (83.3)**
具有以≥5%的相对频率出现的≥1 个 AE 的患者总数	**539 (65.3)**	**544 (65.9)**
损伤、中毒和程序并发症	**155 (18.8)**	**333 (40.4)**
输注相关的反应	80(9.7)	283 (34.3)
一般性病症和施用部位病况	**396 (47.9)**	**173 (21.0)**
流感样疾病	177 (21.4)	38 (4.6)
注射部位红斑	127 (15.4)	1 (0.1)
疲劳	64 (7.7)	64 (7.8)
注射部位反应	45 (5.4)	2 (0.2)
感染和侵害	**433 (52.4)**	**482 (58.4)**
上呼吸道感染	87 (10.5)	125 (15.2)
鼻咽炎	84 (10.2)	122 (14.8)
尿路感染	100 (12.1)	96 (11.6)
鼻窦炎	45 (5.4)	46 (5.6)
支气管炎	29 (3.5)	42 (5.1)
神经系统病症	**252 (30.5)**	**224 (27.2)**
头痛	124 (15.0)	93 (11.3)
精神疾病	**144 (17.4)**	**149 (18.1)**
抑郁	54.(6.5)	64 (7.8)
失眠	38 (4.6)	46 (5.6)

[0581]

肌肉骨骼和结缔组织病症	**207 (25.1)**	**204 (24.7)**
背痛	37 (4.5)	53 (6.4)
关节痛	51 (6.2)	46 (5.6)

[0582] 表6A仅包括在至少一个治疗组和相应系统器官类别中≥5%的患者中发生的合并的AE。

[0583] 表6B.总的严重不良事件

[0584]

n (%)	IFN β-1a 44μg (n = 826)	Ocrelizumab 600 mg (n = 825)
≥SAE 的所有患者	72 (8.7)	57 (6.9)
感染和侵害	24 (2.9)	11 (1.3)
神经系统病症	11 (1.3)	8 (1.0)
损伤、中毒和程序并发症	10 (1.2)	6 (0.7)

[0585] 在研究I和研究II(合并的)中,在8.7%的IFN β-1a治疗的患者以及6.9%的ocrelizumab治疗的患者中报告了严重的不良事件。(参见表6B)。

[0586] 感染

[0587] 感染发生率在研究I中对于IFN β-1a为52.8%且对于ocrelizumab为56.6%,并且在研究II中对于IFN β-1a为52.0%且对于ocrelizumab为60.2%。最常见的感染(在至少一个治疗组中至少10%的患者中报告)是尿路感染、上呼吸道感染和鼻咽炎。在这两项研究中严重感染的发生率都很低(在这两项研究中,所有治疗组中为1.2%至2.9%)。在96周研究期间,在任何组中未报告严重的机会性感染。总体而言,在用IFN β-1a治疗的28位患者和用ocrelizumab治疗的50位患者中报告了疱疹病毒感染。所有病例均为轻度或中度的,除了研究I中用ocrelizumab治疗的患者中1例单纯疱疹病例(分级为严重的)以外。

[0588] 输注相关的反应

[0589] 相对于IFN β-1a,具有至少一次输注相关反应(IRR)的患者的数量在用ocrelizumab治疗的患者中更高(在研究I中为30.9%(对于ocrelizumab)比上7.3%(IFN β-1a);在研究II中为37.6%(对于ocrelizumab)比上12.0%(IFN β-1a);在研究I和研究II两者(合并的)中为34.3%(对于ocrelizumab)比上9.7%(IFN β-1a))。一名OCR治疗的患者在第一次输注时具有严重的IRR(具有危及生命的支气管痉挛);尽管事件得到解决,但是依据方案退出了随后的治疗。仅在ocrelizumab组中的11名患者(1.3%)中发生了第一次输注期间由于IRR导致的治疗退出。ocrelizumab治疗的患者的IRR发生率在第一次输注时最高(27.5%),并且随着后续给药显著降低(在剂量2时为13.7%)。大多数输注有关反应是1或2级,并且在第一次输注剂量1时报告(参见图23A和23B,研究I和研究II的合并数据)。柱中的

数字代表经历IRR等级的患者的比例。依照通用术语标准的分级(Grading per Common Terminology Criteria,CTCAE):1级,轻度;无症状或轻度症状;2级,中度;指示最小限度的、局部的或非侵袭性的干预;3级,严重或者医学上显著但不会立即危及生命;4级,危及生命的后果;指示紧急干预;5级,与AE相关的死亡。注意:所有人都接受100-mg i.v.甲泼尼龙。在96周中,9.7%的IFNβ-1a患者(n=80/826)和34.3%的ocrelizumab治疗患者(n=283/825)具有至少1次IRR;大多数为轻度至中度的严重程度(分别为99%[n=79]和93%[n=262])。ocrelizumab治疗的大多数IRR症状包括瘙痒、皮疹、咽喉刺激和发红。IRR主要发生于输注期间(IFNβ-1a,46.3%;ocrelizumab,80.6%),并且通过输注调整和对症治疗进行管理(42.5%的患者IFNβ-1a组中42.5%的患者具有IRR,并且在接受治疗的ocrelizumab组中为65.4%)。

[0590] 恶性肿瘤

[0591] 总计,对于IFN β-1a报告了两例恶性肿瘤(一例套细胞癌和一例鳞状细胞癌),并且在用ocrelizumab治疗的患者中报告了四例恶性肿瘤(两例浸润性乳腺导管乳腺癌、一例肾细胞癌和一例恶性黑素瘤)。

[0592] 实验室评价

[0593] 在实验室评价中,到第2周,在用ocrelizumab治疗的患者中平均CD19水平已经降低至可忽略不计。对CD3-、CD4、CD8-、CD16-和CD56-阳性细胞没有影响,这支持了先天免疫和总T细胞数量不受ocrelizumab影响。ocrelizumab对腮腺炎、风疹、水痘和肺炎球菌的现有抗体滴度没有影响。

[0594] 尽管在研究I和研究II中仅测量了ocrelizumab与IFN β-1a的直接比较,但是这些功效和安全性结果表明ocrelizumab在2年期的收益/风险特征优于可用于治疗RMS患者的所有减轻疾病的疗法(DMT)。

[0595] ocrelizumab时体液免疫标记的影响

[0596] 在纳入研究之前,建议医师检查患者免疫状态并且遵循疫苗接种的当地指导;治疗前≥6周完成免疫。在基线和第12周、第24周、第48周、第72周和第96周时测量针对腮腺炎、风疹、水痘和肺炎链球菌(Streptococcus pneumoniae)的抗体(Ab)滴度。对于每个治疗组,随着时间评价具有可被认为是保护性的Ab水平的患者比例。

[0597] 腮腺炎:在基线时,94.1%的IFNβ-1a治疗的患者和93.6%的ocrelizumab治疗的患者具有阳性水平的腮腺炎Ab。(在96周研究治疗期间进行的六次测量中,该比例的范围(最小-最大)为92.7-94.8%(IFNβ-1a)和91.8-93.5%(ocrelizumab))。

[0598] 风疹:87.9%的IFNβ-1a治疗的患者和89.0%的ocrelizumab治疗的患者具有阳性水平的风疹Ab,并且在治疗期间进行的六次测量中的范围(最小-最大)为89.8-90.8%(IFNβ-1a)和88.7-89.4%(ocrelizumab)。

[0599] 水痘:两个治疗组中的95.5%均在基线具有阳性水平的水痘Ab,并且在治疗期间进行的六次测量中的范围(最小-最大)为96.2-97.5%(IFNβ-1a)和94.8-95.6%(ocrelizumab)。

[0600] 肺炎链球菌:在可评价的患者中,在基线时,肺炎链球菌Ab的平均(标准偏差)水平对于IFNβ-1a治疗的患者为53.67(54.13)mg/mL,并且对于ocrelizumab治疗的患者为55.35(67.00)mg/mL。在第96周,肺炎链球菌Ab的平均(标准偏差)水平对于IFNβ-1a治疗的患者为

51.74(42.50)mg/mL，并且对于ocrelizumab治疗的患者为54.06(80.98)mg/mL。在96周时，从基线的平均变化对于IFNβ-1a治疗的患者为-1.13(40.25)mg/mL，并且对于ocrelizumab治疗的患者为-1.99(59.60)mg/mL。

[0601] ocrelizumab对免疫应答的影响

[0602] 感染可以导致MS疾病恶化并且可能引起目前采用的治疗方法的治疗并发症。因此，针对感染的疫苗接种是MS患者管理的一部分。本研究评价了ocerlizumab治疗的患者是否可以针对临床相关疫苗产生保护性免疫应答。本研究使用以下疫苗来评价不同的免疫应答途径：

[0603] a)含破伤风类毒素(TT)的疫苗接种，以评价T细胞依赖性回忆性体液应答；

[0604] b)23价肺炎球菌多糖疫苗(23-PPV)，以评价大部分非T细胞依赖性或纯B细胞体液应答；

[0605] c)钥孔�avi血蓝蛋白(KLH)，以探索对新抗原的B细胞依赖性免疫应答；

[0606] d)加强型13价结合肺炎球菌疫苗(13-PCV)，以评价与单独的23-PPV疫苗相比，23-PPV疫苗随后是加强型13-PCV的临床功效；

[0607] e)流感疫苗，以检测对临床相关疫苗产生体液应答的能力。

[0608] 在用于评价ocrelizumab对复发型多发性硬化症患者的免疫应答的影响的III期开放标签研究中，大约100名患者被随机化(2:1)以接受ocrelizumab 600mg(作为第1天和第15天的两次300mg静脉内输注)。A组患者在免疫前接受ocrelizumab。B组患者保持初治治疗/在免疫期间继续干扰素治疗。

[0609] 在第一次ocrelizumab施用后＞85天/12周免疫A组患者。简言之，A组在第12周用含破伤风类毒素(TT)的吸附疫苗免疫，在第16周用23价肺炎球菌多糖疫苗(23-PPV)免疫，以及在第12周、第16周和第20周用钥孔�avi血蓝蛋白(KLH)免疫。进一步细分A组患者以接受：

[0610] ●加强型13-肺炎球菌结合疫苗(13-PCV;A1组)；或者

[0611] ●流感疫苗(A2组)。

[0612] B组患者在第1天用TT免疫，在第28天用23-PPV免疫，在第1天、第28天和第56天用(KLH)免疫，以及在第1-12周期间用流感疫苗免疫。

[0613] 该研究的关键的纳入标准是：RMS诊断(2010修订的McDonald标准，Polman等人，Ann Neurol 2011;69:292-302)；年龄为18-55岁；接受≥1次针对TT或破伤风和白喉(DT/Td)或者破伤风、白喉和无细胞百日咳(DTaP/Tdap)的先前免疫；EDSS评分为0.0-5.5。关键的排除标准是：已知对含有TT的吸附疫苗、肺炎球菌多糖/结合疫苗或流感疫苗的任何组分过敏；筛选前＜5年内接受任何PPV或在随机化前＜6周内接种活疫苗；在筛选前＜2年内先前暴露于LKH或者用任何含破伤风的疫苗免疫。

[0614] 所有组进行免疫后评价直至研究期结束(A组：第169天/第24周；B组：第84天/第12周)。关键的合格标准包括针对TT、破伤风或白喉；或者破伤风、白喉和无细胞百日咳的至少1次先前免疫。主要结果测量将免疫后8周具有阳性TT应答(IgG)的A1组和A2组患者的比例与B组中的患者进行比较。对加强型免疫的阳性应答被定义为抗体滴度≥0.2IU/mL(免疫前滴度＜0.1IU/mL)或抗体滴度增加到4倍(免疫前滴度≥0.1IU/mL)。

[0615] 关键的次要结果测量列出如下：

[0616] TT应答

[0617] ●用ocrelizumab治疗的患者(A1组和A2组)相比于未用ocrelizumab治疗的患者(B组),在免疫后4周时对TT疫苗具有阳性应答(IgG)的患者的比例;

[0618] ●用ocrelizumab治疗的患者(A1组和A2组)相比于未用ocrelizumab治疗的患者(B组),在免疫后4周时破伤风抗体滴度增加到2倍(免疫前滴度≥0.1IU/mL),或具有≥0.2IU/mL的破伤风抗体滴度(免疫前滴度<0.1IU/mL);

[0619] ●在紧邻加强型疫苗之前和加强型疫苗之后4周时测量的用OCR治疗(A1组和A2组)和未用OCR治疗(B组)的患者的抗破伤风抗体平均水平。

[0620] 23 PPV

[0621] ●用ocrelizumab治疗的患者(A1组和A2组)相比于未用ocrelizumab治疗的患者(B组),在23-PPV后4周时对个体抗肺炎球菌抗体血清型具有阳性应答的患者的比例。阳性应答被定义为与免疫前水平相比,水平增加到2倍或水平增加>1μg/mL。

[0622] KLH

[0623] ●用ocrelizumab治疗的患者(A1组和A2组)相比于未用ocrelizumab治疗的患者(B组),在紧邻第一次施用KLH之前和最后一次施用KLH之后4周时测量的患者中的抗KLH抗体(IgG)的平均水平。

[0624] A1组和B组中的肺炎球菌结合加强型应答

[0625] ●A1组中用ocrelizumab治疗的患者(A1组)相比于未用ocrelizumab治疗的患者(B组),在加强型13-PCV疫苗之后4周时测量的对个体抗肺炎球菌抗体血清型(23个血清学)具有阳性应答的患者的比例。阳性应答被定义为与免疫前水平相比,水平增加到2倍或水平增加>1μg/mL。

[0626] 流感疫苗应答

[0627] ●与未用ocrelizumab治疗的患者(B组)相比,用ocrelizumab治疗的患者(A2组)在免疫后4周达到血清保护作用(特异性血凝抑制滴度>1:40)的比例。

[0628] 免疫分型结果测量

[0629] ●体液和细胞免疫的测量包括:(a)总B细胞($CD19^+$);(b)B细胞亚群(记忆;幼稚;血浆);(c)总T细胞($CD3^+$);(d)T辅助细胞($CD3^+CD4^+$);(e)细胞毒性T淋巴细胞($CD3^+CD8^+$);和(f)天然杀伤细胞($CD3^-CD16/56^+$)。

[0630] 开放标签延长阶段

[0631] 在完成双盲的受控治疗阶段研究I和研究II之后,患者可能有资格进入开放标签延长(OLE)阶段以评价ocrelizumab的长期安全性、耐受性和功效。以下描述了开放标签研究I和研究II延长研究的设计,其评价了OCR在复发型MS中的长期安全性和功效。

[0632] 进入OLE阶段的患者进入OLE筛选阶段,持续长达4周。在OLE筛选期间,患者接受IFN β-1A或IFN β-1A安慰剂,直到第一次输注剂量5为止。依照当地法规或者直到发起人决定终止ocrelizumab MS程序,否则持续OLE阶段直到ocrelizumab在患者所在国家是可商购的。OLE阶段不超过4年(208周)。OLE不是强制性的。不愿意进入OLE阶段的患者进入安全性随访阶段,该阶段从患者最近一次访问的日期开始以12周为间隔进行。如果B细胞不充足,则继续监测。

[0633] OLE阶段的关键纳入标准包括:完成盲法治疗期;获得研究者的同意,该研究者基于治疗期间进行的评价确定患者可能从OCR治疗中受益;待进入OLE阶段的患者的书面知情

同意书;以及符合ocrelizumab治疗/再治疗标准,其中患者不具有以下情况和实验室异常:(a)在先前的OCR输注期间发生的危及生命(CTCAE 4级)的输注相关事件;(b)任何重大或不受控制的医疗状况或者治疗突发的临床显著的实验室异常;(c)活动性感染;(d)嗜中性粒细胞绝对计数＜1.5×10^3/μL;(e)CD4细胞计数＜250/μL;和(f)低丙种球蛋白血症免疫球蛋白G＜3.3g/L。

[0634] 关键的安全性和功效评价的时间表如下表6C所示。

[0635] 表6C

[0636]

	OLE阶段											延迟的给药访问[a]	计划外的访问[b]	退出治疗访问
周期	5			6		7		8		N				
访问	12	13	14	15	16	17	18	19	20	…	…			
OLE周	0	2 (±2)	12 (±7)	22 (±7)	24 (±5)	46 (±7)	48 (±5)	70 (±7)	72 (±5)	n-2周 (±7)	n (±7)			
神经检查[c]	x		x		x		x		x		x		x	x
EDSS[d]	x		x		x		x		x		x		x	x
潜在的复发[e]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
EQ-5D[f]	x					x					(x)			
MRI[g]						x					(x)			
常规安全性实验室[h]	x		x	x		x		x		x			x	x
伴行的治疗[i]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
抗体滴度[j]	x		x	x		x		x		x				x

[0637] [a]当在定期给药访问中不能施用给药时,进行延迟的给药访问。此时,其它测试/评价可以适当地进行。

[0638] [b]根据患者的临床需求进行计划外的访问(非给药)评价。此时,其它测试/评价可以适当地进行。

[0639] [c]每次计划和计划外的访问时都会进行神经检查。具有暗示MS恶化或复发的新神经症状的所有患者应该具有由检查研究者进行的EDSS。

[0640] [d]每12周由检查研究者对所有患者进行EDSS评分。额外的EDSS评价可以在访问之间进行(即,在MS复发、神经恶化等期间)。能力丧失进展被定义为不由其它病因引起的从基线EDSS评分增加≥1.0分(基线EDSS≤5.5时)或≥0.5分(基线EDSS>5.5时)。

[0641] 在每次定期访问时由治疗研究者评价患者的复发,并且如有需要,进行计划外访问以确认访问之间发生复发。如果怀疑复发,则除了完成MS复发电子病例报告表以外,应该进行EDSS。

[0642] [f]EQ-5D(EuroQol五维问卷)评价每年进行一次。

[0643] [g]在OLE阶段开始时(如果先前12周中没有进行)、定期访问的(±)4周内和OLE退出访问时(如果在先前4周中没有进行)进行年度脑MRI扫描。

[0644] [h]常规安全性实验室评价包括血液学、化学和尿分析。

[0645] [i]在每次定期和计划外访问时报告伴行的药物和程序。

[0646] [i]进行针对常见抗原(腮腺炎、风疹、水痘和肺炎链球菌)的抗体滴度。

[0647] 总结

[0648] 研究I和研究II表明,在患有复发型MS的患者中,与IFN β-1a相比,ocrelizumab显著降低了年复发率以及12周和24周确认的能力丧失进展的风险。ocrelizumab是在两项单独的III期研究中并且针对活性药物对照组显著降低12周和24周确认的能力丧失进展两者的第一种多发性硬化治疗,这显示了效果的一致性。与IFN β-1a相比,在接受ocrelizumab的患者中实现确认的能力丧失改善达至少12周的患者的比例也显著增加。这些临床发现得到了MRI终点的支持,经由所述MRI终点,与IFN β-1a相比,ocrelizumab显著减少了脑中T1钆增强性损伤和新的/扩大的T2损伤的数量。对T1钆增强性损伤和新的/扩大的T2损伤的影响程度大到表明局灶性炎症和疾病活动性在很大程度上停止/暂停。

[0649] 在探索性分析中,与IFN β-1a相比,ocrelizumab降低了脑体积损失(参见图21A-D)。在另外的探索性分析中,与IFN β-1a相比,ocrelizumab增加了EDSS>2.0的ITT人群中无疾病活动迹象(NEDA)的患者的比例(参见表4和5)。与IFN β-1a相比,ocrelizumab增加了ITT人群的所有患者中无疾病活动迹象(NEDA)的患者的比例(参见图34A和34B)。在图34A和34B中,使用按地理区域(美国比上世界其它地区)和基线EDSS评分(<4.0比上≥4.0)分层的Cochran-Mantel-Haenszel X2检验来比较具有NEDA的患者的比例。

[0650] 这些功效结果证明了能力丧失恶化得以降低并且在一些患者中提高了功能,这支持ocrelizumab影响炎症和神经元损伤的标志物的假设。ocrelizumab对炎症和退行性结果的功效表明,使用ocrelizumab治疗具有有效的抗炎作用,并且还可以具有神经保护作用。

[0651] 早期观察到研究I和研究II中使用ocrelizumab的益处,并且在96周治疗期间持续;根据早期经验,停止治疗后不预期出现反弹风险(Kappos等人,(2012)“ocrelizumab在复发缓和型多发性硬化患者中的长期安全性和功效:II期随机多中心试验的第144周结果(Long-term safety and efficacy of ocrelizumab in patients with relapsing-remitting multiple sclerosis:Week 144 results of a phase II,randomized,multicentre trial)”欧洲多发性硬化治疗和研究委员会第28次代表大会(28th Congress of the European Committee for Treatment and Research in Multiple Sclerosis))。

[0652] 接受ocrelizumab的患者中观察到的不良事件与在ocrelizumab的II期研究(Kappos等人,(2011)Lancet.378,1779-87)中报道的不良事件一致。在96周研究期间没有报告严重的机会性感染的病例。迄今为止的结果表明ocrelizumab对免疫监视不存在明显影响。总体而言,在研究I和研究II两者中,与IFN β-1a相比,ocrelizumab在96周中具有相似的安全性特征。

[0653] 作为一类药物,$CD20^+$靶向治疗具有进行性多灶性脑白质病的风险很低(25,000中<1)(Clifford等人,(2011)Arch Neurol.68,1156-64)。迄今为止的研究中并未报告进行性多灶性脑白质病的病例。

[0654] 如所预期的,使用ocrelizumab更常见的是输注相关反应。与使用600mg剂量的ocrelizumab的II期试验一致,这些反应主要在第一次剂量时观察到并且在随后的剂量时下降(Hauser等人,(2008)N Engl J Med.358,676-88)。这为将首次600mg剂量分成间隔约两周的两次300mg的单独输注提供了理论依据。输注相关反应可使用前驱用药、输注调整和对症治疗进行管理。

[0655] 总的来说,临床和MRI数据表明ocrelizumab至少与任何其它测试的多发性硬化治疗一样有效,包括被认为是高效疗法的那些治疗。尽管在研究I和研究II中仅测量了与IFN β-1a的直接比较,但是这些功效和安全性结果表明ocrelizumab在2年期的收益/风险特征优于可用于治疗RMS患者的所有减轻疾病的疗法。与IFN β-1a相比,高功效与安全性风险的增加无关。其它益处包括治疗益处的较早开始以及停用ocrelizumab后没有反弹迹象。

[0656] 从研究I和研究II获得的数据显示,靶向$CD20^+$B细胞可以有效治疗复发型MS。

[0657] 亚组分析

[0658] 为了在进入研究之前评价具有不同程度的疾病活动性的相对收益/风险和对治疗的先前应答,确定四个另外的亚组:

[0659] 活跃的初治治疗患者:定义为未接受先前治疗,其在此前2年经历至少2次复发和在随机化之前的最后一年经历至少1次复发。

[0660] 活跃的不充分应答者:定义为先前用IFN β-1a或醋酸格拉默治疗至少1年的患者,并且(a)在前一年经历至少一次复发或(b)经历至少1次基线钆增强性损伤。

[0661] 高度活跃的初治治疗患者;定义为未接受过治疗的患者,该患者在随机化之前的最后一年中经历至少2次复发,并且与先前的MRI相比,经历了(a)至少1次基线钆损伤或(b)基线访问时T2损伤计数的增加(绝对变化从0-5至6-9个损伤或从6-9个损伤至>9个损伤)。

[0662] 高度活跃的不充分应答者;定义为先前用干扰素或醋酸格拉默治疗至少1年的患者,在前一年具有至少一次复发,并且(a)具有至少9个T2损伤或(b)在基线具有至少一个Gd损伤。

[0663] 合并研究I和研究II以增加检测4个亚组之间差异的能力。每个亚组中存在至少100名患者以能够充分检测治疗差异。

[0664] 询问每组/每组的年复发率以确定不充分应答者的趋势是否由ocrelizumab IFN β-1a绝对结果所驱动。总体而言,与IFN β-1a相比,ocrelizumab在所有亚组中在降低年复发率方面显示出显著效果(参见图43)。干扰素组中的不充分应答者的ARR高于ITT组,而同一组的ocrelizumab患者的ARR低于ITT组。在干扰素组和ocrelizumab组中,活跃的初治治疗组均具有更高的ARR。

[0665] 在所有4个亚组中，与IFN β-1a相比，对于ARR、CDP达至少12周、CDP达至少24周、CDI和MRI，ocrelizumab具有更大的治疗效果(参见上图43-47)。对于ARR和MRI，所有4个亚组间的差异具有统计学显著性。对于CDP达至少12周，在活跃的不充分应答者和高度活跃的不充分应答者中均具有统计学显著性，证明与IFN β-1a相比ocrelizumab具有一致的治疗效果。对于CDP达至少24周，这些组显示了朝着显著性的趋势(分别为p＝0.075和p＝0.082)。当比较客观测量如T1 Gd增强性损伤(指示急性炎症效应，与OCR作用机制一致)时，与IFN β-1a相比，ocrelizumab的强大和一致的效果在所有亚组中都是明显的。

[0666] 类似地，对4个亚组的安全性结果进行分析，并且与总体安全性人群进行比较。由于组规模相对较小，组之间的比较受到限制，以便不会根据少量事件得出不适当的结论。4个亚组中的安全性结果彼此一致，并且与总人群(“安全性人群”定义为接受任何OCR暴露的任何患者)类似，通常经历导致退出的任何AE、SAE和AE的患者比例较低。与安全性人群的观察结果一致，与干扰素组相比，ocrelizumab组中感染更多，而在所有亚组中，ocrelizumab组中经历严重感染的患者比例与干扰素相似或更低。

[0667] 在每个亚组中，总体ocrelizumab功效和安全性结果都与ITT/安全性人群相当。因此，ocrelizumab在所有亚组中均显示出有利的收益/风险特征。亚组间的数据的致性进一步支持了总人群中观察到的效果。

[0668] 实施例2：原发进行性多发性硬化患者中ocrelizumab的III期研究

[0669] 进行随机、平行组、双盲、安慰剂对照的研究以评价与安慰剂相比，ocrelizumab在原发性进行性多发性硬化患者中的功效和安全性。

[0670] 由于疾病进展率的异质性，进行性MS的临床试验的持续时间通常大于2年，其在历史上有时低于预期，导致无法显示治疗效果。本研究具有事件驱动的设计，这意味着如果由于疾病进展缓慢而未能在第120周达到确认的能力丧失进展事件的预计数目，则治疗期延长直至发生足够的进展事件从而维持统计效力以检测治疗差异。因此，盲法治疗期距纳入最后一名患者最少120周；平均治疗时间为3.5年。大多数患者在盲法治疗阶段继续观察3到4年。

[0671] 方法

[0672] 合格和排除标准

[0673] 关键的合格标准包括：年龄为18至55岁；依照2005年修订的McDonald标准(Polman等人，(2011)“Diagnostic criteria for multiple sclerosis:2005 revisions to the ‘McDonald criteria.”’Ann Neurol 58,840-846)诊断为原发进行性多发性硬化；筛选时扩展的能力丧失状态量表(EDSS)评分为3至6.5分；在功能系统量表(FSS)的金字塔形功能要素上具有至少2.0的评分；具有以下的有记录的历史或在筛选时存在以下：脑脊液(CSF)样本中升高的IgG指数和/或通过脑脊液(CSF)样本中的等电聚焦检测的一个或多个IgG寡克隆条带；不具有复发缓和型多发性硬化(RRMS)、继发进行性多发性硬化(SPMS)或进行性复发型多发性硬化(PRMS)的历史；从MS症状发病的疾病持续时间(a)在筛选时EDSS＞5.0的患者中小于15年或(b)在筛选时EDSS≤5.0的患者中小于10年；以及筛选时未使用其它减轻多发性硬化疾病的治疗进行治疗。

[0674] 关键的排除标准包括：筛选时具有复发缓和型多发性硬化、激发进行性多发性硬化或进行性复发型多发性硬化的历史；对磁共振成像(MRI)的禁忌症；已知存在其他神经病

症;已知的活动性感染或者复发性或慢性感染的历史或者存在复发性或慢性感染;癌症的历史,包括实体瘤和血液恶性肿瘤(除已经切除并解决的皮肤的基底细胞癌、原位鳞状细胞癌和宫颈的原位癌以外);使用靶向B细胞的疗法(例如,利妥昔单抗、ocrelizumab、阿塞西普、贝利木单抗或奥法木单抗)的先前治疗;使用淋巴细胞运输阻断剂(例如,那他珠单抗、FTY720)的任何先前治疗,使用阿仑单抗(CAMPATH®、LEMTRADA™)、抗-CD4、克拉屈滨、环磷酰胺、米托蒽醌、硫唑嘌呤、麦考酚酸吗乙酯、环孢菌素、甲氨喋呤、全身照射或骨髓移植的任何先前治疗;随机化前12周内使用β干扰素、醋酸格拉默(i.v.)、免疫球蛋白、血浆去除术或其它免疫调节疗法;筛选前4周内的系统性皮质类固醇;以及在研究过程期间可能需要使用系统性皮质类固醇或免疫抑制剂的慢性治疗的任何伴行疾病。对于在筛选前对其MS已经使用系统性皮质类固醇的患者,可以延长筛选期(但是不能超过8周)。对于合格的患者,不应在筛选和基线之间施用系统性皮质类固醇。

[0675] 研究设计

[0676] 患者被随机化(2:1)以接受ocrelizumab(300mg静脉内,2次输注,在每个治疗周期(即暴露)中间隔14天)或安慰剂。按年龄(≤45比上>45)和地区(美国比上世界其它地区)将患者分层。

[0677] 研究由以下时期组成(总结于图24中):

[0678] 筛选长达4周。

[0679] 盲法治疗期:所有患者接受至少120周的研究治疗,代表五个治疗周期,每个周期持续24周。随着患者在12-18个月内被招募,对于纳入研究的第一组患者,该盲法治疗期延长至3.5-4年。如图24所示,Ocrelizumab剂量作为分开14天的300mg×2的双重静脉内输注施用。每次ocrelizumab或安慰剂输注开始前30分钟,患者静脉内接受100mg甲泼尼龙。还推荐使用口服止痛剂/解热剂(例如对乙酰氨基酚)和口服抗组胺剂(例如苯海拉明)进行输注前治疗。根据原始样本大小假设,盲法治疗期设计为在达到约253个事件时结束。如果到第120周时还未达到事件数量,则将继续研究直到达到目标事件数量。

[0680] 开放标签治疗期:初步非盲后,可以从进一步治疗获益的患者接受开放标签ocrelizumab。随机分至ocrelizumab组的患者继续开放标记治疗,每24周使用双重ocrelizumab输注(300mg i.v.输注,间隔14天施用)。随机分至安慰剂的患者接受第一次开放标记治疗周期的双重ocrelizumab输注(即,两个i.v.输注,间隔14天施用),从临时数据库锁定和初步分析后的下一次定期访问开始。

[0681] 研究过程

[0682] 主要功效终点是在治疗期内确认的能力丧失进展的发病时间,定义为持续至少12周的EDSS增加(基于定期访问)。确定的能力丧失进展(CDP)是指如果基线评分不大于5.5,则持续至少12周的增加为至少1.0(从基线EDSS),或者如果基线评分大于5.5,则增加至少0.5。

[0683] 次要终点包括:在治疗期内确认的能力丧失进展的发病时间,定义为持续至少24周的EDSS增加(即24周CDP)(基于定期访问);从基线至第120周的计时25英尺行走(T25-FW)的变化;从基线至第120周的MRI总T2损伤体积的百分比变化;从第24周至第120周的MRI总脑体积的百分比变化;以及从基线至第120周的简式-36(SF-36)健康药物评分的变化,以及ocerlizumab的安全性和耐受性。

[0684] 探索性终点包括:12周和24周确认的复合能力丧失进展的发病时间(定义为EDSS进展的首次确认发生,或计时25英尺行走增加至少20%,和/或9孔钉测试(9-HPT)时间增加至少20%),在计时25英尺行走中持续进展至少20%的时间,治疗期期间在9孔钉测试中持续进展至少20%的时间,以及从基线至第120周的通过脑MRI检测的新的或扩大的T2损伤的总数。9-HPT是上肢功能和精细手灵巧度的标准化定量测试。它是MSFC(多发性硬化功能综合)的第二个组分。

