

INEBILIZUMAB AND METHODS OF USING THE SAME FOR SUSTAINED B-CELL DEPLETION

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 **[0001]** This application claims priority to U.S. Provisional Patent Application No. 63/369,590, filed July 27, 2022, incorporated by reference herein in its entirety for all purposes.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

- 10 **[0002]** The contents of the electronic sequence listing (HOPA_045_01WO_SeqList_ST26.xml; Size: 10,611 bytes; and Date of Creation: September 19, 2022) is herein incorporated by reference in its entirety.

TECHNICAL FIELD

- 15 **[0003]** The present disclosure is related to compositions comprising Inebilizumab and methods of using the same for sustained B-cell depletion.

BACKGROUND

- 20 **[0004]** Inebilizumab is an anti-CD19 monoclonal antibody approved in Japan and the USA for the treatment of neuromyelitis optica spectrum disorder (NMOSD). However, the relationship between B-cell, plasma cell, and immunoglobulin depletion after inebilizumab treatment, and reductions in NMOSD disease activity after inebilizumab treatment remains unclear and further elucidation of the relationship warrants analysis to elucidate optimized methods of treatment
25 for subjects with NMOSD.

BRIEF SUMMARY

- 30 **[0005]** The description provides methods of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, thereby treating the NMOSD. In aspects, the subject in need's Fc Gamma Receptor IIIa (*FCGR3A*) gene comprises a rs396991 polymorphism. In aspects, the subject in need comprises a V allele as determined by a V/F or V/V genotype.

[0006] Provided are methods of treatment, the methods comprising assaying a blood sample from a subject in need to determine presence of a rs396991 polymorphism of the *FCGR3A*

gene and administering Inebilizumab to the subject in need if the determination is positive for the rs396991 polymorphism. In aspects, the subject in need has an autoimmune disease. In aspects, the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD. In aspects, the autoimmune disease is NMOSD. In aspects, the autoimmune disease is MG. In aspects, the autoimmune disease is IgG4RD. In aspects, the subject in need has at least two autoimmune diseases. In aspects, the at least two autoimmune diseases are selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.

10 **[0007]** Provided are methods of treating Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that comprises a rs396991 polymorphism of the *FCGR3A* gene, wherein the administering is more effective in reducing B-cell or plasma-cell levels in the subject in need that comprises the rs396991 polymorphism of the *FCGR3A* gene as compared to an otherwise comparable subject lacking
15 the rs396991 polymorphism.

[0008] In aspects, levels of B-cells or plasma-cells are determined at least 1, 2, 4, 8, 12, 16, 22, 28, and/or 32 weeks after the administering. In aspects, the administering is effective in reducing a criteria selected from the group consisting of: Annualized Attack Rate (AAR), incidence of disability as assessed by Expanded Disability Status Scale (EDSS) score,
20 incidence of hospitalization, magnetic resonance imaging (MRI) lesions, and combinations thereof. In aspects, the administering is effective in reducing the AAR, wherein the reduction is at least about 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%. In aspects, the administering is effective in reducing the MRI lesions, wherein the reduction is at least about 20%, 30%, 40%, 50%, 60%, 70%, 73%, 75%, 80%, 85%, 90%, 95%,
25 97%, or up to about 100%. In aspects, the administering is repeated. In aspects, the administering is repeated daily, monthly, or yearly. In aspects, the administering is repeated monthly. In aspects, the administering is repeated every 6 months. In aspects, the administering is continued for at least about 1 year, 2 years, 3 years, 4 years, 5 years, or up to about the lifetime of the subject in need. In aspects, the subject in need was previously administered
30 Inebilizumab. In aspects, administering is intravenous. In aspects, the administering is by intravenous infusion. In aspects, Inebilizumab is administered at a dose of about 300 mgs.

[0009] Provided are methods of treating or preventing an autoimmune disease, the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6

months. In aspects, the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD. In aspects, the autoimmune disease is NMOSD. In aspects, the autoimmune disease is MG. In aspects, the autoimmune disease is IgG4RD.

5 **[0010]** Provided are methods of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months.

[0011] Provided are methods of treating or preventing an autoimmune disease, the method
10 comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months, and wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4 related disease (IgG4RD). In aspects, the administering is continued for at least about 2.5 years.

15 **[0012]** Provided are methods of treating neuromyelitis optica spectrum disorder, the methods comprising administering Inebilizumab to a subject in need, wherein the subject has no anti-aquaporin 4 immunoglobulin G antibodies (AQP4-IgG $-$) and has immunoglobulin G antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG $+$). In aspects, about 300 mg of Inebilizumab is administered. In aspects, the administration is continued for at least 6
20 months, 1 year, 2 years, or 3 years. In aspects, the administration is continued indefinitely. In aspects, the administration is continued for at least 2 years. In aspects, the subject in need has increased serum autoreactivity to an autoantibody that is not related to NMOSD as compared to a healthy control subject.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1A** is a graphic of a study design. **FIG. 1B** shows attack free probability of newly diagnosed subjects during RCP. **FIG. 1C** shows attack free probability of subjects with ≥ 2 prior NMOSD relapses.

[0014] **FIG. 2A** shows serum autoreactivity to at least one autoantigen in subjects with
30 NMOSD in all subclasses, IgG subclass, IgM subclass, IgA subclass and IgE subclass. **FIG. 2B** shows subjects with NMOSD who were IFNGS (type 1 interferon-inducible gene signature)-positive at baseline. **FIG. 2C** shows the proportion of IFNGS-negative and IFNGS-positive subjects with NMOSD reactive to at least one anti-ribonucleoprotein (RNP) IgG. **FIG.**

2D shows proportion of ANA (anti-nuclear antibody) score by IFNGS status. **FIG. 2E** shows (left) ANA score, (middle) autoantibody score and (right) IFNGS positivity in subjects with NMOSD alone or in addition to other coexisting inflammatory diseases.

[0015] FIG. 3A shows kinetics of B-cell depletion following treatment with inebilizumab.

5 **FIG. 3B** shows long-term (> 2.5 years) B-cell depletion kinetics across subgroups. **FIG. 3C** shows AAR. **FIG. 3D** shows annualized rate of new/enlarging T2 lesions. **FIG. 3E** shows annualized rate of EDSS score worsening. **FIG. 3F** shows annualized rate of in-patient hospitalizations in subjects with B-cell counts ≤ 4 cells/ μ L versus > 4 cells/ μ L. aB-cell counts were measured after the first inebilizumab dosing interval (at 6 months after treatment initiation). **FIG. 3G** shows AAR and new/emerging T2 lesions. For each of **FIG. 3C- FIG. 3G** plots shows rates in placebo-treated subjects during the RCP (in grey), then in both inebilizumab-treated subject groups during the first 6 months of inebilizumab treatment (first dosing interval), and yearly afterwards (in green and light blue). The final bar in each plot displays the combined rate of each endpoint after 2.5 years or more of continued inebilizumab treatment. Error bars represent 95% CI estimated by negative binomial regression. AAR, annualized attack rate; CI, confidence interval; EDSS, Expanded Disability Status Scale; RCP, randomized controlled period.

[0016] FIG. 4A-FIG. 4C show effects of long-term (>2.5 years) inebilizumab-related B-cell depletion on metrics of disease activity.

20 **[0017] FIG. 5A** is a graphic of CD16a-mediated ADCC. **FIG. 5B** (left) AARs and (right) EDSS scores from subjects grouped by FCGR3A genotype. **FIG. 5C** shows attack risk (left), new/enlarging T2 lesion rate (middle), and RCP hospitalization rate in placebo-treated subjects (right). **FIG. 5D** shows attack risk (left) and new/enlarging T2 lesion rate in inebilizumab-treated subjects (right). **FIG. 5E** shows longitudinal CD20+ B-cell count (left), longitudinal plasma-cell signature (middle), and longitudinal IgG by FCGR3A genotype (right). **FIG. 5F** shows annualized attack rate, expanded disability status scale, in-patient hospitalizations, and new lesions.

25 **[0018] FIG. 6A-FIG. 6F** show kinetics of B-cell and immunoglobulin depletion. **FIG. 6A** shows plasma cell gene signature normalized to a pool of 10 healthy donors using ddCT method, **FIG. 6B** shows total immunoglobulin, **FIG. 6C** shows immunoglobulin G, **FIG. 6D** shows immunoglobulin M, **FIG. 6E** immunoglobulin A, and **FIG. 6F** shows immunoglobulin E over time during the RCP in placebo (in grey) and inebilizumab-treated (in red) subjects. Points represent medians, shaded region represents IQR. Dashed black line represents LLOQ of 0.2 cells/ μ L for FACS assays. Samples with counts below LLOQ imputed to 0.05 cells/ μ L.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Mann–Whitney U Test between dose groups. Vertical dashed line represents when subjects were transitioned to OLP and placebo cohort was administered two inebilizumab doses; inebilizumab cohort was given additional maintenance dose. ddCT=delta delta cycle threshold. FACS= fluorescence-activated cell sorting. Ig=immunoglobulin. IQR=interquartile range. LLOQ=lower limit of quantification. OLP=open-label period. RCP=randomized controlled period.

[0019] FIG. 7A-FIG. 7D show decreases in NMOSD progression observed with long-term inebilizumab treatment. FIG. 7A shows annualized Attack Rate (AAR). FIG. 7B shows annualized rate of new/enlarging T2 lesions. FIG. 7C shows annualized rate of EDSS worsening. FIG. 7D shows annualized in-patient hospitalization rate in placebo treated subjects during the RCP, in all subjects during the first dosing period of inebilizumab treatment (first dosing interval), and yearly afterwards. The final bar in each plot displays the combined rate of each endpoint after 2.5 years or more of continued inebilizumab treatment. Error bars represent 95% CI estimated by negative binomial regression.

[0020] FIG. 8A-FIG. 8C show temporal relationship between CD20+ B-cell counts and attacks. FIG. 8A shows profiles of CD20+ B-cell counts in subjects who experienced attacks after inebilizumab treatment. FIG. 8B is a boxplot of CD20+ B-cell counts in all samples drawn from subjects who never experienced attacks (left), in samples drawn preceding attacks (sample draws range 15-115 days preceding attacks), during attack assessment, after attacks (sample draws range 8-200 days after attack). FIG. 8C is a boxplot of CD20 B-cell counts in subset of samples drawn after 3rd dose was administered.

[0021] FIG. 9A-FIG. 9B show B-cell kinetics in the first dosing interval of inebilizumab predict long-term deep B-cell depletion. FIG. 9A is a profile plot of B-cell counts in individual subjects throughout the first dosing period with inebilizumab. FIG. 9B shows total CD20 counts over 3 years of inebilizumab treatment in subjects with B-cell counts >4 and ≤ 4 cells/ μ L at the end of the first dosing period. Points represent medians $\pm$ IQR. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Mann–Whitney U Test). IQR=interquartile range. RCP=randomized controlled period.

[0022] FIG. 10 shows annualized in-patient hospitalization rate split by subjects with > 4 cells/ μ L after the first inebilizumab dosing interval (6 months post treatment).

[0023] FIG. 11 is an exemplary graphic showing that CD19 is a differentiated target for B-cell depletion.

[0024] FIG. 12A-FIG. 12B show B-cell counts for individual subjects at the time of attacks. FIG. 12A shows total CD20+ and FIG. 12B shows CD20+ memory B-cell counts over time

of subjects with more than 2 inebilizumab doses who experienced attacks. Each sub-panel contains data of five subjects; each subject is represented by a differently colored line.

[0025] FIG. 13A-FIG. 13D are plots showing rate ratios of disease activity based on cut-off points of post-first dosing interval B-cell level. Rate ratio (+/- 95% CI shaded region) versus W26 B-cell cutpoint of attacks (FIG. 13A), new or enlarging T2 lesions (FIG. 13B), EDSS worsening (subjects/year) (FIG. 13C), In-patient hospitalizations that occurred after the first dosing period with inebilizumab (FIG. 13D). Rate ratio was calculated by comparing rate at which each endpoint occurred in subjects below versus above each B-cell cutpoint. AAR=annualized attack rate. CI=confidence interval. EDSS=Expanded Disability Status Scale. RR=rate ratio.

[0026] FIG. 14A shows annualized attack rate. FIG. 14B shows annualized rate of new/enlarging T2 lesions. FIG. 14C shows annualized rate of EDSS worsening and FIG. 14D shows annualized rate of in-patient hospitalizations split by subjects above or below 4 cells/ μ L after the first inebilizumab dosing interval (6 months post-treatment) in AQP4- seropositive subjects.

[0027] FIG. 15 shows pharmacokinetics of inebilizumab in those with 6-month B-cell counts above or below 4 cells/ μ L. Subjects with B-cells $\leq$ 4 cells/ μ L (blue) and Median serum inebilizumab concentration in subjects with B-cells $\leq$ 4 cells/ μ L (blue) or subjects with $>$ 4 cells/ μ L (red) during the first dosing period with inebilizumab (top panel). Serum inebilizumab concentration across both groups during at week 22 of the RCP (bottom panel).

[0028] FIG. 16A-FIG. 16C show baseline B-cell count, plasma cell gene signature, and total Ig of subjects divided by B-cell count after first dosing period with inebilizumab. FIG. 16A shows baseline blood CD19+ B-cell counts. FIG. 16B shows plasma cell gene signature, and FIG. 16C shows total immunoglobulin across the two B-cell depletion subgroups. FC=fold change. HD=healthy donor. Ig=immunoglobulin.

[0029] FIG. 17A shows median B-cell counts in subjects with B-cell counts $\leq$ 4 cells/ μ L after the first dosing period of inebilizumab treatment (blue), and in those with B-cell counts $>$ 4 cells/ μ L at the end of the first dosing period further subdivided by whether subjects displayed depletion $\leq$ 4 cells/ μ L at any point during this interval (red and blue). FIG. 17B is a bar plot displaying % of subjects within each subgroup who were rs396991 F/F homozygotes.

[0030] FIG. 18A-FIG. 18C show the relationship between CD20+ B-cell counts and ADA. FIG. 18A is a profile plot of ADA titres as a function of time since start of inebilizumab treatment. FIG. 18B is a chart displaying percentage of subjects from different ADA subgroups with B-cell counts $>$ 4 cells/ μ L at W26-W28. FIG. 18C is a boxplot displaying median B-cell

counts in subjects who were persistently ADA negative, at intervals >90 days from ADA positivity in the subjects who intermittently displayed ADA positivity, and at intervals within 90 days of ADA positivity from these subjects. ADA=anti-drug antibody. BP=baseline positive. LLN=lower limit of normal. LLOQ=lower limit of quantification. TU=treatment unaffected. TE=treatment emergent. W=week.

[0031] FIG. 19A shows an exemplary study design. FIG. 19B shows time from first dose of inebilizumab to first Adjudication Committee (AC)-determined attack. FIG. 19C shows CD19 B-cell levels in seven subjects with serotype AQP4-IgG-/MOG-IgG+.

DETAILED DESCRIPTION

[0032] Described herein is Inebilizumab and methods of utilizing the same for reducing a B-cell-mediated immune response in a subject having a disease or disorder. Also provided are methods that comprise subject/subject stratification to optimize treatment of NMOSD with Inebilizumab based on presence of biomarkers such as a polymorphism of the *FCGR3A* gene. Also provided are therapeutic regimens comprising Inebilizumab for the treatment of NMOSD.