[0685] 另外的敏感性分析与初步结果一致,并且进行该分析通过排除经历医师报告的临床复发(包括方案定义的复发)的患者来评价研究中的复发对确认的能力丧失进展数据的影响(表12A)。安慰剂组中11%的患者和ocrelizumab组中5%的患者报告了方案定义的复发。在基线具有或不具有T1钆增强性损伤的患者的关键临床和MRI功效终点的亚组分析与总研究人群一致(下表12C-12E)。

[0686] 表12A:探索临床复发影响的主要终点和第一个次要终点的敏感性分析

[0687]

	排除具有临床复发的患者	
终点	**安慰剂 (n=204)**	**Ocrelizumab 600 mg (n=456)**
主要终点		
确认的能力丧失进展达至少 12 周 危险比，相比于安慰剂(95% CI) p 值	 0.74 (0.56–0.98) P=0.0324	
次要终点		
确认的能力丧失进展达至少 24 周 危险比，相比于安慰剂(95% CI) p 值	 0.71 (0.53-0.95) P=0.0188	

[0688] .统计学分析

[0689] 图25中提供了统计学层级结构。

[0690] 结果

[0691] 患者

[0692] 如图12B所示,患者的多发性硬化疾病历史和特征相当平衡。

[0693] 表12B.患者的人口统计学和基线疾病特征

[0694]

	安慰剂 n=244	Ocrelizumab 600 mg N= 488
年龄，岁，平均值(SD) 中值(范围)	44.4 (8.3) 46.0 (18–56)	44.7 (7.9) 46.0 (20–56)
女性，n (%)	124 (50.8)	237 (48.6)
症状发作以来的时间，年，平均值(SD) 中值(范围)	6.1 (3.6) 5.5 (0.9–23.8)	6.7 (4.0) 6.0 (1.1–32.9)
诊断以来的时间，年，平均值(SD) 中值(范围)	2.8 (3.3) 1.3 (0.1–23.8)	2.9 (3.2) 1.6 (0.1–16.8)
未接受 MS 的减轻疾病的治疗，n (%)	214 (87.7)	433 (88.7)

[0695]

EDSS，平均值(SD) 中值(范围)	4.7 (1.2) 4.5 (2.5–6.5)	4.7 (1.2) 4.5 (2.5–7.0)
MRI 发现		
具有 0 个 Gd+ T1 损伤的患者，n (%)	183 (75.3)	351 (72.5)
具有 Gd+ T1 损伤的患者，n (%)	60 (24.7%)	133 (27.5)
1 个损伤	29 (11.9)	62 (12.8)
2 个损伤	15 (6.2)	22 (4.5)
3 个损伤	5 (2.1)	17 (3.5)
≥4 个损伤	11 (4.5)	32 (6.6)
Gd^+ T1 损伤的数量，平均值(SD)	0.6 (1.6)	1.2 (5.1)
T2 损伤的数量，平均值(SD) 中值(范围)	48.15 (39.31) 43 (0-208)	48.71 (38.16) 42.0 (0-249)
T2 损伤体积，cm^3，平均值(SD) 中值(范围)	10.9 (13.0) 6.17 (0-81.1)	12.7 (15.1) 7.31 (0-90.3)
正常的脑体积，cm^3，平均值(SD) 中值 (范围)	1469.9 (88.7) 1464.51 (1216.3-1701.7)	1462.9 (83.9) 1462.23 (1214.3-1711.1)

[0696] 如图26所示，意向治疗人群的特征相当平衡。在用于初步分析的数据库锁定中，在ocrelizumab组中390名(80％)治疗患者仍在治疗中，而在安慰剂组中为159名(67％)(中值研究持续时间为2.8年(安慰剂)和2.9年(ocrelizumab))。在数据库锁定之前退出研究的患者中，ocrelizumab组中61例(64％)进入安全性随访，而安慰剂组中为45例(56％)。

[0697] 能力丧头

[0698] 图27中提供了与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中持续至少12周的确认的能力丧失进展的时间(HR-危险比)。图28中提供了与接受安慰剂的患者

相比，接受600mg ocrelizumab的患者中持续至少24周的确认的能力丧失进展的时间。早期停止治疗而不具有确定的EDSS评价的具有初始能力丧失进展的ITT患者被认为具有确认的能力丧失进展，p值基于按地理区域和年龄分层的对数秩检验。相比于安慰剂，ocrelizumab使CDP持续≥12周的风险降低24％，并且相比于安慰剂，CDP持续≥24周的风险降低25％。图27和28中的分析基于ITT人群；p值基于按地理区域和年龄分层的对数秩检验。早期停止治疗而不具有确定的EDSS评价的具有初始能力丧失进展的ITT患者被认为具有确认的能力丧失进展。

[0699] 在基线具有T1 Gd^+损伤的接受ocrelizumab的患者中12周CDP的风险降低35％(危险比，0.65；95％CI，0.40-1.06；p＝0.0826)，而在基线不具有T1 Gd^+损伤的接受ocrelizumab的患者中为16％(危险比，0.84；95％CI，0.62-1.13；p＝0.2441)。参见下表12C。

[0700] 表12C

[0701]

	总计	安慰剂 (N=244)		Ocrelizumab 600mg (N=488)		危险比	95% CI	p 值
	n	n	事件	n	事件			
总人数	731	244	96	487	160	0.76	(0.59, 0.98)	731
基线处的 T1 Gd^+增强性损伤	193	60	27	133	43	0.65	(0.40, 1.06)	0.0826
基线处无 T1 Gd^+增强性损伤	533	183	68	350	115	0.84	(0.62, 1.13)	0.2441

[0702] 在基线具有T1 Gd^+损伤的接受ocrelizumab的患者中24周CDP的风险降低33％(危险比，0.67；95％CI，0.40-1.14；p＝0.1417)，而在基线不具有T1 Gd^+损伤的接受ocrelizumab的患者中为19％(危险比，0.81；95％CI，0.59-1.10；p＝0.1783)。参见下表12D。

[0703] 表12D

[0704]

	总计	安慰剂 (N=244)		Ocrelizumab 600mg (N=488)		危险比	95% CI
	n	n	事件	n	事件		
总人数	731	244	87	487	144	0.75	(0.58, 0.98)
基线处的 T1 Gd 增强性损伤	193	60	23	133	39	0.67	(0.40, 1.14)
基线处无 T1 Gd 增强性损伤	533	183	63	350	103	0.81	(0.59, 1.10)

[0705] 下表12E中总结了在基线的MRI扫描中具有或不具有钆增强性损伤的患者中关键的临床和MRI终点的另外的亚组分析。

[0706] 表12E

[0707]

终点	安慰剂 (n=244)		Ocrelizumab 600 mg (n=488)			
	Gd 损伤	无 Gd 损伤	Gd 损伤	P 值	无 Gd 损伤	P 值

[0708]

主要终点						
确认的能力丧失进展达至少 12 周 具有事件的患者– n 危险比，相比于安慰剂 (95% CI)	27/60	68/183	43/133 0.65 (0.40–1.06)	P=0.0826	115/350 0.84 (0.62–1.13)	P=0.2441
次要终点						
确认的能力丧失进展达至少 24 周 具有事件的患者– n 危险比，相比于安慰剂 (95% CI)	23/60	63/183	39/133 0.67 (0.40–1.14)	P=0.1417	103/350 0.81 (0.59–1.10)	P=0.1783
从基线到第 120 周的 T25FW 组中的数量 调整几何平均值的比率 (95% CI)	39	134	106 0.859 (0.655–1.112)	P=0.2464	288 0.922 (0.801–1.060)	P=0.2536
从基线到第 120 周的 T2 损伤体积 组中的数量 调整几何平均值的比率 (95% CI)	39	144	107 0.859 (0.818–0.901)	P<0.0001	291 0.913 (0.885–0.943	P<0.0001
第 24 周-第 120 周的全脑体积 组中的数量 调整平均值的差异，相比于安慰剂 – % (95% CI)	31	119	83 18.1 (–21.1 至 57.2)	P=0.3625	241 20.6 (3.3 至 37.9)	P=0.0198
探索性终点						
12 周确认的综合能力损伤进展* 具有事件的患者– n 危险比，相比于安慰剂 (95% CI)	43/60	127/183	78/133 0.77 (0.52–1.13)	P=0.1824	206/351 0.75 (0.60–0.94)	P=0.0109
T25FW 中的 12 周确认的进展达至少 20% 具有事件的患者– n (%) 危险比，相比于安慰剂 (95% CI)	38/60	106/183	65/133 0.67 (0.45–1.02)	P=0.0591	170/351 0.78 (0.61–0.99)	P=0.0431
9HPT 中的 12 周确认的进展达至少 20% 具有事件的患者– n (%) 危险比，相比于安慰剂	22/60	43/183	24/133 0.42	P=0.0044	58/351 0.64	P=0.0254

[0709]

(95% CI)			(0.23–0.76)		(0.43–0.95)	

[0710] 图29显示了与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中从基线至第120周的行走速度的下降速率(即行走时间的进展率的降低)(如通过计时25英尺行走所测量的)。相比于安慰剂，ocrelizumab使行走速度下降的速率降低了29％。分析基于ITT人群；p值基于在第120周访问时的分级(ranked)ANCOVA，针对基线25英尺计时行走、地理区域和年龄调整，具有由末次观察推进法(即LOCF)推算的缺失值。评分估计值和95％CI均基于对数变换数据。图19中的分析基于ITT(意向治疗人群)；p值基于在第120周访问时的分级ANCOVA，针对基线计时25英尺行走、地理区域和年龄调整，具有由LOCF(末次观察推进法)推算的缺失值。评分估计值和95％CI(置信区间)基于MMRM分析和对数变换数据，针对基线计时25英尺行走、地理区域和年龄调整。在基线具有Gd^+损伤(参见图35A)和在基线不具有T1 Gd^+损伤(参见图35B)的患者中，使用ocrelizumab(相对于安慰剂)的从基线行走时间至第120周的百分比变化的降低与总研究人群一致(参见图35C)。图35A-C中的分析基于意向治疗人群；p值基于在第120周访问时的分级ANCOVA，针对基线计时25英尺行走、地理区域和年龄调整，具有由LOCF(末次观察推进法)推算的缺失值。评分估计值和95％置信区间基于对数变换数据的MMRM分析，针对基线计时25英尺行走、地理区域和年龄调整。

[0711] 图30显示了与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中第120周行走速度相对于基线的下降速率。相比于安慰剂，ocrelizumab使行走速度的下降速率降低10％(p＝0.404)。

[0712] 图40A显示了与接受安慰剂的总研究群体中的患者相比，接受600mg ocrelizumab的总研究群体中的患者中从基线至第120周的SF-36健康要素总结评分的变化。图40B显示了与接受安慰剂的在基线处具有T1钆增强型损伤的患者相比，接受600mg ocrelizumab的在基线处具有T1钆增强型损伤的患者中从基线至第120周的SF-36健康要素总结评分的变化。图40C显示了与接受安慰剂的在基线处不具有T1钆增强型损伤的患者相比，接受600mg ocrelizumab的在基线处不具有T1钆增强型损伤的患者中从基线至第120周的SF-36健康要素总结评分的变化。

[0713] MRI相关终点

[0714] 图31显示了与接受安慰剂的患者相比，接受600mg ocrelizumab的患者中从第24周至第96周的全脑体积的百分比变化。与安慰剂相比，ocrelizumab使从第24周至第96周的全脑体积损失的速率降低17.5％(p＝0.0206)。分析基于第24周的ITT人群和至少一个第24周后的评价；p值基于第120周访问的MMRM，针对第24周脑体积、地理区域和年龄调整)。在基线具有T1 Gd^+损伤(参见图36A)和在基线不具有T1 Gd^+损伤(参见图36B)的OCR治疗的患者中，从第24周至第120周的全脑体积损失速率与总人群数据一致(参见图36C)。图36A-C中的分析基于第24周的意向治疗人群和至少一个第24周后的评价；p值基于第120周访问的MMRM，针对第24周脑体积、地理区域和年龄调整。

[0715] 与接受安慰剂的患者相比，接受600mg ocrelizumab的患者表现出从基线至第120周的T2损伤体积的实质性减少(p＜0.0001)。如图32所示，安慰剂组中的患者的T2损伤体积增加7.4％，而接受ocrelizumab的患者的T2损伤体积减少3.4％。分析基于意向治疗人群；p值基于在第120周访问时的分级ANCOVA，针对基线T2损伤体积、地理区域和年龄调整，具有由LOCF(末次观察推进法)推算的缺失值。评分估计值和95％CI(置信区间)基于对数变换数据的MMRM分析，针对基线T2损伤体积、地理区域和年龄调整。

[0716] ocrelizumab治疗减少了在基线具有和不具有T1 Gd^+损伤的患者中从基线至第120周的脑T2高强度损伤的总体积，而在安慰剂组中在基线具有和不具有T1 Gd^+损伤的患者的总损伤体积增加。参见图37A。

[0717] 在基线具有T1 Gd^+损伤的患者中，ocrelizumab治疗的患者的总T2损伤体积减少3.8％(95％CI，-7.0至-0.5)。相比之下，接受安慰剂的患者的总T2损伤体积增加12.0％(95％CI，7.2-17.1；p＜0.001)。参见图37B。在基线不具有T1 Gd^+损伤的患者中，ocrelizumab治疗的患者的总T2损伤体积减少3.1％(95％CI，-5.0至-1.1)。相比之下，接受安慰剂的患者的总T2损伤体积增加6.1％(95％CI，3.3-9.0；p＜0.001)。参见图37C。图37A-C中的分析基于ITT人群；p值基于在第120周访问时的分级ANCOVA，针对基线T2损伤体积、地理区域和年龄调整，具有由LOCF(末次观察推进法)推算的缺失值。评分估计值和95％CI基于对数变换数据的MMRM分析，针对基线T2损伤体积、地理区域和年龄调整。

[0718] 与接受安慰剂的患者相比，接受600mg ocrelizumab的患者显示出T1钆增强性损伤减少97.6％(p＜0.0001)。与接受安慰剂的患者相比，接受600mg ocrelizumab的患者还显示出新的和/或扩大的T2损伤减少91.9％(p＜0.0001)。将T1 Gd^+损伤总数和新的和/或扩大的T2损伤总数除以治疗组中所有患者的第24周、第48周和第120周的个体损伤数的总和，以脑MRI扫描的总数计。通过负二项回归计算平均值进行调整，并且针对*T1Gd^+损伤(存在与否)、基线年龄(≤45岁比上＞45岁)和地理区域(US比上世界其他地区)调整。

[0719] 功效

[0720] 本研究的功效终点总结于表13A中。临床和MRI终点(ITT人群)总结于表13B中。

[0721] 表13A：功效总结

[0722]

终点	风险降低	P 值
至 CDP 12 周的时间	24%	0.0321
至 CDP 24 周的时间	25%	0.0365
计时 25 英尺行走的变化 (基线至第 120 周)	29%	0.0404
MRI 总 T2 损伤体积的百分比变化 (基线至第 120 周)	安慰剂：7.4%提高 ocrelizumab: 3.4%降低	<0.0001
MRI 总脑体积的百分比变化 (第 24 周至第 120 周)	17.5%	0.0206
SF-36 PCS 的变化 (基线至第 120 周)		

[0723] 表13B：临床和MRI终点(ITT人群)

[0724]

终点	安慰剂 (n=244)	Ocrelizumab 600 mg (n=488)
临床终点		
主要终点		
确认的能力丧失进展达至		

[0725]

少 12 周 具有事件的患者：n (%) 危险比，相比于安慰剂 (95% CI) P 值	96 (39.3)		160 (32.9) 0.76 (0.59–0.98) P=0.0321	
Secondary endpoints				
确认的能力丧失进展达至少 24 周 具有事件的患者 – n (%) 危险比，相比于安慰剂 (95% CI) P 值	87 (35.7)		144 (29.6) 0.75 (0.58–0.98) P=0.0365	
从基线到第 120 周的 T25FW 平均百分比变化 相对降低，相比于安慰剂 – % (95% CI) P 值	55.10		39.93 29.337 (–1.618 至 51.456) P=0.0404	
探索性终点	**12 周 CDP**	**24 周 CDP**	**12 周确认的**	**24 周确认的**
复合能力丧失进展* 具有事件的患者 – n (%) 危险比，相比于安慰剂 (95% CI) P 值	171 (70.1)	155 (63.5)	287 (58.8) 0.74 (0.61–0.89) P=0.0014	251 (51.4) 0.71 (0.58–0.87) P=0.0008
T25FW 中的进展达至少 20% 具有事件的患者 – n (%) 危险比，相比于安慰剂 (95% CI) P 值	145 (59.4)	127 (52.0)	238 (48.8) 0.75 (0.61–0.92) P=0.0053	202 (41.4) 0.73 (0.59–0.91) P=0.0055
9HPT 中的进展达至少 20% 具有事件的患者 – n (%) 危险比，相比于安慰剂 (95% CI) P 值	66 (27.0)	57 (23.4)	83 (17.0) 0.56 (0.41–0.78) P=0.0004	69 (14.1) 0.55 (0.38–0.77) P=0.0006
MRI 终点				
次要终点				
从基线到第 120 周的 T2 损伤体积 调整几何平均值百分比变化(95% CI)调整几何平均值的比率，相比于安慰剂 (95% CI) P 值	7.426 (4.967–9.942)		-3.366 (-4.987 至-1.718) 0.900 (0.876–0.924) P<0.0001	
第 24 周-第 120 周的总脑体积	–1.093±0.072		–0.902±0.052	

[0726]

平均百分比变化相对降低，相比于安慰剂–% (95% CI) P 值		17.475 (3.206–29.251) P=0.0206
探索性终点		
新的和/或扩大的 T2 损伤损伤的平均数量/MRI 扫描 (95% CI)[3] 百分比变化(95% CI)[3] P 值	3.880 (2.841–5.299)	0.313 (0.246–0.397) 0.081 (0.058–0.111) -91.9 (-88.9 至 -94.2) P<0.0001
患者报告结果		
次要终点		
从基线到第 120 周的 SF-36 健康要素总评评分 调整平均值变化(95% CI) 调整平均值的差异(95% CI) P 值	–1.108 (–2.394 至 0.177)	–0.731 (–1.655 至 0.193) 0.377 (–1.048 至 1.802) P=0.6034

[0727] 在初步分析时，与安慰剂相比，ocrelizumab使12周和24周确认的能力丧失进展的风险分别显著降低24％(HR＝0.76，95％CJ[0.59，0.98]；P＝0.0321；表13B；图27)和25％(0.75，95％CI[0.58，0.98]；P＝0.0365；表13B；图28)。在第120周，与安慰剂相比，ocrelizumab使计时25英尺行走的进展速率降低29％(P＝0.0404)(表13B)。到双盲治疗期结束时，与安慰剂相比，ocrelizumab还使12周和24周确认的复合能力丧失进展(即，EDSS进展，或计时25英尺行走测试中的至少20％进展，和/或9孔钉测试中的至少20％进展)分别显著降低26％(HR＝0.74[0.61-0.89]，P＝0.0014；表13B；图41)和29％(HR＝0.71[0.58-0.87]，P＝0.0008；表13B；图42)。关于复合能力丧失进展评价的各个组分，与安慰剂相比，ocrelizumab一贯地且显著地降低了计时25英尺行走(表13B)和9孔钉测试中12周和24周确认的20％进展的风险(表13B)。

[0728] 另外的敏感性分析与初步结果一致，并且进行该分析通过排除经历医师报告的临床复发(包括方案定义的复发)的患者来评价研究中的复发对确认的能力丧失进展数据的影响(表S1)。安慰剂组中11％的患者和ocrelizumab组中5％的患者报告了方案定义的复发。在基线具有或不具有T1钆增强性损伤的患者的关键临床和MRI功效终点的亚组分析与总研究人群一致(表S2)。然而，ORATORIO研究未能证明这些亚组之间的功效差异。

[0729] 安全性

[0730] 输注相关的反应

[0731] 安全性分析中包括725名患者(即，安慰剂组中239名和ocrelizumab组中486名)。在大约3年的平均治疗持续时间内，具有至少一次输注相关反应(IRR)的患者的比例对于给予安慰剂的患者为25.5％(n＝61)并且对于ocrelizumab治疗的患者为39.9％(n＝194)；大多数患者的严重程度为轻度至中度(分别为93.4％[n＝57]和96.9％[n＝188])。最常见的IRR症状包括皮肤和皮下组织病症(对于ocrelizumab治疗的患者为45.9％[n＝89]，并且对于给予安慰剂的患者为13.1％[n＝8])。不存在危机生命或致命的IRR。ocrelizumab治疗的

患者的IRR发生率在第一次输注时最高(27.4%),并且随着后续给药显著降低(在剂量2的第一次输注时为11.6%)。总体而言,IRR主要在ocrelizumab治疗的患者中在输注期间发生(61.3%,相比于给予安慰剂的患者组中的37.7%)。一名患者(0.2%)在首次输注期间由于IRR而退出ocrelizumab治疗。

[0732] 不良事件

[0733] 表14A提供了直到临床截止日期时,每个治疗组中≥10%的患者报告的不良事件。

[0734] 表14A.

[0735]

n (%)	安慰剂 (n=239)	Ocrelizumab 600 mg (n=486)
≥1 AE 的所有患者	215 (90.0)	462 (95.1)
感染和侵害*	162 (67.8)	339 (69.8)
鼻咽炎	65 (27.2)	110 (22.6)
尿路感染	54 (22.6)	96 (19.8)
流感	21 (8.8)	56 (11.5)
上呼吸道感染	14 (5.9)	53 (10.9)
支气管炎	12 (5.0)	30 (6.2)
胃肠炎	12 (5.0)	20 (4.1)
损伤、中毒和程序并发症	104 (43.5)	263 (54.1)
肌肉骨骼和结缔组织病症	98 (41.0)	181 (37.2)
神经系统病症	79 (33.1)	174 (35.8)
一般性病症和施用部位病况	60 (25.1)	130 (26.7)
胃肠道病症	60 (25.1)	126 (25.9)

[0736]

精神疾病	59 (24.7)	89 (18.3)
皮肤和皮下组织病症	44 (18.4)	99 (20.4)
呼吸、胸廓和纵隔病症	35 (14.6)	87(17.9)
代谢和营养病症	28 (11.7)	56 (11.5)
肾和泌尿病症	30 (12.6)	51 (10.5)
血管病症	26 (10.9)	54 (11.1)
调查	20 (8.4)	58 (11.9)

[0737] *仅对于感染和侵害SOC:呈现由一个治疗组中至少5%的患者报告的事件。

[0738] 与治疗组中的20.4%相比,安慰剂组中经历多于1次严重不良事件的总患者百分比为22.2%。参见表14B。这两组中的严重不良事件总数都很低,并且类似。

[0739] 与ocrelizumab相关的最常见的不良事件是输注相关反应(39.9%,而对于安慰剂为25.5%)。一名患者(0.2%)在首次输注时由于IRR而退出ocrelizumab治疗。如图33所示,输注相关反应(IRR)的严重程度随着每个连续疗程以及随着相同疗程内的每个剂量而降低。ocrelizumab与安慰剂具有类似的安全性特征。

[0740] 表14B.总的严重不良事件

[0741]

n (%)	安慰剂 n = 239	Ocrelizumab n = 486
≥1 SAE 的所有患者	53 (22.2)	99 (20.4)
感染和侵害	14 (5.9)	30 (6.2)
损伤、中毒和程序并发症	11 (4.6)	19 (3.9)
神经系统病症	9 (3.8)	18 (3.7)
赘生物(neoplasms)：良性、恶性和不明(包括囊肿和息肉)	7 (2.9)	8 (1.6)
胃肠道病症	3 (1.3)	10 (2.1)
肌肉骨骼和结缔组织病症	6 (2.5)	6 (1.2)
一般性病症和施用部位病况	3 (1.3)	6 (1.2)
肾和泌尿病症	3 (1.3)	5 (1.0)

[0742] 从该研究获得的数据表明，B细胞可能在PPMS病理生理学中起作用。与安慰剂相比，ocrelizumab是在大型III期研究中对PPMS显示一致的、统计学显著的临床效果的第一项研究性治疗。ocrelizumab显著降低了持续12周的临床能力丧失进展(主要终点)、持续24周的临床能力丧失进展、计时25英尺行走的变化、T2损伤体积的变化和脑体积损失。接受ocrelizumab的患者始终如一地符合关键的次要终点。在大约3年的平均治疗持续时间内，ocrelizumab显示出有利的安全性特征。与ocrelizumab相关的不良事件的总发生率与安慰剂相似。最常见的不良事件是轻度至中度的输液相关反应。与ocrelizumab相关的严重不良事件(包括严重感染)的发生率也与安慰剂相似。

[0743] 开放标签延长阶段

[0744] 在随机化、平行组、双盲、安慰剂对照的研究之后，患者可能有资格进入开放标签延长(OLE)阶段以评价ocrelizumab的长期安全性、耐受性和功效。以下描述了开放标签延长研究的设计，其将评价OCR在复发型MS中的长期安全性和功效。

[0745] 在完成盲法治疗期时的初步数据库锁定后，可以从进一步治疗获益的患者接受开放标签ocrelizumab。随机分至ocrelizumab组的患者继续开放标记治疗，每24周使用双重ocrelizumab输注(300mg i.v.输注，间隔14天施用)。随机分至安慰剂的患者接受第一次开放标记治疗周期的双重ocrelizumab输注(即，两个i.v.输注，间隔14天施用)，从临时数据库锁定和初步分析后的下一次定期访问开始。

[0746] 依照当地法规或者直到发起人决定终止ocrelizumab MS程序，否则持续OLE阶段直到ocrelizumab在患者所在国家是可商购的。OLE阶段不超过4年(208周)。OLE不是强制性的。不愿意进入OLE阶段的患者进入安全性随访阶段，该阶段从患者最近一次访问的日期开始以12周为间隔进行。如果B细胞不充足，则继续监测。

[0747] OLE阶段的关键纳入标准包括：完成盲法治疗期；获得研究者的同意，该研究者基于治疗期间进行的评价确定患者可能从OCR治疗中受益；待进入OLE阶段的患者的书面知情同意书；以及符合ocrelizumab治疗/再治疗标准，其中患者不具有以下情况和实验室异常：

(a)在先前的OCR输注期间发生的危及生命(CTCAE 4级)的输注相关事件;(b)任何重大或不受控制的医疗状况或者治疗突发的临床显著的实验室异常;(c)活动性感染;(d)嗜中性粒细胞绝对计数＜1.5×10^3/μL;(e)CD4细胞计数＜250/μL;和(f)低丙种球蛋白血症免疫球蛋白G＜3.3g/L。

[0748] 关键的安全性和功效评价的时间表如下表15所示:

[0749] 表15

[0750]

评价	OLE阶段周期[a]			延迟的给药访问b	计划外的访问c	退出治疗访问
	第1天OLE周期(±5天)	第15天OLE周期(±5天)	第1天输注后12周访问第1天OLE周期(±7天)			
神经检查[d]	x	x	x		x	x
EDSS[e]	x		x		x	x
潜在的复发[f]	x	x	x	x	x	x
EQ-5D[g]	x					x
MRI[h]	x					x
常规安全性实验室[i]	x		x		x	x
伴行的治疗[j]	x	x	x	x	x	x
抗体滴度[k]						x

[0751] [a]患者继续盲法24周治疗周期,直到最后一名患者接受安排于第98周的他/她的最终疗程。那时,所有患者都继续到当前(24周)周期结束。如果在第120周还未达到确认的能力丧失进展事件的预计总数,则所有患者继续进行另外的盲法24周治疗周期,直至达到确认的能力丧失事件的预计数量。

[0752] [b]当在定期给药访问中不能施用给药时,进行延迟的给药访问。此时,其它测试/评价可以适当地进行。

[0753] [c]根据患者的临床需求进行计划外的访问(非给药)评价。此时,其它测试/评价可以适当地进行。

[0754] [d]每次计划和计划外的访问时都会进行神经检查。具有暗示MS恶化或复发的新神经症状的所有患者应该由检查研究者进行EDSS。

[0755] [e]每12周由检查研究者对所有患者进行扩展的能力丧失状态量表评分。额外的EDSS评价可以在访问之间进行(即,在MS复发、神经恶化等期间)。能力丧失进展被定义为不由其它病因引起的从基线EDSS评分增加≥1.0分(基线EDSS≤5.5时)或≥0.5分(基线EDSS＞5.5时)。

[0756] [f]在每次定期访问时由治疗研究者评价患者的复发,并且如有需要,进行计划外访

问以确认访问之间发生复发。如果怀疑复发，则除了完成MS复发电子病例报告表以外，应该进行EDSS。

[0757] [g]EQ-5D(EuroQol五维问卷)评价将每年进行一次。

[0758] [h]在OLE阶段开始时(如果先前12周中没有进行)、定期访问的(±)4周内和OLE退出访问时(如果在先前4周中没有进行)进行年度脑MRI扫描。

[0759] [i]常规安全性实验室评价包括血液学、化学和尿分析。

[0760] [j]在每次定期和计划外访问时报告伴行的药物和程序。

[0761] [k]进行针对常见抗原(腮腺炎、风疹、水痘和肺炎链球菌)的抗体滴度。

[0762] 实施例(其目的为单纯示例性描述本发明并因而不应认为以任何方式限制本发明)也描述和详细说明了以上讨论的本发明的多个方面和实施方式。通过示例性方式而非限制性方式提供前述实施例和详细说明。

序列表

<110> 秀记·加雷恩
让-保罗·普费芬
安妮-玛丽·李桂璋
米凯莱·利博纳蒂

<120> 治疗多发性硬化的方法

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<150> US 62/238,103
<151> 2015-10-06

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<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 12
Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
1 5 10

<210> 13
<211> 213
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 13
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Leu Ile Tyr
35 40 45
Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr

85 90 95
Phe Gly Glu Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 14
<211> 452
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 14
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
[0005] Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

```
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu
            340                 345                 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
        355                 360                 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        435                 440                 445
Ser Pro Gly Lys
    450
```

<210> 15
<211> 452
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 15

[0006]

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
    130                 135                 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
        195                 200                 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
    210                 215                 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
```

```
          275                 280                 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ala Thr
     290                 295                 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335
Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
        355                 360                 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        435                 440                 445
Ser Pro Gly Lys
    450
```

<210> 16
<211> 213
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

[0007]

```
<400> 16
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1                  5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Leu
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Leu Ile Tyr
        35                  40                  45
Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ala Phe Asn Pro Pro Thr
                85                  90                  95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205
Asn Arg Gly Glu Cys
    210
```

<210> 17
<211> 452

<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 17
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Val Tyr Tyr Ser Tyr Arg Tyr Trp Tyr Phe Asp Val Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205
[0008]
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ala Thr
290 295 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala Ala Leu Pro Ala Pro
325 330 335
Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445
Ser Pro Gly Lys
450

<210> 18
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<220>
<221> 变体
<222> 9
<223> Xaa = Met或Leu

<400> 18
Arg Ala Ser Ser Ser Val Ser Tyr Xaa His
1 5 10

<210> 19
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<220>
<221> 变体
<222> 4
<223> Xaa = Ser或Ala

<400> 19
[0009] Gln Gln Trp Xaa Phe Asn Pro Pro Thr
1 5

<210> 20
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<220>
<221> 变体
<222> 8
<223> Xaa = Asp或Ala

<400> 20
Ala Ile Tyr Pro Gly Asn Gly Xaa Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15
Gly

<210> 21
<211> 13
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<220>

<221> 变体
<222> 6
<223> Xaa = Asn, Ala, Tyr, Trp, 或Asp

<220>
<221> 变体
<222> 7
<223> Xaa = Ser或Arg

<400> 21
Val Val Tyr Tyr Ser Xaa Xaa Tyr Trp Tyr Phe Asp Val
1 5 10

<210> 22
<211> 449
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 22

[0010]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Trp Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Trp Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Ala Lys Gly Glu Pro Arg Glu Pro Gln Val Tyr

```
            340                 345                 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445
Gly
```

<210> 23
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 23
```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Leu
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Leu Ile Tyr
        35                  40                  45
Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ala Phe Asn Pro Pro Thr
                85                  90                  95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

[0011]

<210> 24
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 24
```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Val Val Tyr Tyr Ser Tyr Arg Tyr Trp Tyr Phe Asp Val Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
```

115 120

<210> 25
<211> 451
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 25

[0012]

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Val Val Tyr Tyr Ser Tyr Arg Tyr Trp Tyr Phe Asp Val Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
    130                 135                 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190
Val Pro Ser Ser Ser Leu Gly Thr Glu Thr Tyr Ile Cys Asn Val Asn
        195                 200                 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
    210                 215                 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        275                 280                 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Gln Gln Tyr Asn Ala Thr
    290                 295                 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala Ala Leu Pro Ala Pro
                325                 330                 335
Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
        355                 360                 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430
```

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445
Ser Pro Gly
450

<210> 26
<211> 451
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 26
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
[0013] Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190
Val Pro Ser Ser Ser Leu Gly Thr Glu Thr Tyr Ile Cys Asn Val Asn
195 200 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr

405 410 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445
Ser Pro Gly
450

<210> 27
<211> 451
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 27
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
100 105 110
[0014] Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Gln Gln Tyr Asn Ala Thr
290 295 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335
Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

[0015]

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445
Ser Pro Gly
450

可变轻链结构域的序列比对

```
                  FR1                  CDR1
                  10        20        30        40
2H7               QIVLSQSPAILSASPGEKVTMTC [ RASSSVS- YMH ] WYQQKP
                  * ***  **  * ** *  *
hu2H7.v16         DIQMTQSPSSLSASVGDRVTITC [ RASSSVS- YMH ] WYQQKP
                                 ** * ** **
humkl             DI QMTQSPSSLSASVGDRVTI TC [ RASQSISNYLA ] WYQQKP

                  FR2       CDR2                  FR3
                  50        60        70        80
2H7               GSSPKPWIY [ APSNLAS ] GVPARFSGSGSGTSYSLTISRVEA
                  ** *  *   *          *** **** ****
hu2H7.v16         GKAPKPLIY [ APSNLAS ] GVPSRFSGSGSGTDFTLTISSLQP
                  *         * ** *
humkl             GYAPKLLIY [ AASSLES ] GVPSRFSGSGSGTDFTLTISSLQP