Definitions

[0033] While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques and/or substitutions of equivalent techniques that would be apparent to one of skill in the art.

[0034] Any ranges listed herein are intended to be inclusive of endpoints. For example, a range of 2-4 includes 2 and 4 and values between.

[0035] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise.

[0036] The term “about” or “approximately” when immediately preceding a numerical value means a range plus or minus 10% of that value. For example, “about 50” can mean 45 to 55, “about 25,000” can mean 22,500 to 27,500, etc., unless the context of the disclosure indicates otherwise, or is inconsistent with such an interpretation. In the context of a list of numerical values such as “about 49, about 50, about 55, ...”, “about 50” means a range extending to less than half the interval(s) between the preceding and subsequent values, e.g., more than 49.5 to

less than 52.5. Furthermore, the phrases “less than about” a value or “greater than about” a value should be understood in view of the definition of the term “about” provided herein.

[0037] When referring to a nucleic acid sequence or protein sequence, the term “identity” is used to denote similarity between two sequences. Unless otherwise indicated, percent identities described herein are determined using the BLAST algorithm available at the world wide web address: blast.ncbi.nlm.nih.gov/Blast.cgi using default parameters.

[0038] As used herein, the term “subject” refers to any individual, e.g., a human or a non-human mammal, for whom diagnosis, prognosis, or therapy is desired. The term “subject” may mean a human or non-human mammal affected, likely to be affected, or suspected to be affected with a disease, e.g., an autoimmune disease or condition. The terms “subject” and “patient” are used interchangeably herein. Although the compositions provided herein are principally directed to compositions which are suitable for administration to humans, the skilled artisan will understand that such compositions are generally suitable for administration to subjects of all sorts. In aspects, a subject is a mammal. A mammal includes primates, such as humans, monkeys, chimpanzee, and apes, and non-primates such as domestic animals, including laboratory animals (such as rabbits and rodents, e.g., guinea pig, rat, or mouse) and household pets and farm animals (e.g., cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic animals, such as wildlife, birds, reptile, fish, or the like.

[0039] As used herein, “treating” or “treat” describes the management and care of a subject for the purpose of combating a disease, condition, or disorder and includes the administration of a composition used in the methods described herein to alleviate the symptoms or complications of a disease, condition, or disorder, or to eliminate the disease, condition, or disorder. Thus, the term “treat” or “treating” refers to both therapeutic measures and prophylactic or preventative measures, wherein the objective is to prevent, slow down (lessen), or ameliorate the progression of a disease (e.g., an autoimmune disease). Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishing the extent of the disease, stabilized (i.e., not worsening) state of the disease, delaying or slowing of disease progression, amelioration, or palliation of the disease state, and reversing the disease (whether partial or total). The term “treat” can also include treatment of a cell *in vitro* or an animal model.

Inebilizumab

[0040] Inebilizumab (also known as MEDI-551) is a humanized, affinity-optimized, afucosylated IgG1 kappa monoclonal antibody (mAb) known as 16C-aFuc that binds to the B-cell specific surface antigen CD19, resulting in reduction or elimination of CD19+ B-cells.

Inebilizumab is glycoengineered by expression of mAb 16C4 in a fucosyltransferase deficient Chinese hamster ovary producer cell line (BioWa Potelligent® Technology), which generates a homogenously afucosylated antibody with enhanced antibody-dependent cellular cytotoxicity. In contrast to the anti-CD20 mAb rituximab, Inebilizumab does not mediate
5 complement-dependent cytotoxicity but eliminates B-cells via antibody-dependent cellular cytotoxicity and antibody-mediated cellular phagocytosis mechanisms.

[0041] In aspects, Inebilizumab comprises a CDR sequence from **Table 1**. In aspects, Inebilizumab comprises the variable heavy chain CDR1-CDR3 corresponding to SEQ ID NO: 2-4. In aspects, Inebilizumab comprises the variable light chain CDR1-CDR3 corresponding
10 to SEQ ID NO: 6-8. In aspects, Inebilizumab comprises the VH amino acid sequence and a VL amino acid sequence of **Table 1** corresponding to SEQ ID NO: 1 and SEQ ID NO: 5 respectively. In aspects, Inebilizumab comprises the heavy chain amino acid sequence and light chain amino acid sequence of **Table 1** corresponding to SEQ ID NO: 9 and SEQ ID NO: 10 respectively.

[0042] In aspects, Inebilizumab or a derivative of Inebilizumab comprises at least about or at most about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity with any one of SEQ ID NO: 1 – SEQ ID NO: 10 shown in **Table 1**. In aspects, any one of the sequences from **Table 1** can be modified. In aspects, a modification comprises one or more truncations, deletions, insertions, and combinations thereof. In aspects,
20 a modification does not alter the function of Inebilizumab. A modification can occur at any of the residues provided in **Table 1** and in any number of residues from **Table 1**. In aspects, a modification can comprise from 1-3, 1-5, 1-10, 5-20, 1-3, 1-5, 1-10, 1-20, 3-8, 3-10, 3-15, 5-8, 5-10, or 5-20 residues. In aspects, a modification can occur in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 450 residues. In aspects,
25 all the residues of **Table 1** comprise a modification.

Table 1: Exemplary Inebilizumab sequences

SEQ ID NO	Description	Sequence
1	Variable Heavy (VH)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSWMN WVRQAPGKGLEWVGRIYPGDGDTNYNVKFKGRFT ISRDDSKNSLYLQMNSLKTEDTAVYYCARSGFITTV RDFDYWGQGLTVTVSS
2	VH CDR1	SSWMN
3	VH CDR2	RIYPGDGDTNYNVKFKG
4	VH CDR3	SGFITTVRDFDY
5	Variable Light (VL)	EIVLTQSPDFQSVTPKEKVTITCRASESVDTFGISFM NWFQQKPDQSPKLLIHEASNQSGVPSRFSGSGSGT DFTLTINSLEAEDAATYYCQQSKEVPFTFGGGTKVE IK
6	VL CDR1	RASESVDTFGISFMN
7	VL CDR2	EASNQGS
8	VL CDR3	QQSKEVPFT
9	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSWMN WVRQAPGKGLEWVGRIYPGDGDTNYNVKFKGRFT ISRDDSKNSLYLQMNSLKTEDTAVYYCARSGFITTV RDFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
10	Light Chain	EIVLTQSPDFQSVTPKEKVTITCRASESVDTFGISFM NWFQQKPDQSPKLLIHEASNQSGVPSRFSGSGSGT DFTLTINSLEAEDAATYYCQQSKEVPFTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC

[0043] Inebilizumab and methods of making thereof are described in International PCT Patent Application PCT/US2007/077916, published as WO 2008/031056, which is hereby
 5 incorporated by reference (PCT/US2007/077916 refers to VIB551 as “16C4”). In aspects, VIB551 (also referred to HZN551, MEDI551, UPLIZNA™ or Inebilizumab; disclosed in U.S.

Appl. No. 11/852,106 and Int'l Appl. No. PCT/US2020/029613, which are incorporated herein by reference in their entireties) is administered in any of the methods disclosed herein.

[0044] In aspects, Inebilizumab is a clear to slightly opalescent, colorless to slightly yellow solution, free from or practically free from visible particles. Inebilizumab is a sterile liquid drug product (100 mg Inebilizumab per vial, nominal) intended for IV infusion following dilution in normal saline. In aspects, Inebilizumab for IV administration can be supplied as a sterile liquid in a 10R glass vial at a nominal fill volume of 10 mL with 20 mm stopper and flip-off cap overseal. In aspects, a formulation may be preservative-free. In aspects, Inebilizumab for IV administration is supplied as a sterile liquid filled at a nominal volume of 10 mL in 10R vials. In aspects, a vial contains Inebilizumab formulated at 10 mg/mL, in 20 mM histidine/histidine hydrochloride, 70 mM NaCl, 106 mM (4% [w/v]) trehalose dihydrate, and 0.01% (w/v) polysorbate 80, pH 6.0. Alternate formulations are also contemplated and further described below.

15 **Methods**

[0045] In aspects, Inebilizumab is used to treat an autoimmune disease or disorder. In aspects, the disclosure provides a method of treating neuromyelitis optica spectrum disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need of treatment for NMOSD. In aspects, the Inebilizumab may be administered as a first-line therapy.

20 [0046] In aspects, the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD. In aspects, the autoimmune disease or disorder comprises Neuromyelitis Optica Spectrum Disorder (NMOSD). In aspects, the autoimmune disease or disorder comprises MG. In aspects, the autoimmune disease or disorder comprises IgG4RD.

25 [0047] The treatment of the autoimmune disease or disorder may be in the form of suppressing a B-cell- or T cell-mediated immune response, which may be a reduction of class-switched antibodies, a reduction in circulating B-cell subsets, a reduction in plasma activity or a reduction in plasma cells and plasma cell gene signature. The treatment of the autoimmune disease or disorder may be a reduction in markers of inflammation. The markers of inflammation may be one or more of autoantibody levels, plasma cell (PC) or PC gene signature (signature characterized by expression of genes IGHA1, IGJ, IGKC, IGKV4-1 and TNFRSF17), circulating B-cell subsets and class-switched antibodies. The treatment of the autoimmune disease or disorder may be a reduction of clinical signs and symptoms, such as those measured by a subject or physician global assessment. In aspects, administration is

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effective in reducing one or more of: Annualized Attack Rate (AAR), incidence of disability as assessed by Expanded Disability Status Scale (EDSS) score, incidence of hospitalization, magnetic resonance imaging (MRI) lesions, and combinations thereof.

5 [0048] The treatment of the autoimmune disease or disorder may be characterized by a reduction of at least 20% of clinical symptoms of the disease or disorder, or by a reduction in inflammation, or by a reduction in biomarkers of the disease or disorder, relative to their levels prior to the treatment with Inebilizumab. The reduction of any of these symptoms, or inflammation, or biomarkers, may be a reduction in the symptoms, or inflammation or biomarkers of at least 50% relative to their levels prior to the initiation of treatment with
10 Inebilizumab. The reduction may be such that the autoimmune disease or disorder is characterized as being in remission.

[0049] In aspects, the autoimmune disease or disorder is NMOSD. NMOSD is a rare autoimmune disease of the central nervous system (CNS) in which approximately 75-90% of subjects have pathogenic immunoglobulin (Ig) G autoantibodies against the aquaporin-4 water
15 channel (AQP4-IgG). Recurrent, severe attacks of optic neuritis and myelitis are typical manifestations of the disease, although brain and brainstem lesions also occur. B-cells play a role in NMOSD pathogenesis, acting through several mechanisms. Exemplary mechanisms comprise: B-cell secretion of pro-inflammatory cytokines, such as interleukin (IL)-6 and IL-17, that may stimulate pathogenic, pro-inflammatory immune responses. Second, B-cells can
20 act as antigen-presenting cells and promote the development and activation of autoimmune T cells. And third, B-cell maturation leads to the generation of AQP4-IgG-producing plasmablasts and plasma cells.

[0050] If Inebilizumab is used to treat NMOSD, it may treat the NMOSD by reducing the worsening of the subject's Kurtzke Expanded Disability Severity Scale (EDSS), or by reducing
25 the number of active magnetic resonance imaging (MRI) lesions in the subject, or by reducing the worsening of the modified Rankin score of the subject, or by reducing the frequency of in-patient hospitalization of the subject related to NMOSD, or by reducing the risk of an NMOSD-related attack of the subject, or by reducing optic neuritis, or by reducing the severity of the subject's NMOSD-related attacks, or by decreasing the subject's pain, or by reducing
30 NMOSD-related damage in the subject, or by reducing NMOSD-related attacks in the subject.

[0051] If the Inebilizumab treats the subject's NMOSD by reducing the worsening of the subject's EDSS score, and the subject has a baseline EDSS score of 0, then the subject's EDSS score may worsen by fewer than 2 points, or worsen by fewer than 1 point, or worsen by fewer than .5 points. This reduction of worsening of EDSS score for the subject with the baseline

score of 0 may be over a period of at least 6 months, 9 months, 1 year, 2 years, 3 years, 4 years, 5 years, 7.5 years, or 10 years. If the Inebilizumab treats the subject's NMOSD by reducing the worsening of the subject's EDSS score, and the subject has a baseline score of 1 to 5, then the subject's EDSS score may worsen by fewer than 1 point, or worsen by fewer than .5 point.

- 5 This reduction of worsening for the subject with the baseline EDSS score of 1 to 5 may be reduction of worsening over a time period of over 6 months, 9 months, 1 year, 2 years, 3 years, 4 years, 5 years, 7.5 years, or 10 years. If the Inebilizumab treats the subject's NMOSD by reducing the worsening of the subject's EDSS score, and the subject has a baseline EDSS score of 5.5 or more, then the subject's EDSS score may worsen by fewer than 0.5 point or worsen
- 10 by fewer than .25 point. This reduction of worsening for the subject with the baseline score of 5.5 or more may be reduction of worsening of EDSS score over a period of over 6 months, 9 months, 1 year, 2 years, 3 years, 4 years, 5 years, 7.5 years, or 10 years.

- [0052]** If Inebilizumab treats the subject's NMOSD by reducing the number of active MRI lesions, then the treating may be a reduction in the number of enlarging T2 MRI lesions, gadolinium-enhancing (Gd+) lesions, or may be a reduction in the number of new MRI lesions,
- 15 gadolinium-enhancing (Gd+) lesions, or may be a reduction in the number of enlarging T2 MRI lesions, gadolinium-enhancing (Gd+) lesions, and the number of new MRI lesions. The reduction in lesions may be a reduction in brain lesions, a reduction in brainstem lesions, a reduction in spinal cord lesions, a reduction in optic nerve lesions, or a reduction lesions in a combination of any two or more of brain,
- 20 brainstem, spinal cord, and optic nerve. The new MRI lesions may not be clinically symptomatic.

- [0053]** If Inebilizumab treats the subject's NMOSD by reducing the risk of an NMOSD-related attack, then the subject's risk of attack may be reduced by between 60% and 85%, or may be reduced by between 65% and 75%, or may be reduced by between 70% and 80%. The subject's
- 25 risk of attack may be reduced by at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79% or at least 80%. The subject's risk of attack may be reduced by 70%, 75%, 76%, 77%, 78%, 79% or 80%.

- [0054]** If Inebilizumab treats the subject's NMOSD by reducing the risk of an NMOSD-related attack, then as a result of the reduction in risk of an NMOSD-related attack, the treated subject's
- 30 probability of having no NMOSD-related attack may be greater than 70% over at least 6 months following treatment, or greater than 70% over at least 12 months following treatment, or greater than 70% over at least 18 months following treatment. As a result of the reduction in risk of an NMOSD-related attack, the treated subject's probability of having no NMOSD-related attack may be greater than 75% over at least 6 months following treatment, or greater than 75%

over at least 12 months following treatment, or greater than 75% over at least 18 months following treatment. In addition, as a result of the reduction in risk of an NMOSD-related attack, the treated subject's probability of having no NMOSD-related attack may be greater than 80% over at least 6 months following treatment, or greater than 80% over at least 12 months following treatment, or greater than 80% over at least 18 months following treatment. Also, as a result of the reduction in risk of an NMOSD-related attack, the treated subject's probability of having no NMOSD-related attack may be greater than 85% over at least 6 months following treatment, or greater than 85% over at least 12 months following treatment, or greater than 85% over at least 18 months following treatment.