                                 CDR3      FR4
                  90        100
2H7               EDAATYYC [ QQWSFNPPT ] FGAGTKLELKR
                  **       **  * **      * * *
hu2H7.v16         EDFATYYC [ QQWSFNPPT ] FGQGTKVEIKR
                           ********  *
humkl             EDFATYYC [ QQYNSLPWT ] FGQGTKVEIKR
```

图1A

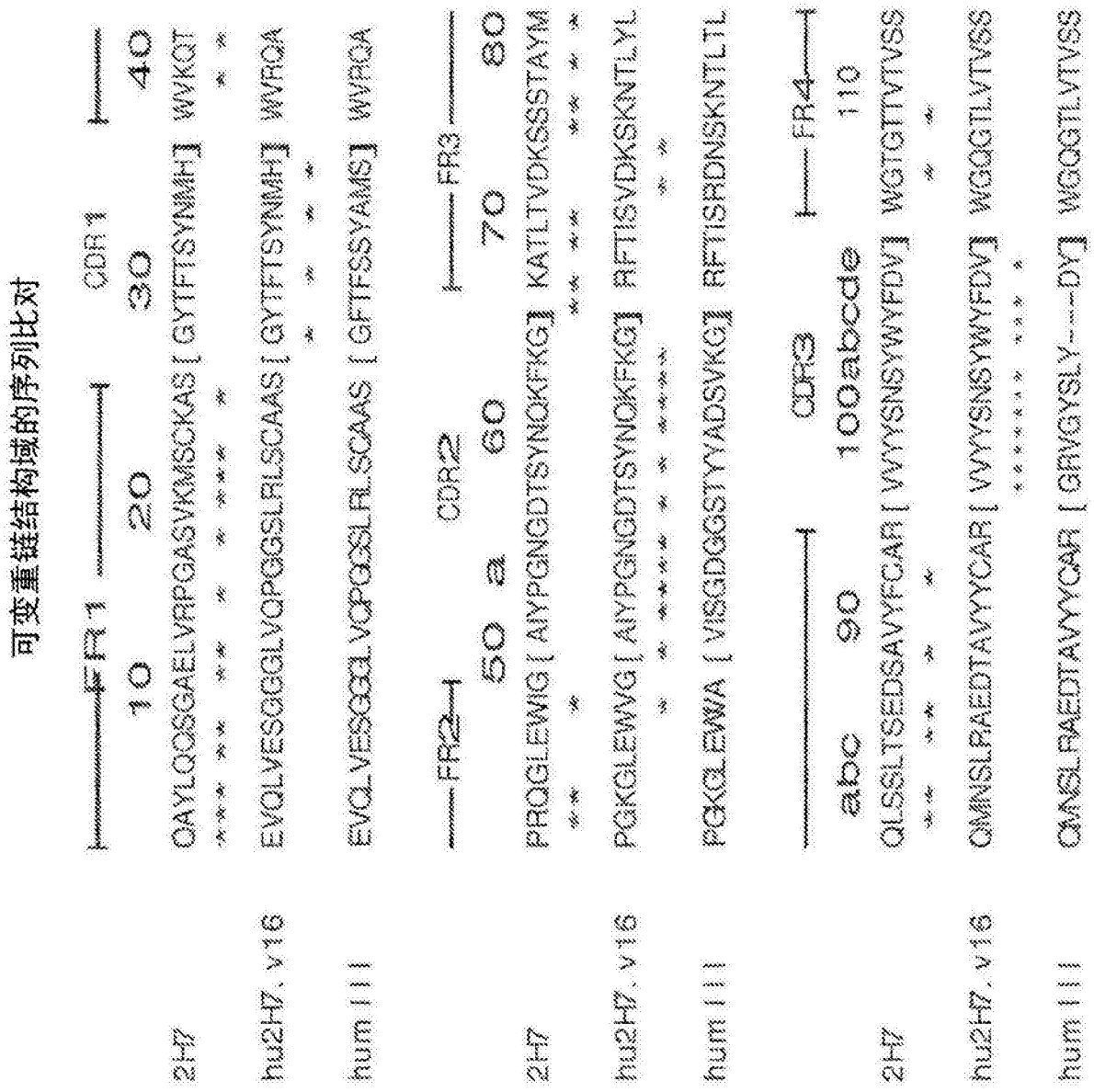


图1B

人源化 2H7.v16 轻链

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

图2

人源化 2H7.v16 重链

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

图3

人源化 2H7.v31 重链

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

图4

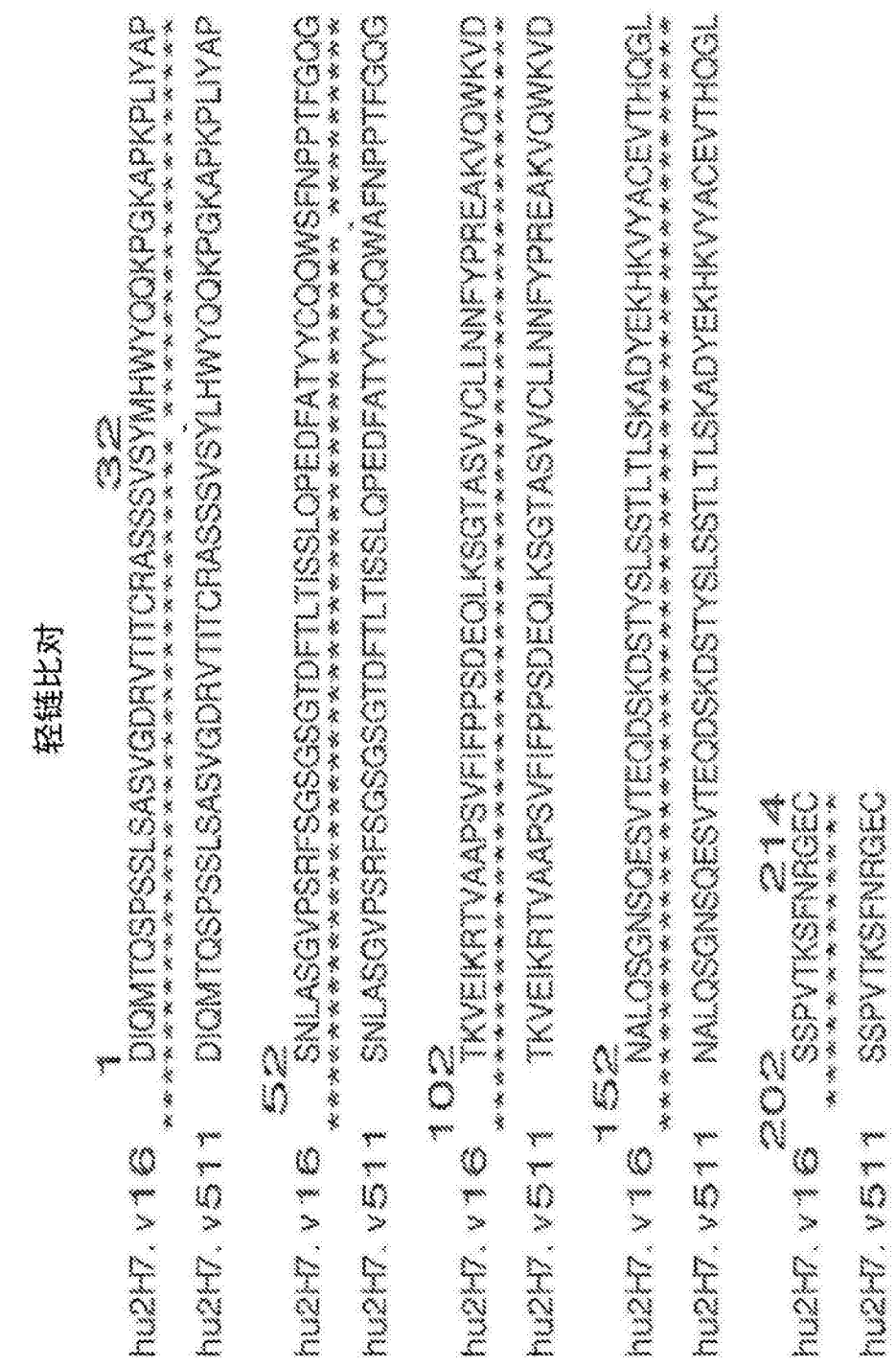

轻链比对
1
32
hu2H7.v16 DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP
hu2H7.v511 DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP
52
hu2H7.v16 SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG
hu2H7.v511 SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG
102
hu2H7.v16 TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
hu2H7.v511 TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
152
hu2H7.v16 NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
hu2H7.v511 NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
202
214
hu2H7.v16 SSPVTKSFNRGEC
hu2H7.v511 SSPVTKSFNRGEC

图5

重链比对

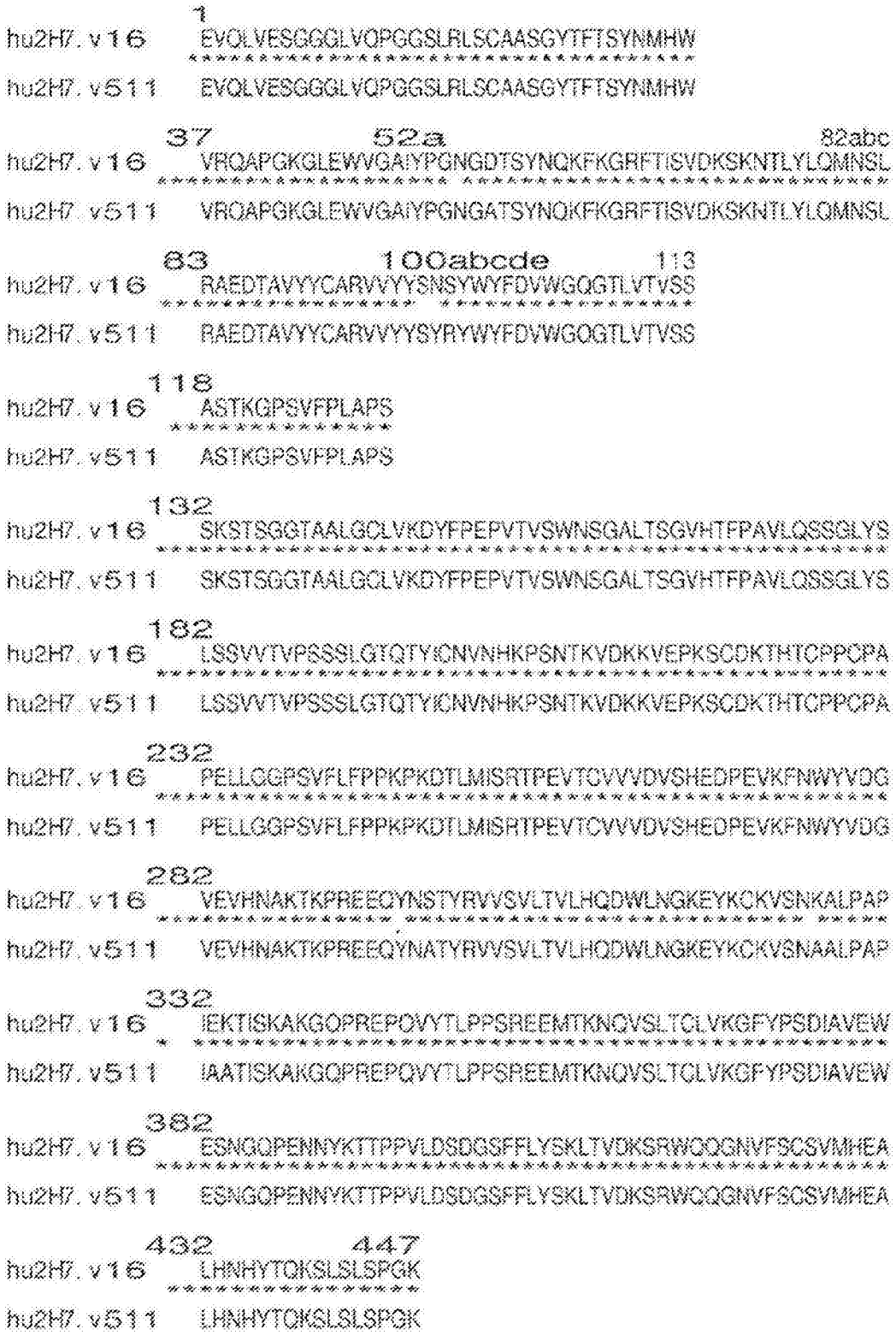

1
hu2H7.v16 EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
hu2H7.v511 EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
37 52a 82abc
hu2H7.v16 VRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSL
hu2H7.v511 VRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL
83 100abcde 113
hu2H7.v16 RAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS
hu2H7.v511 RAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS
118
hu2H7.v16 ASTKGPSVFPLAPS
hu2H7.v511 ASTKGPSVFPLAPS
132
hu2H7.v16 SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
hu2H7.v511 SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
182
hu2H7.v16 LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
hu2H7.v511 LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
232
hu2H7.v16 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
hu2H7.v511 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
282
hu2H7.v16 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
hu2H7.v511 VEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAP
332
hu2H7.v16 IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
hu2H7.v511 IAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
382
hu2H7.v16 ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
hu2H7.v511 ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
432 447
hu2H7.v16 LHNHYTQKSLSLSPGK
hu2H7.v511 LHNHYTQKSLSLSPGK

图6

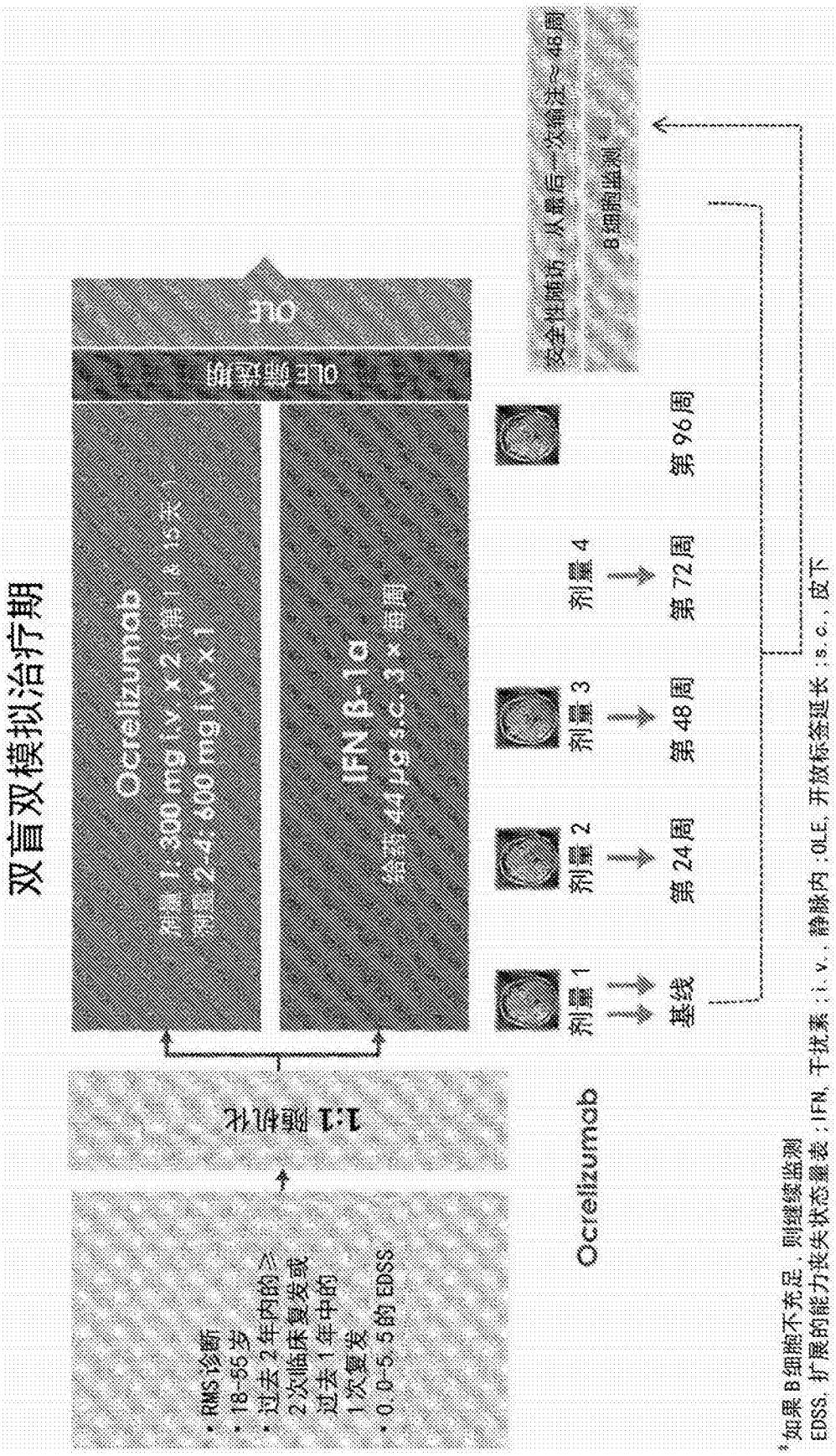

双盲双模拟治疗期
• RMS诊断
• 18-55岁
• 过去2年内的≥2次临床复发或过去1年中的1次复发
• 0.0-5.5的EDSS
1:1 随机化
Ocrelizumab
静脉 1 / 300 mg IV x 2，第 14 ± 2 天
随后 2 / 4 / 600 mg IV x 1
IFN β-1a
给药 44 μg 3 x 1 w 皮下
OLE 筛选期
OLE
安全性随访，从最后一次输注≈ 46 周
B 细胞监测*
Ocrelizumab
剂量 1
剂量 2
剂量 3
剂量 4
基线
第 24 周
第 48 周
第 72 周
第 96 周
*如果 B 细胞不充足，则继续监测
EDSS，扩展的能力丧失状态量表；IFN，干扰素；i. v.，静脉内；OLE，开放标签延长；s. c.，皮下

图7

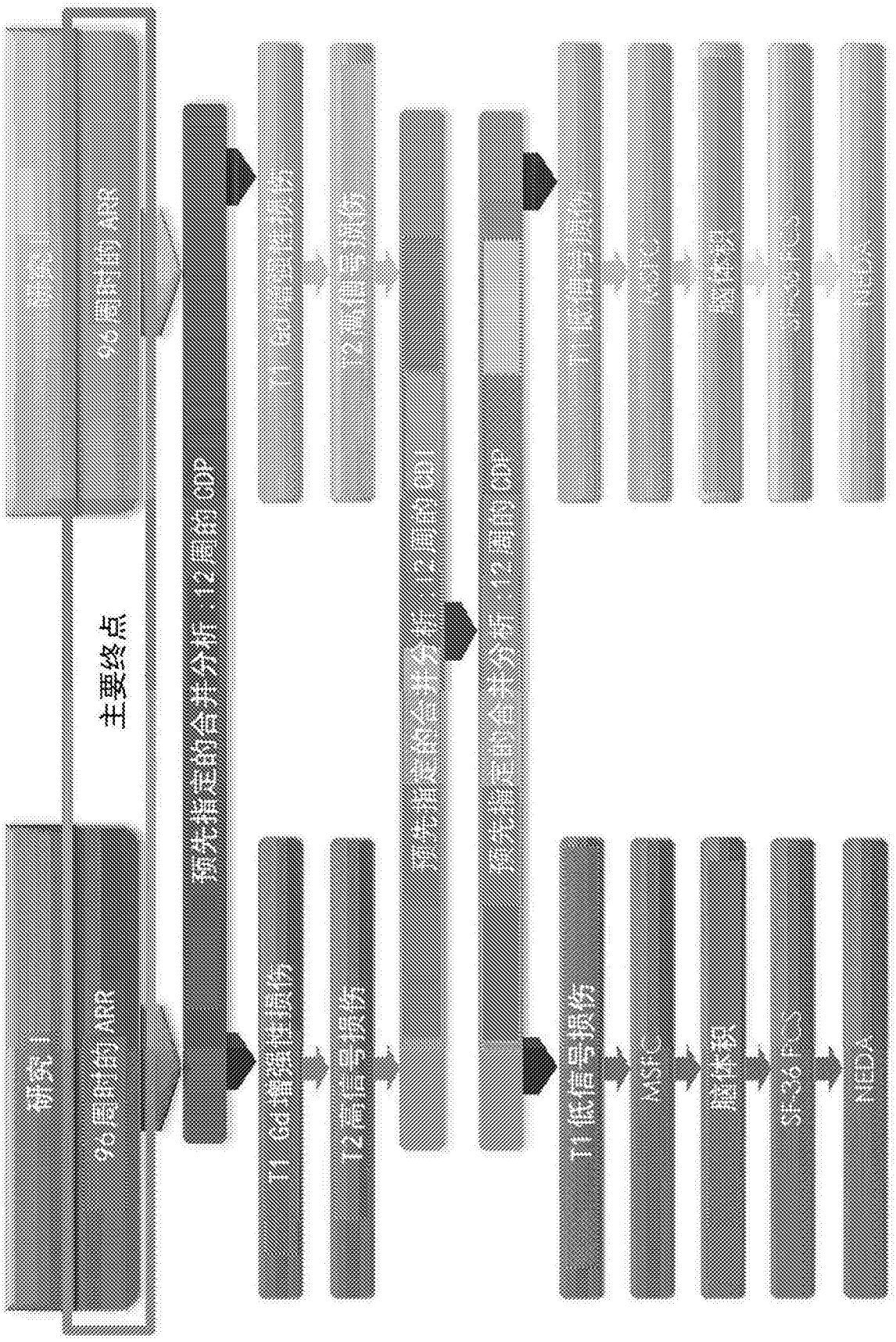
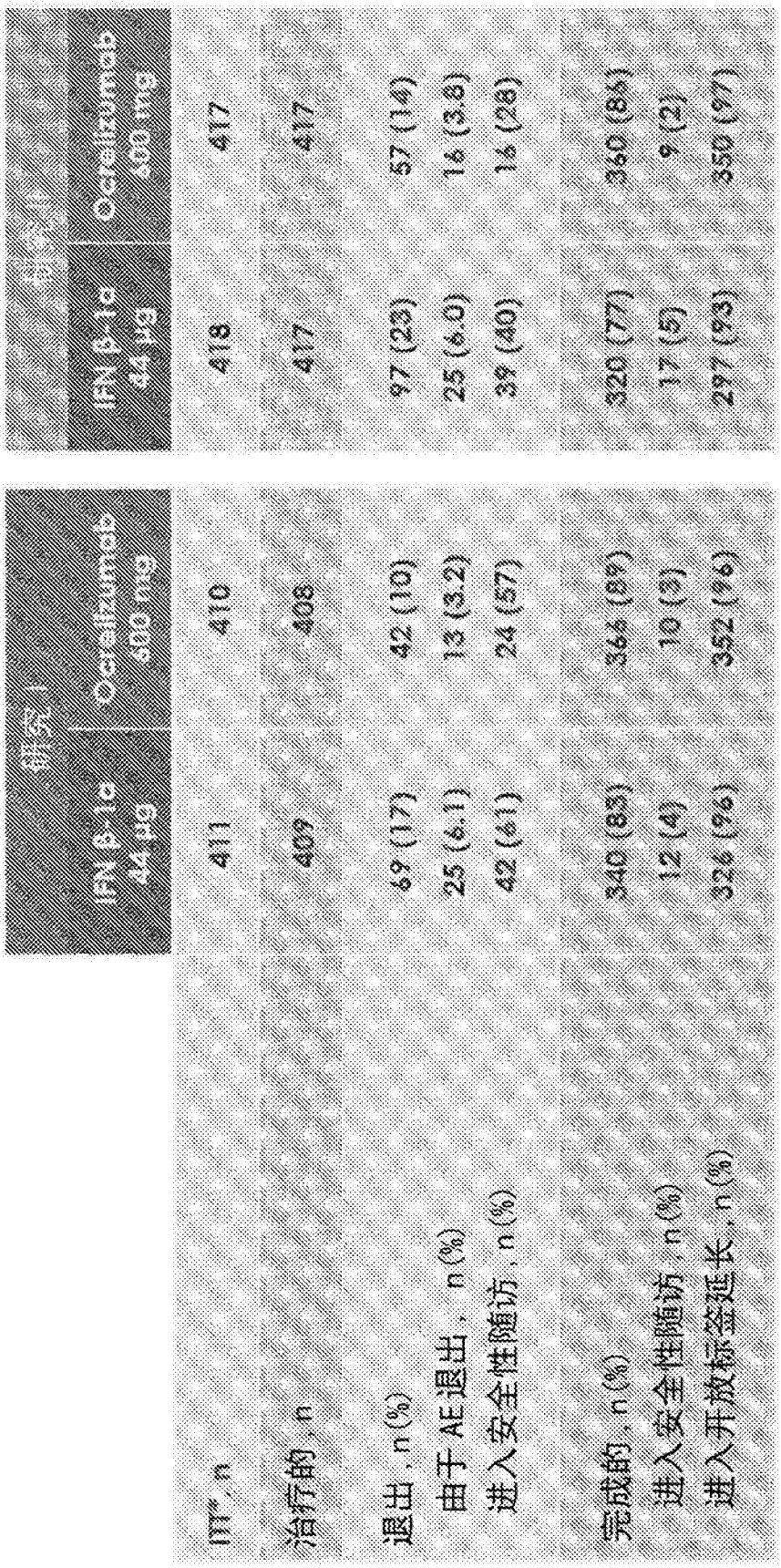

研究1
96周时的ABR
主要终点
预先指定的合并分析、12周的QIP
11/64强效性损伤
12高信号损伤
预先指定的合并分析、12周的QIP
11低信号损伤
MSK
脑体积
评分
NEDA
研究2
96周时的ABR
11/64强效性损伤
12高信号损伤
预先指定的合并分析、12周的QIP
11低信号损伤
MSK
脑体积
评分
NEDA

图8



	第52周		第104周	
	IFN β-1a 44 mcg	Ocrelizumab 600 mg	IFN β-1a 44 mcg	Ocrelizumab 600 mg
ITT*, n	411	410	418	417
治疗的，n	409	408	417	417
退出，n (%)	69 (17)	42 (10)	97 (23)	57 (14)
由于 AE 退出，n (%)	25 (6.1)	13 (3.2)	25 (6.0)	14 (3.8)
进入安全性随访，n (%)	42 (41)	24 (57)	39 (40)	14 (25)
完成的，n (%)	340 (83)	366 (89)	320 (77)	360 (86)
进入安全性随访，n (%)	12 (4)	10 (3)	17 (5)	9 (2)
进入开放标签延长，n (%)	326 (96)	352 (96)	297 (93)	350 (97)

图9

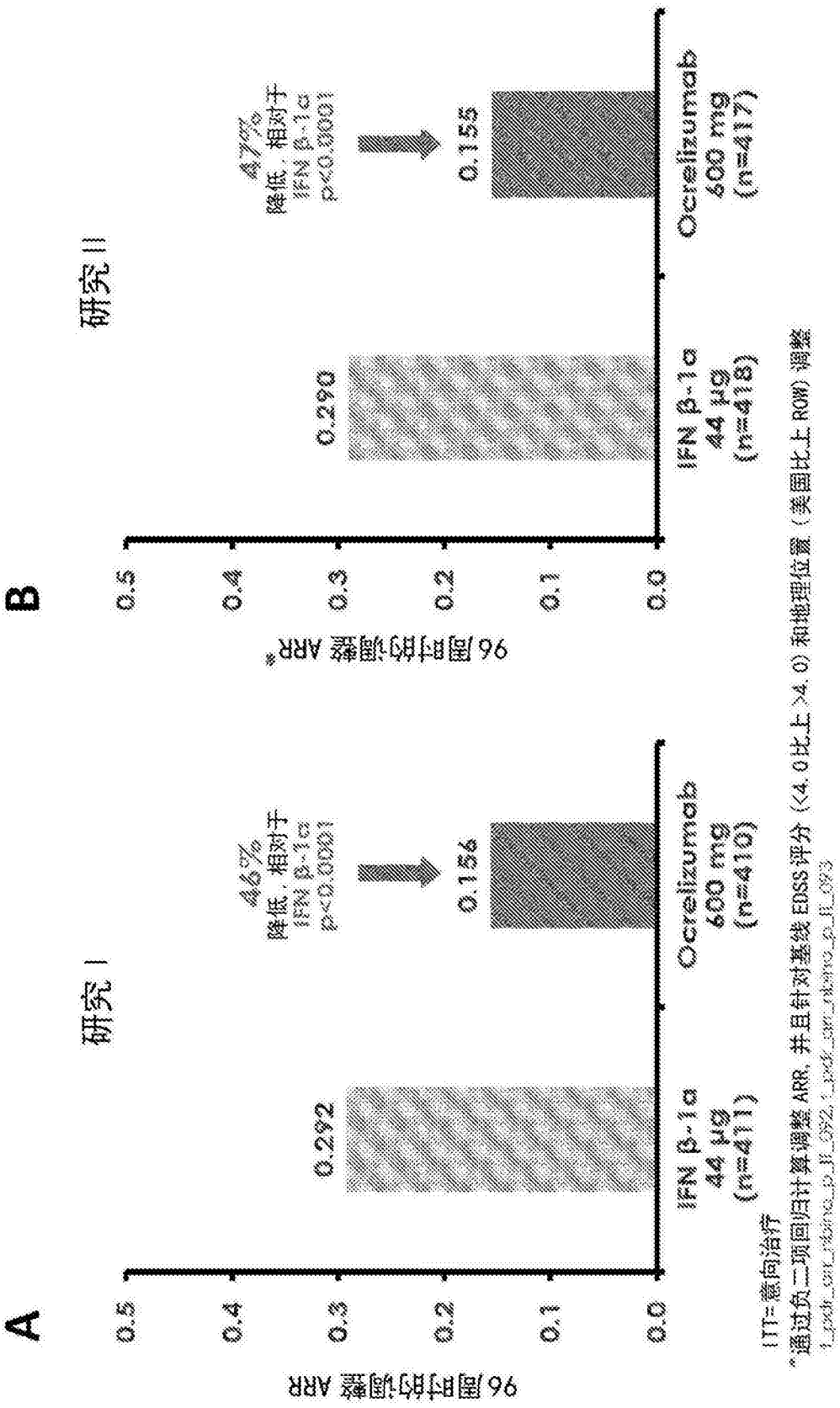

A
研究 I
96 周时的调整 ARR
0.5
0.4
0.3
0.2
0.1
0.0
0.292
0.156
46%
降低，相对于
IFN β-1a
p<0.0001
IFN β-1a
44 μg
(n=411)
Ocrelizumab
600 mg
(n=410)
B
研究 II
96 周时的调整 ARR*
0.290
0.155
47%
降低，相对于
IFN β-1a