- 10 **[0055]** Furthermore, if Inebilizumab treats the subject's NMOSD by reducing the risk of an NMOSD-related attack, then as a result of the reduction in risk, the treated subject's annualized risk of an NMOSD-related attack may be reduced to between 0.18 and 0.08, or it may be reduced to between 0.15 and 0.08, or it may be reduced to 0.14, or 0.13, or 0.12, or 0.11, or 0.10, or 0.09, or 0.08, or 0.07. If the subject in treatment for NMOSD is AQP4-IgG seropositive, then the subject's annualized risk of an NMOSD-related attack may be reduced to between 0.15 and 0.11, or reduced to between 0.14 and 0.12, or it may be reduced to 0.14, 0.13, 0.12, or 0.11. If the subject in treatment for NMOSD is AQP4-IgG seronegative, then the subject's annualized risk of an NMOSD-related attack may be reduced to between 0.09 and 0.07, or it may be reduced to 0.09, 0.08, or 0.07. The NMOSD-related attack, the risk of which may be reduced as in the treating of the NMOSD subject, may be an attack characterized by the appearance of a new NMOSD symptom or the worsening of an existing NMOSD symptom. The new or existing symptom may be an eye symptom. If the new or existing symptom is an eye symptom it may be eye pain, a new optic nerve lesion, an enlarging optic nerve lesion, blurred vision, loss of vision, or a 5 or more character drop in low-contrast Landolt C Broken Rings Chart. The new or existing symptom may be a spinal cord symptom. If the new or existing symptom is a spinal cord symptom, it may be a deep or radicular pain, extremity paraesthesia, weakness, sphincter dysfunction, Lhermitte's sign, a new spinal cord lesion, or an enlarging spinal cord lesion. The new or existing symptom may be a brain or brain stem symptom. If the new or existing symptom is a brain or brain stem symptom, it may be nausea, double vision, oculomotor palsy, vertigo, intractable vomiting, intractable hiccups, dysarthria, dysphagia, weakness, encephalopathy, hypothalamic dysfunction, a new brain or brain stem lesion, or an enlarging brain or brain stem lesion. The new or worsening symptom may be a combination of any two or more of the eye, spinal cord, or brain/brain stem symptoms. It may be a combination of any two, three, or four of these symptoms.

[0056] In aspects, Inebilizumab treats the subject's NMOSD by reducing optic neuritis, then the subject may experience reduced eye pain, reduced vision loss, reduced visual field loss, reduced loss of color vision, or reduced flashing or flickering of lights with movement of the eye. The reduction in optic neuritis may result in improved vision, and/or relief from eye pain.

5 [0057] In aspects, Inebilizumab treats the subject's NMOSD by reducing the severity of the subject's NMOSD-related attacks, then the severity of any NMOSD-related attack suffered by the subject may be graded mild or moderate as opposed to graded as severe. A mild attack may be an attack that is transient, may be an attack that requires only minimal treatment or therapeutic intervention, and/or is an attack that may not interfere with usual activities of daily
10 living. A moderate attack may be an attack that may be alleviated with specific additional therapeutic interventions. Any moderate attack may be one that interferes with usual activities of daily living, and/or causes discomfort, but that poses no significant or permanent risk of harm to the subject. The reduction of severity of the subject's NMOSD-related attacks may be a reduction in the attacks suffered by the subject as being graded as major. Such a major attack
15 may be an attack that requires intensive therapeutic intervention, interrupts usual activities of daily living, or that significantly affects the clinical status of the subject. Such a major attack may require in-patient hospitalization.

[0058] In aspects, a method comprises subject stratification. Subject stratification can be based on a level of B-cells of less than about 4 cells/ μ L of blood in a subject after an initial or prior
20 administration of Inebilizumab. In aspects, a method comprises repeating administration of Inebilizumab in a subject with B-cell counts less than 4 cells/ μ L after a first dosing period. In aspects, a method comprises maintaining suppression or reduction of B cells in a subject in need thereof.

[0059] Inebilizumab may also be used in a method of reducing AQP4-IgG titers in a AQP4-IgG+ subject in need of treatment for NMOSD. Provided are methods comprising
25 administering Inebilizumab to a subject with a high AQP4-IgG titer. In aspects, the titer is greater than about: 1:20, 480. In aspects, the disclosure provides a method of reducing AQP4-IgG titers in a AQP4-IgG+ subject in need of treatment for NMOSD, the method comprising administering the anti-CD19 antibody Inebilizumab to a subject in need of treatment for
30 NMOSD, wherein the subject had been previously treated with an anti-CD20 antibody, wherein the subject had an NMOSD attack while being treated with the anti-CD20 antibody; and wherein Inebilizumab is administered intravenously at a dose of 300 mg every 6 months. In aspects, the disclosure provides a method of reducing AQP4-IgG titers in a AQP4-IgG+ subject in need of treatment for NMOSD, the method comprising: administering the anti-CD19

antibody Inebilizumab to a subject in need of treatment for NMOSD, wherein the subject had been previously treated with an anti-CD20 antibody, wherein the subject had an NMOSD attack within 6 months of the last dose of the anti-CD20 antibody; and, wherein the Inebilizumab is administered intravenously at a dose of 300 mg every 6 months. In aspects, the administration can be continued indefinitely. If the Inebilizumab is used in a method of reducing AQP4-IgG titers in a AQP4-IgG+ subject in need of treatment for NMOSD, then the Inebilizumab may reduce the AQP4-IgG titers by 75% to 100%, or by 75% to 90%, or by 75% to 85%, or by 80% to 100%, or by 85% to 100%, or by 90% to 95%, or by 75%, 80%, 85%, 90%, 95 or 100%. The Inebilizumab may reduce the AQP4-IgG titers for a sustained period of time of at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months or at least 12 months following administration of a Inebilizumab dose. In aspects, provided are methods of treating neuromyelitis optica spectrum disorder. In aspects, the methods comprise administering Inebilizumab to a subject that has no anti-aquaporin 4 immunoglobulin G antibodies (AQP4-IgG-) and has immunoglobulin G antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG+). In aspects about 300 mg of the Inebilizumab is administered. In aspects, the administration is continued for at least about 6 months, 1 year, 2 years, 3 years, or indefinitely. In aspects, the administration is continued for at least 2 years. In aspects, the subject in need has increased serum autoreactivity to an autoantibody that is not related to the NMOSD as compared to a healthy control subject. In aspects, the administering is effective in reducing AAR. An attack comprises the presence of one or more new symptoms or worsening of one or more existing symptoms related to NMOSD that met at least one of the 18 protocol-defined neurological examination criteria for an attack comprising myelitis, optic neuritis, and the brain/brainstem domain symptoms. In aspects the reduction of AAR is at least about 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%.

[0060] In aspects, the administering is effective in reducing MRI lesions. In aspects, the reduction in MRI lesions is at least about 20%, 30%, 40%, 50%, 60%, 70%, 73%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%.

[0061] In aspects, administration of Inebilizumab is effective in preventing disease progression. For example, a single or repeated dosing scheme of Inebilizumab can be effective in delaying NMOSD progression by at least about 1 month, 2 months, 3 months, 5 months, 6 months, 8 months, 10 months, 12 months, 1.5 years, 2 years, 3 years, 5 years, or over 5 years. In aspects, a subject administered Inebilizumab has decreased NMOSD progression as

determined by a reduced AAR, annualized rate of new/enlarging T2 lesions detection or gadolinium-enhancing (Gd+) lesion detection, hospitalization rate, and/or annualized rate of EDSS worsening.

[0062] In aspects, a method comprises determining a level of B-cell levels in peripheral blood.

- 5 In aspects, a method comprises B-cell monitoring. Monitoring can be performed on any subject sample including but not limited to whole food, PBMC, bone marrow, tissue, tumor, and combinations thereof. In aspects, B-cell monitoring is performed on whole blood. Monitoring can be performed once or repeatedly. In aspects, B-cell monitoring is performed daily, weekly, monthly, semi-monthly, or annually. In aspects, levels of B-cells or plasma-cells are
10 determined at least 1, 2, 4, 8, 12, 16, 22, 28, and/or 32 weeks after the administering of Inebilizumab.

- [0063]** Flow cytometry and/or FACS analysis can be utilized to determine B-cell levels. In aspects, B-cells are counted using CD20 as a FACS marker (bound inebilizumab interferes with CD19-based FACS). In aspects, B-cell subsets are also measured by way of CD20+ B-
15 cells defined as CD45hi [CD3⁻, CD56⁻, CD14⁻], CD33⁻, CD20. In addition, an analysis of plasmablast/plasma cell counts (CD45hi [CD3⁻, CD14⁻, CD56⁻], CD27⁺, HLA-DR^{hi/low}, CD38⁺) can also be performed. In aspects, a level of CD20+ B-cells and CD27+ memory B-cells is determined. In aspects, a level of B-cell subsets is determined in peripheral blood.

- [0064]** In aspects, a treated subject comprises reduced levels of CD20+ B-cells, CD27+
20 memory B-cells, or both as compared to an otherwise comparable subject lacking treatment with Inebilizumab. In aspects, a treated subject comprises reduced levels of CD27⁺, and CD20+ B-cells of at least about 1-fold, 3-fold, 5-fold, 10-fold, 20-fold, 40-fold, 60-fold, 80-fold, 100-fold, 130-fold, 150-fold, 175-fold, 200-fold, 300-fold, 400-fold, or up to about 500-fold.

- 25 **[0065]** In aspects, a method comprises maintaining the reduced levels of B-cells in a subject in need thereof. In aspects, the reduced levels of B-cells are maintained for at least about 1 month, 3 months, 5 months, 6 months, 8 months, 10 months, 1 year, 1.5 years, 2 years, or up to about 3 years post treatment with Inebilizumab.

- [0066]** In aspects, a method comprises determining plasma-cell specific gene expression. Gene
30 expression can be determined using quantitative reverse transcription PCR of blood RNA. The plasma-cell gene signature can be based on expression analysis of genes (IGHA1, IGJ, IGKV4-1, and TNFRSF17) that are expressed predominantly by plasma cells in blood. The signature can be calculated as the average expression of the four-plasma cell-specific genes minus the average expression of five control genes (B2M, GAPDH, TFRC, GUSB, and UBC) at time

points of interest. The fold-change in plasma-cell gene expression signature at each time point can be calculated relative to a pool of healthy donor samples and interpreted as plasma cell abundance relative to expected prevalence under a non-activated immune state.

[0067] In aspects, single nucleotide polymorphism analysis is conducted on subject samples.

- 5 TaqMan single nucleotide polymorphism profiling can be used to investigate possible associations between the rs396991 polymorphism in the FCGR3A gene and impaired B-cell depletion. In aspects, an analysis comprises determining the presence of a single nucleotide substitution (SNP) from G to T at cDNA nucleotide position 559 of the FCGR3A gene. This SNP can generate two different FcγRIIIa allotypes: one with a valine (V) and one with a
- 10 phenylalanine (F) at amino acid position 158, known as FcγRIIIA-V158F polymorphism (rs396991). The rs396991 polymorphism encodes a valine/phenylalanine substitution at position 158 of FCGR3A that is associated with decreased binding affinity for Ig Fc, thus potentially impairing ADCC mechanisms for which the Fc region of Inebilizumab is optimized. In aspects, a method comprises polymorphism analysis to ascertain subject eligibility for
- 15 dosing with Inebilizumab.

- [0068]** In aspects, a method comprises determining the presence of a V allele in a subject. A V allele can be determined by a V/F or V/V genotype. The presence of the V allele can inform administration of Inebilizumab. In aspects, a method comprises stratifying a subject population based on the presence of the V allele for dosing with Inebilizumab. In aspects, a method
- 20 comprises administering Inebilizumab to a subject in need that comprises a rs396991 polymorphism of the FCGR3A gene. In aspects, treatment with Inebilizumab can be effective in reducing B-cell or plasma-cell levels in a subject in need that comprises the rs396991 polymorphism of the FCGR3A gene as compared to an otherwise comparable subject lacking the rs396991 polymorphism.

- 25 **[0069]** Provided are also methods that comprise determining autoreactivity to a nuclear antigen. An antinuclear antibody (ANA) test can determine the presence of an autoimmune disease or be used to monitor status of an autoimmune disease in a subject. Antibodies to extractable nuclear antigens (ENAs) are ribonucleoproteins (e.g., protein antigens not containing DNA) that are extractable from cell nuclei. Exemplary ENAs comprise Ro (SS-A),
- 30 La (SS-B), Sm and RNP. In aspects, method comprises determining a level of autoreactivity in a subject prior to treatment and monitoring the level during treatment. In aspects, autoreactivity is determined weekly, monthly, twice a year, or yearly.

[0070] Provided are also methods that comprise determine a level or presence of an autoantigen in a subject. In aspects, responses (e.g., ANA and/or RNP) against an autoantigen by one or

more of IgG, IgM, IgA, and IgE are evaluated. In aspects, the autoantigen is related to the presence of an autoimmune disease. Exemplary autoantigens comprise: Mi-2 (CHD4), Signal recognition particle (SRP), Transcriptional intermediary factor 1-gamma (TIF1- γ ; TRIM 33), Nuclear matric protein-2 (NXP2; MORC3), 3-hydroxy-3-methylglutaryl-coA reductase (HMGCR), Melanoma-associated differentiation gene-5 (MDA5), Small ubiquitin-like modifier activating enzymes SAE-1 and SAE-2, Topoisomerase-1, Centromere proteins A, B & C ("CENPs"), Fibrillarin, Component of the small nucleolar U3 (snoRNP) complex, Nucleophosmin (NPM; B23), RNA polymerases I, II & III, multiple components PMSCL; RNA exosome complex consisting of 10 proteins, the most commonly targeted are EXOSC9 & EXOSC10, Ku 70/80 components of DNA-dependent protein kinase, Ro52 (TRIM 21), Ro60, La (SS-B), Gamma interferon-inducible protein-16 (IFI16), Proteinase-3 (PR3), Myeloperoxidase (MPO), ACPA (anti-citrullinated protein antibodies), Peptidyl arginine-deiminase-4 (PAD4), ds DNA, Components of the Sm splicing ribonucleoprotein (subunits A-G, most commonly targeted are B, B' & D), U1-RNP, Ribosomal protein P, Cardiolipin, Anionic phospholipid/protein complexes, N-methyl-D-aspartate (NMDA) receptor, CENP-A, CENP-B, Core Histone, DNA Polymerase beta (POLB), dsDNA, EBNA1, Genomic DNA, Histone H1, Histone H2A, Histone H2B, Histone H3, Histone H4, Jo-1, KU (P70/P80), La/SSB, Mi-2, PCNA, PL-7, PL-12, PM/Scl-75, PM/Scl 100, Ribo Phosphoprotein P0, Ribo Phosphoprotein P1, Ribo Phosphoprotein P2, Ro/SSA (52 Kda), Ro/SSA (60 Kda), S100, Scl-70/Topoisomerase I, Sm, Sm/RNP, SmD, SmD1, SmD2, SRP54, ssDNA, ssRNA, U1-snRNP 68/70, U1-snRNP A, U1-snRNP B/B, U1-snRNP C, Histone-total, Histone-total citrullinated human genomic DNA, KU(P70/P80), Nup 62, Ro/SSA (52+60), U1-snRNP and the like. In aspects, an autoantigen is selected from the group consisting of: CENP-A, CENP-B, Core Histone, DNA Polymerase beta (POLB), dsDNA, EBNA1, Genomic DNA, Histone H1, Histone H2A, Histone H2B, Histone H3, Histone H4, Jo-1, KU (P70/P80), La/SSB, Mi-2, PCNA, PL-7, PL-12, PM/Scl-75, PM/Scl 100, Ribo Phosphoprotein P0, Ribo Phosphoprotein P1, Ribo Phosphoprotein P2, Ro/SSA (52 Kda), Ro/SSA (60 Kda), S100, Scl-70/Topoisomerase I, Sm, Sm/RNP, SmD, SmD1, SmD2, SRP54, ssDNA, ssRNA, U1-snRNP 68/70, U1-snRNP A, U1-snRNP B/B, U1-snRNP C, Histone-total, Histone-total citrullinated human genomic DNA, KU(P70/P80), Nup 62, Ro/SSA (52+60), and U1-snRNP. In aspects, administration of Inebilizumab is effective in reducing a level of an autoantigen in a treated subject as compared to the baseline level in the same subject or the level of a comparable untreated control subject. The level can be reduced by at least about 5%, 10%, 20%, 30%, 40%, 50%, 75%, 100%, 150%, 200%, 300%, 350%, or up to about 400%.

[0071] Provided are also methods that comprise determining a level of interferon-inducible gene expression. In aspects, gene expression profiling, e.g., using microarrays, can be performed to determine levels of mRNA transcripts in a biologic sample of a subject. In aspects, the gene expression profiling evaluates type I interferon- α/β (IFN α/β)–inducible genes.

- 5 The up-regulation of these genes can be correlated with disease activity, with down-regulation occurring when disease was controlled with treatment, for example with Inebilizumab. In aspects, the up-regulation or down-regulation is of about 5%, 10%, 20%, 30%, 40%, 50%, 75%, 100%, 150%, 200%, 300%, 350%, or up to about 400%. In aspects, administration of Inebilizumab is effective in reducing a level of interferon-inducible gene expression in a treated
- 10 subjected as compared to the baseline level in the same subject or the level of a comparable untreated control subject.

Pharmaceutical Composition

- [0072] Provided are pharmaceutical compositions. Also provided are pharmaceutical compositions that comprise Inebilizumab. In aspects, a therapeutic regimen comprises a pharmaceutical composition, such as one comprising Inebilizumab. Therapeutic regimens can also comprise additional pharmaceutical compositions that are not Inebilizumab.

- [0073] Inebilizumab can be administered at any dose. In aspects, Inebilizumab is administered at a dose from about 0.5 mg to 1000 mg. In aspects, Inebilizumab is administered at a dose
- 20 from about: 10 mg to 30 mg, 20 mg to 100 mg, 50 mg to 200 mg, 100 mg to 300 mg, 150 mg to 350 mg, 200 mg to 400 mg, 250 mg to 450 mg, 300 mg to 500 mg, 300 mg to 700 mg, or 400 mg to 1000 mg. In aspects, Inebilizumab is administered at a dose from about 50mg, 55mg, 60mg, 65mg, 70mg, 75mg, 80mg, 85mg, 90mg, 95mg, 100mg, 105mg, 110mg, 115mg, 120mg, 125mg, 130mg, 135mg, 140mg, 145mg, 150mg, 155mg, 160mg, 165mg, 170mg,
- 25 175mg, 180mg, 185mg, 190mg, 195mg, 200mg, 205mg, 210mg, 215mg, 220mg, 225mg, 230mg, 235mg, 240mg, 245mg, 250mg, 255mg, 260mg, 265mg, 270mg, 275mg, 280mg, 285mg, 290mg, 295mg, 300mg, 305mg, 310mg, 315mg, 320mg, 325mg, 330mg, 335mg, 340mg, 345mg, 350mg, 355mg, 360mg, 365mg, 370mg, 375mg, 380mg, 385mg, 390mg, 395mg, 400mg, 405mg, 410mg, 415mg, 420mg, 425mg, 430mg, 435mg, 440mg, 445mg,
- 30 450mg, 455mg, 460mg, 465mg, 470mg, 475mg, 480mg, 485mg, 490mg, 495mg, 500mg, 505mg, 510mg, 515mg, 520mg, 525mg, 530mg, 535mg, 540mg, 545mg, 550mg, 555mg, 560mg, 565mg, 570mg, 575mg, 580mg, 585mg, 590mg, 595mg, 600mg, 605mg, 610mg, 615mg, 620mg, 625mg, 630mg, 635mg, 640mg, 645mg, 650mg, 655mg, 660mg, 665mg, 670mg, 675mg, 680mg, 685mg, 690mg, 695mg, 700mg, 705mg, 710mg, 715mg, 720mg,

725mg, 730mg, 735mg, 740mg, 745mg, 750mg, 755mg, 760mg, 765mg, 770mg, 775mg, 780mg, 785mg, 790mg, 795mg, 800mg, or up to about 1000 mg. In aspects, a subject in need thereof is administered 300mg.

[0074] In aspects, Inebilizumab is administered repeatedly. The administration can be daily, monthly, or yearly. The dose Inebilizumab may be administered about every other week or may be administered twice per month. The dose Inebilizumab may also be administered about every week or about once a month. The dose Inebilizumab may be administered every 7 days, every 10 days, every 14 days, every 15 days, every 16 days, every 14-10 days, every 14-16 days, or every 30 days. In aspects, Inebilizumab is administered every 6 months. Inebilizumab can be administered for at least about 1 year, 2 years, 2.5 years, 3 years, 4 years, 5 years, or up to about the lifetime of a subject in need. In aspects, the administering is continued for at least about 2.5 years.

[0075] If the dose of Inebilizumab administered is one of 1000 mg, 1500 mg, or between approximately 1000 mg and approximately 1500 mg, then the dose may be administered every other week, or it may be administered twice per month. If the dose Inebilizumab is 3000 mg, then the dose Inebilizumab may be administered once per month. If the dose Inebilizumab is 500 mg or 750 mg, then the dose Inebilizumab may be administered once every other week, or, alternatively, be administered twice per month. In aspects, a subject receives from 0-2, 0-3, 0-5, 0-10, 1-3, 1-5, or 1-10 administrations.

[0076] In aspects, Inebilizumab may be administered at a dose of about 300 mg. In aspects, the Inebilizumab may be administered at a dose of about 250 mg to about 350 mg, about 275 mg to about 325 mg, about 290 mg to about 310 mg, about 205 mg to about 305 mg, or it may be a dose of 300 mg. In aspects, the subject may receive one or more initial doses of Inebilizumab. In aspects, the subject may receive, one, two, three or more initial doses. In aspects, the initial dose may be about 300 mg. In aspects, the Inebilizumab may be administered at an initial dose of about 250 mg to about 350 mg, about 275 mg to about 325 mg, about 290 mg to about 310 mg, about 205 mg to about 305 mg, or an initial dose of 300 mg. In aspects, Inebilizumab may be administered intravenously with a first initial dose of about 300 mg, a second initial dose of about 300 mg two weeks after the first initial dose, and subsequent doses of about 300 mg every 6 months following the first initial dose.

[0077] The dose of Inebilizumab that may be used in a method of treating a subject in need thereof, may be a dose administered intravenously at an interval of approximately once every 6 months, or once every 7 months, or once every 8 months, or once every 9 months, or once every 10 months, or once every 11 months, once a year, and/or indefinitely. In aspects,

- Inebilizumab administered in the methods disclosed herein may be at an interval of approximately every 6 months. The approximately every 6 months may be administration every 6 months, every 180 days, every between 170 and 190 days, every between 175 and 185 days, every between 175 and 190 days, or every between 170 and 185 days. The approximately every 6 months may be administration every 26 weeks, every 25 weeks, every 27 weeks, every between 25 and 27 weeks, every between 25 and 26 weeks, or every between 26 and 27 weeks. In aspects, the administration is continued indefinitely. Prior to the administering the Inebilizumab every approximately 6 months in the methods disclosed herein, an initial Inebilizumab dose may be administered to a subject in need. The initial Inebilizumab dose may be administered approximately 2 weeks before the approximately every 6-month Inebilizumab dosing. The administering the initial Inebilizumab dose approximately 2 weeks before every approximately 6-month Inebilizumab dosing may be the administering of the initial Inebilizumab dose 12 days, 13 days, 14 days, 15 days, or 16 days before the approximately 6 months Inebilizumab dosing.
- [0078]** The dose and dosing regimen of Inebilizumab may be such that any therapeutic effect achieved from administration of Inebilizumab to treat any autoimmune/inflammatory disease or disorder, may be "long-lasting." A "long-lasting" effect of Inebilizumab in the treatment of an autoimmune/inflammatory disease or disorder is one in which the therapeutic effect achieved by Inebilizumab is maintained (although Inebilizumab is no longer administered) over at least 4 weeks, at least 6 weeks, at least 8 weeks, at least 10 weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, or at least 24 weeks following administration of the last dose of a course of Inebilizumab. The course of Inebilizumab may be administration of a dose of Inebilizumab of between 500 mg and 3000 mg (*e.g.*, 500 mg, 750 mg, 1000 mg, 1250 mg, 1500 mg, 1750 mg, 2000 mg, 2250 mg, 2500 mg, 2750 mg or 3000 mg) over a period of time of approximately between 8 and 24 weeks (*e.g.*, 8 weeks, or 10 weeks, or 12 weeks, or 14 weeks, or 16 weeks, or 18 weeks, or 20 weeks, or 22 weeks, or 24 weeks, or 2 months or 4 months, or 6 months) at a dosing interval of once every 7 to 31 days (*e.g.*, every 7 days, every 10 days, every 14 days, every 15 days, every 16 days, every 14-10 days, every 14-16 days, or every 30 days).
- [0079]** In aspects, an administration is intravenous. In aspects, an administration is by infusion.
- [0080]** A pharmaceutical composition can comprise an immunosuppressive agent. Immunosuppression therapy may comprise the administration of one or more immunosuppressive agents such as inhibitors of cytokine transcription (*e.g.*, cyclosporin A, tacrolimus), nucleotide synthesis (*e.g.*, azathiopurine, mycophenolate mofetil), growth factor

signal transduction (e.g., sirolimus, rapamycin), and the T cell interleukin 2 receptor (e.g., daclizumab, basiliximab). In a particular embodiment, an immunosuppressant agent used in combination with compositions and methods of the invention includes one or more of the following: adriamycin, azathiopurine, busulfan, cyclophosphamide, cyclosporin A ("CyA"),
5 cytoxin, fludarabine, 5-fluorouracil, methotrexate, mycophenolate mofetil (MOFETIL), nonsteroidal antiinflammatories (NSAIDs), rapamycin, and tacrolimus (FK506). Immunosuppressive agents may also comprise inhibitors of complement, for example, soluble complement receptor- 1, anti-C5 antibody, or a small molecule inhibitor of CIs, for example as described in Buerke et al. (J. Immunol, 167:5375-80 (2001)).

- 10 **[0081]** In aspects, a pharmaceutical composition comprises an immunosuppressive agent in an amount effective to immunosuppress a treated subject. In aspects, a subject is pre-treated or has previously received an immunosuppressive prior to treatment with Inebilizumab. Any immunosuppressive may be administered. In aspects, a subject was previously treated with one or both of azathioprine (AZA) and/or mycophenolate mofetil (MMF).
- 15 **[0082]** Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific aspects described herein. Such equivalents are intended to be encompassed by the following claims.

INCORPORATION BY REFERENCE

- [0083]** This patent application incorporates by reference in its entirety for all purposes the
20 following patent publications: WO2008031056 and WO2020219743.

[0084] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated herein by reference.

25

NUMBERED EMBODIMENTS

- [0085]** Embodiment 1. A method of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, thereby treating the NMOSD.
- 30 **[0086]** Embodiment 2. The method of embodiment 1, wherein the subject in need's Fc Gamma Receptor IIIa (FCGR3A) gene comprises a rs396991 polymorphism.
- [0087]** Embodiment 3. The method of embodiment 2, wherein the subject in need comprises a V allele as determined by a V/F or V/V genotype.

- [0088] Embodiment 4. A method of treatment, the method comprising assaying a blood sample from a subject in need to determine presence of a rs396991 polymorphism of the FCGR3A gene and administering Inebilizumab to the subject in need if the determination is positive for the rs396991 polymorphism.
- 5 [0089] Embodiment 5. The method of embodiment 4, wherein the subject in need has an autoimmune disease.
- [0090] Embodiment 6. The method of embodiment 5, wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
- 10 [0091] Embodiment 7. The method of embodiment 6, wherein the autoimmune disease is NMOSD.
- [0092] Embodiment 8. The method of embodiment 6, wherein the autoimmune disease is MG.
- [0093] Embodiment 9. The method of embodiment 6, wherein the autoimmune disease is IgG4RD.
- 15 [0094] Embodiment 10. The method of embodiment 4, wherein the subject in need has at least two autoimmune diseases.
- [0095] Embodiment 11. The method of embodiment 10, wherein the at least two autoimmune diseases are selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
- 20 [0096] Embodiment 12. A method of treating Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that comprises a rs396991 polymorphism of the FCGR3A gene, wherein the administering is more effective in reducing B-cell or plasma-cell levels in the subject in need that comprises the rs396991 polymorphism of the FCGR3A gene as compared to an otherwise comparable subject
- 25 lacking the rs396991 polymorphism.
- [0097] Embodiment 13. The method of embodiment 12, wherein levels of B-cells or plasma-cells are determined at least 1, 2, 4, 8, 12, 16, 22, 28, and/or 32 weeks after the administering.
- [0098] Embodiment 14. The method of any one of embodiments 1-13, wherein the administering is effective in reducing a criterion selected from the group consisting of:
- 30 Annualized Attack Rate (AAR), incidence of disability as assessed by Expanded Disability Status Scale (EDSS) score, incidence of hospitalization, magnetic resonance imaging (MRI) lesions, and combinations thereof.