p<0.0001
IFN β-1a
44 μg
(n=418)
Ocrelizumab
600 mg
(n=417)
ITT= 意向治疗
*通过负二项回归计算调整 ARR，并且针对基线 EDSS 评分（<4.0 比上 >4.0）和地理位置（美国比上 ROW）调整

图10

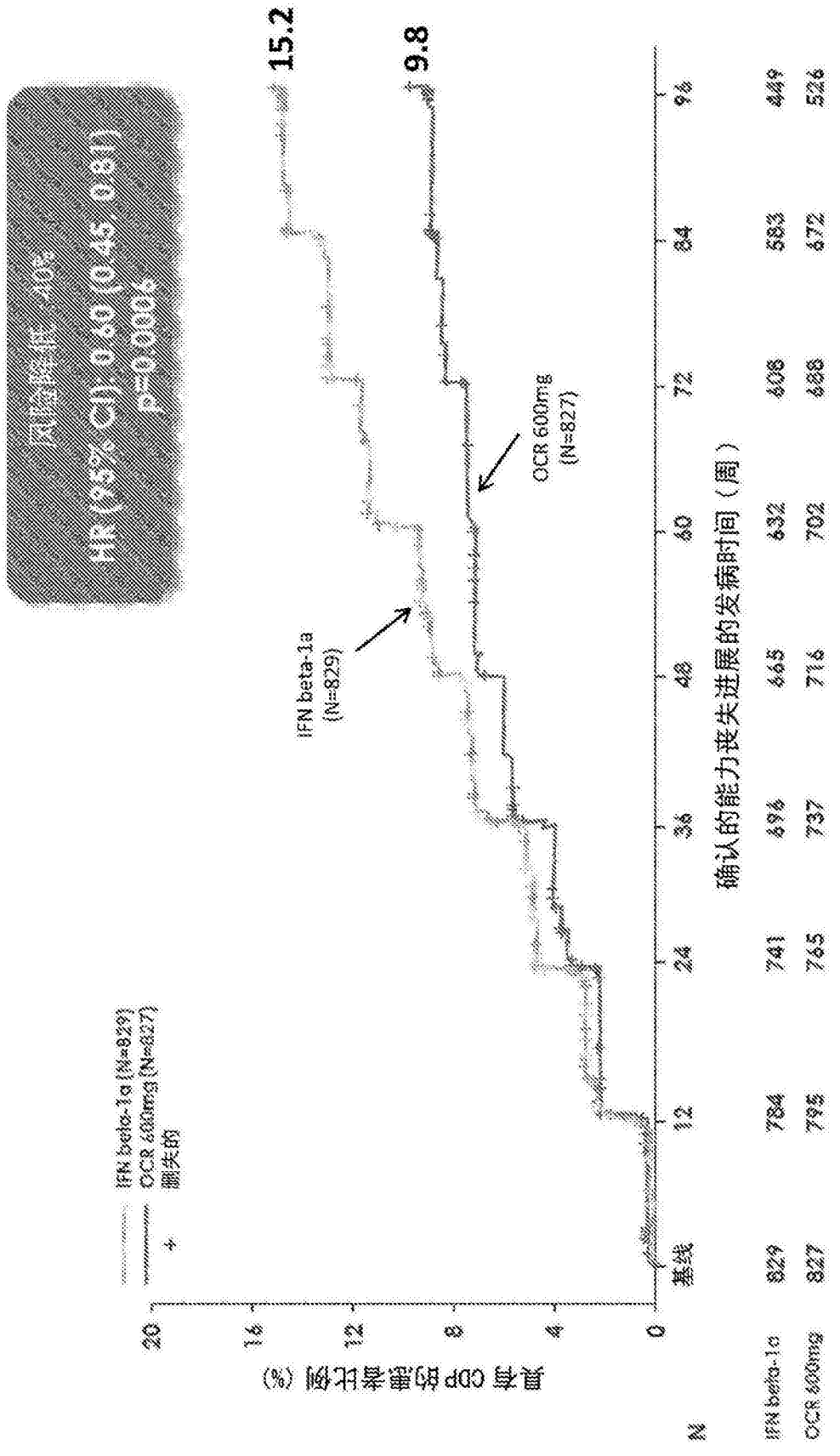

风险降低 40%
HR=0.60 (0.45-0.81)
p=0.0004
15.2
9.8
IFN beta-1a
(N=829)
OCR 600mg
(N=827)
具有 CDP 的患者比例 (%)
确认的能力丧失进展的发病时间（周）
IFN beta-1a (N=829)
OCR 600mg (N=827)
+ 删失的
N
基线 12 24 36 48 60 72 84 96
IFN beta-1a 829 784 741 696 665 632 608 583 449
OCR 600mg 827 795 765 737 716 702 688 672 526

图11

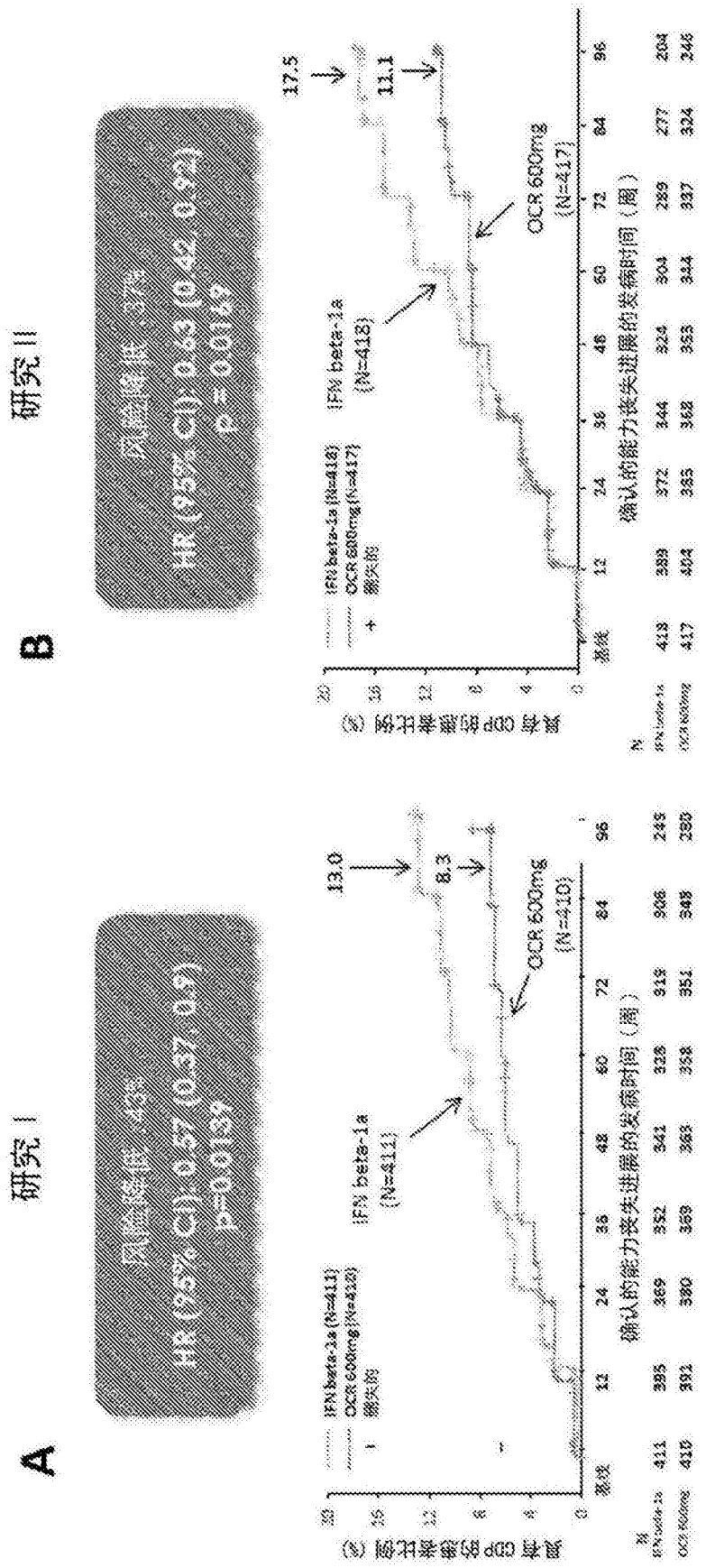

A
研究 I
风险降低 25%
HR (95% CI) 0.37 (0.27, 0.51)
p=0.0167
B
研究 II
风险降低 37%
HR (95% CI) 0.63 (0.49, 0.82)
p = 0.0167
IFN beta-1a (N=411)
OCR 600mg (N=410)
+ 删失的
IFN beta-1a (N=418)
OCR 600mg (N=417)
13.0
8.3
17.5
11.1
IFN beta-1a (N=411)
IFN beta-1a (N=418)
OCR 600mg (N=410)
OCR 600mg (N=417)
基线
确认的能力丧失进展的发病时间（周）
患有 CDP 的患者比例 (%)
N

图12

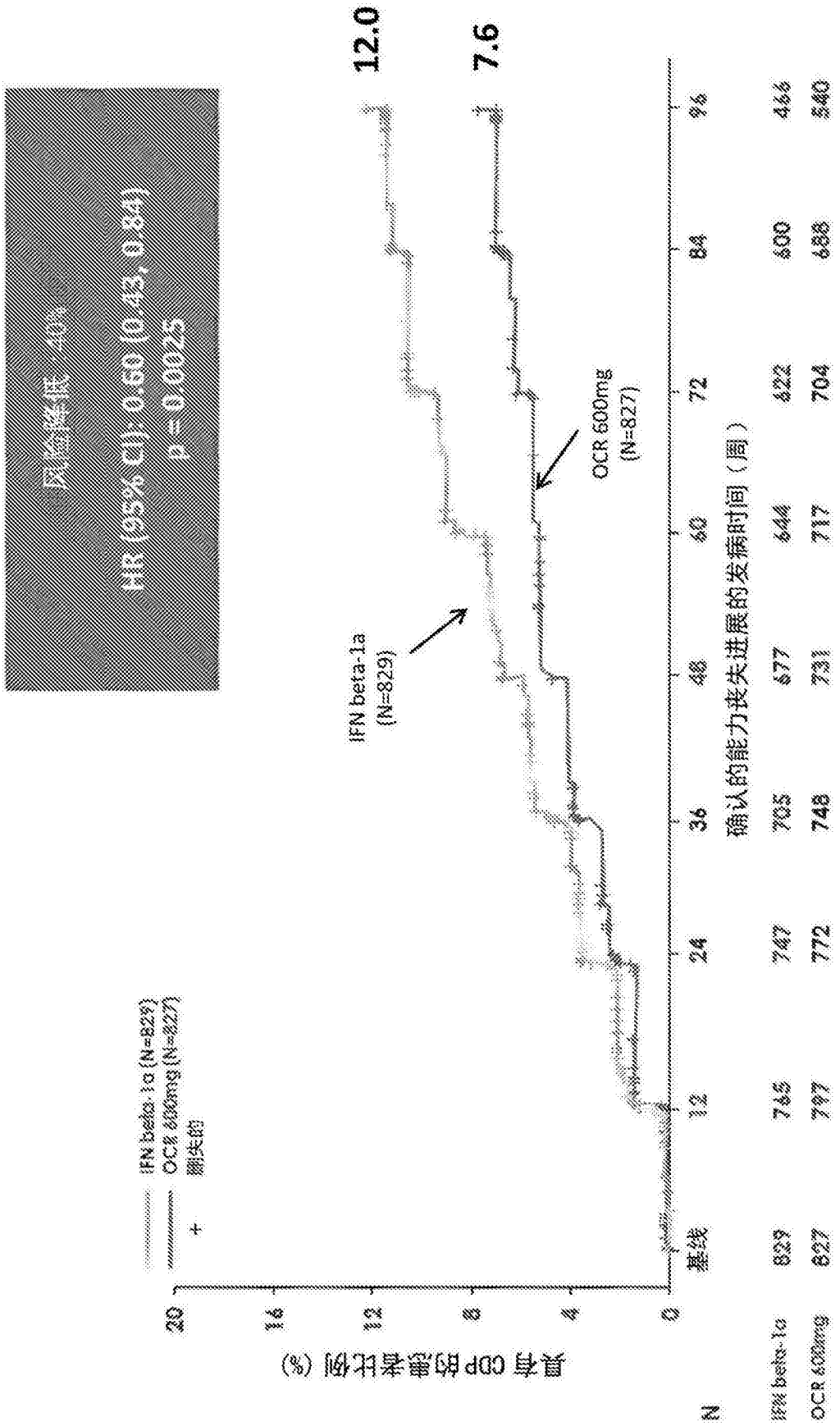

风险降低 40%
HR (95% CI): 0.60 (0.43, 0.84)
p = 0.0025
12.0
7.6
IFN beta-1a (N=829)
OCR 600mg (N=827)
具有 CDP 的患者比例 (%)
确认的能力丧失进展的发病时间（周）
IFN beta-1a (N=829)
OCR 600mg (N=827)
删失的
N
IFN beta-1a 829 785 747 705 677 644 622 600 466
OCR 600mg 827 797 772 748 731 717 704 686 540

图13

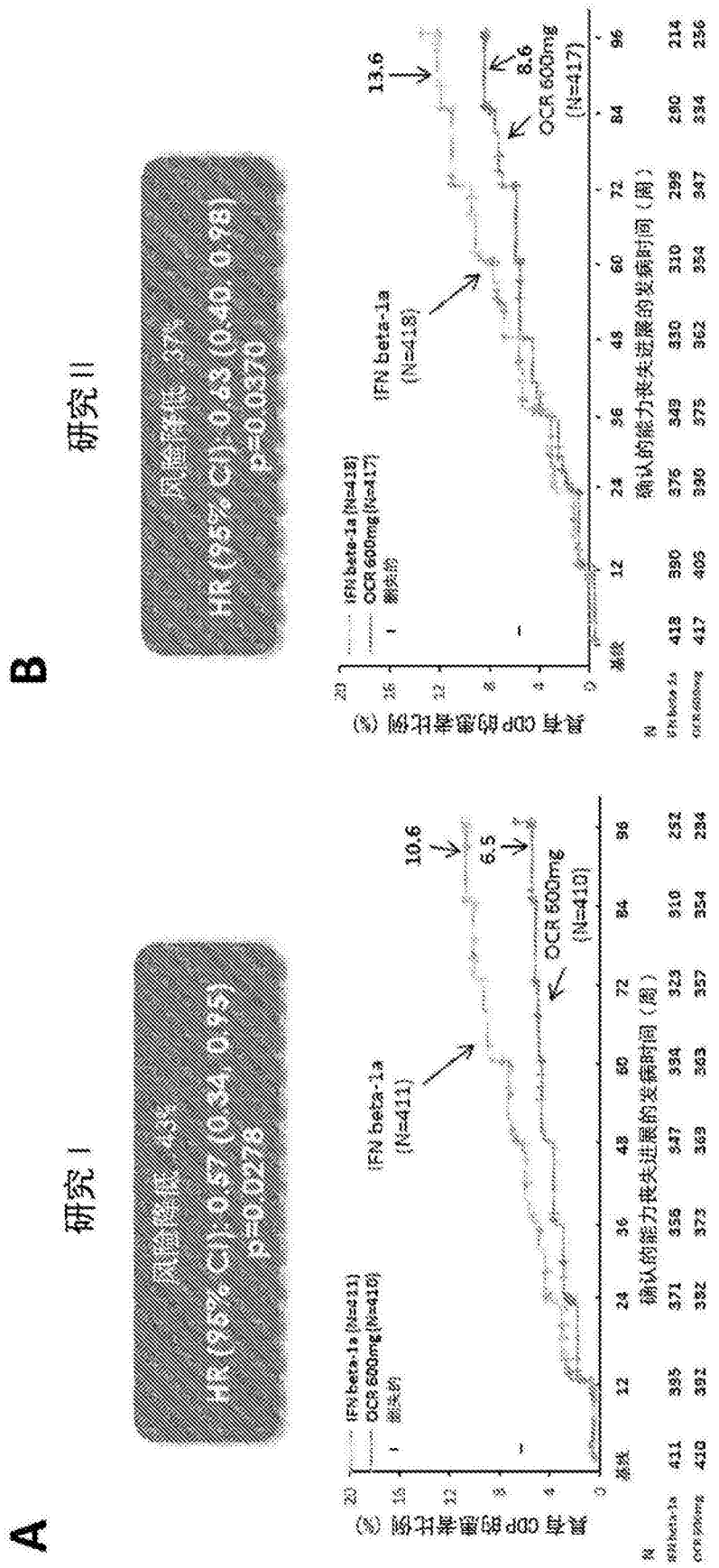

A
研究 I
风险降低 43%
HR (95% CI) 0.57 (0.44, 0.75)
p=0.0078
(FN beta-1a (N=411)
OCR 600mg (N=410)
基线
(FN beta-1a
(N=411)
10.6
6.5
OCR 600mg
(N=410)
确认的能力丧失进展的发病时间（周）
B
研究 II
风险降低 37%
HR (95% CI) 0.63 (0.49, 0.81)
p=0.0370
(FN beta-1a (N=418)
OCR 600mg (N=417)
基线
(FN beta-1a
(N=418)
13.6
8.6
OCR 600mg
(N=417)
确认的能力丧失进展的发病时间（周）

图14

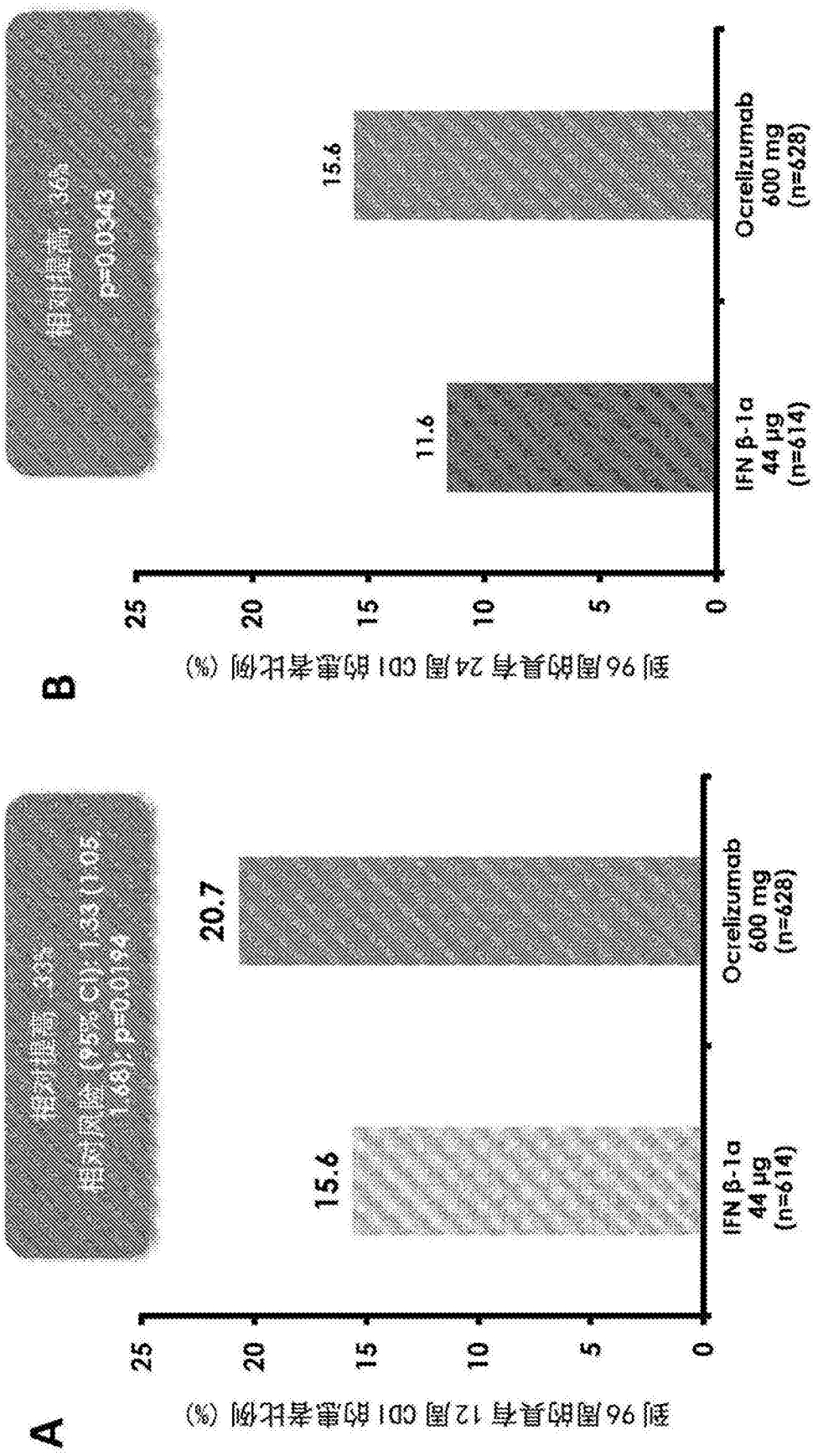

A
到 96 周的具有 12 周 CDI 的患者比例 (%)
25
20
15
10
5
0
15.6
20.7
IFN β-1a
44 µg
(n=614)
Ocrelizumab
600 mg
(n=628)
相对风险 0.75
相对风险 (95% CI) 1.33 (1.05, 1.68)
p=0.0194
B
到 96 周的具有 24 周 CDI 的患者比例 (%)
25
20
15
10
5
0
11.6
15.6
IFN β-1a
44 µg
(n=614)
Ocrelizumab
600 mg
(n=628)
相对风险 .765
p=0.0442

图15

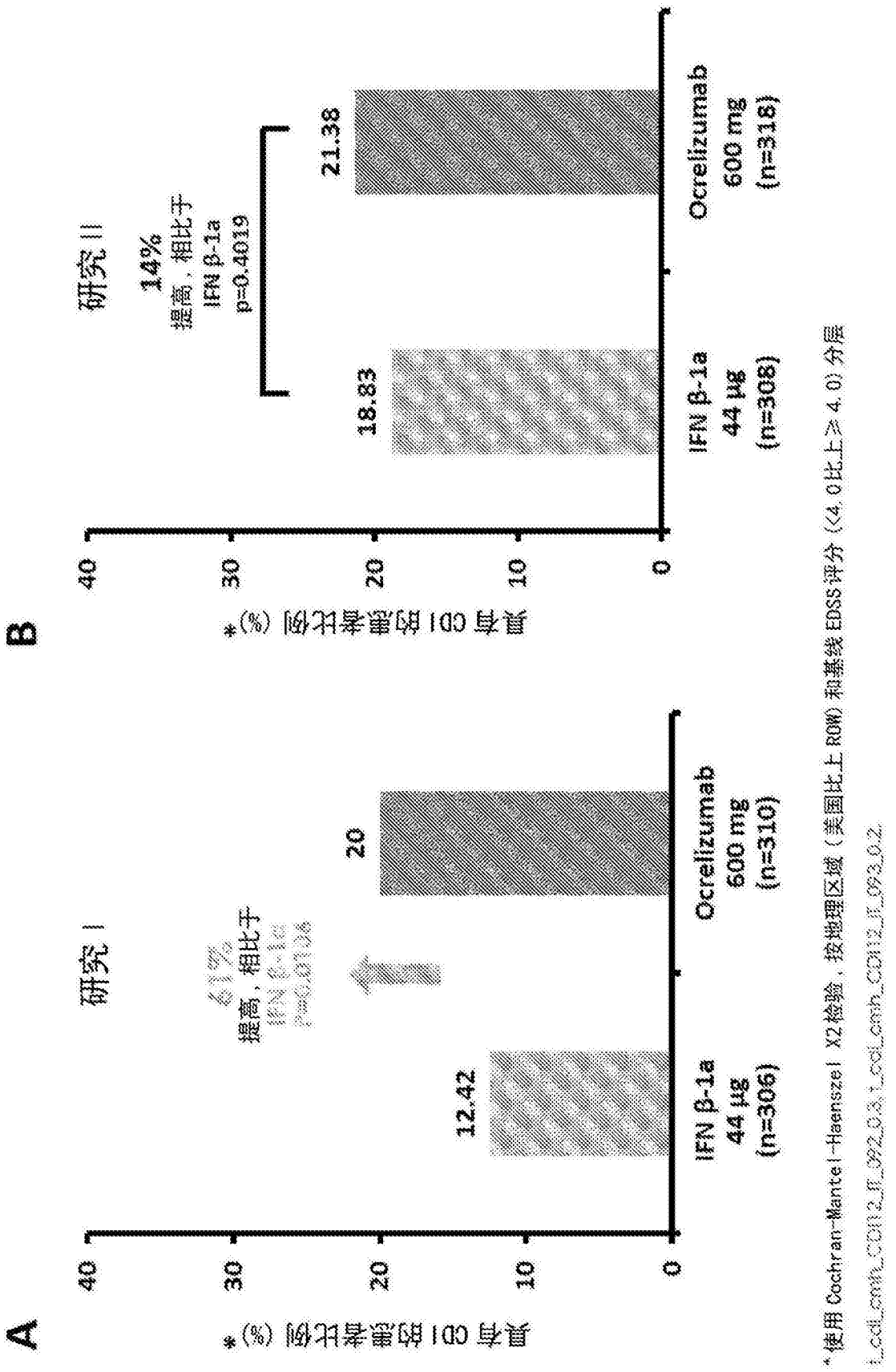

A
研究 I
具有 CDI 的患者比例 (%)*
40
30
20
10
0
12.42
20
61%
提高，相比于
IFN β-1a
p=0.0136
IFN β-1a
44 µg
(n=306)
Ocrelizumab
600 mg
(n=310)
B
研究 II
具有 CDI 的患者比例 (%)*
40
30
20
10
0
18.83
21.38
14%
提高，相比于
IFN β-1a
p=0.4019
IFN β-1a
44 µg
(n=308)
Ocrelizumab
600 mg
(n=318)
* 使用 Cochran-Mantel-Haenszel X2 检验，按地理区域（美国比上 ROW）和基线 EDSS 评分（<4.0 比上≥ 4.0）分层
t_cdi_cmh_CD12_ff_092_03.1_cdi_cmh_CD12_ff_093_0.2

图16

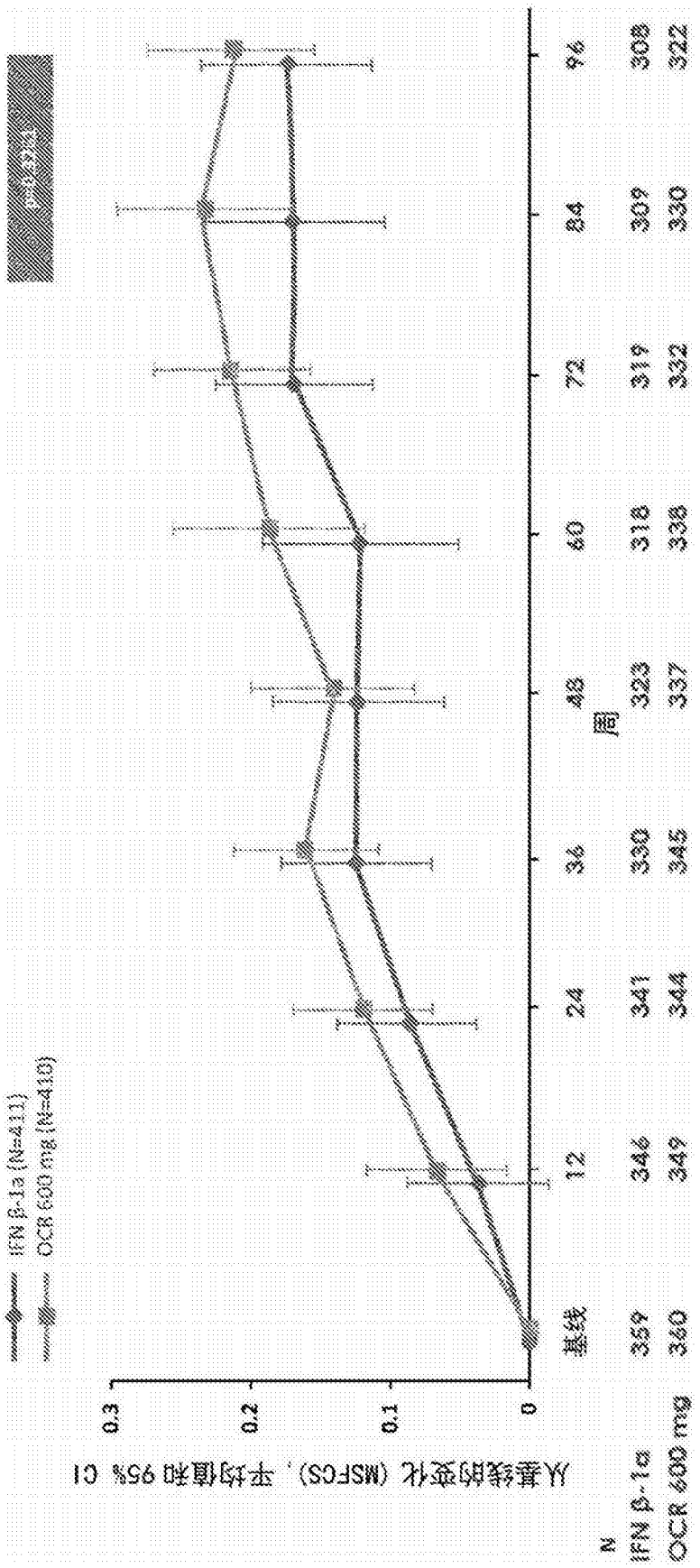

IFN β-1a (N=411)
OCR 600 mg (N=410)
从基线的变化（MSFCS），平均值和 95% CI
0.3
0.2
0.1
0
周
基线
12
24
36
48
60
72
84
96
N
IFN β-1a 359 346 341 330 323 318 319 309 308
OCR 600 mg 360 349 344 345 337 338 332 330 322

图16C

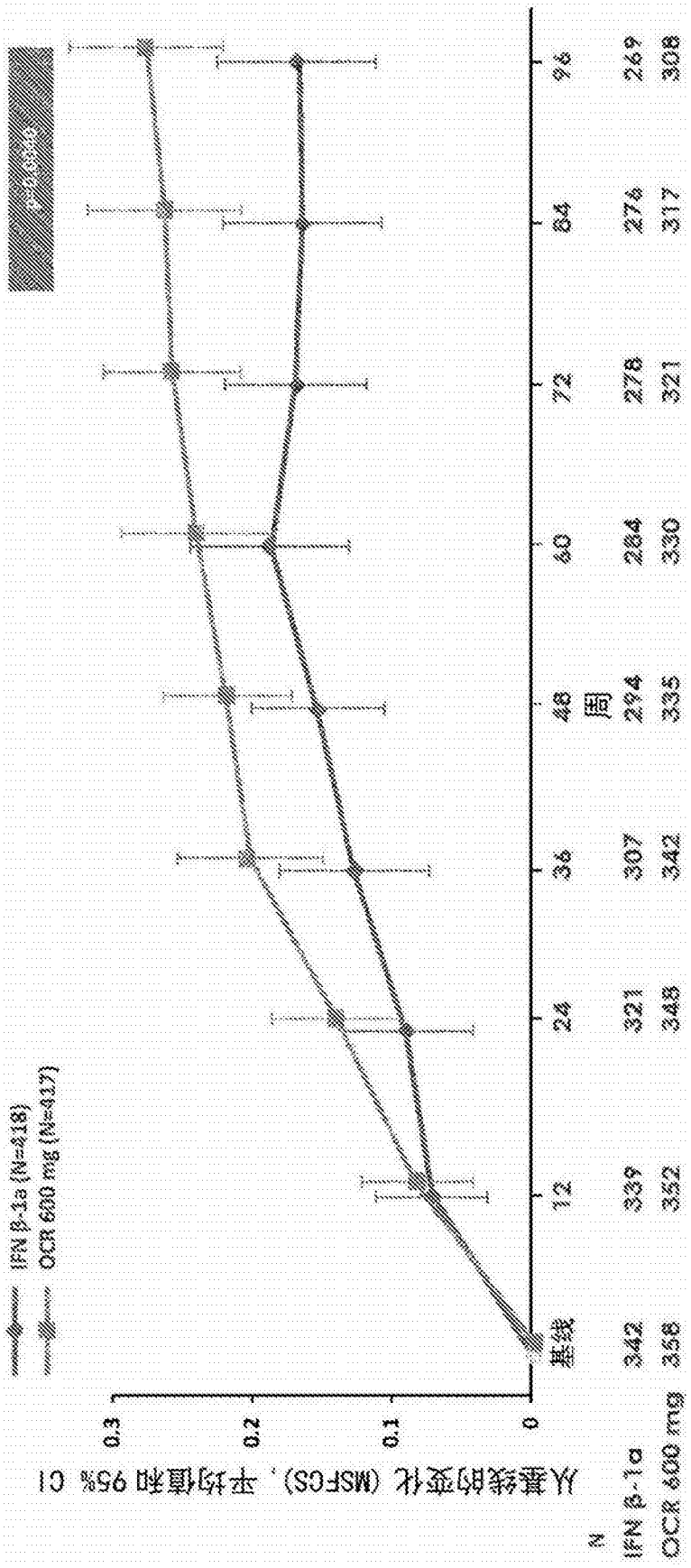

IFN β-1a (N=418)
OCR 600 mg (N=417)
从基线的变化 (MSFCS)，平均值和 95% CI
0.3
0.2
0.1
0
基线
12
24
36
48
60
72
84
96
周
N
IFN β-1a 342 339 321 307 294 284 278 274 269
OCR 600 mg 358 352 348 342 335 330 321 317 308

图16D

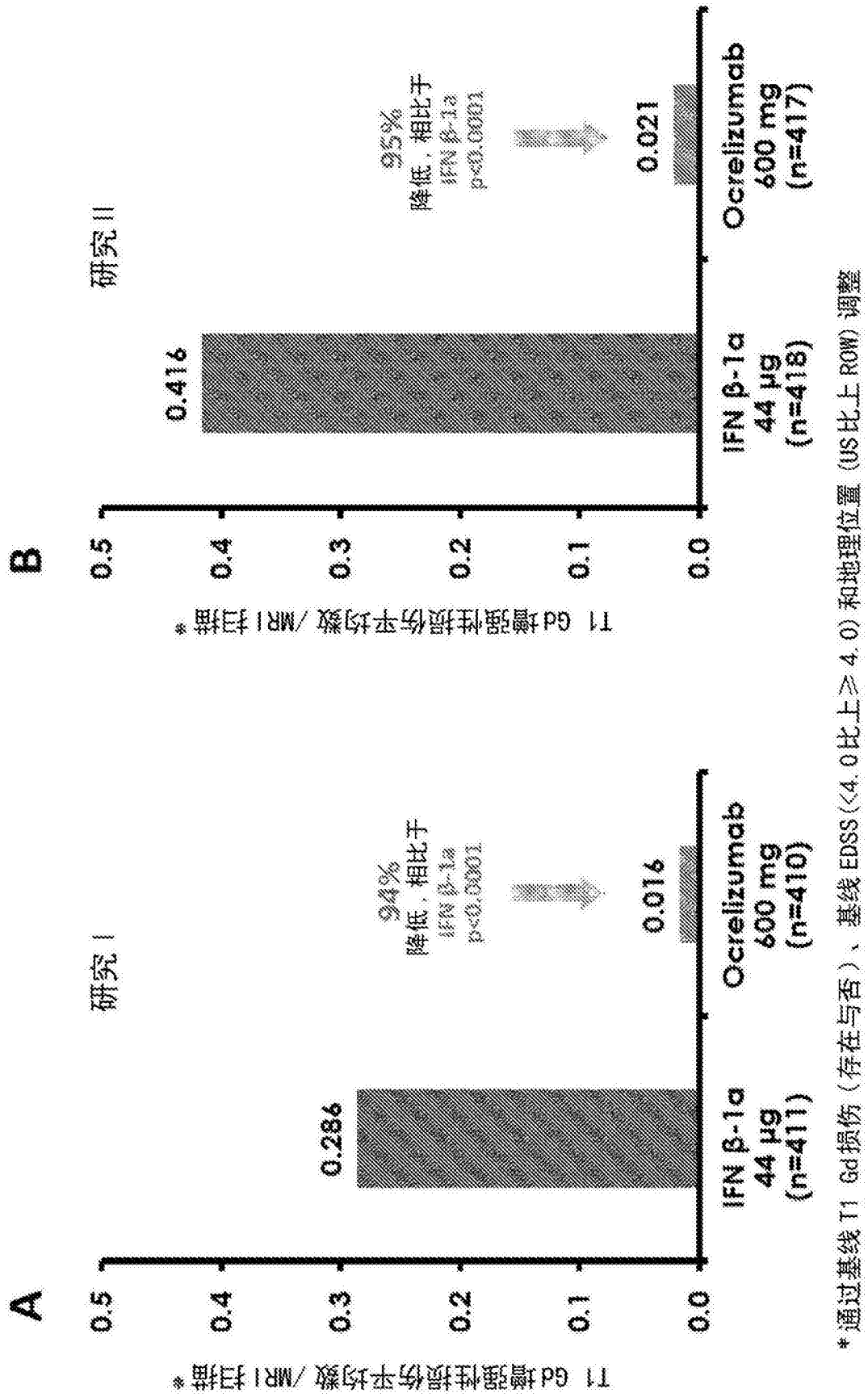

A
研究 I
T1 Gd增强性损伤平均数 /MRI扫描*
0.5
0.4
0.3
0.2
0.1
0.0
0.286
0.016
94%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=411)
Ocrelizumab
600 mg
(n=410)
B
研究 II
T1 Gd增强性损伤平均数 /MRI扫描*
0.5
0.4
0.3
0.2
0.1
0.0
0.416
0.021
95%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=418)
Ocrelizumab
600 mg
(n=417)
* 通过基线 T1 Gd损伤（存在与否）、基线 EDSS（<4.0比上≥4.0）和地理位置（US比上 ROW）调整

图17

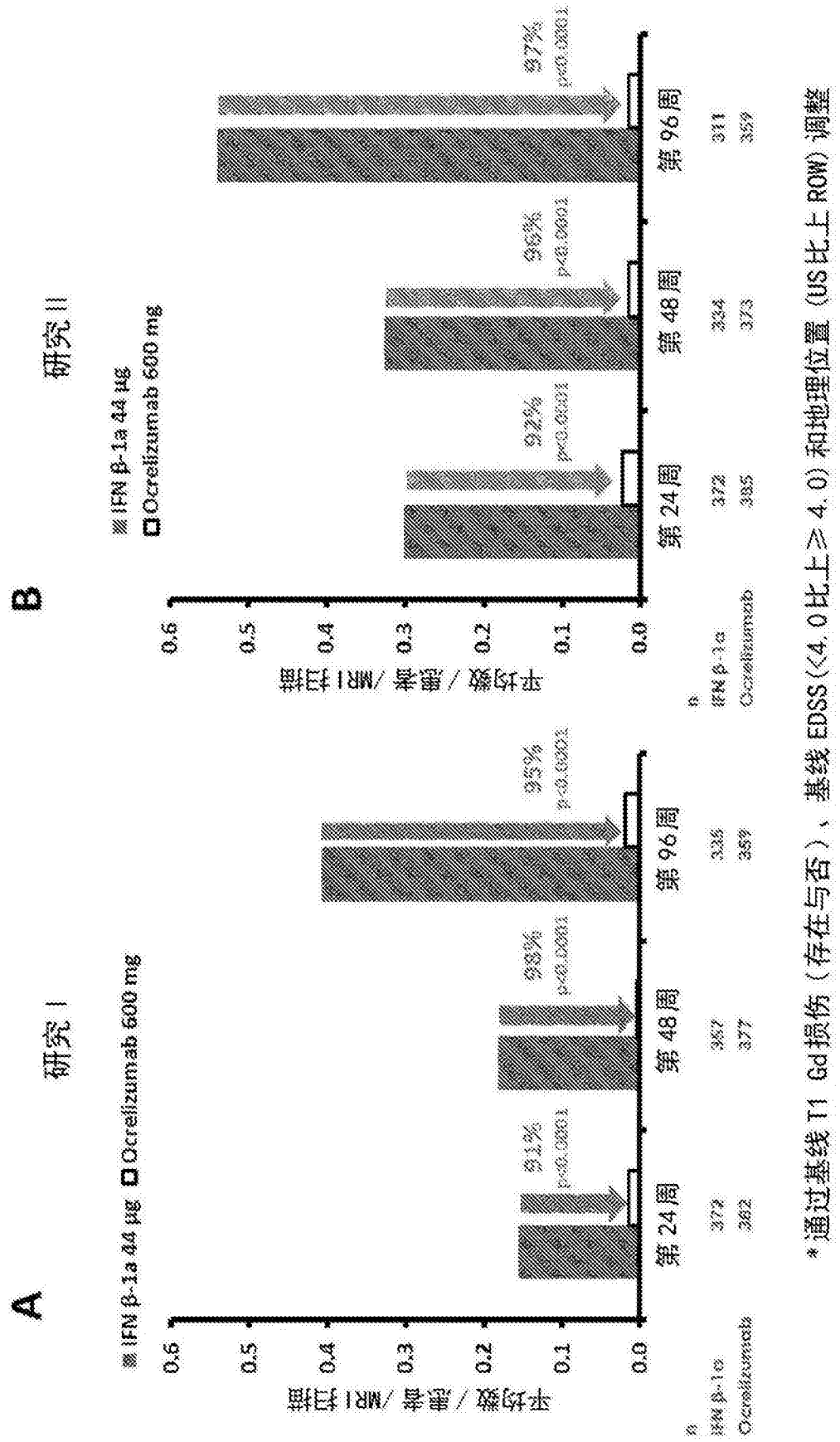

A
研究 I
IFN β-1a 44 μg
Ocrelizumab 600 mg
平均数 / 患者 /MRI 扫描
0.6
0.5
0.4
0.3
0.2
0.1
0.0
第 24 周
第 48 周
第 96 周
91%
p<0.0001
93%
p<0.0001
93%
p<0.0001
IFN β-1a 372 367 326
Ocrelizumab 362 377 359
B
研究 II
IFN β-1a 44 μg
Ocrelizumab 600 mg
平均数 / 患者 /MRI 扫描
第 24 周
第 48 周
第 96 周
92%
p=0.0031
96%
p<0.0001
97%
p<0.0001
IFN β-1a 372 324 311
Ocrelizumab 385 373 359
* 通过基线 T1 Gd 损伤（存在与否）、基线 EDSS（<4.0 比上 ≥ 4.0）和地理位置（US 比上 ROW）调整

图18

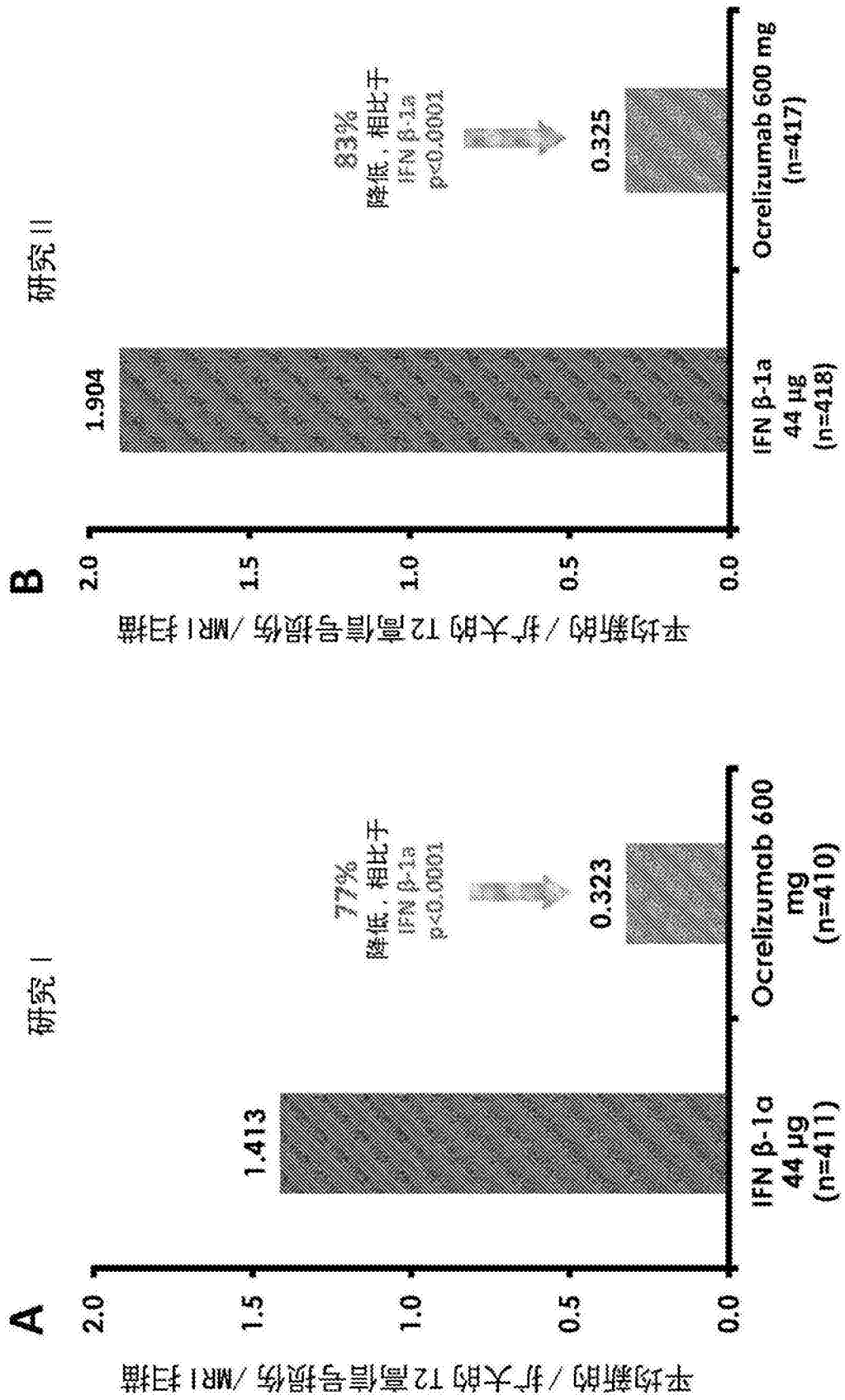

A
研究 I
平均新的 / 扩大的 T2 高信号损伤 /MRI 扫描
2.0
1.5
1.0
0.5
0.0
1.413
0.323
77%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=411)
Ocrelizumab 600
mg
(n=410)
B
研究 II
平均新的 / 扩大的 T2 高信号损伤 /MRI 扫描
2.0
1.5
1.0
0.5
0.0
1.904
0.325