- [0099] Embodiment 15. The method of embodiment 14, wherein the administering is effective in reducing the AAR, wherein the reduction is at least about 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%.
- 5 [0100] Embodiment 16. The method of embodiment 14, wherein the administering is effective in reducing the MRI lesions, wherein the reduction is at least about 20%, 30%, 40%, 50%, 60%, 70%, 73%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%.
- [0101] Embodiment 17. The method of embodiment 14, wherein the administering is effective in reducing EDSS.
- 10 [0102] Embodiment 18. The method of embodiment 14, wherein the administering is effective in reducing incidence of hospitalization.
- [0103] Embodiment 19. The method of any one of embodiments 1-18, wherein the administering is repeated.
- [0104] Embodiment 20. The method of embodiment 19, wherein the administering is repeated daily, monthly, or yearly.
- 15 [0105] Embodiment 21. The method of embodiment 20, wherein the administering is repeated monthly.
- [0106] Embodiment 22. The method of embodiment 21, wherein the administering is repeated every 6 months.
- [0107] Embodiment 23. The method of any one of embodiments 19-22, wherein the
20 administering is continued for at least about 1 year, 2 years, 3 years, 4 years, 5 years, or up to about the lifetime of the subject in need.
- [0108] Embodiment 24. The method of any one of embodiments 1-23, wherein the subject in need was previously administered Inebilizumab.
- [0109] Embodiment 25. The method of any one of embodiments 1-24, wherein the
25 administering is intravenous.
- [0110] Embodiment 26. The method of embodiment 25, wherein the administering is by intravenous infusion.
- [0111] Embodiment 27. The method of any one of embodiments 1-26, wherein the Inebilizumab is administered at a dose of about 300 mgs.
- 30 [0112] Embodiment 28. A method of treating or preventing an autoimmune disease, the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months.

[0113] Embodiment 29. The method of embodiment 28, wherein the administering is repeated every 6 months for at least about 1 year.

[0114] Embodiment 30. The method of any one of embodiments 28-29, wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.

[0115] Embodiment 31. The method of embodiment 30, wherein the autoimmune disease is NMOSD.

[0116] Embodiment 32. The method of embodiment 30, wherein the autoimmune disease is MG.

[0117] Embodiment 33. The method of embodiment 30, wherein the autoimmune disease is IgG4RD.

[0118] Embodiment 34. A method of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months.

[0119] Embodiment 35. A method of treating or preventing an autoimmune disease, the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months, and wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.

[0120] Embodiment 36. The method of any one of embodiments 34-35, wherein the administering is continued for at least about 2.5 years.

[0121] Embodiment 37. A method of treating neuromyelitis optica spectrum disorder, the method comprising administering Inebilizumab to a subject in need, wherein the subject has no anti-aquaporin 4 immunoglobulin G antibodies (AQP4-IgG⁻) and has immunoglobulin G antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG⁺).

[0122] Embodiment 38. The method of embodiment 37, wherein about 300 mg of the Inebilizumab is administered.

[0123] Embodiment 39. The method of any one of embodiments 37-38, wherein the administration is continued for at least 6 months, 1 year, 2 years, or 3 years.

[0124] Embodiment 40. The method of embodiment 39, wherein the administration is continued for at least 2 years.

[0125] Embodiment 41. The method of any one of embodiments 1-40, wherein the subject in need has increased serum autoreactivity to an autoantibody that is not related to NMOSD as compared to a healthy control subject.

5

EXAMPLES

Example 1- Safety and Efficacy of Inebilizumab as Treatment for Newly Presenting Neuromyelitis Optica Spectrum Disorder

[0126] A study was completed to evaluate the safety and efficacy of inebilizumab in newly diagnosed subjects compared with those who have had ≥ 2 prior NMOSD attacks.

10 *Methods*

[0127] A double-masked, placebo-controlled, randomized phase 2/3 trial that assessed the efficacy and safety of inebilizumab in adults with NMOSD (**FIG. 1A**) was performed and comprised periods: Randomized controlled period (RCP; 3:1 to inebilizumab [intravenous, 300 mg] or placebo) for up to 28 weeks or to an adjudicated attack; Optional open-label period (OLP; inebilizumab every 28 weeks) for ≥ 2 years. No immunosuppressants were used, except for oral corticosteroids during the first 2 weeks with a 1-week taper. The study included adults with NMOSD who had received treatment for ≥ 1 attack in the past year or ≥ 2 attacks in the past 2 years, and who had an EDSS score ≤ 8.0 . Primary endpoint was time to first adjudicated attack during the RCP. Secondary endpoints included annualized attack rate (AAR) and disability progression assessed via the Expanded Disability Status Scale (EDSS). Safety assessments include treatment-emergent adverse events (TEAEs) and treatment-emergent AEs of special interest. Subjects who received inebilizumab at any point during the study were pooled into the 'any inebilizumab' group for data analysis purposes. For the present study, post hoc analyses were conducted for subjects with AQP4+ NMOSD enrolled after their first attack.

25 **Baseline characteristics and demographics**

[0128] A total of 37 subjects with newly diagnosed AQP4+ NMOSD and 176 subjects with ≥ 2 pre-study attacks were included in this analysis (**Table 2**).

Table 2: Summary of baseline demographics and characteristics

Demographic/characteristic	Newly diagnosed (n = 37)	Subjects with ≥ 2 prior relapses (n = 176)
Age, years (mean [SD])	48.9 (10.19)	41.8 (12.35)
Sex, women (n [%])	36 (97.3)	164 (93.2)
Time to 1 st inebilizumab administration, years (median [range])	0.55 (0.1–1.3)	4.13 (0.2–27.4)

Number of MRI lesions (mean [SD])	0.9 (0.95)	1.2 (1.15)
EDSS score (median [range])	3.50 (1.0–6.5)	4.00 (0.0–8.0)
BMI, kg/m ² (mean [SD])	25.94 (6.46)	25.75 (5.95)
Disease duration, years (mean [SD])	0.60 (0.57)	3.01 (3.62)
Race (n [%])		
American Indian or Alaskan Native	2 (5.4)	14 (8.0)
Asian	7 (18.9)	38 (21.6)
Black or African American	5 (13.5)	14 (8.0)
White	20 (54.1)	90 (51.1)
Multiple Categories Checked	0	1 (0.6)
Other	3 (8.1)	19 (10.8)
ARR prior to 1 st IP administration (mean [SD])	2.34 (1.63)	1.48 (1.38)
EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; IQR, interquartile range; SD, standard deviation.		

[0129] Of these, 37 newly diagnosed subjects and 173 with ≥ 2 pre-study attacks are included in the respective ‘any inebilizumab’ groups. Subject characteristics were similar between newly diagnosed subjects and those with ≥ 2 pre-study attacks.

5 Results

NMOSD attack risk decreases with inebilizumab treatment

[0130] Attack risk was reduced in all subjects treated with inebilizumab. Newly diagnosed subjects treated with inebilizumab had a lower incidence of adjudicated attacks, lower AAR, and higher probability of remaining attack-free at the end of RCP than those in the placebo group, irrespective of the number of pre-study attacks (**FIG. 1B** and **FIG. 1C**, **Table 3**). Similar results were observed for those with ≥ 2 pre-study attacks. In the OLP, AAR continued to decrease over time with inebilizumab; AARs were low for ‘any inebilizumab’ subjects who were newly diagnosed and those with ≥ 2 pre-study attacks.

Table 3: Results Summary

	Newly diagnosed subjects			Subjects with ≥ 2 prior relapses		
	RCP		Any inebilizumab exposure (n = 37)	RCP		Any inebilizumab exposure (n = 173)
	Placebo (n = 13)	Inebilizumab (n = 24)		Placebo (n = 39)	Inebilizumab (n = 137)	
Adjudicated attacks	3 (23.1)	1 (4.2)	---	19 (48.7)	17 (12.4)	---

during RCP, n (%)						
AAR (95% CI)	0.359 (0.074–1.049)	0.082 (0.002–0.456)	0.093 (0.052–0.168)	1.201 (0.723–1.876)	0.263 (0.153–0.421)	0.112 (0.079–0.157)
Probability of being attack-free after						
6 months	72.7	95.7	---	51.1	86.2	---
2 years	---	---	94.3	---	---	78.3
4 years	---	---	90.9	---	---	73.7

EDSS worsening is stabilized with inebilizumab treatment

[0131] During RCP, fewer subjects had EDSS worsening with inebilizumab treatment compared to placebo in both newly diagnosed subjects and those with ≥ 2 pre-study attacks (Table 4).

Table 4: Summary of results

	Newly diagnosed subjects		Subjects with ≥ 2 prior relapses	
	Placebo (n = 13)	Inebilizumab (n = 24)	Placebo (n = 39)	Inebilizumab (n = 137)
Subjects with EDSS worsening, n (%)	3 (23.1)	3 (12.5)	15 (38.5)	21 (15.3)
Odds ratio (95% CI)	0.503 (0.082–3.080)		0.309 (0.138–0.691)	

[0132] Stabilization of EDSS was for both groups seen throughout the OLP.

10 Safety and tolerability of inebilizumab treatment

[0133] During the RCP, rates of treatment-emergent AEs and AESIs were not higher in newly diagnosed subjects than in subjects with ≥ 2 pre-study attacks when divided by treatment group (Table 5).

Table 5: Summary of treatment-emergent AEs and AESIs occurring in newly diagnosed subjects and subjects with ≥ 2 prior relapses by treatment group during the RCP and following any exposure to inebilizumab during the whole study

	Newly diagnosed subjects			Subjects with ≥ 2 prior relapses		
	RCP		Any inebilizumab exposure (n = 37)	RCP		Any inebilizumab exposure (n = 173)
	Placebo (n = 13)	Inebilizumab (n = 24)		Placebo (n = 39)	Inebilizumab (n = 137)	
Exposure (subject-years)	8.36	12.20	111.11	15.82	64.64	366.07
Events, n (%); rate/PY						
≥ 1 AE	10 (76.9); 1.20	19 (79.2); 1.56	34 (91.9); 0.31	27 (69.2); 1.71	100 (73.0); 1.55	161 (93.1); 0.44
≥ 1 study drug-related AE	4 (30.8); 0.48	10 (41.7); 0.82	17 (45.9); 0.15	9 (23.1); 0.57	30 (21.9); 0.46	66 (38.2); 0.18
≥ 1 AE of $\geq$ grade 3 severity	1 (7.7); 0.12	2 (8.3); 0.16	10 (27.0); 0.09	6 (15.4); 0.38	12 (8.8); 0.19	45 (26.0); 0.12
≥ 1 SAE	1 (7.7); 0.12	0	8 (21.6); 0.07	5 (12.8); 0.32	7 (5.1); 0.11	34 (19.7); 0.09
≥ 1 SAE and/or AE of $\geq$ grade 3 severity	2 (15.4); 0.24	2 (8.3); 0.16	13 (35.1); 0.12	7 (17.9); 0.44	14 (10.2); 0.22	54 (31.2); 0.15
≥ 1 study drug-related SAE	0	0	2 (5.4); 0.02	0	1 (0.7); 0.02	8 (4.6); 0.02
≥ 1 AE leading to dose interruption	0	1 (4.2); 0.08	2 (5.4); 0.02	0	3 (2.2); 0.05	6 (3.5); 0.02
≥ 1 AE leading to discontinuation of study drug	0	0	1 (2.7); 0.01	0	2 (1.5); 0.03	5 (2.9); 0.01
Death	0	0	0	0	0	3 (1.7); 0.01
Events of special interest, n (%); rate/PY						
≥ 1 AE	5 (38.5); 0.60	13 (54.2); 1.07	30 (81.1); 0.27	19 (48.7); 1.20	62 (45.3); 0.96	139 (80.3); 0.38

Cytopenia	0	2 (8.3); 0.16	3 (8.1); 0.03	0	6 (4.4); 0.09	10 (5.8); 0.03
Hepatic function abnormality	0	0	2 (5.4); 0.02	2 (5.1); 0.13	8 (5.8); 0.12	14 (8.1); 0.04
Infection	5 (38.5); 0.60	11 (45.8); 0.90	29 (78.4); 0.26	18 (46.2); 1.14	54 (39.4); 0.84	130 (75.1); 0.36
Infusion-related reaction	0	0	9 (24.3); 0.08	0	0	17 (9.8); 0.05

[0134] In summary, Inebilizumab was efficacious and generally well tolerated in newly diagnosed AQP4+ adults, similar to that seen in those previously treated with other immunotherapies. In both subgroups, subjects receiving inebilizumab had fewer attacks, higher probability of remaining attack-free and less worsening of EDSS than those receiving placebo.

Example 2-Extent of B-cell depletion is associated with disease activity reduction in neuromyelitis optica spectrum disorder

[0135] The study characterized the relationship between B-cell depletion and outcomes in subjects receiving long-term inebilizumab in the double-blind, placebo-controlled, phase 2/3 trial.

Methods

[0136] The study comprised a randomized, placebo-controlled period (RCP) of up to 28 weeks, followed by an optional open-label period (OLP) of at least 2 years. B-cell counts were determined using high-resolution flow cytometry (lower limit of quantification, 0.2 cells/ μ L). Disease activity was measured using annualized attack rates (AARs) and number of new/enlarging T2 lesions.

Results

[0137] Inebilizumab induced rapid B-cell depletion that was sustained with multiple dosing compared with placebo (normal range, 74.4–441.1 cells/ μ L; mean $\pm$ standard deviation [SD], RCP week [W]4: inebilizumab, 6.1 $\pm$ 9.6 cells/ μ L; placebo, 147.7 $\pm$ 89.4 cells/ μ L; OLP W156: inebilizumab, 10.4 $\pm$ 37 cells/ μ L). B-cell levels at the end of the first inebilizumab dosing period (W28) were predictive of stable, deep depletion with long-term exposure. Clinical and imaging metrics of disease activity were lower in subjects with B- L (n=139/200) than in those with >4 cells/ μ L (n=61/200) at W28; rate ratios (95% confidence interval [CI]) were 0.4 (0.16–0.98), p=0.049 for AAR and 0.36 (0.23–0.56), p<0.0001 for new/enlarging T2 lesions. AARs (mean [standard error]) in both groups were lower than those in the RCP placebo group; L: AAR, 0.03

(0.02 0.04); new/enlarging T2 lesions, 0.49 (0.43 0.56); >4 cells/ μ L: AAR, 0.09 (0.06 0.12); new/enlarging T2 lesions, 1.36 (1.12 1.61); placebo: AAR, 1.01 (0.79 1.23); new/enlarging T2 lesions, 2.72 (1.99 3.46). After 2.5 years, long-term inebilizumab treatment provided sustained B-cell depletion and decreased metrics of NMOSD disease activity, with significant reductions in AAR (97%) and new/enlarging T2 lesions (73%) versus the RCP placebo group (both $p < 0.05$).

[0138] Inebilizumab provides rapid and durable B-cell depletion in subjects with NMOSD. These findings suggest that deep, persistent B-cell depletion is beneficial in NMOSD, and that monitoring B-cell counts may be informative with inebilizumab treatment.

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Example 3 – Subjects with neuromyelitis optica spectrum disorder display hallmarks of systemic autoimmunity: broad serum autoreactivity to nuclear antigens and elevated interferon-inducible gene expression

[0139] A study was completed to profile serum autoreactivity and type 1 interferon-inducible gene signature (IFNGS) in blood and to characterize their relationship with disease activity in individuals with NMOSD in the study.