83%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=418)
Ocrelizumab 600 mg
(n=417)

图19

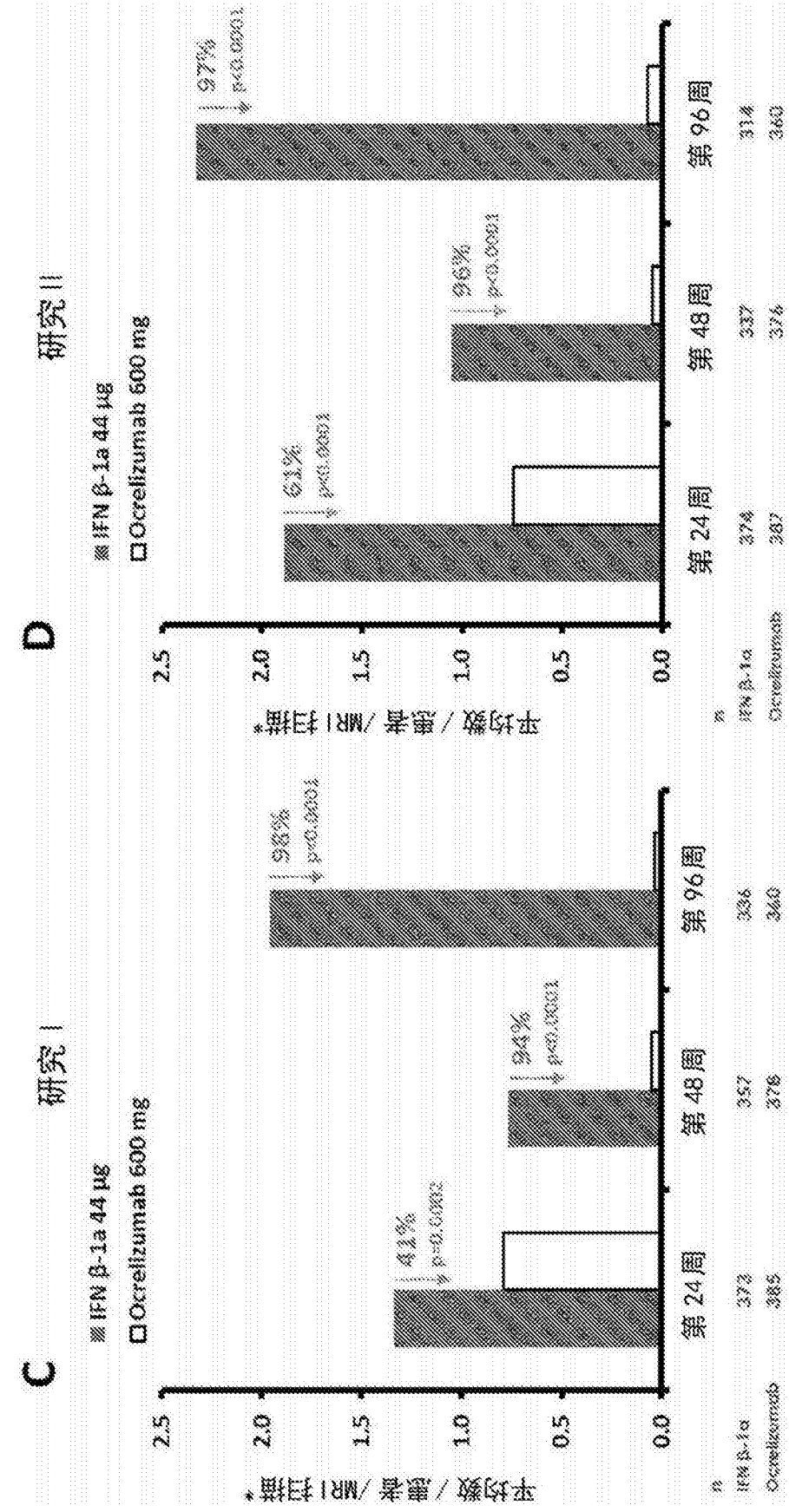

C
研究 I
IFN β-1a 44 µg
Ocrelizumab 600 mg
平均数 / 患者 /MRI 扫描*
2.5
2.0
1.5
1.0
0.5
0.0
42%
p=0.0002
94%
p<0.0001
93%
p<0.0001
第 24 周
第 48 周
第 96 周
n
IFN β-1a 373 357 336
Ocrelizumab 385 378 360
D
研究 II
IFN β-1a 44 µg
Ocrelizumab 600 mg
平均数 / 患者 /MRI 扫描*
2.5
2.0
1.5
1.0
0.5
0.0
61%
p<0.0001
96%
p<0.0001
97%
p<0.0001
第 24 周
第 48 周
第 96 周
n
IFN β-1a 378 337 314
Ocrelizumab 387 374 360

图19(续)

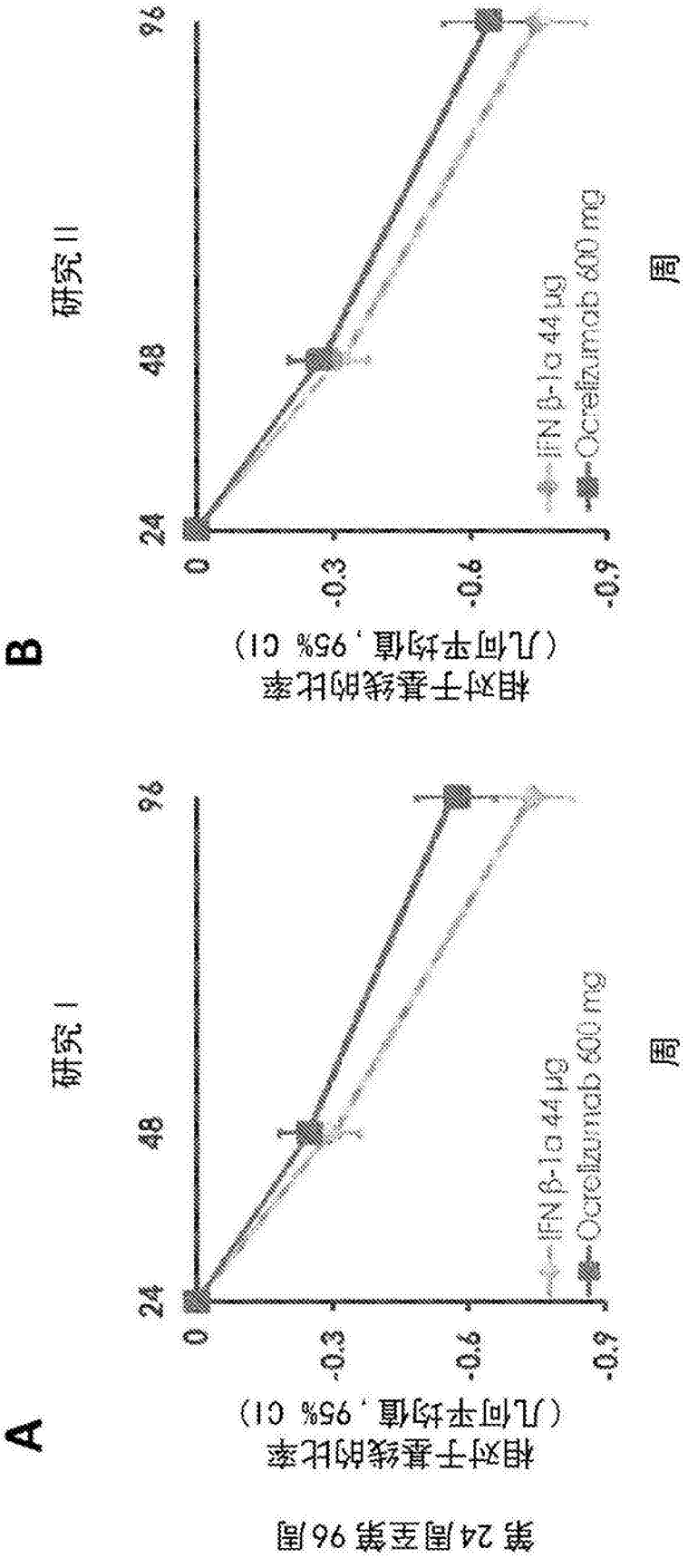

A
研究 I
相对于基线的比率
(几何平均值，95% CI)
0
-0.3
-0.6
-0.9
24
48
96
周
IFN β-1a 44 µg
Ocrelizumab 600 mg
第 24 周至第 96 周
B
研究 II
相对于基线的比率
(几何平均值，95% CI)
0
-0.3
-0.6
-0.9
24
48
96
周
IFN β-1a 44 µg
Ocrelizumab 600 mg

图20

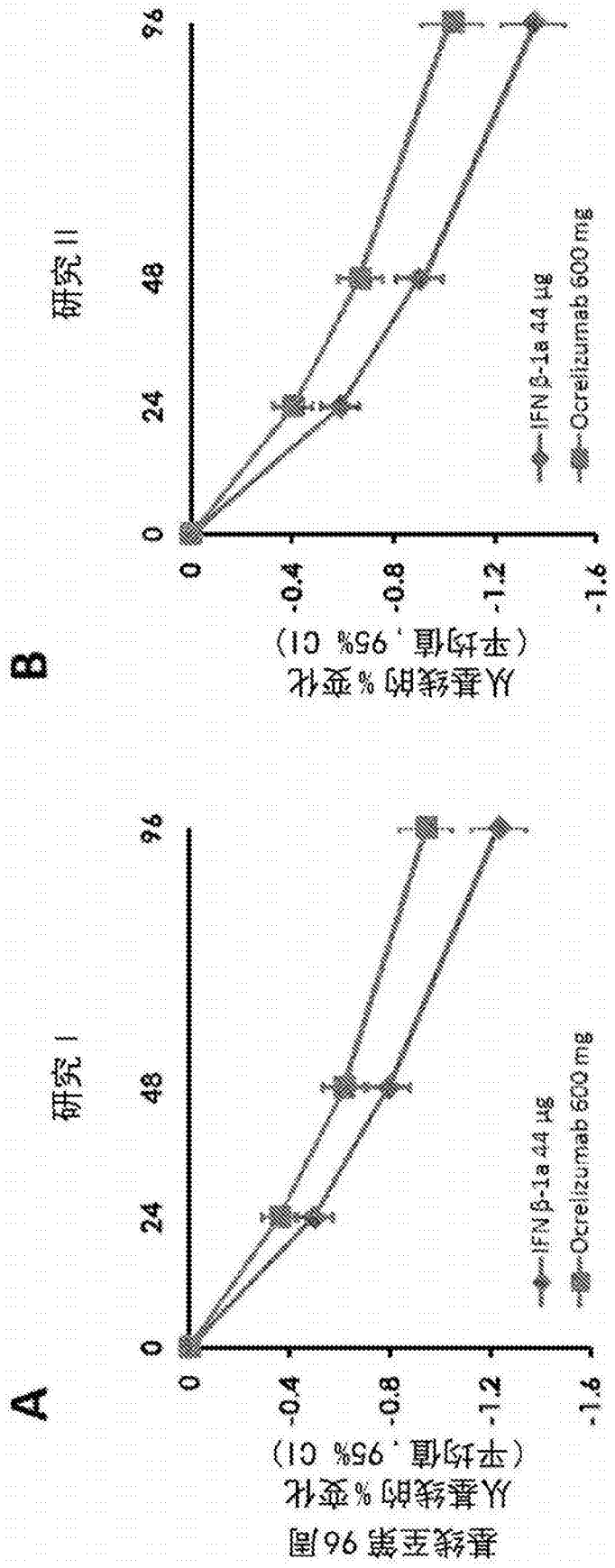

A
研究 I
基线至第 96周
从基线的 % 变化
（平均值，95% CI）
0
-0.4
-0.8
-1.2
-1.6
0
24
48
96
IFNβ-1a 44 µg
Ocrelizumab 600 mg
B
研究 II
从基线的 % 变化
（平均值，95% CI）
0
-0.4
-0.8
-1.2
-1.6
0
24
48
96
IFN β-1a 44 µg
Ocrelizumab 600 mg

图21

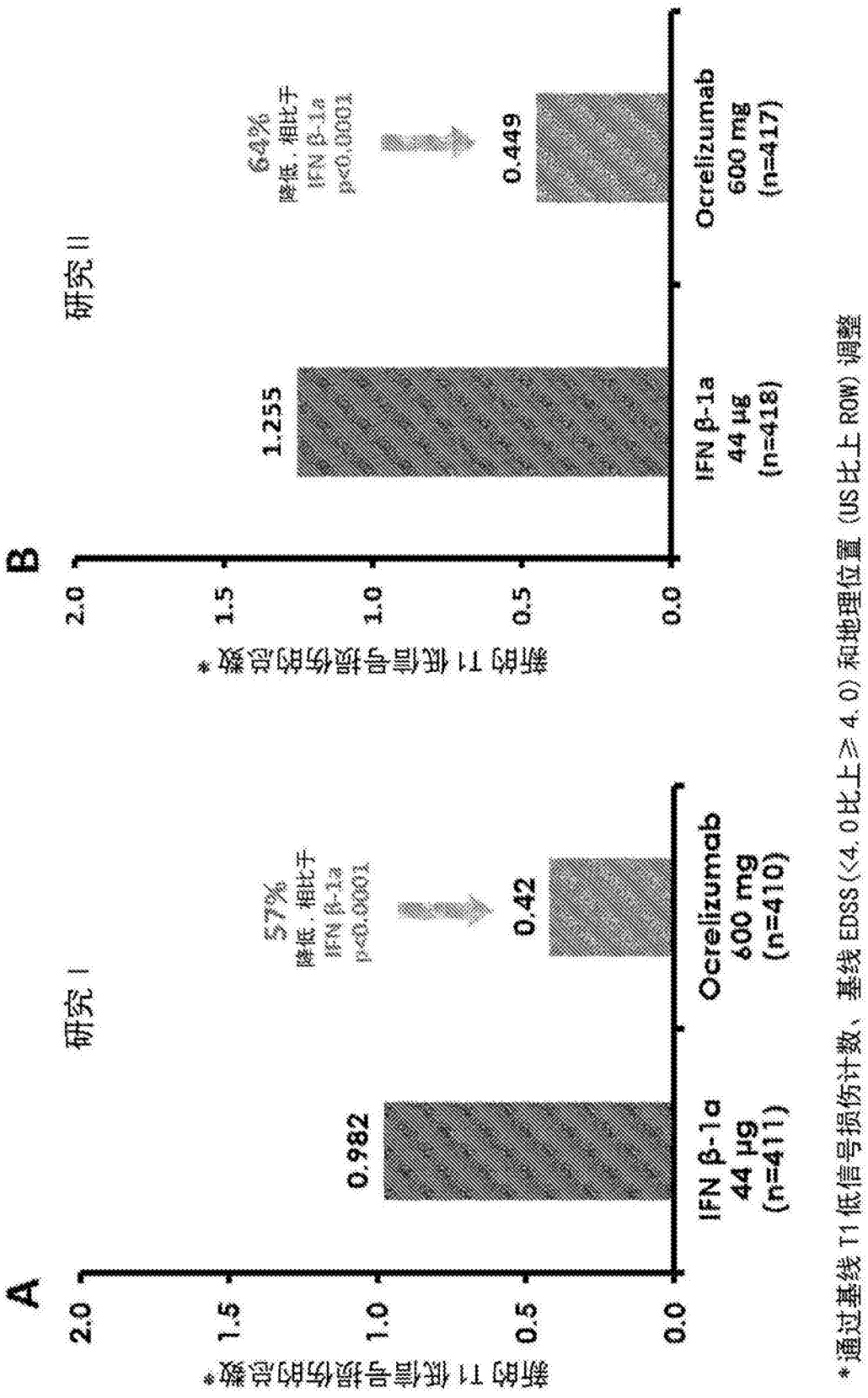

A
研究 I
新的 T1 低信号损伤的总数*
2.0
1.5
1.0
0.5
0.0
0.982
0.42
57%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=411)
Ocrelizumab
600 mg
(n=410)
B
研究 II
新的 T1 低信号损伤的总数*
2.0
1.5
1.0
0.5
0.0
1.255
0.449
64%
降低，相比于
IFN β-1a
p<0.0001
IFN β-1a
44 µg
(n=418)
Ocrelizumab
600 mg
(n=417)
*通过基线 T1 低信号损伤计数、基线 EDSS(<4.0 比上 ≥ 4.0) 和地理位置 (US 比上 ROW) 调整

图22

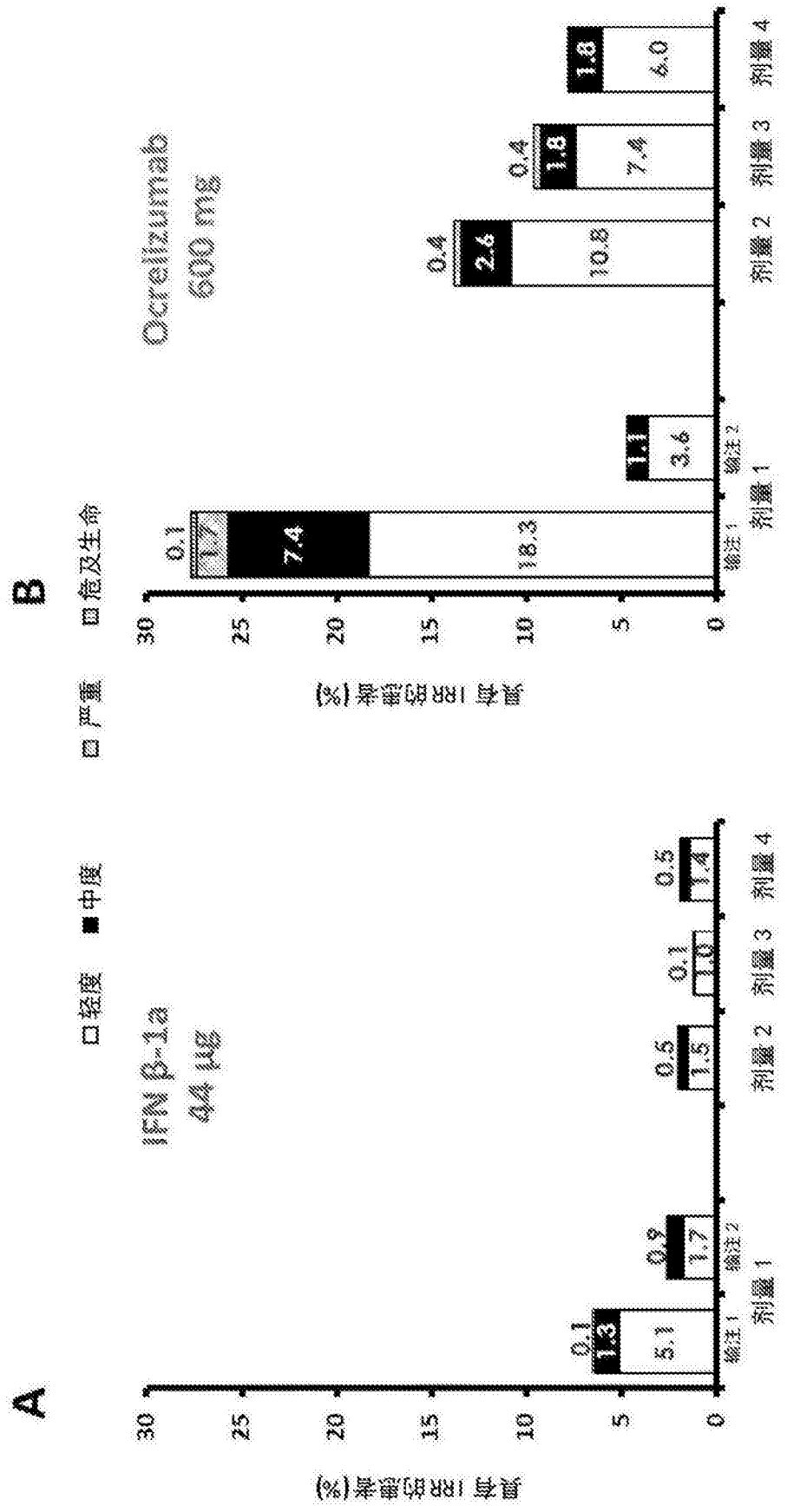

A
B
□轻度 ■中度 □严重 □危及生命
IFN β-1a
44 μg
Ocrelizumab
600 mg
具有 IRR的患者(%)
输注 1
输注 2
剂量 1
剂量 2
剂量 3
剂量 4
0.1
1.3
5.1
0.2
1.7
0.5
1.5
0.1
1.0
0.5
1.4
0.1
1.7
7.4
18.3
1.1
3.6
0.4
2.6
10.8
0.4
1.8
7.4
1.8
4.0

图23

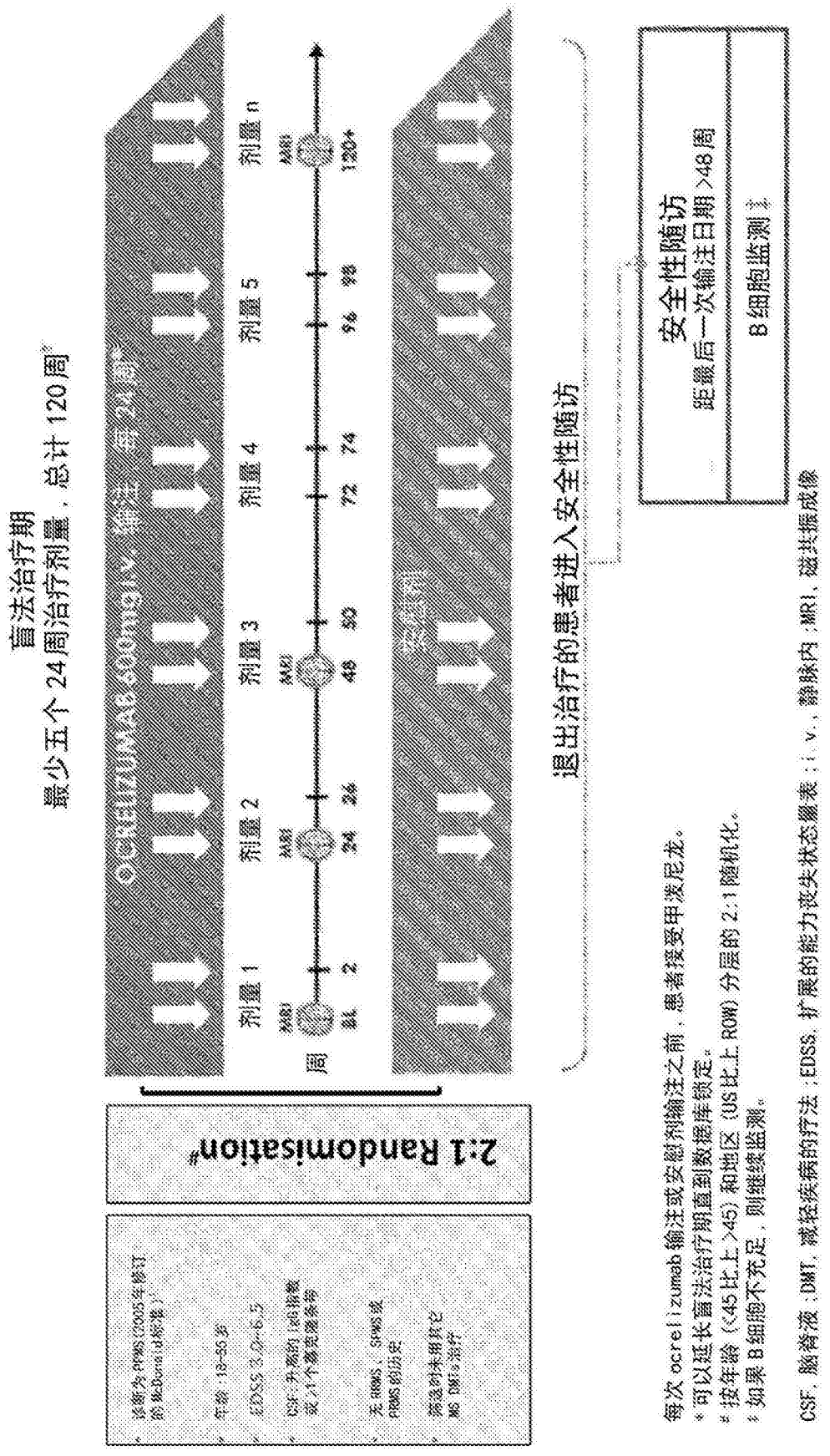

盲法治疗期
最少五个 24 周治疗剂量，总计 120 周
ocrelizumab 输注 每 24 周
剂量 1
剂量 2
剂量 3
剂量 4
剂量 5
剂量 n
周
1 2 24 26 46 50 72 74 96 98 120+
MRI
2:1 Randomisation
退出治疗的患者进入安全性随访
安全性随访
距最后一次输注日期 >48 周
B 细胞监测 ‡
每次 ocrelizumab 输注或安慰剂输注之前，患者接受甲泼尼龙。
* 可以延长盲法治疗期直到数据库锁定。
† 按年龄（<45 比上 >45）和地区（US 比上 ROW）分层的 2:1 随机化。
‡ 如果 B 细胞不充足，则继续监测。
CSF，脑脊液；DMT，减轻疾病的疗法；EDSS，扩展的能力丧失状态量表；i. v.，静脉内；MRI，磁共振成像

图24

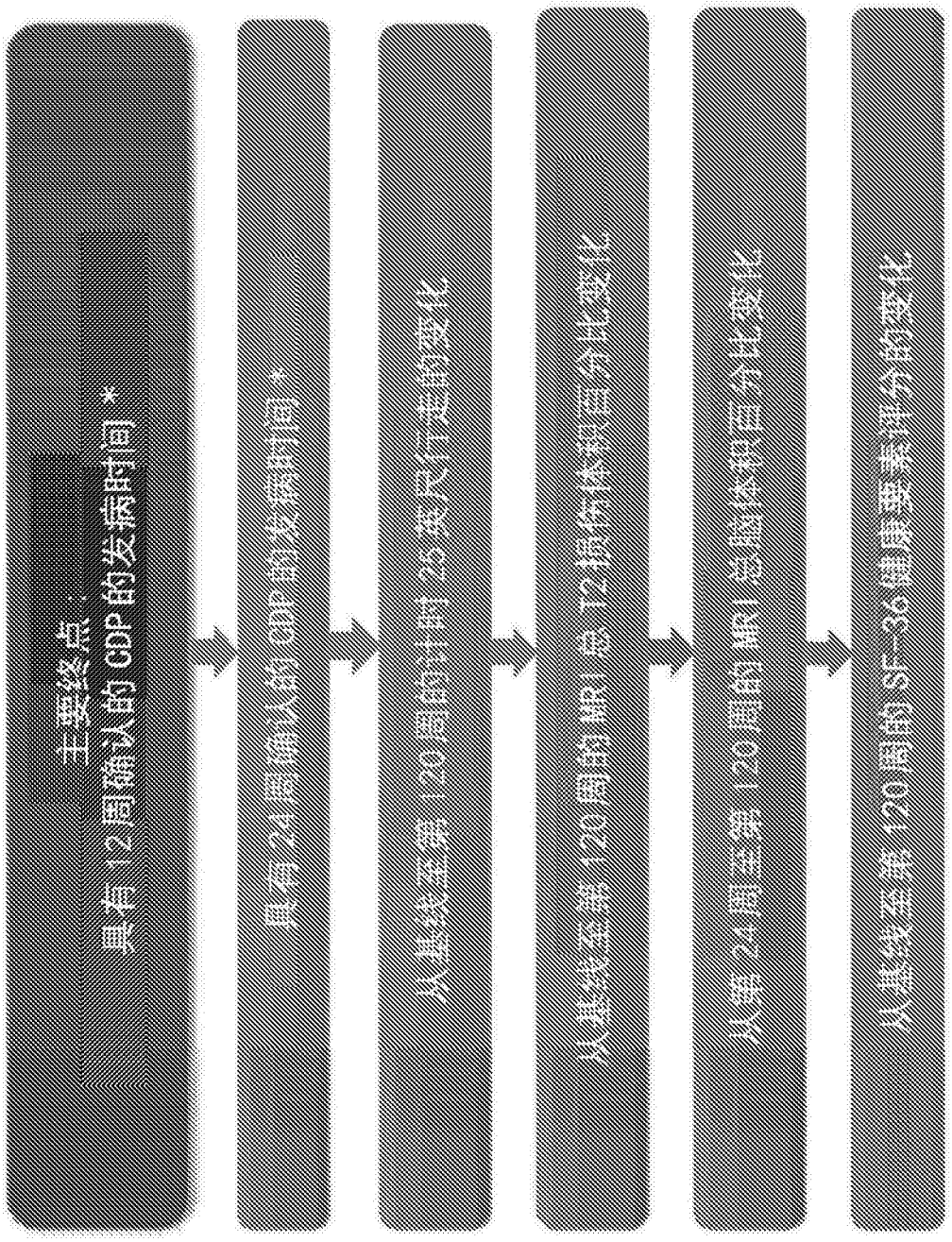

主要终点：
基准12周确认的CDP的发病时间*
从基线至第24周确认的CDP的发病时间*
从基线至第120周的计时25英尺行走的改变
从基线至第120周的MRI的T2病灶体积百分比改变
从基线至第120周的MRI总脑容积百分比改变
从基线至第120周的SF-36健康要素评分的改变

图25

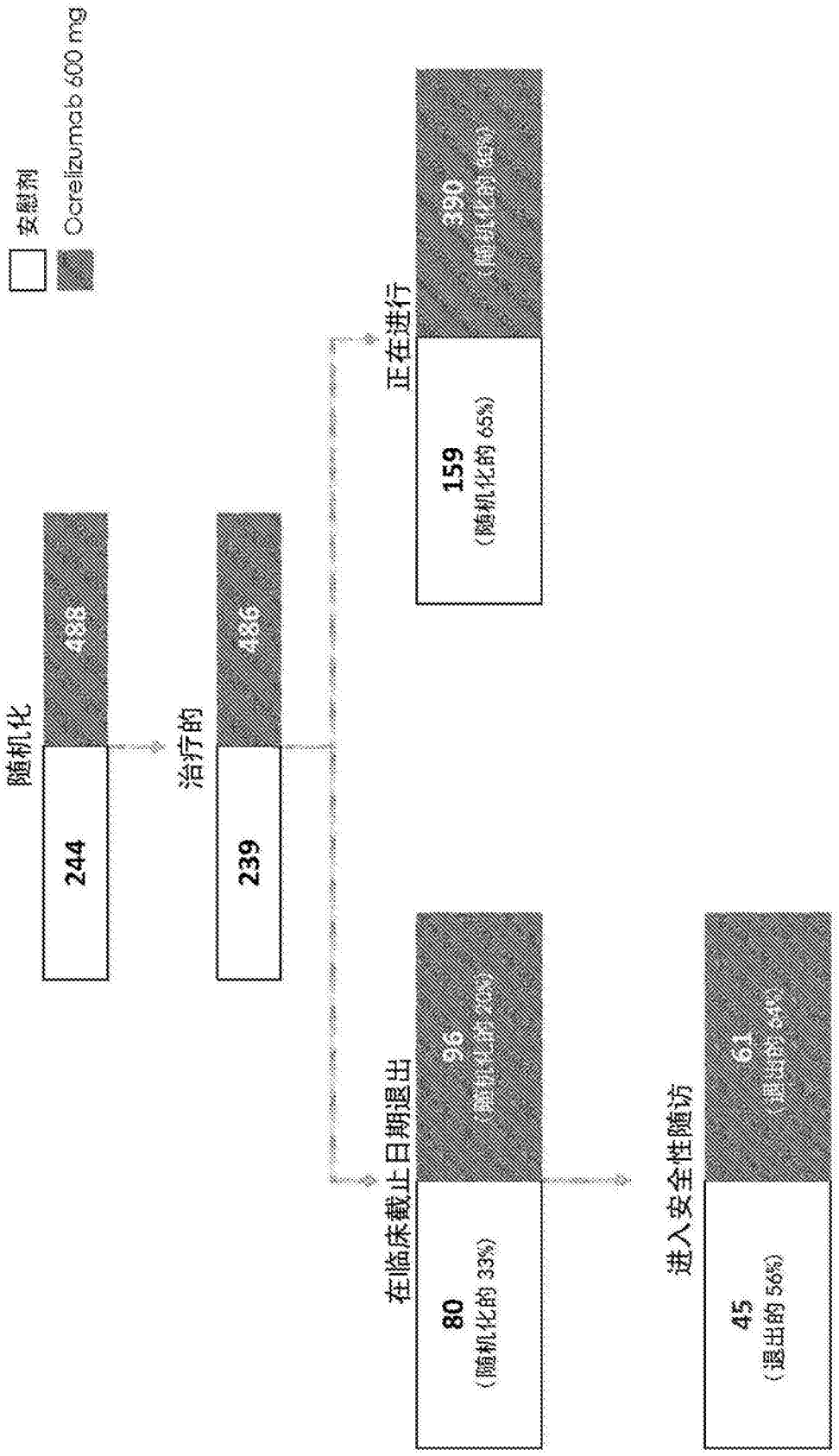

安慰剂
Cerelizumab 600 mg
随机化
244
治疗的
239
正在进行
159
(随机化的 65%)
在临床截止日期退出
80
(随机化的 33%)
进入安全性随访
45
(退出的 56%)

图26

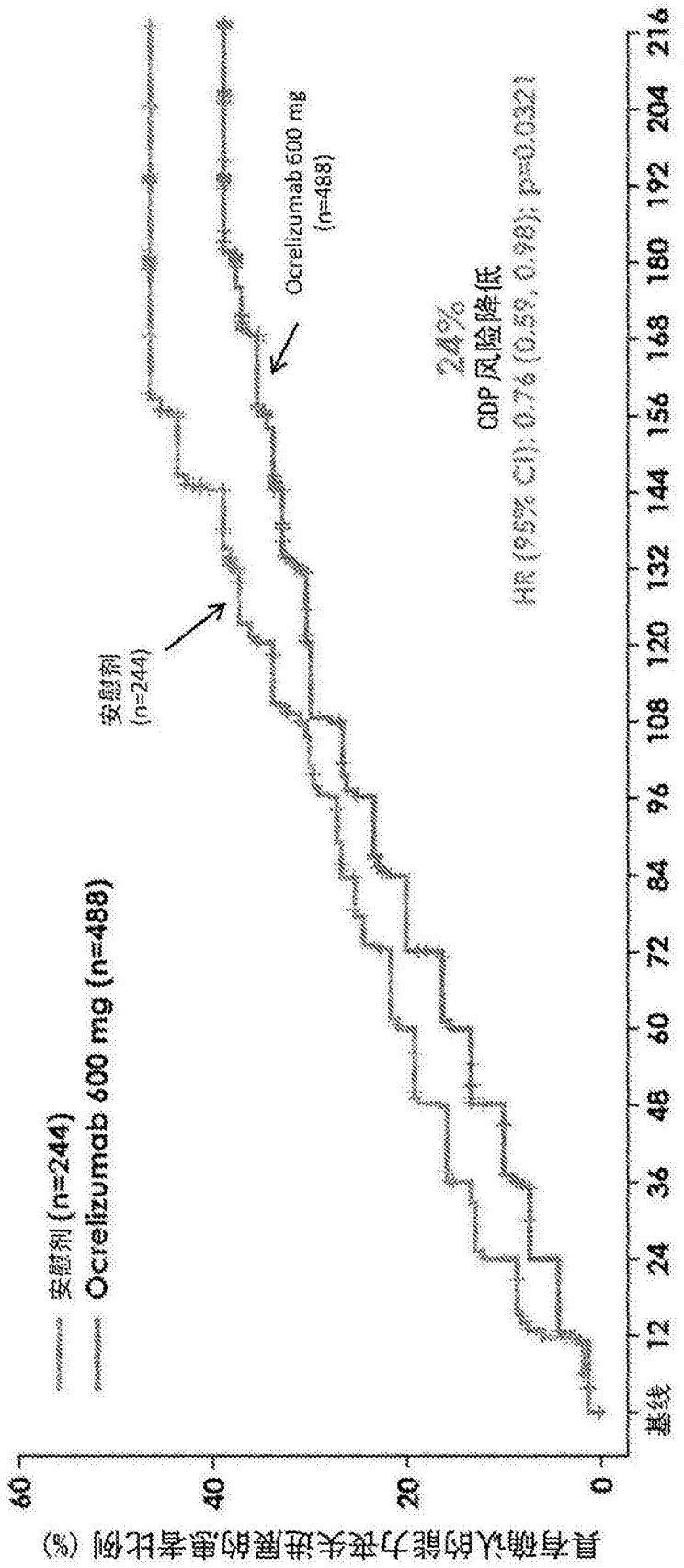

具有确认的能力丧失进展的患者比例（%）
60
40
20
0
安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
安慰剂
(n=244)
Ocrelizumab 600 mg
(n=488)
24%
CDP 风险降低
HR (95% CI), 0.76 (0.59, 0.98); p=0.032
基线 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
到确认的能力丧失进展的时间（周）

图27

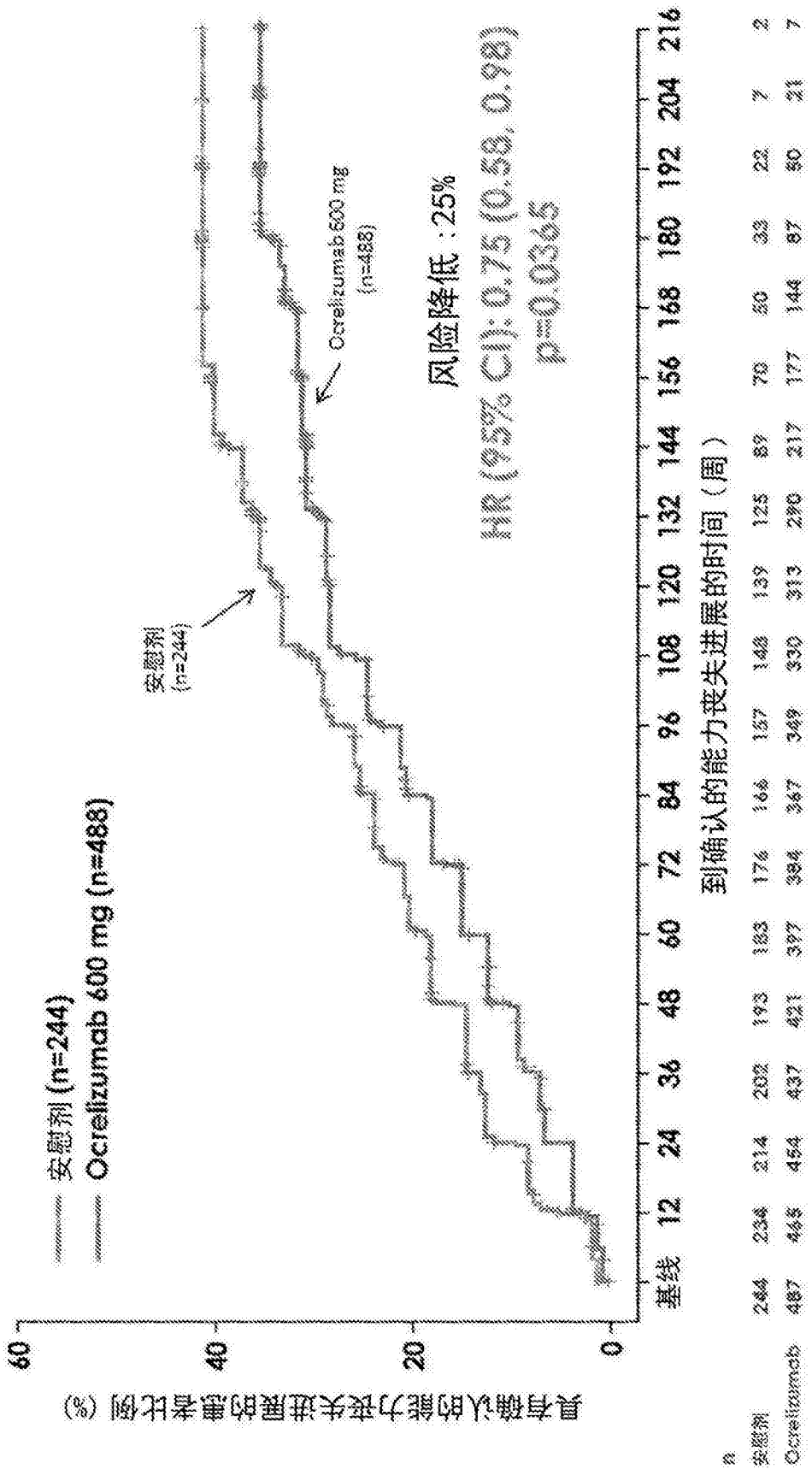

具有确认的能力丧失进展的患者比例（%）
60
40
20
0
安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
风险降低：25%
HR (95% CI): 0.75 (0.58, 0.98)
p=0.0345
基线 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
到确认的能力丧失进展的时间（周）
n
安慰剂 244 234 214 202 193 183 176 166 157 148 139 125 89 70 50 33 22 7 2
Ocrelizumab 487 466 454 437 421 397 384 367 349 330 313 290 217 177 144 87 60 21 7

图28

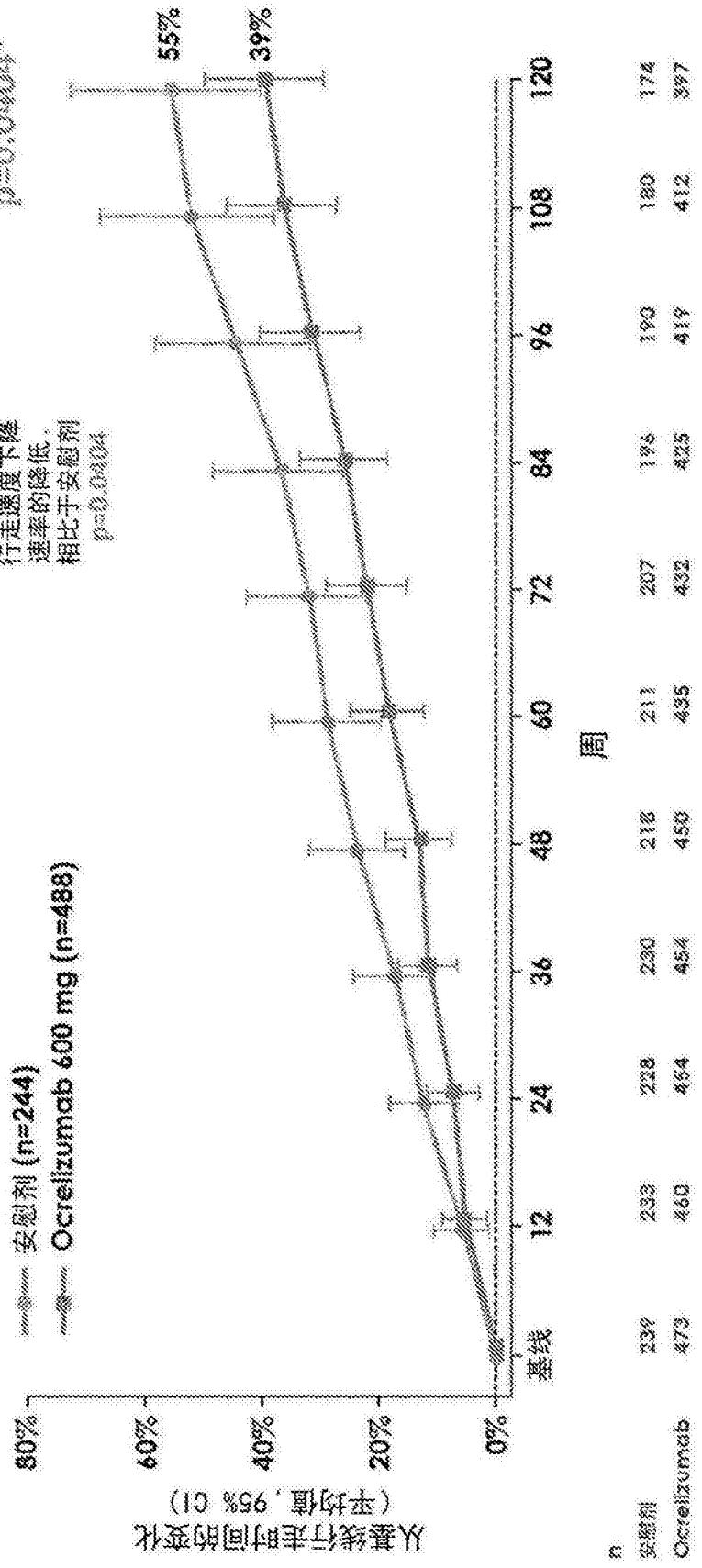

从基线行走时间的变化
（平均值，95% CI）
80%
60%
40%
20%
0%
基线
12
24
36
48
60
72
84
96
108
120
周
55%
39%
p=0.0004*
2.9%
行走速度下降
速率的降低，
相比于安慰剂
p=0.0404
安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
n
安慰剂 239 233 228 230 216 211 207 196 190 180 174
Ocrelizumab 473 460 464 454 450 435 432 425 419 412 397

图29

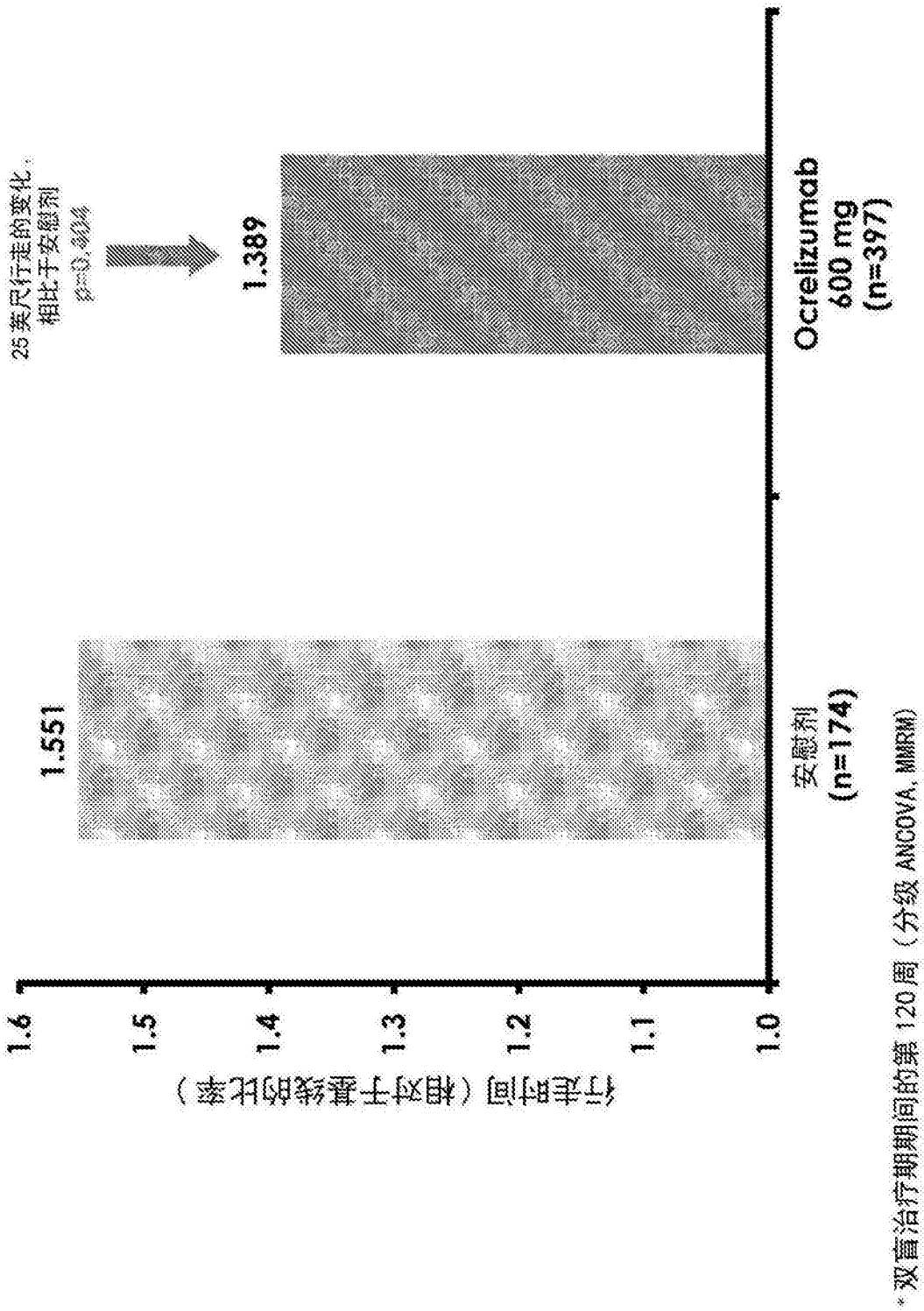

10%
25 英尺行走的变化，
相比于安慰剂
p=0.404
1.389
1.551
Ocrelizumab
600 mg
(n=397)
安慰剂
(n=174)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
行走时间（相对于基线的比率）
*双盲治疗期期间的第 120 周（分级 ANCOVA, MMRM）

图30

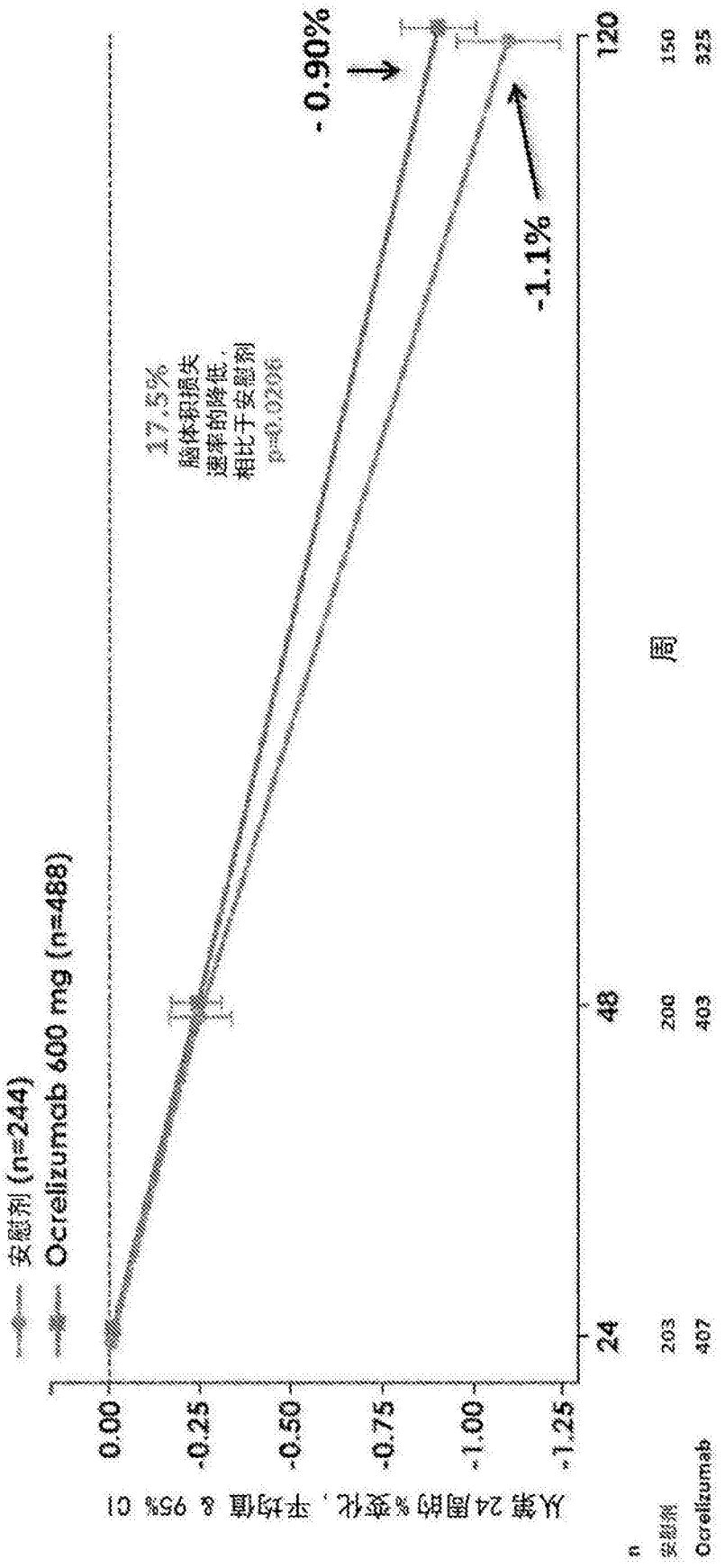

安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
从第 24 周的 % 变化，平均值 & 95% CI
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
24
48
120
周
-0.90%
-1.1%
17.5%
脑体积损失
速率的降低，
相比于安慰剂
p=0.0206
n
安慰剂 203 200 150
Ocrelizumab 407 403 325

图31

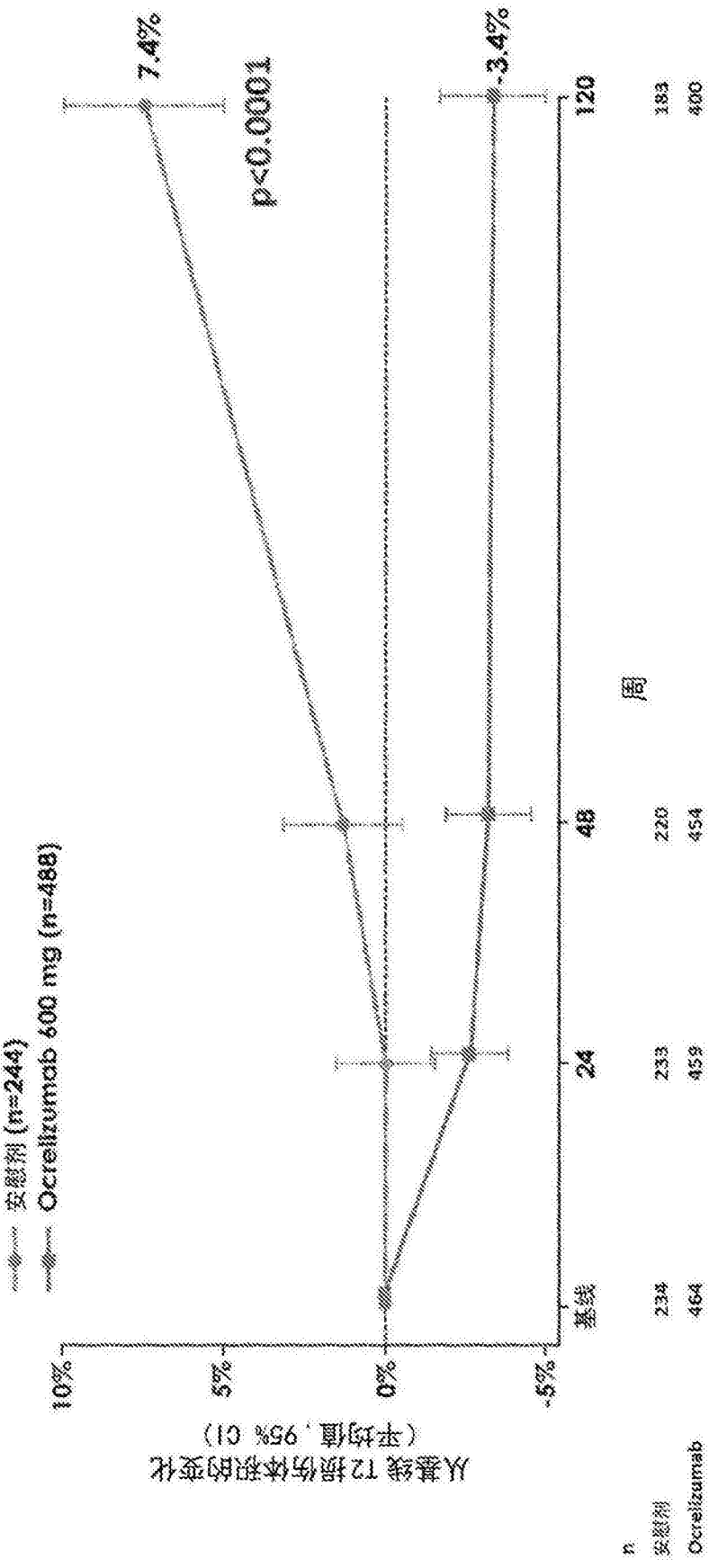

7.4%
p<0.0001
-3.4%
安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
10%
5%
0%
-5%
从基线 T2 损伤体积的变化
(平均值，95% CI)
基线
24
48
120
周
n
安慰剂 234 233 220 183
Ocrelizumab 464 459 454 400

图32

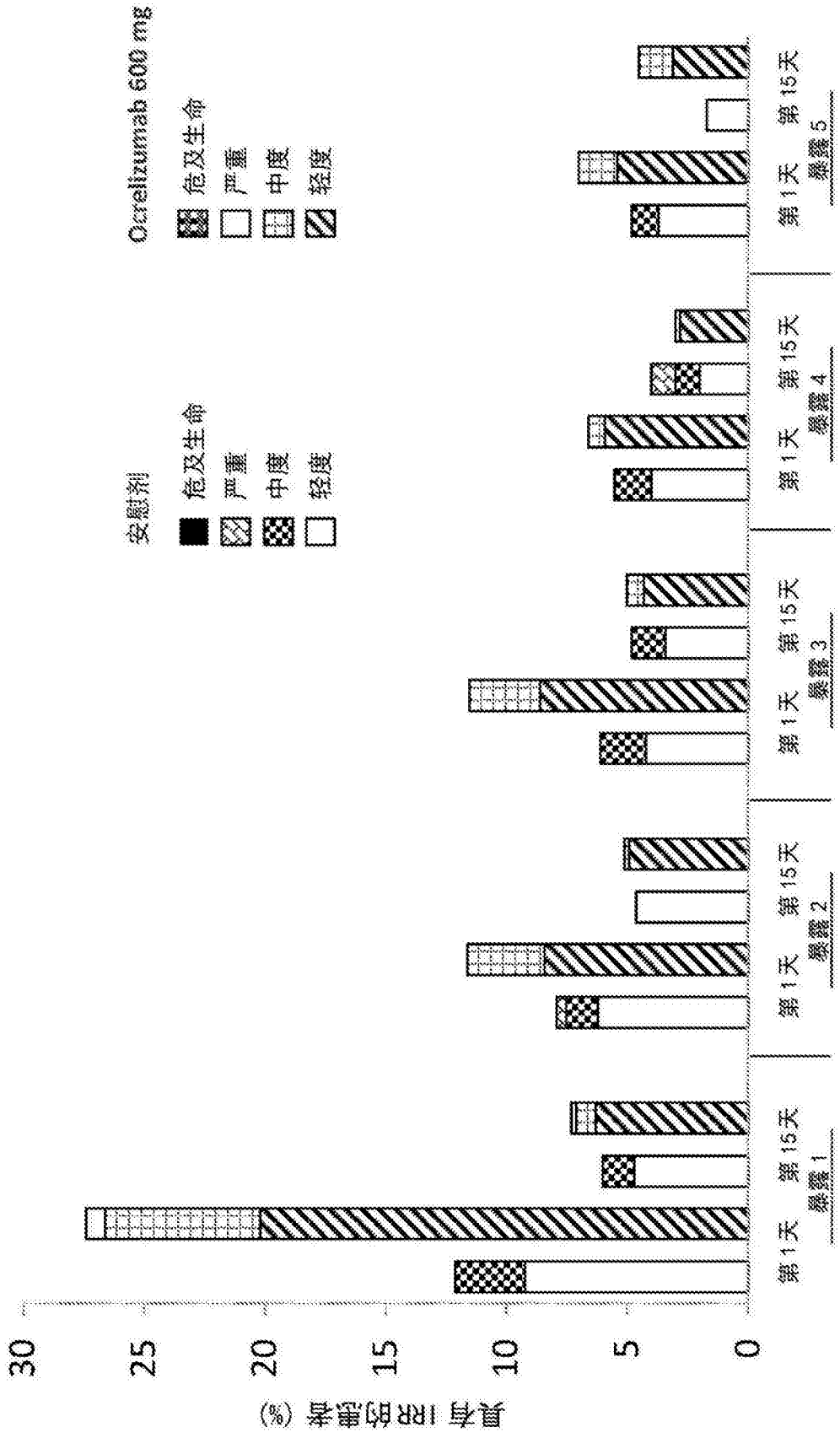

Ocrelizumab 600 mg
危及生命
严重
中度
轻度
安慰剂
危及生命
严重
中度
轻度
具有 IRR的患者 (%)
30
25
20
15
10
5
0
第1天 第15天
暴露 1
第1天 第15天
暴露 2
第1天 第15天
暴露 3
第1天 第15天
暴露 4
第1天 第15天
暴露 5

图33

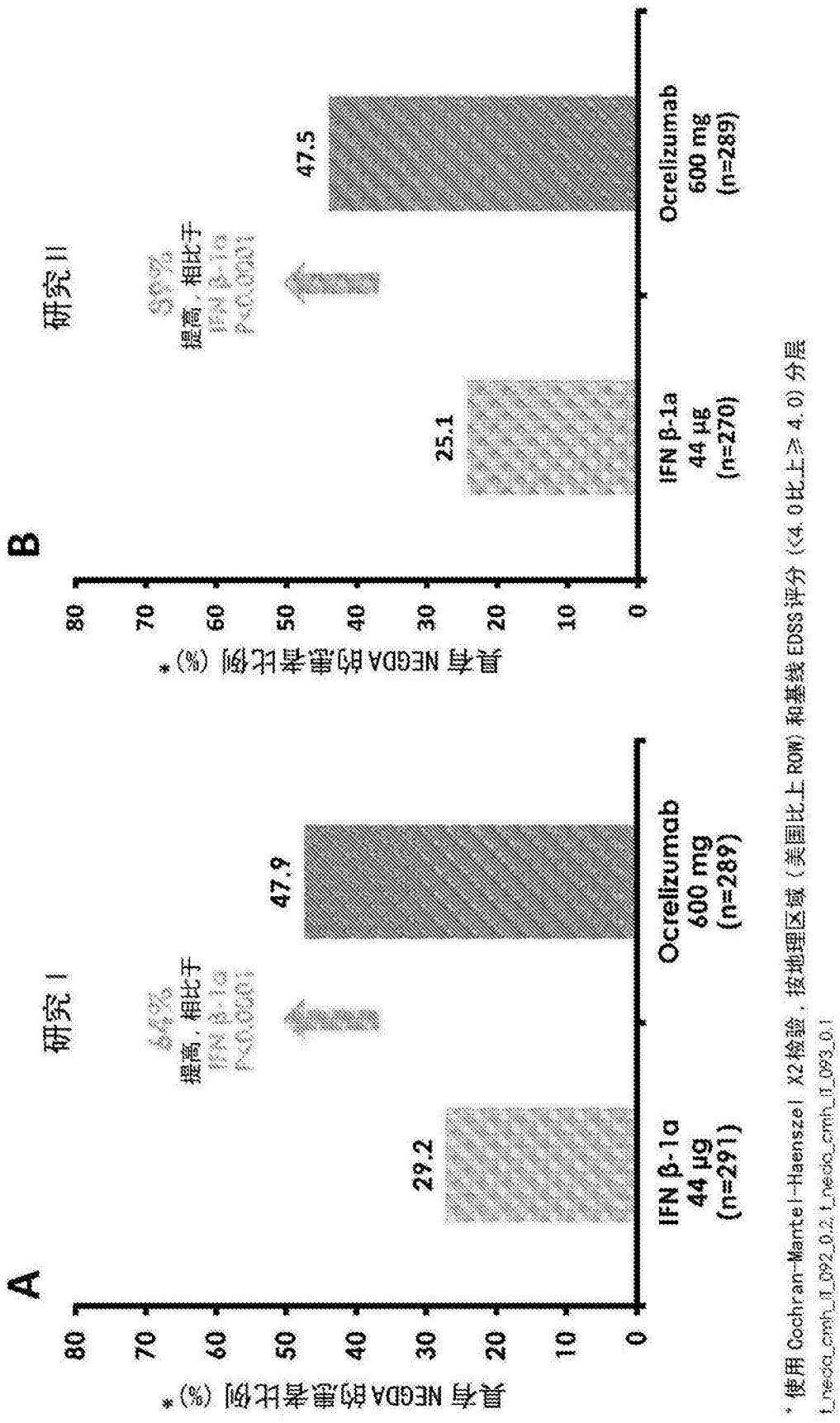

A
研究 I
具有 NEGDA 的患者比例 (%)*
80
70
60
50
40
30
20
10
0
29.2
47.9
64%
提高，相比于
IFN β-1a
P<0.0001
IFN β-1a
44 µg
(n=291)
Ocrelizumab
600 mg
(n=289)
B
研究 II
具有 NEGDA 的患者比例 (%)*
80
70
60
50
40
30
20
10
0
25.1
47.5
90%
提高，相比于
IFN β-1a
P<0.0001
IFN β-1a
44 µg
(n=270)
Ocrelizumab
600 mg
(n=289)
* 使用 Cochran-Mantel-Haenszel χ2 检验，按地理区域（美国比上 ROW）和基线 EDSS 评分（≤4.0 比上≥ 4.0）分层
t_nedo_cmh_IL092_0.2 t_nedo_cmh_IL093_0.1

图34

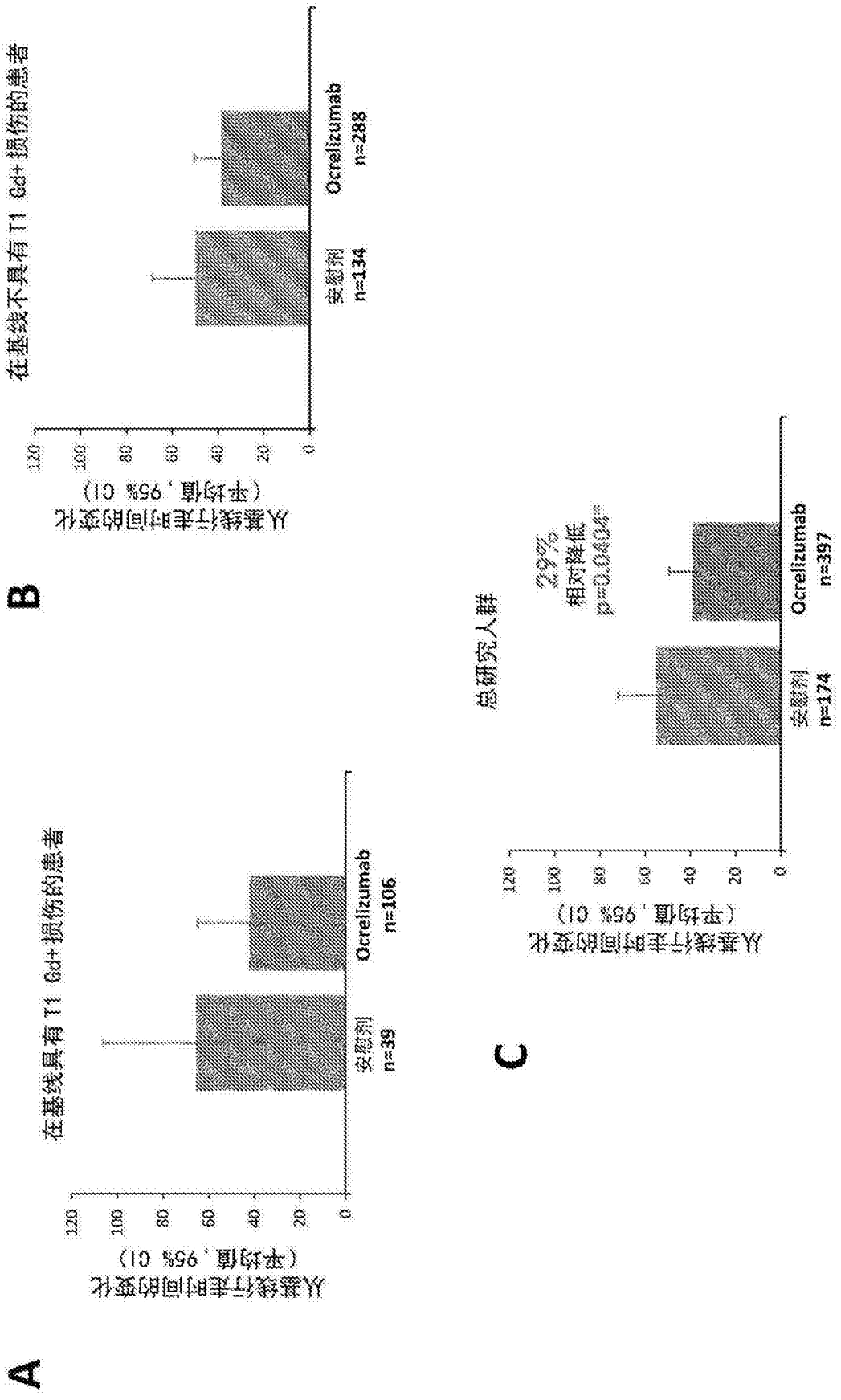

A
在基线具有 T1 Gd+ 损伤的患者
从基线行走时间的变化
（平均值，95% CI）
120
100
80
60
40
20
0
安慰剂
n=39
Ocrelizumab
n=106
B
在基线不具有 T1 Gd+ 损伤的患者
从基线行走时间的变化
（平均值，95% CI）
120
100
80
60
40
20
0
安慰剂
n=134
Ocrelizumab
n=288
C
总研究人群
29%
相对降低
p=0.0404*
从基线行走时间的变化
（平均值，95% CI）
120
100
80
60
40
20
0
安慰剂
n=174
Ocrelizumab
n=397

图35

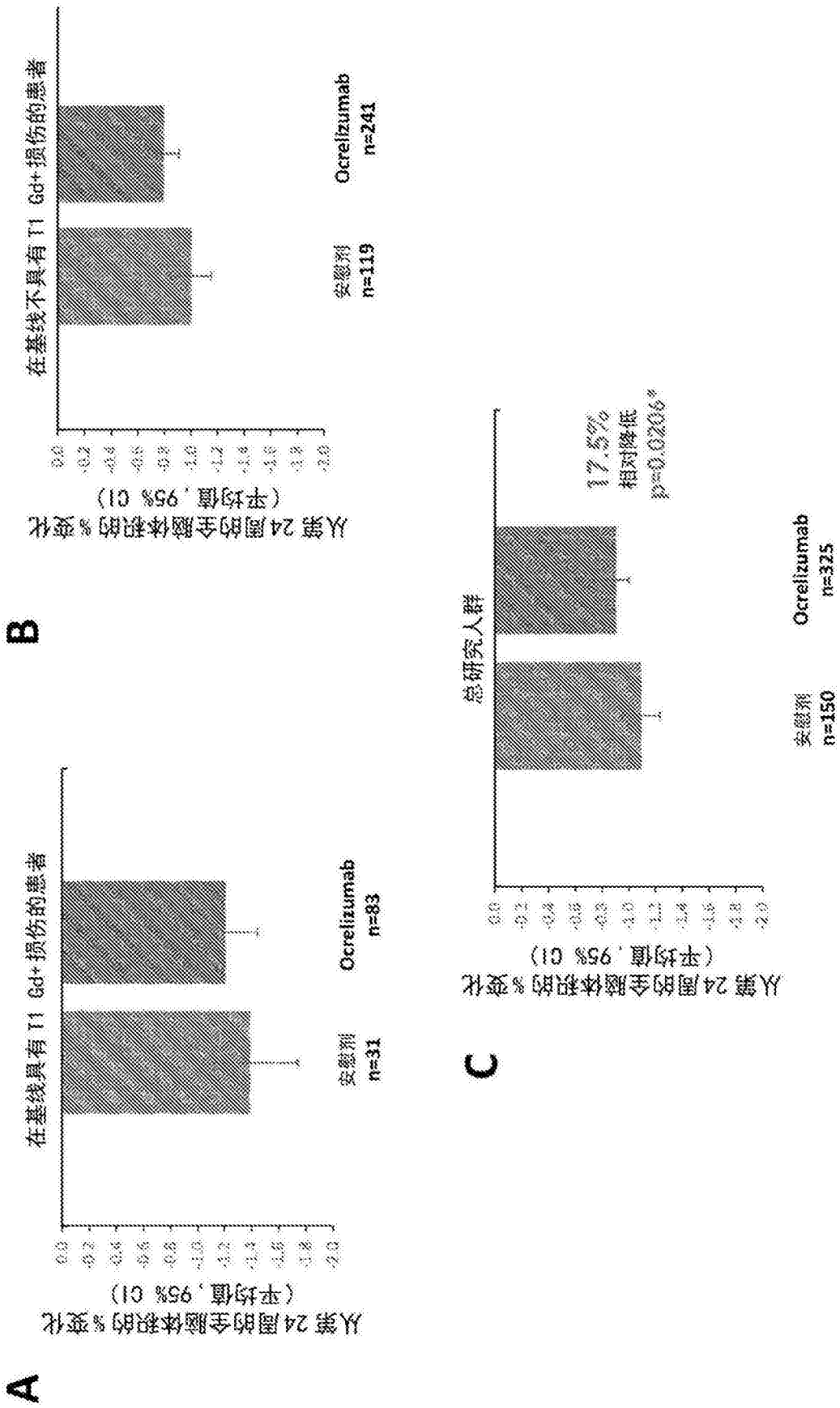

A
在基线具有 T1 Gd+损伤的患者
从第 24 周的全脑体积的 % 变化
（平均值，95% CI）
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
安慰剂
n=31
Ocrelizumab
n=83
B
在基线不具有 T1 Gd+损伤的患者
从第 24 周的全脑体积的 % 变化
（平均值，95% CI）
安慰剂
n=119
Ocrelizumab
n=241
C
总研究人群
从第 24 周的全脑体积的 % 变化
（平均值，95% CI）
17.5%
相对降低
p=0.0206*
安慰剂
n=150
Ocrelizumab
n=325

图36

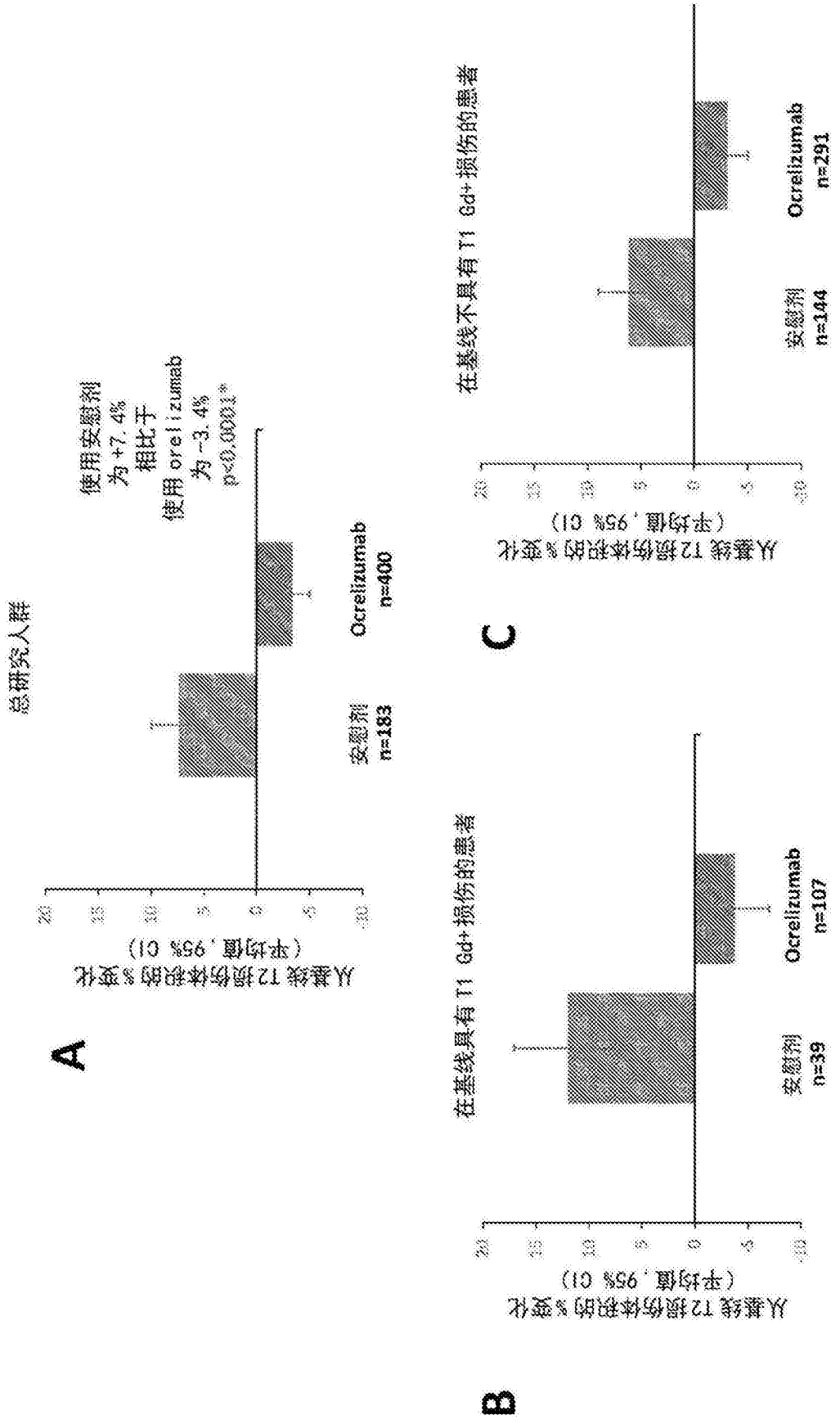
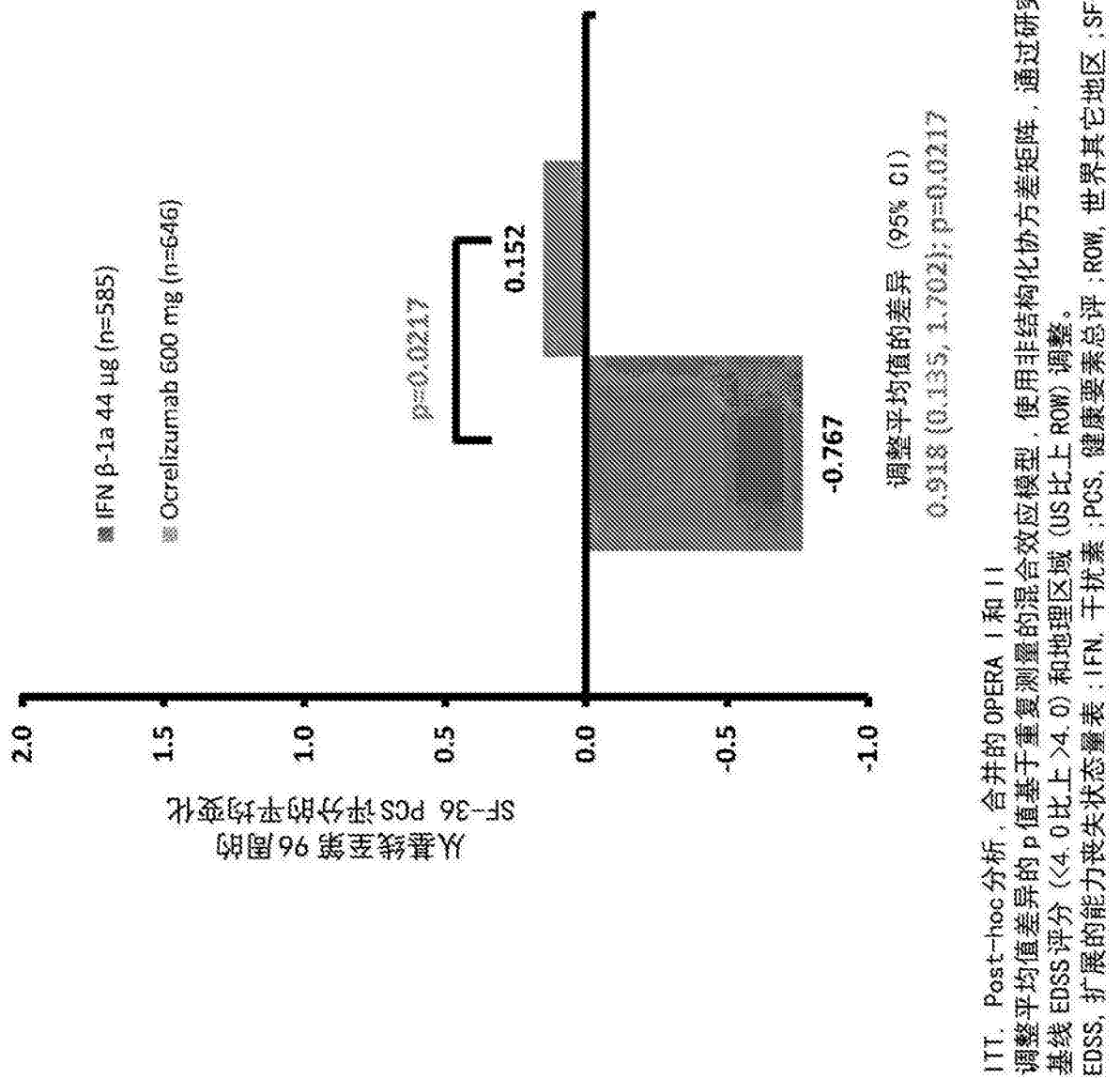
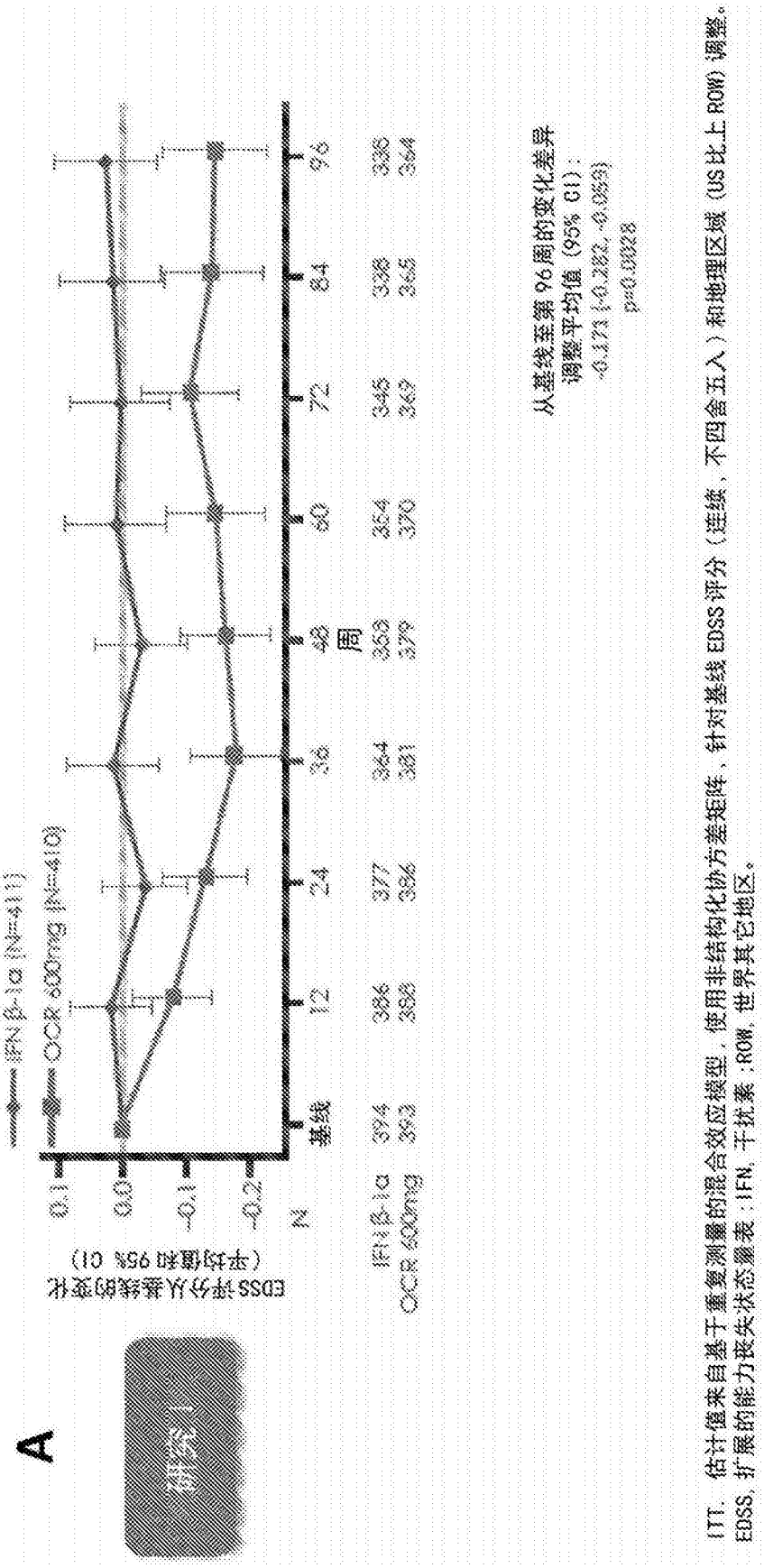

A
总研究人群
使用安慰剂
为+7.4%
相比于
使用 ocrelizumab
为-3.4%
p<0.0001*
从基线 T2 损伤体积的 %变化
(平均值,95% CI)
安慰剂
n=183
Ocrelizumab
n=400
B
在基线具有 T1 Gd+损伤的患者
从基线 T2 损伤体积的 %变化
(平均值,95% CI)
安慰剂
n=39
Ocrelizumab
n=107
C
在基线不具有 T1 Gd+损伤的患者
从基线 T2 损伤体积的 %变化
(平均值,95% CI)
安慰剂
n=144
Ocrelizumab
n=291

图37



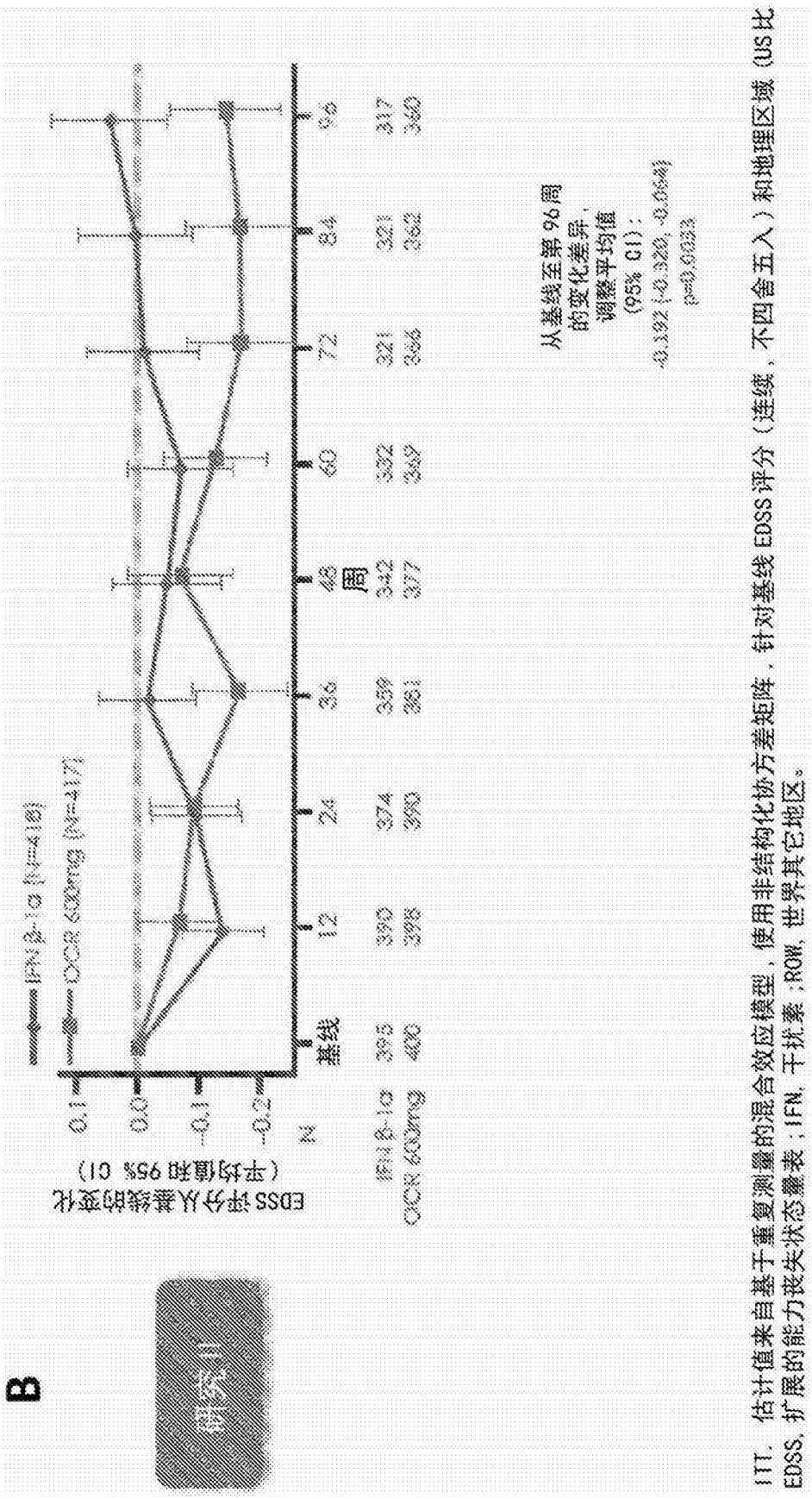
ITT, Post-hoc 分析, 合并的 OPERA I 和 II
调整平均值差异的 p 值基于重复测量的混合效应模型, 使用非结构化协方差矩阵, 通过研究、基线 PCS 评分、基线 EDSS 评分 (<4.0 比上 >4.0) 和地理区域 (US 比上 ROW) 调整。
EDSS, 扩展的能力丧失状态量表; IFN, 干扰素; PCS, 健康要素总评; ROW, 世界其它地区; SF-36, 简式 (36) 健康调查。

图38



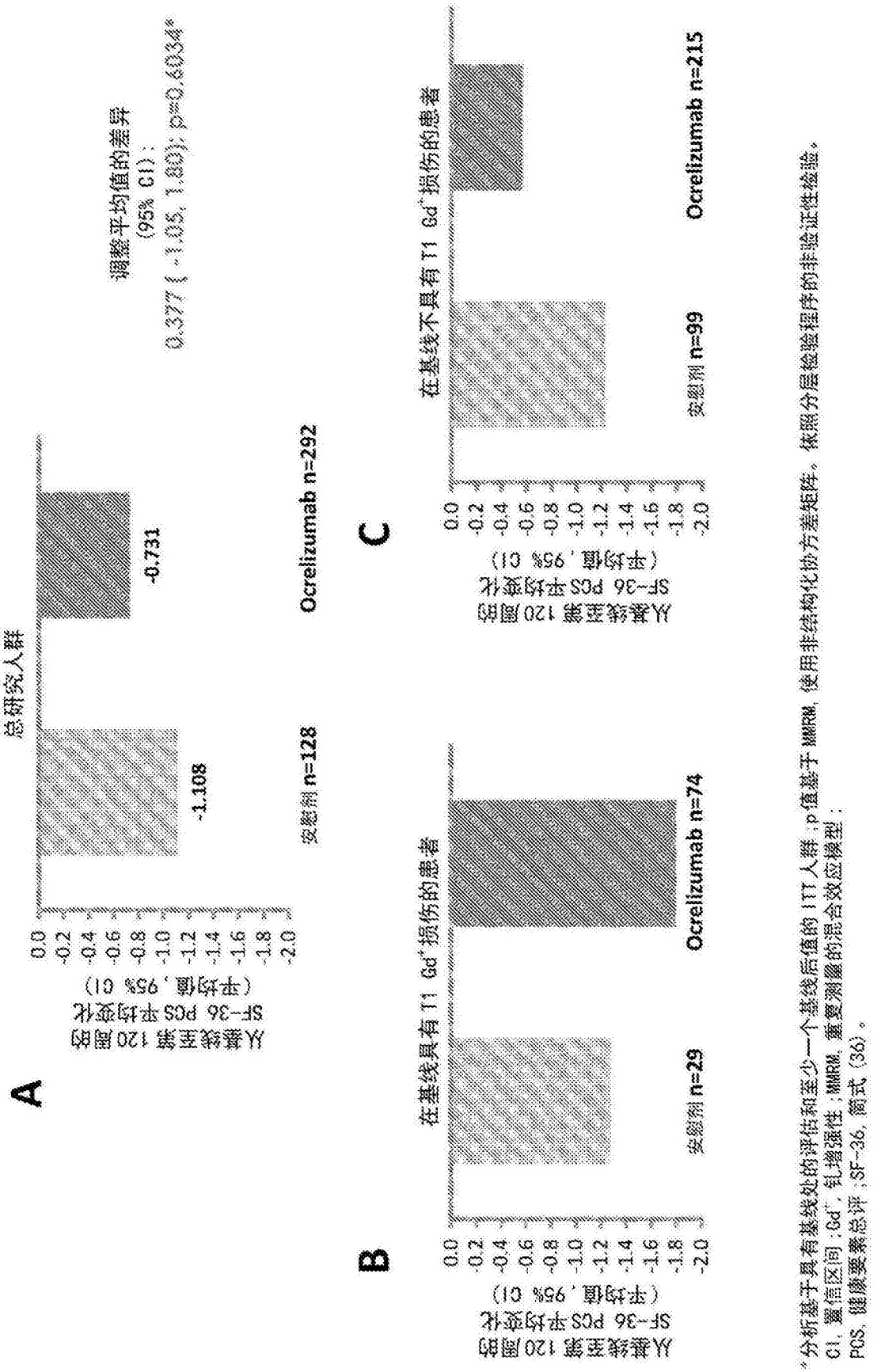
ITT，估计值来自基于重复测量的混合效应模型，使用非结构化协方差矩阵，针对基线 EDSS 评分（连续，不四舍五入）和地理区域（US 比上 ROW）调整。
EDSS，扩展的能力丧失状态量表；IFN，干扰素；ROW，世界其它地区。

图39



ITT，估计值来自基于重复测量的混合效应模型，使用非结构化协方差矩阵，针对基线 EDSS 评分（连续，不四舍五入）和地理区域（US 比 EDSS，扩展的能力丧失状态量表；IFN，干扰素；ROW，世界其它地区。

图39



*分析基于具有基线处的评估和至少一个基线后值的 ITT 人群；p 值基于 MMRM，使用非结构化协方差矩阵。依照分层检验程序的非验证性检验。
CI，置信区间；Gd+，钆增强性；MMRM，重复测量的混合效应模型；
PCS，健康要素总评；SF-36，简式（36）。

图40

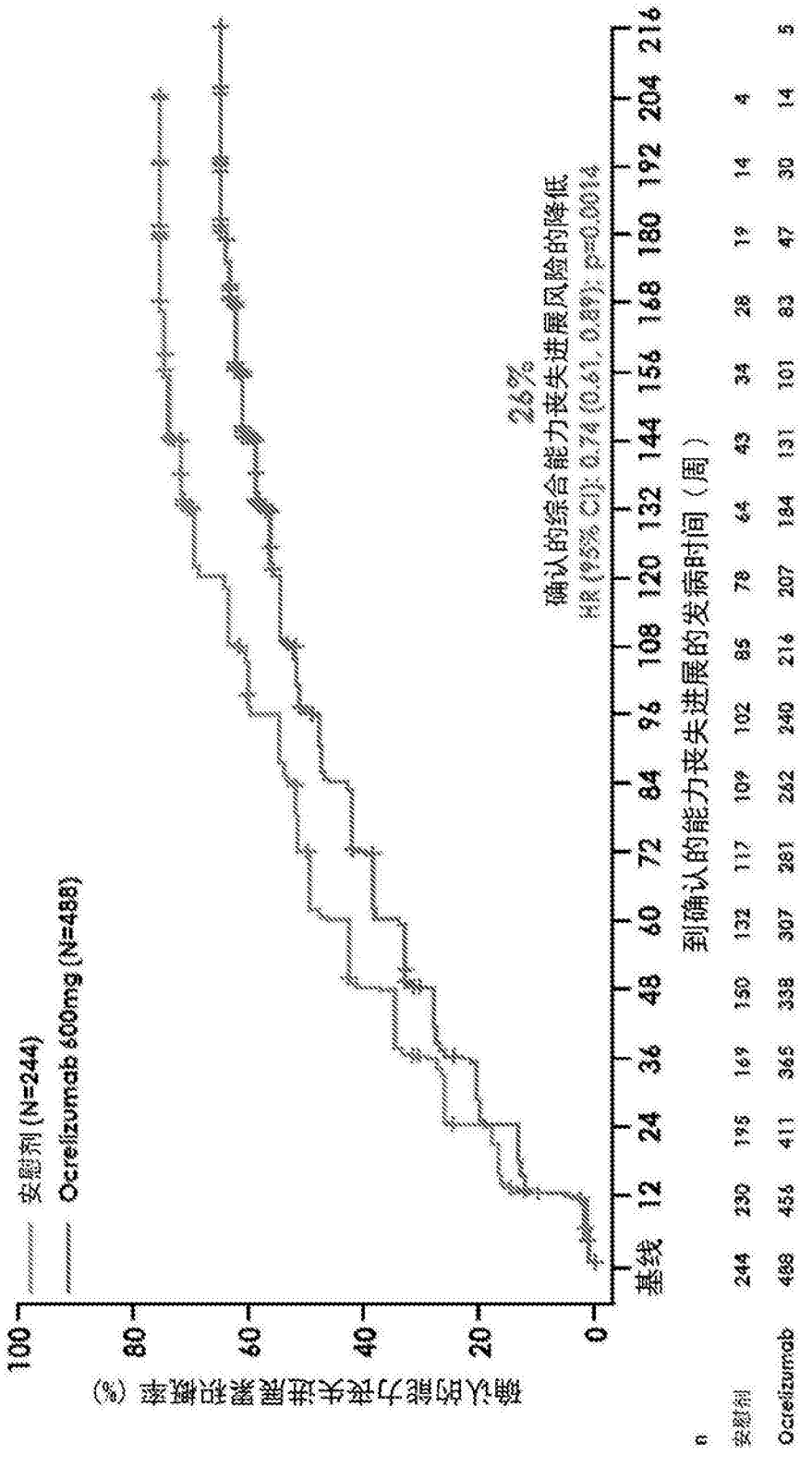

安慰剂 (N=244)
Ocrelizumab 600mg (N=488)
确认的能力丧失进展累积概率 (%)
100
80
60
40
20
0
26%
确认的综合能力丧失进展风险的降低
HR (95% CI): 0.74 (0.61, 0.89); p=0.0014
到确认的能力丧失进展的发病时间（周）
基线 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
安慰剂 244 230 195 169 150 132 117 109 102 85 78 44 43 34 28 19 14 4
Ocrelizumab 488 456 411 365 338 307 281 262 240 216 207 184 131 101 83 47 30 14 5

图41

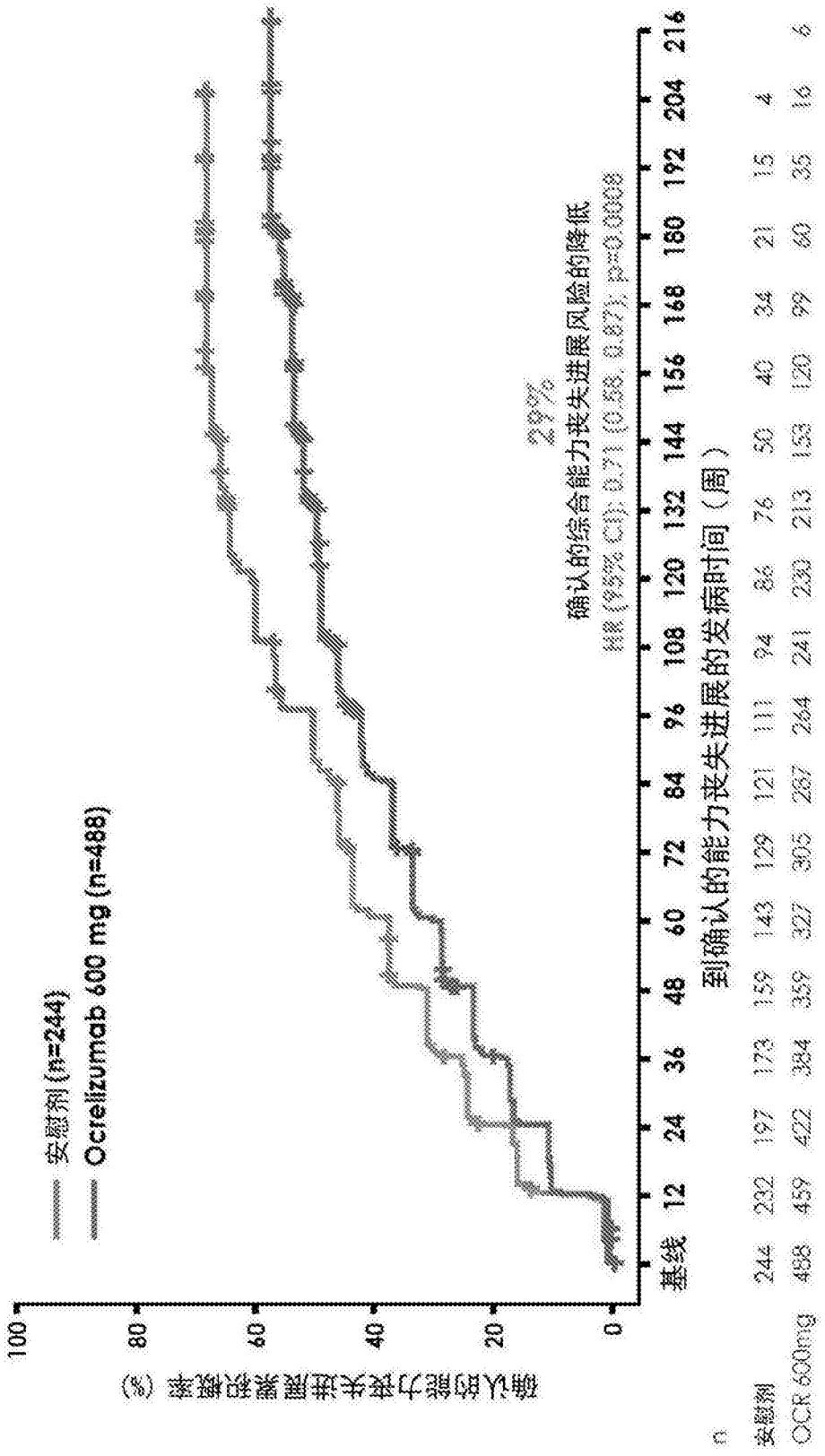

安慰剂 (n=244)
Ocrelizumab 600 mg (n=488)
确认的能力丧失进展累积概率 (%)
100
80
60
40
20
0
29%
确认的综合能力丧失进展风险的降低
HR (95% CI) 0.71 (0.58, 0.87); p=0.0008
到确认的能力丧失进展的发病时间（周）
基线 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216
n
安慰剂 244 232 197 173 159 143 129 121 111 94 86 76 50 40 34 21 15 4
OCR 600mg 488 459 422 384 359 327 305 287 264 241 230 213 153 120 99 60 35 16 6

图42

亚组的到第96周的方案定义的年复发率（负二项模型）-不充分应答者和初治治疗患者
意向治疗人群
合并的：WA21092和WA21093

基线危险因子	IFN beta-1a (N=[illegible])			OCR 600mg (N=[illegible])			比率比	95% CI	p值
	组数据	复发数据	患者年龄	组数据	复发数据	患者年龄			
所有患者	[illegible]	2054	[illegible]	[illegible]	1394	[illegible]	[illegible]	[illegible]	[illegible]
活跃的不充分应答者									
是	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
否	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
活跃的初治治疗患者									
是	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
否	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