Methods

The study was a multicenter, double-blind, randomized, placebo-controlled, phase 2/3 trial that tested the efficacy and safety of inebilizumab, a humanized, affinity-optimized, anti-CD19 monoclonal antibody, for the treatment of NMOSD. The study comprised a randomized controlled period (RCP) of up to 28 weeks, followed by an optional open-label period of at least 2 years. Serum samples were obtained from: 220/230 subjects with NMOSD randomized in the study, 25 healthy controls (HCs) collected from an independent commercial source. Baseline samples were hybridized to autoantigen microarrays to profile serum immunoglobulin (Ig)G, IgM, IgA and IgE autoreactivity against 122 autoantigens (excluding AQP4) implicated in human autoimmune diseases: CENP-A, CENP-B, Core Histone, DNA Polymerase beta (POLB), dsDNA, EBNA1, Genomic DNA, Histone H1, Histone H2A, Histone H2B, Histone H3, Histone H4, Jo-1, KU (P70/P80), La/SSB, Mi-2, PCNA, PL-7, PL-12, PM/Scl-75, PM/Scl 100, Ribo Phosphoprotein P0, Ribo Phosphoprotein P1, Ribo Phosphoprotein P2, Ro/SSA (52 Kda), Ro/SSA (60 Kda), S100, Scl-70/Topoisomerase I, Sm, Sm/RNP, SmD, SmD1, SmD2, SRP54, ssDNA, ssRNA, U1-snRNP 68/70, U1-snRNP A, U1-snRNP B/B, U1-snRNP C, Histone-total, Histone-total citrullinated human genomic DNA, KU(P70/P80), Nup 62, Ro/SSA (52+60), and U1-snRNP. Autoreactivity was confirmed by a signal more than five standard deviations above HC mean. Autoantibody score was calculated by summing the

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number of spots with signal more than five standard deviations above HC mean. Baseline IFNGS was simultaneously analyzed in blood RNA samples from 219 subjects with NMOSD and 10 HCs. IFNGS positivity was confirmed by a fourfold change in signal above HC mean. Possible associations of baseline autoreactivity and IFNGS positivity with pre-study NMOSD disease activity were estimated using negative binomial regression. Disease activity was measured by annualized attack rate (AAR) or mean annual disability worsening on the Expanded Disability Status Scale (EDSS) between diagnosis and study baseline, in subjects with more than 4 years of medical history.

Results

Baseline autoreactivity in subjects with NMOSD

[0140] At baseline, 197/220 subjects with NMOSD (90%) had serum autoreactivity to at least one autoantigen, primarily ribonucleoproteins (RNPs), in line with previous findings (FIG. 2A). By contrast, HCs did not display autoreactivity to any of the antigens studied. Serum autoreactivity was mainly observed in the IgG subclass (83% [n = 183]).

Baseline IFNGS in subjects with NMOSD

[0141] In all, 27% of subjects with NMOSD (59/219) were IFNGS-positive at baseline (> 4-fold change from HC mean) (FIG. 2B). Proportionally more IFNGS-positive than IFNGS-negative subjects were reactive to one or more RNP (68% vs 46%) (FIG. 2C). Statistically significant differences were observed in ANA score between IFNGS-positive and IFNGS-negative subjects (FIG. 2D). IgG RNP immunoreactive subjects had increased IFNGS at baseline.

NMOSD and prior diagnoses of inflammatory diseases

[0142] Most subjects with NMOSD who had high autoantigen immunoreactivity and IFNGS positivity had no prior diagnosis of other coexisting inflammatory diseases, as reported by the investigators (FIG. 2E). In total, 23 subjects (10%) had a diagnosis of a systemic inflammatory disease in addition to NMOSD. Eight subjects (4%) had a prior diagnosis of two or more systemic inflammatory diseases in addition to NMOSD (Table 6).

Table 6: Subjects diagnosed with at least two coexisting inflammatory diseases in addition to NMOSD

Subject 1	pSS, MG	Subject 5	pSS, MG
Subject 2	SLE, RA	Subject 6	Fibromyalgia, thyroid
Subject 3	pSS, thyroid	Subject 7	SLE, MG
Subject 4	RA, thyroid	Subject 8	pSS, thyroid

[0143] Baseline autoantibody and IFNGS positivity and NMOSD disease activity IgG autoantibodies and IFNGS positivity at baseline were not associated with increased NMOSD disease activity pre-study (**Table 7** and **Table 8**).

Table 7: Autoantibody positivity, IFNGS vs pre-study AAR in subjects with more than 4 years of medical history

Y	X	RR (95% CI)	Subjects, n	Attacks, n	Total years of observation	p value	BH FDR
Pre-study – AAR	IFNGS	0.96 (0.70–1.33)	84	595	834	0.86	1.00
Pre-study – AAR	Autoantibody score (any subclass)	0.99 (0.97–1.01)	86	609	867	0.24	1.00
Pre-study – AAR	IgG autoantibody score	1.00 (0.97–1.03)	86	609	867	0.80	1.00
Pre-study – AAR	Anti-RNP score (any subclass)	0.97 (0.94–1.01)	86	609	867	0.22	1.00
Pre-study – AAR	IgG anti-RNP score	0.99 (0.94–1.06)	86	609	867	0.86	1.00

Table 8: Autoantibody positivity, IFNGS vs pre-study EDSS progression in subjects with more than 4 years of medical history

Y	X	RR (95% CI)	Subjects, n	EDSS points worsened, n	Total years of observation	p value	BH FDR
Pre-study – EDSS progression	IFNGS	1.08 (0.81–1.44)	84	364.5	835	0.61	1.00
Pre-study – EDSS progression	Autoantibody score (any subclass)	1.00 (0.99–1.02)	86	373	867	0.56	1.00
Pre-study – EDSS progression	IgG autoantibody score	1.01 (0.99–1.04)	86	373	867	0.36	1.00

Pre-study -- EDSS progression	Anti-RNP score (any subclass)	1.01 (0.97– 1.04)	86	373	867	0.69	1.00
Pre-study -- EDSS progression	IgG anti-RNP score	1.02 (0.97– 1.07)	86	373	867	0.51	1.00

[0144] Similar results were observed for IgM, IgA and IgE autoantibodies. In summary, subjects with NMOSD show greater serum autoreactivity to autoantibodies known to be associated with other systemic autoimmune diseases than do HCs. Moreover, subjects with NMOSD and elevated serum immunoreactivity to at least one RNP tended to show increased blood IFNGS at baseline. The majority of subjects with NMOSD with high serum immunoreactivity to autoantigens and high blood IFNGS showed no co-existing autoimmune disease. Neither serum autoreactivity nor IFNGS positivity at baseline appear to be associated with increased NMOSD disease activity.

Example 4- Extent of B-cell depletion is associated with disease activity reduction in neuromyelitis optica spectrum disorder

[0145] A study was completed to characterize the relationship between B-cell depletion and outcomes in subjects receiving long-term inebilizumab in the study.

[0146] The relationship between B-cell, plasma cell, and immunoglobulin depletion after inebilizumab treatment, and longitudinal reductions in NMOSD disease activity after inebilizumab treatment was characterized in the study.

Methods

[0147] Peripheral blood (PB) B-cells, plasma-cell signature, and immunoglobulin levels were regularly assessed throughout the study and analyzed for correlation with clinical metrics and magnetic resonance imaging (MRI) lesion activity.

[0148] The study comprised a randomized controlled period (RCP) of up to 28 weeks in which subjects were allocated (3:1) to intravenous inebilizumab 300 mg or placebo, and an optional open-label period (OLP), during which all subjects received intravenous inebilizumab 300 mg every 6 months for at least 2 years (**FIG. 1**).

Evaluation of B-cell pharmacodynamics

[0149] During the RCP, blood samples were collected during study visits at baseline and weeks 1, 2, 4, 8, 12, 16, 22, and 28 to assess B-cell counts; at baseline and weeks 2, 4, 8, 12, 16, and 28 to evaluate plasma cell-specific gene expression; and at weeks 12 and 28 to evaluate Ig

levels. During the OLP, blood samples were collected every 13 weeks for assessment of B-cell counts, plasma-cell specific gene expression, and Ig levels. Blood samples to assess B-cell counts and plasma-cell gene expression were also collected during any assessment visit for new or worsening NMOSD symptoms during the RCP and OLP. Samples for attack assessments were taken before initiation of treatment for NMOSD attacks.

[0150] B-cell levels in peripheral blood were assayed by flow cytometry using fluorescence-activated cell sorting (FACS), conducted at a central laboratory within 72 hours of sample collection. Whole blood samples were collected in Streck Cytochex cell preservative tubes and shipped to the central laboratory on day of collection. A minimum of 100,000 lymph events were acquired. B-lineage cells were counted using CD20 as a FACS marker (bound inebilizumab interferes with CD19-based FACS). The B-cell subsets measured included CD20+ B-cells defined as CD45hi [CD3-, CD56-, CD14-], CD33-, CD20. In addition, an analysis of plasmablast/plasma cell counts (CD45hi [CD3-, CD14-, CD56-], CD27+, HLA-DRhi/low, CD38+) was performed on a subset of subjects using FACS. The lower limit of quantification (LLOQ) for all cell populations was set at 0.2 cells/ μ L. Samples with cell counts less than the LLOQ for any given cell population were imputed to 0.05 cells/ μ L.

[0151] Plasma-cell specific gene expression was assessed by quantitative reverse transcription polymerase chain reaction of blood RNA samples. The plasma-cell gene signature was based on expression analysis of four genes (IGHA1, IGJ, IGKV4-1, and TNFRSF17) that are expressed predominantly by plasma cells in blood.²¹ The signature was calculated as the average expression of the four plasma cell-specific genes minus the average expression of five control genes (B2M, GAPDH, TFRC, GUSB, and UBC) at each time point. The fold-change in plasma-cell gene expression signature at each time point was calculated relative to a pool of ten healthy donor samples and interpreted as plasma cell abundance relative to expected prevalence under a non-activated immune state.

[0152] TaqMan single nucleotide polymorphism profiling was used to investigate possible associations between the rs396991 polymorphism in the FCGR3A gene and impaired B-cell depletion in a subgroup of subjects who provided consent for genomic analysis. The rs396991 polymorphism is widespread and encodes a valine/phenylalanine substitution at position 158 of FCGR3A that is associated with decreased binding affinity for Ig Fc, thus impairing ADCC mechanisms for which the Fc region of inebilizumab has been optimized.

Results

[0153] Compared with placebo, inebilizumab provided rapid B-cell and plasma cell depletion (reference range of normal concentration, 74–441 cells/ μ L) that was sustained with multiple

dosing (**FIG. 3A**). All subjects had B-cell reductions at 1 week from first treatment. At week 4 of the RCP, median (interquartile range [IQR]) B-cell counts were 2.5 (1.0–7.6) cells/ μ L in the inebilizumab group and 112.3 (96.3–176.9) cells/ μ L in the placebo group. At week 156 of the OLP, median (IQR) B-cell count was 0.33 (< LLoQ–1.0) cells/ μ L with inebilizumab.

5 **Effects of inebilizumab on long-term B-cell depletion**

[0154] B-cell levels at the end of the first inebilizumab dosing period (28 weeks) were predictive of stable, deep depletion with long-term exposure (**FIG. 3B**). Of the 200 subjects, 70% (n = 139) had B-cell depletion to ≤ 4 cells/ μ L and durable B-cell depletion with multiple doses of inebilizumab. Subjects with B-cell counts ≤ 4 cells/ μ L had persistently deeper B-cell
10 depletion compared with those with B-cell counts > 4 cells/ μ L (**FIG. 3B**). Sustained B-cell depletion was also observed in the > 4 cells/ μ L subgroup with continued inebilizumab treatment.

[0155] Inebilizumab treatment significantly reduced circulating levels of B-cells and plasma cells versus placebo during the RCP (**FIG. 3A, FIG. 6A-FIG. 6F**). Total Ig levels were also
15 decreased with the greatest reductions seen in IgE, IgA, and IgM classes. Overall NMOSD disease activity, as measured by annualized attack rate [AAR], EDSS worsening, NMOSD-related hospitalizations, and new or enlarging T2 MRI lesions, decreased significantly with inebilizumab treatment during the RCP18 and continued to decline with further doses of inebilizumab (**FIG. 7A-FIG. 7D**). After 2.5 years of inebilizumab treatment, there was
20 progressive reduction in AAR, annualized rate of new/enlarging T2 lesions, EDSS worsening, and NMOSD-related in-patient hospitalizations.

Association between B-cell kinetics and long-term NMOSD outcomes

[0156] Since prolonged inebilizumab treatment resulted in a progressive reduction in NMOSD disease activity, it was hypothesized that the depth of B-cell depletion would correlate with
25 therapeutic efficacy. CD20+ peripheral blood B-cell counts were examined in subjects, with and without attacks, through the RCP and OLP, during attack-independent, pre-attack, attack, and post-attack epochs (**FIG. 8A-FIG.8C**). Significant increases were not observed in peripheral blood CD20+ B-cell levels at the time of attack in samples from all subjects or from the subgroups of subjects receiving 3 or more doses of inebilizumab (**FIG. 8A-FIG.8C** and
30 **FIG. 12A-FIG. 12B**). No significant increases in B-cell counts were observed during attack assessments, regardless as to whether the attacks occurred during or after the first dosing period.

[0157] At 6 months, clinical and imaging metrics of NMOSD disease activity were generally lower in subjects with B-cell counts ≤ 4 cells/ μ L (n/N = 139/200) than in those with > 4 cells/ μ L

(n/N = 61/200) (**FIG. 3C-FIG. 3F**). Subsequent doses of inebilizumab further decreased the metrics of NMOSD activity in both subgroups over time, although subjects in the ≤ 4 cells/ μ L subgroup achieved faster decreases in metrics of NMOSD activity. All subjects showed similar levels of reduced metrics of NMOSD activity after 2.5 years of inebilizumab exposure, regardless of their B-cell counts after the first dosing interval.

[0158] Therefore, whether the extent of B-cell depletion within the first dosing interval might predict the subsequent depth of B-cell depletion and reduction in NMOSD disease activity was investigated. A negative binomial regression revealed a linear association between B-cell counts at the conclusion of the first 6-month dosing interval and disease activity that occurred after the first dosing period with inebilizumab (**Table 9**). Multiple additional timepoints besides 6 months were also correlative of future disease activity in our regression analysis. Indeed, B-cell counts from samples drawn as early as 3 months after inebilizumab treatment initiation correlated with future NMOSD disease activity after the first dosing interval. However, B-cell counts from these early timepoints did not correlate with NMOSD disease activity within the first dosing period of treatment.

Table 9: 6-month B-cell counts vs long term disease activity

		RR (95% CI)	Number of subjects	Total years follow-up	p-value
Attack rate	Log ₁₀ Memory B-cells	2.06 (1.11-3.81)	170	527	2.15e-02
	Log ₁₀ 6-month CD20 B-cells	1.61 (1.04-2.49)	197	582	3.12e-02
Total T2 Lesion Count	Log ₁₀ Memory B-cells	1.93 (1.44-2.59)	145	392	1.21e-05
	Log ₁₀ 6-month CD20 B-cells	1.62 (1.32-1.99)	166	432	4.99e-06
EDSS worsening rate	Log ₁₀ Memory B-cells	1.56 (0.99-2.47)	169	508	5.80e-02
	Log ₁₀ 6-month CD20 B-cells	1.24 (0.89-1.74)	196	562	2.10e-01
Hospitalization rate	Log ₁₀ Memory B-cells	1.87 (1.01-3.45)	170	533	4.65e-02

	Log ₁₀ 6-month CD20 B-cells	1.56 (0.99- 2.45)	197	589	5.55e-02
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Negative binomial regression analysis of B-cell counts at 6 months post-treatment (W28 RCP for inebilizumab treated who completed the RCP, W26 OLP for subjects randomized to placebo and inebilizumab treated subjects who exited RCP early) versus the annualized attack rate, rate of new/enlarging T2 lesions, EDSS worsening (subjects/year), and in-patient hospitalizations that occurred after the first dosing period of inebilizumab treatment.

[0159] A cut-point of 4 cells/ μ L separated subjects with decreased risk of NMOSD activity during subsequent inebilizumab dosing. Sensitivity analyses showed a cut-off of 4 cells/ μ L was the highest threshold that maintained a favorable rate ratio across the multiple NMOSD activity metrics (**FIG. 13A-FIG. 13D**). In total, 139/200 subjects (70%) had B-cell counts ≤ 4 cells/ μ L at the end of the first dosing interval, and they maintained durable B-cell depletion with continued treatment (**FIG. 9A-FIG. 9B**). When compared with subjects with B-cell counts > 4 cells/ μ L, those with ≤ 4 cells/ μ L had persistently lower B-cell numbers (**FIG. 9B**), lower AAR (0.034 vs 0.086; $p=0.0494$), fewer new/enlarging T2 lesions (0.49 vs 1.36; $p<0.0001$), less EDSS worsening (0.076 vs 0.14; $p=0.09$) and trended toward fewer hospitalizations (0.08 vs 0.18; $p=0.11$; **Table 10**). Similar disease activity outcomes were observed when the AQP4 seropositive subject population was analyzed separately (**Table 10, FIG. 14A-FIG. 14D**).

Table 10: Long-term outcomes in subjects with 6-month B-cell counts ≤ 4 vs > 4 cells/ μ L

	Rate/year Placebo RCP	Rate/year ≤ 4 cells/ μ L after 1 st dosing period	Rate/year > 4 cells/ μ L after 1 st dosing period	RR (95% I)	p value
Post-6-month NMOSD attacks	1.05	0.034	0.086	0.40 (0.16–0.98)	0.045
Post-6-month NMOSD attacks (AQP4+ only)	1.1	0.038	0.088	0.44 (1.07,0.18)	0.07
Post-6-month new T2 lesions	2.75	0.49	1.36	0.36 (0.23–0.56)	<0.0001
Post-6-month new T2 lesions (AQP4+ only)	3.18	0.51	1.38	0.37 (0.58,0.24)	<0.0001
Post-6-month EDSS worsening (subjects/year)	1.63	0.076	0.14	0.55 (0.27–1.11)	0.093
Post-6-month EDSS worsening (subjects/year; AQP4+ only)	1.58	0.07	0.15	0.50 (1.05,0.24)	0.07
Post-6-month in-patient hospitalization	0.53	0.08	0.18	0.45 (0.17–1.18)	0.11

Post-6-month in-patient hospitalization (AQP4+ only)	0.51	0.08	0.19	0.41 (1.08,0.15)	0.07
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[0160] With continued inebilizumab administration, durable B-cell depletion was still observed in the >4 cells/ μ L subgroup (**FIG. 9B**) and by week 117, median CD20+ B-cell counts were similar among subjects in both the >4 cells/ μ L and the ≤ 4 cells/ μ L groups.

- 5 [0161] Notably, increases in overall B-cell counts at the time of attacks that occurred during this time frame were not observed (**FIG. 8A-FIG. 8C** and **FIG. 12A-FIG. 12B**).

Effect of long-term inebilizumab on NMOSD disease activity

- 10 [0162] Because NMOSD disease activity decreased longitudinally with long-term inebilizumab treatment, the short and long-term relationship was evaluated between clinical and imaging metrics and subgroups with 6-month B-cell counts >4 cells/ μ L or ≤ 4 cells/ μ L. During the RCP, NMOSD disease activity (measured by AAR, new/enlarging T2-hyperintense MRI lesions, EDSS score worsening, and rate of in-patient hospitalizations) decreased in both subgroups after the first dosing period of inebilizumab treatment compared with placebo.
- 15 Subsequent doses of inebilizumab further decreased disease activity in both subgroups over time. NMOSD activity decreased more rapidly among subjects in the ≤ 4 cells/ μ L than in the >4 cells/ μ L subgroup, especially new T2-hyperintense lesions. However, after 2.5 years of inebilizumab exposure, all subjects showed similar levels of NMOSD activity (**FIG. 3C-FIG. 3E** and **FIG. 10**).

- 20 [0163] After 2.5 years, treatment with inebilizumab was associated with decreased NMOSD disease activity compared with placebo during the RCP (**FIG. 3G**). AAR was reduced by 97%. Annualized rate of new or enlarging T2 lesions was reduced by 73%.

- [0164] In summary, treatment with inebilizumab provided rapid and durable depletion of B-cells in subjects with NMOSD compared with placebo. Greater B-cell depletion was associated with improved outcomes with long-term treatment with inebilizumab. These findings suggest, at least, that deep and persistent B-cell depletion is beneficial in preventing attacks in subjects with NMOSD and/or regular monitoring of B-cell counts may provide a valuable approach to ensure the most effective use of inebilizumab in managing NMOSD.

30 Assessment of subject subgroups

- [0165] Three separate, but not mutually exclusive, mechanisms could impact B-cell counts at the end of the first inebilizumab dosing interval:

Pharmacokinetic loss of inebilizumab may lead to B-cell repletion

[0166] First, pharmacokinetic (PK) loss of inebilizumab after administration of the two loading doses could have led to repletion of B-cells in some subjects by the end of the first dosing interval. PK profiles of inebilizumab were compared in the subgroups with B-cell counts >4 cells/ μ L and $\leq$ 4 cells/ μ L at the end of the first dosing interval. Subjects with less B-cell depletion had lower inebilizumab concentrations relative to those with stable B-cell depletion: inebilizumab was undetectable at week 22 in serum of most subjects with B-cell counts >4 cells/ μ L (LLOQ: 100·5 pg/mL, **FIG. 15**). Additional analysis of baseline covariates revealed that age, sex, race, weight, and BMI were not significantly associated with higher B-cell counts (defined as > 4 cells/ μ L at W28. Prior rituximab use was also not associated with higher B-cell counts. These subjects displayed significantly elevated CD19+ B-cell counts, plasma-cell signature, and Ig concentrations on day 1 of the RCP (false discovery rate <0·10), (**FIG. 16A-FIG. 16C**).

15 *Shallow depletion may be related to the rs396991 polymorphism in FCGR3A*

[0167] Second, increased B-cell counts at the end of the first dosing interval may be caused by shallow cellular depletion attributable, in part, to the rs396991 polymorphism in *FCGR3A*. rs396991 F/F homozygosity was profiled within a subgroup of subjects who consented to genomic analysis. F/F homozygosity was noted in 100·0% (10/10) of subjects who never achieved CD20+ $\leq$ 4 cells/ μ L during the first dosing interval, in 43% (12/28) of subjects whose B-cell levels depleted to $\leq$ 4 cells/ μ L but repleted to >4 cells/ μ L by the end of the first 6-month treatment period, and in 45·2% (38/84) with sustained B-cell depletion $\leq$ 4 cells/ μ L (**FIG. 17A-FIG. 17B**).

25 *Anti-drug antibodies may alter B-cell depletion*

[0168] Third, anti-drug antibodies (ADAs) that bind to or neutralize inebilizumab could affect changes in B-cell counts. In total, 33/225 subjects (14·7%) treated with inebilizumab tested positive for ADAs intermittently on study (lowest titre 1:50 dilution; median of maximum titre = 100), and these titres decreased overall with long-term inebilizumab treatment (**FIG. 18A**). In general, B-cell counts were modestly increased with ADA positivity, but no significant overlap was found between subjects positive for ADAs and subjects with B-cell levels >4 cells/ μ L before completing the first dosing interval (**FIG. 18B**). Median B-cell counts in samples drawn 90 days from periods of ADA positivity were higher than in samples from subjects who were persistently ADA negative (1·5 cells/ μ L vs 0·4 cells/ μ L; **FIG. 18C**).

Example 5- Sustained B-cell depletion by inebilizumab is associated with decreased disease activity in aquaporin-4 seropositive neuromyelitis optica spectrum disorder

[0169] A study was completed to characterize the relationship between B-cell depletion and outcomes in subjects receiving long-term inebilizumab in the N-MOMentum trial as previously described in Example 3 and FIG. 1.

Effects of Inebilizumab on B-cell depletion

[0170] Compared with placebo, inebilizumab provided rapid B-cell depletion. All subjects had B-cell reductions at 1 week after first treatment; reductions were maintained over time with multiple doses. After the first dosing interval (6 months), 126/186 (68%) of subjects had B-cell counts ≤ 4 cells/ μ L. The remaining subjects (n/N=60/186 [32%]) had >4 cells/ μ L, but well below the lower limit of normal. Long-term (>2.5 years) inebilizumab treatment provided sustained B-cell depletion in all subjects, irrespective of the depth of short-term B-cell depletion

Short-term effects of B-cell depletion on metrics of disease activity

[0171] Immediate clinical benefits versus placebo were seen in both groups; however, subjects with ≤ 4 cells/ μ L had greater improvements in metrics of disease activity than those with >4 cells/ μ L during the first dosing interval. After the first dosing interval, 111/124 (90%) of subjects with ≤ 4 cells/ μ L and 50/59 (85%) of subjects with >4 cells/ μ L were attack-free. Subsequent doses of inebilizumab further decreased metrics of NMOSD activity in both subgroups over time, although subjects in the ≤ 4 cells/ μ L achieved faster decreases in metrics of NMOSD activity.

Table 11: Summary of metrics of disease activity

Outcomes	Placebo RCP Rate/year	First dosing interval Rate/year	≤ 4 cells/ μ L after first dosing interval Rate/year	>4 cells/ μ L after first dosing interval Rate/year	RR (95% confidence interval)	P value
NMOSD attacks	1.1 (0.86–1.34)	0.27 (0.22–0.33)	0.04 (0.02–0.07)	0.09 (0.05–0.17)	0.44 (1.07–0.78)	0.07
New T2 lesions	3.18 (2.28–4.07)	1.65 (1.38–1.91)	0.51 (0.39–0.67)	1.38 (0.97–1.97)	0.37 (0.58–0.24)	1.5e- 5
EDSS worsening	1.58 (1.20–1.97)	0.43 (0.34–0.49)	0.07 (0.05–0.12)	0.15 (0.08–0.26)	0.50 (1.05–0.24)	0.07
In-patient hospitalization	0.51 (0.29–0.74)	0.37 (0.29–0.45)	0.08 (0.04–0.14)	0.19 (0.09–0.40)	0.41 (1.08–0.15)	0.07

Effect of long-term (>2.5 years) inebilizumab-related B-cell depletion on metrics of disease activity

[0172] When compared with placebo during the RCP, long term treatment with inebilizumab was associated with: decreased AAR in both subgroups (**FIG. 4A**), decreased EDSS worsening
5 in both subgroups (**FIG. 4B**), and depth of B-cell depletion after the first dosing interval did not correlate with risk of infection (**FIG. 4C**).

[0173] In summary, inebilizumab provided a rapid, durable B-cell depletion in subjects with NMOSD. There was a difference in B-cell depletion and subsequent decrease of disease activity between subjects after the first dosing interval. Monitoring B-cell counts may be useful
10 in optimizing inebilizumab treatment. Long-term inebilizumab treatment provided deep B-cell depletion and decreases in disease activity to all subjects, irrespective of the depth of B-cell depletion of their first dosing period. Long-term inebilizumab treatment or depth of B-cell depletion after the first dosing interval did not correlate with risk of infection.

15 **Example 6-Subjects with neuromyelitis optica spectrum disorder display hallmarks of systemic autoimmunity: broad serum autoreactivity to nuclear antigens and elevated interferon-inducible gene expression**

[0174] A study was completed to profile serum autoreactivity and type 1 interferon-inducible gene signatures (IFNGSs) in blood to characterize the relationship with disease activity in
20 NMOSD.

Methods

[0175] Serum samples were obtained from 220/230 subjects with NMOSD randomized in NMomentum and from 25 healthy controls (HCs). Baseline samples were profiled for serum immunoglobulin (Ig)G, IgM, IgA, and IgE autoreactivity by hybridizing samples to
25 microarrays of 122 autoantigens (excluding AQP4) implicated in human autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome). Autoreactivity was confirmed by a signal >5 standard deviations above HC mean. Baseline IFNGS were analyzed in blood RNA samples from 219 subjects and 10 HCs. Those with >4-fold change in signal above HC mean were IFNGS-positive. Possible associations of baseline autoreactivity
30 and IFNGS positivity with pre-study NMOSD disease activity (annualized attack rate or mean annual disability worsening on Expanded Disability Status Scale between diagnosis and baseline, in subjects with >4 years of medical history) were studied by negative binomial regression.

Results

- [0176] At baseline, HCs did not display autoreactivity to any antigens studied, while 90% of subjects with NMOSD (n=197) had autoreactivity to autoantigen, mostly in IgG subclass (83% [n=183]), but also in IgA (41% [n=90]), IgM (25% [n=56]), and IgE (6% [n=14]) subclasses. In total, 27% of subjects (n=59) were IFNGS-positive at baseline and 4% (n=8) had a prior diagnosis of a systemic autoimmune disease. Serum autoreactivity against nuclear antigens (NAs) was greater than that against other antigens. Proportionally more IFNGS-positive than IFNGS-negative subjects were reactive to NAs (68% [40/59] vs 46% [71/153]; area under the ROC curve, 0.63; p = 0.002). Neither serum autoreactivity nor IFNGS-positivity at baseline were associated with increased risk of pre-study NMOSD disease activity.
- [0177] Subjects with NMOSD show greater serum autoreactivity than do healthy controls to autoantibodies known to be associated with other systemic autoimmune diseases. However, this autoreactivity does not appear to be associated with increased NMOSD disease activity.

Example 7-The impact of low affinity immunoglobulin gamma Fc region receptor III-A gene polymorphisms in neuromyelitis optica spectrum disorder and implications for treatment outcomes

[0178] A study was done to characterize the relationship between rs396991 polymorphism, NMOSD disease activity and treatment response.

Methods

- [0179] The study had a randomized controlled period (RCP; inebilizumab 300 mg or placebo on days 1 and 15) of up to 28 weeks, followed by an open-label period (OLP). 142 subjects (104 randomized to inebilizumab, 38 to placebo) consented for polymorphism genotyping via TaqMan qPCR assay.