高度活跃的不充分应答者									
是	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
否	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
高度活跃的初治治疗患者									
是	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
否	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Forest作图
OCR 600mg 更好 | IFN β-1a 更好
0.1 1 10

通过研究、地理区域（US比上ROW）和基线EDSS（<4.0比上>=4.0）调整。
包括对数变换暴露时间作为位移变量。
未对p值进行调整以解释多重治疗比较。

图43

亚组的双盲治疗期中 CDD 达至少 12 周的发病时间 - 不充分应答者和初治治疗患者，意向治疗人群
合并的：WA21092 和 WA21093

基线风险因子	总计 n	IFN beta-1a (N=629) n	事件	CCR 600mg (N=627) n	事件	危险比	95% CI	p 值 (Wald)
所有患者	1635	629	113	627	75	0.60	(0.45, 0.81)	0.0007
活跃的不充分应答者								
是	301	148	22	153	12	0.46	(0.23, 0.93)	0.0318
否	1334	480	91	474	63	0.64	(0.46, 0.88)	0.0066
活跃的初治治疗患者								
是	634	311	39	323	31	0.72	(0.44, 1.16)	0.1750
否	1021	317	74	304	44	0.54	(0.37, 0.79)	0.0015
高度活跃的不充分应答者								
是	283	140	22	143	12	0.47	(0.23, 0.96)	0.0351
否	1372	489	91	484	63	0.63	(0.46, 0.87)	0.0035
高度活跃的初治治疗患者								
是	219	107	15	112	13	0.72	(0.34, 1.52)	0.3833
否	1436	721	98	715	62	0.57	(0.42, 0.79)	0.0007

按研究、地理区域 (US比上 ROW) 和基线 EDSS (<4.0 比上 >=4.0) 分层。
未对 p 值进行调整以解释多重治疗比较

图44

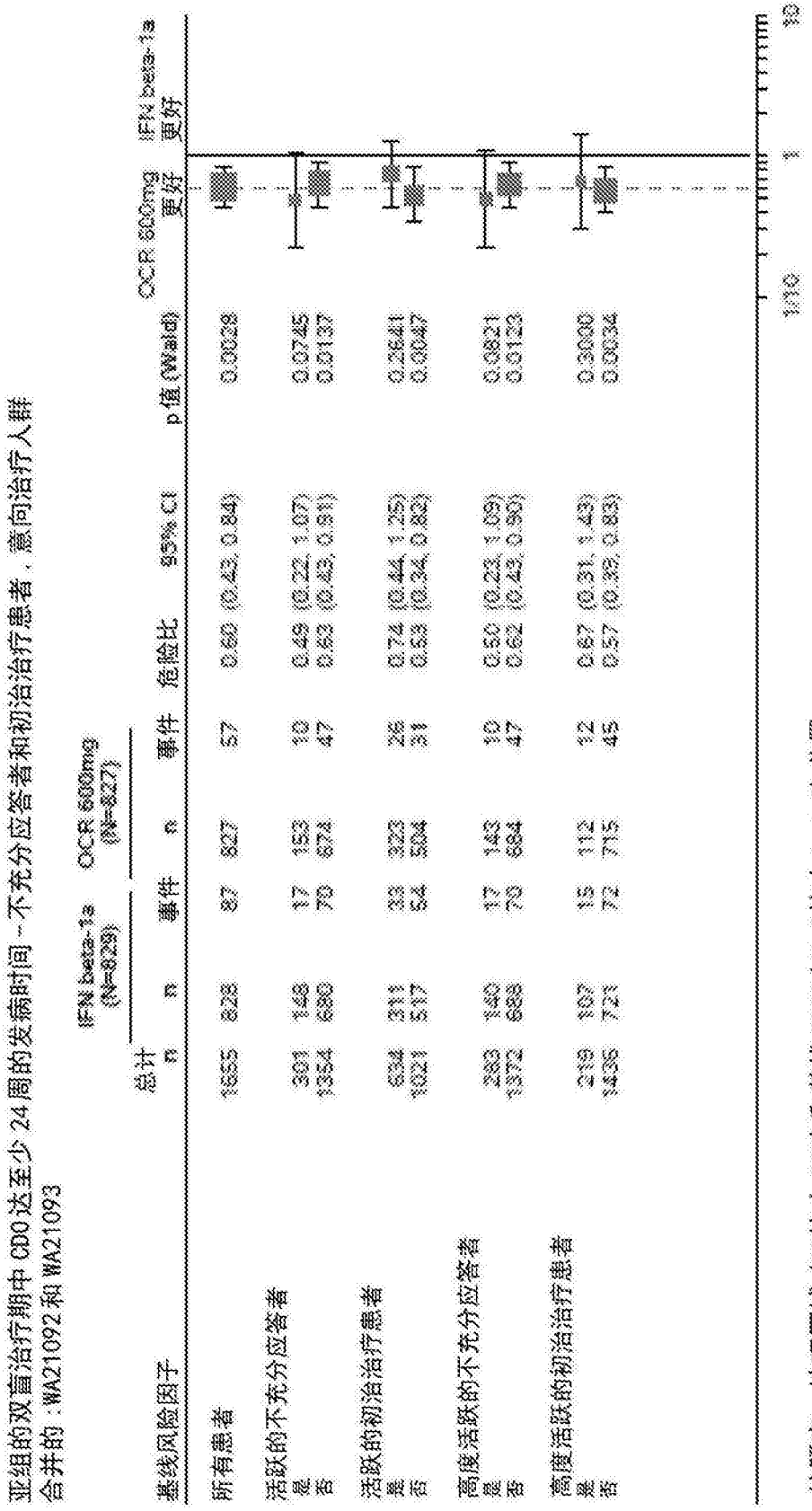

亚组的双盲治疗期中 CDO达至少 24 周的发病时间 - 不充分应答者和初治治疗患者，意向治疗人群
合并的：WA21092和 WA21093
IFN beta-1a (N=629)
CCR 600mg (N=827)
基线风险因子
总计 n
n
事件
n
事件
危险比
95% CI
p值 (Wald)
所有患者 1655 629 87 827 57 0.60 (0.42, 0.84) 0.0028
活跃的不充分应答者
是 301 148 17 153 10 0.49 (0.22, 1.07) 0.0745
否 1354 480 70 674 47 0.63 (0.43, 0.91) 0.0137
活跃的初治治疗患者
是 634 311 33 323 26 0.74 (0.44, 1.25) 0.2641
否 1021 317 54 504 31 0.53 (0.34, 0.82) 0.0047
高度活跃的不充分应答者
是 293 149 17 143 10 0.50 (0.23, 1.09) 0.0821
否 1372 628 70 684 47 0.62 (0.43, 0.90) 0.0123
高度活跃的初治治疗患者
是 219 107 15 112 12 0.67 (0.31, 1.43) 0.3000
否 1435 721 72 715 45 0.57 (0.39, 0.83) 0.0034
1/10
1
10
CCR 600mg 更好
IFN beta-1a 更好
按研究、地理区域 (US比上 ROW) 和基线 EDSS(<4.0比上 >=4.0) 分层。
未对 p 值进行调整以解释多重治疗比较。

图45

亚组的基线 EDSS>=2.0 的患者中双盲治疗期期间具有 GD1 达至少 12 周的患者比例 - 不充分应答者和初治治疗患者，意向治疗人群合并的：WA21092 和 WA21093

基线风险因子	IFN beta-1a (N=614)			OCR 600mg (N=626)			相对风险	95% CI of RR	p 值 (CMH)	Forest 作图 IFN β-1a 更好 OCR 600mg 更好
	组数量	具有 GD1 的患者数量	比例 %	组数量	具有 GD1 的患者数量	比例 %				
所有患者	318	60	18.84	329	120	36.47	1.93	(1.426, 2.612)	0.01006	
活跃的不充分应答者										
否	205	42	20.49	205	69	33.66	1.64	(1.143, 2.348)	0.15365	
是	113	18	15.93	124	51	41.13	2.58	(1.58, 4.209)	0.02122	
活跃的初治治疗患者										
否	279	54	19.35	293	101	34.47	1.78	(1.304, 2.43)	0.00046	
是	226	32	14.16	236	88	37.29	2.63	(1.788, 3.872)	0.00033	
高度活跃的不充分应答者										
否	266	51	19.17	269	100	37.17	1.94	(1.421, 2.646)	0.00011	
是	158	12	7.59	159	39	24.53	3.23	(1.822, 5.728)	0.00003	
高度活跃的初治治疗患者										
否	228	44	19.3	240	87	36.25	1.88	(1.364, 2.591)	0.00004	
是	176	12	6.82	181	52	28.73	4.21	(2.34, 7.569)	0.00003	

0.1 1 10

CMH: Cochran-Mantel-Haenszel 卡方检验。
使用 Pearson-Clopper 法构建比例的 95% CI。
按研究、地理区域 (US 比上 ROW) 和基线 EDSS(<4.0 比上 >=4.0) 分层。
缺少 EDSS 或在能力丧失改善发作后未确诊的患者被计为不具有确认的能力丧失改善。
未对 p 值进行调整以解释多重治疗比较。

图46

亚组的双盲治疗期期间在定期访问时通过脑 MRI 检测的 T1 钆增强性损伤的总数 - 不充分应答者和初治治疗患者，意向治疗人群合并的：WA21092 和 WA21093

基线风险因子	IFN beta-1a (N=420)			OCR 600mg (N=827)			比率比	95% CI	p 值	Forest 作图
	组数量	损伤数量	MRI 数量	组数量	损伤数量	MRI 数量				IFN β-1a 更好 OCR 600mg 更好
所有患者	[illegible]	802	[illegible]	827	42	2235	[illegible]	(0.537,0.882)	<0.0001	
活跃的不充分应答者										
是	145	178	358	183	4	416	[illegible]	(0.097,0.823)	<0.0001	
否	561	624	[illegible]	674	38	1819	[illegible]	(0.541,0.950)	<0.0001	
活跃的初治治疗患者										
是	181	324	789	323	20	826	[illegible]	(0.222,0.726)	<0.0001	
否	318	478	[illegible]	504	22	1387	[illegible]	(0.699,0.879)	<0.0001	
高度活跃的不充分应答者										
是	145	148	350	148	4	407	[illegible]	(0.097,0.823)	<0.0001	
否	499	638	[illegible]	684	38	1648	[illegible]	(0.540,0.898)	<0.0001	
高度活跃的初治治疗患者										
是	107	222	571	112	13	319	[illegible]	(0.222,0.745)	<0.0001	
否	222	560	[illegible]	719	29	1652	[illegible]	(0.621,0.879)	<0.0001	

0.0001 0.1 1.0

负二项模型。通过研究、基线 T1 Gd 损伤（存在与否）、基线 EDSS（<4.0 比上 >=4.0）和地理区域（US 比上 ROW）调整。
包括脑 MRI 扫描的对数变换数量作为位移变量。
未对 p 值进行调整以解释多重治疗比较。

图47