Results

- [0180] Historical annualized attack rates (AAR) and change in Expanded Disability Status Scale (EDSS) scores from NMOSD onset to enrolment were nominally higher in rs396991 V allele subjects (V; V/V or V/F genotype; n=74) than in F/F allele subjects (F/F; n=68): mean ($\pm$ SEM) AAR: V, 1.0 (0.8 1.3); F/F, 0.7 (0.5 0.9); EDSS score change: V, 0.6 (0.5 0.7); F/F, 0.4 (0.3 0.5). In the placebo group, the rates of attacks, new/enlarged T2 MRI lesions and NMOSD-related hospitalizations were higher in V allele (n=22) than in F/F allele subjects (n=16), but not statistically significant: mean ($\pm$ SEM) AAR: V, 1.3 (0.9 1.7); F/F, 0.8 (0.5 1.2); new T2 lesions: V, 4.5 (2.6 6.3); F/F, 2.1 (1.0 3.1); hospitalizations: V, 0.5 (0.2 0.8); F/F, 0.2 (0.0 0.4). At end of RCP, V-allele carriers randomized to inebilizumab (n=52) had greater median (IQR) B-cell, plasma-cell and Ig depletion than F/F homozygotes (n=52): B-cells; V,

0.6 (0.1 3.2) vs F/F, 1.3 (0.5 4.2) cells/ μ L; plasma cells: V, 0.04 (0.02 0.2) vs F/F, 0.05 (0.03 0.2) fold change from control mean; Ig: V, 1050 (867 1284) vs F/F, 1238 (1076 1455) Ig/ μ L. V allele carriers also had lower attack rates and new/enlarged T2 lesions than F/F homozygotes but differences were not statistically significant: mean ($\pm$ SEM): AAR: V, 0.1 (0.1 0.2); F/F, 0.3 (0.2 0.4); new T2 lesions: V, 1.4 (0.9 1.8); F/F, 1.7 (1.2 2.2). By dose 4 in the OLP, there was little difference between the subgroups in clinical metrics or B-cell depletion.

[0181] In summary, V allele carriers may have increased NMOSD disease activity but may have greater early pharmacodynamic response to inebilizumab compared with those with the wild F/F genotype.

10

Example 8- Impact of Low Affinity IgG Fc Region Receptor III-A Gene Polymorphisms on Neuromyelitis Optica Spectrum Disorder Treatment Outcomes

[0182] A study was conducted to characterize the relationship between rs396991 polymorphism, NMOSD disease activity, and treatment response.

15 *Methods*

[0183] The study was a double-blind, randomized, placebo-controlled, phase 2/3 trial of the efficacy and safety of inebilizumab in 230 adults with NMOSD. The study comprised a randomized controlled period (RCP) of up to 28 weeks in which subjects were allocated (3:1) to intravenous (i.v.) inebilizumab 300 mg or placebo, followed by an optional open-label period (OLP; all subjects received inebilizumab 300 mg i.v. every 26 weeks) of at least 2 years. Primary endpoint was risk of NMOSD attack, assessed as time to the onset of an NMOSD attack. In all, 142 subjects (inebilizumab, n = 104; placebo, n = 38) consented to polymorphism genotyping via a TaqMan quantitative polymerase chain reaction assay.

[0184] In a *post hoc* analysis, data from subjects with different *FCGR3A* genotypes were compared for disease severity and response to inebilizumab. Outcomes analyzed included attacks, disability (as assessed by Expanded Disability Status Scale [EDSS] scores), NMOSD-related hospitalizations and magnetic resonance imaging (MRI) lesions.

Results

Pre-study disease severity in V allele subjects

[0185] Historical annualized attack rates (AARs) from NMOSD onset to enrolment were nominally higher in V allele subjects (**FIG. 5B**) (V/F or V/V genotype; n = 25) than in F/F genotype subjects (F/F; n = 25). Median (interquartile range [IQR]): V allele, 0.9 (0.5–1.4); F/F genotype, 0.4 (0.3–0.8). Changes in EDSS scores were nominally higher among V allele subjects than F/F genotype subjects (**FIG. 5B**). Median (IQR): V allele, 0.5 (0.4–0.9); F/F

genotype, 0.4 (0.3–0.7). EDSS score was assumed to be zero at the time of NMOSD disease onset for all subjects for this analysis. No significant differences were observed across the entire cohort. Disease activity among V allele subjects in the study. In the RCP, subjects with the V allele who received placebo had higher AARs and rates of new/enlarging T2 MRI lesions and NMOSD-related hospitalizations than those who were homozygous for the F allele (although these differences did not achieve statistical significance). Mean ($\pm$ standard error of the mean [SEM]) values for these parameters in V allele versus F/F genotype subjects, respectively, were as follows: attacks, 1.3 (0.9–1.7) versus 0.8 (0.5–1.2) (**FIG. 5C**) new/enlarging T2 lesions, 4.5 (2.6–6.3) versus 2.1 (1.0–3.1) (**FIG. 5C**), NMOSD-related hospitalizations, 0.5 (0.2–0.8) versus 0.2 (0.0–0.4) (**FIG. 5C**).

Effects of inebilizumab in V allele subjects

[0186] AARs and annual rates of new/enlarging T2 lesions were lower in inebilizumab-treated V allele subjects than in F allele subjects. The mean ($\pm$ SEM) values for these parameters in V allele versus F/F genotype subjects, respectively, were as follows: AAR, 0.1 (0.1–0.2) versus 0.3 (0.2–0.4) (**FIG. 5D**), annual rate of new/enlarging T2 lesions, 1.4 (0.9–1.8) versus 1.7 (1.2–2.2) (**FIG. 5D**). However, these differences did not achieve statistical significance. B-cell, plasma-cell and IgG depletion was greater for inebilizumab-treated V allele subjects than for F/F allele subjects. At the end of the RCP, the median (IQR) values for these parameters in V allele subjects (n = 52) versus F/F genotype subjects (n = 52), respectively, were as follows: B-cell depletion, 0.6 (0.1–3.2) versus 1.3 (0.5–4.2) cells/ μ L (**FIG. 5E**), plasma-cell depletion, 0.04 (0.02–0.2) versus 0.05 (0.03–0.2) fold-change from control mean (**FIG. 5E**), IgG levels, 1050 (867–1284) versus 1238 (1076–1455) mg/dL (**FIG. 5E**).

Long-term inebilizumab treatment

[0187] With repeated Inebilizumab dosing in the OLP (1.5–2.5 years), there was little difference in the clinical metrics of NMOSD activity or B-cell depletion between V allele and F/F genotype subject subgroups (**FIG. 5F**).

[0188] In summary, the F176V polymorphism (V/F or V/V genotypes) of *FCGR3A* was not associated with clear differences in NMOSD disease activity compared with the F/F genotype. Subjects with the V allele may have greater pharmacodynamic response to inebilizumab than those with the F/F genotype. In subjects treated with inebilizumab, there was a trend for V allele subjects to have greater B-cell and plasma-cell depletion, as well as lower attack rates and fewer new/enlarging T2 lesions. Additionally, V-allele carriers may have increased NMOSD disease activity as compared to F/F-allele homozygotes. In this study of Inebilizumab

treated subjects, no significant differences in outcomes were seen between those with F and V allele genotypes.

Inebilizumab reduces attack risk independent of Low Affinity IgG Fc Region Receptor III-A Gene Polymorphisms in Neuromyelitis Optica Spectrum Disorder

5 [0189] V-allele carriers (V-allele genotype [V/V or V/F], n=74) and F/F-allele homozygotes (n=68) did not demonstrate a significant difference in baseline demographics or disease duration. Depletion of CD20+ B cells was similar in V allele vs F/F subjects (0.6 (0.1–3.2) vs 1.3 (0.5–4.2) cells/ μ l at end of RCP) and was sustained in both groups throughout the duration of the study. No differences in risk of relapse (OR 0.94 (0.39, 2.24)) or Expanded Disability

10 Status Scale worsening (OR: 1.55 (0.54, 4.70)) in V vs F/F subjects were found. Annualized attack rates (SEM) for patients on inebilizumab treatment were V/V 0.00 (0.00), V/F 0.10 (0.04), and F/F 0.06 (0.03). Inebilizumab treated subjects in the N-MOMentum trial did not demonstrate differences in clinical outcomes between those with F and V allele genotypes.

15 **Example 9-Myelin oligodendrocyte glycoprotein immunoglobulin g in aquaporin-4 seronegative patients with neuromyelitis optica spectrum disorder: outcomes in the study of inebilizumab**

[0190] A study was conducted to report outcomes among subjects with serotype AQP4-IgG-/MOG-IgG+, receiving long-term inebilizumab in the double-blind, placebo-controlled,

20 phase 2/3 study

Methods

[0191] The study comprised a randomized controlled period (RCP) of up to 28 weeks in which subjects were allocated (3:1) to intravenous inebilizumab (300 mg) or placebo, followed by an optional open-label period (OLP) in which all subjects received inebilizumab for at least 2

25 years (**Fig. 19A**). Data for any exposure to inebilizumab included time in the RCP and the OLP.

[0192] An expert eligibility committee reviewed all screened AQP4-IgG- subjects; those meeting the 2006 NMOSD diagnostic criteria (Wingerchuk DM et al. Neurology 2006; 66:1485–9) were randomized.

[0193] Endpoints comprised: (1) time to onset of an NMOSD attack determined by an adjudication committee (AC; annualized attack rate [AAR] was also determined); (2)

30 worsening of Expanded Disability Status Scale (EDSS) score; (3) cumulative total number of active lesions or new/enlarging T2 lesions detected by magnetic resonance imaging; (4) number of NMOSD-related hospitalizations; (5) B-cell counts in peripheral blood; (6) serum immunoglobulin levels; (7) adverse events (AEs); and (8) AEs of special interest (AESIs).

*Results***Efficacy in AQP4-IgG⁻/MOG-IgG⁺ subjects**

[0194] In all, 17/231 subjects (7.4%) with NMOSD were AQP4-IgG⁻; of these, 7 (3.0%) were MOG-IgG⁺ (inebilizumab, n = 6; placebo, n = 1). In those randomized to inebilizumab there was (each occurring in a separate subject) (**Table 12, FIG. 19B**): one AC-determined attack 78 days after the first dose; one case of disability worsening; and one hospitalization.

Table 12: Time from first dose of inebilizumab to first AC-determined attack

	Inebilizumab (n = 6)	Placebo (n = 6)
Subjects with AC-determined attacks	1 (16.7)	0
EDSS worsening	1 (16.7)	0
MRI lesions		
0	3 (50.0)	1 (100)
1	2 (33.3)	0
3	1 (16.7)	0
Inpatient hospitalization	1 (16.7)	0

Data are n (%). EDSS worsening was defined as an increase in score of ≥ 2 from a baseline score of 0, an increase of ≥ 1 from a baseline score of 1–5, or an increase of ≥ 0.5 from a baseline score of ≥ 5.5 .
 AC, adjudication committee; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging;
 RCP, randomized controlled period.

[0195] For the 2 years before enrollment, mean (95% confidence interval) AAR for AQP4-IgG⁻/MOG-IgG⁺ subjects was 3.12 (1.52–4.72); during the study, it was 0.05 (0.01–0.35). Inebilizumab exposure in RCP and OLP was 20.05 person-years (n = 7).

CD19 B-cell and immunoglobulin levels in AQP4-IgG⁻/MOG-IgG⁺ subjects

[0196] In most AQP4-IgG⁻/MOG-IgG⁺ subjects, B-cell depletion with inebilizumab was rapid and sustained, see **FIG. 19C**. Levels of IgG and IgM were below the lower limit of normal in at least half of subjects with serotype AQP4-IgG⁻/MOG-IgG⁺ from 1 year after their first dose of inebilizumab, see **Table 13**.

Table 13: Subjects with serotype AQP4-IgG[−]/MOG-IgG⁺ at baseline and total serum immunoglobulin levels below the lower limit of normal during the study.

	1 year (n = 7)	2 years (n + 6)
IgG	4 (57.1)	3 (50.0)
IgM	6 (85.7)	5 (83.3)

Data are n (%).

^a Thresholds for lower limit of normal were: IgG, 565 mg/dL; IgM, 40 mg/dL.

- 5 AQP4, aquaporin-4; Ig, immunoglobulin; MOG, myelin oligodendrocyte glycoprotein.

- 10 **[0197]** Considering subjects with any exposure to inebilizumab, the safety profile was similar to that seen in the RCP; one subject had several mild (grade 1) infusion-related reactions (**Table 14**). No neutrophil- or lymphocyte-related AEs were reported, and no new safety signals were observed.

Table 14: AEs and AESIs during any exposure to inebilizumab

	Inebilizumab (n = 7)
At least 1 AE	7 (100)
Study drug-related	3 (42.9)
Severity ≥ grade 3	1 (14.3)
At least 1 serious AE	3 (42.9)
Study drug-related	1 (14.3)
At least one AE-related dose interruption	1 (14.3)
At least one AESI	5 (71.4)
Infusion-related reaction	1 (14.3)
Infection	5 (71.4)
Bronchitis	2 (28.6)
Urinary tract infection	2 (28.6)

Data are n (%).

AE, adverse event; AESI adverse event of special interest.

- 15 *Conclusion*

[0198] These *post hoc* analyses from the study suggest that inebilizumab was generally well tolerated and reduces NMOSD attacks in subjects with NMOSD and serotype AQP4-IgG – /MOG-IgG+.

5 **Example 10- Association of B cell Subsets and Aquaporin-4 antibody titers with Disease Activity in Subjects Receiving Inebilizumab Treatment**

[0199] A study was conducted to evaluate B-cell subsets and AQP4 IgG titers in peripheral blood of subjects receiving treatment with Inebilizumab.

Methods

10 [0200] Subjects received 300 mg of Inebilizumab or placebo (PBO), on days 1 and 15 during the randomized controlled period (RCP) and every 6 months in the optional open-label period (OLP). Absolute counts of CD20+ B cells and CD27+ memory B cells were assessed by flow cytometry (RCP + OLP). Plasma cell (PC) gene expression was assessed by qRT-PCR (RCP only). AQP4 IgG titers were determined by cell-based assay (RCP only). All measurements
15 were done in peripheral blood.

Results

[0201] In the placebo group, significant increases in CD20+ B-cells and CD27+ memory B-cells were seen at time of attack relative to the preceding visit ($p < 0.05$). Increases in PC subset were already observed at the preceding visit relative to baseline ($p < 0.01$). During attack, a
20 >2-fold increase from baseline was seen in 4/20 (20%) for CD20+ B-cells, 3/19 (16%) for memory B-cells, and 11/20 (55%) for PC. Inebilizumab significantly decreased all B-cell subsets. No significant increases in any B-cell subsets at time of attack were observed in the Inebilizumab group relative to the preceding visit. Changes in AQP4 IgG titer from baseline to attack were not significantly different between treatment groups ($p = 0.15$). At RCP end, 9/50
25 (18%) of PBO subjects vs 59/159 (37%) of Inebilizumab subjects ($p=0.014$) had ≥ 2 -fold decrease in AQP4-IgG (0% vs 11% ≥ 8 -fold decrease, $p = 0.008$).

[0202] Increased levels of B-cell subsets at time of attack were observed in the placebo group but not Inebilizumab group, particularly in the plasma cell subset. Inebilizumab treatment was associated with reduction in AQP4-IgG in a subset of subjects.

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Example 11- Safety and Efficacy of Inebilizumab in AQP4+ NMOSD Subjects with history of Immunosuppression Treatment

[0203] A study was completed to evaluate long-term outcomes of inebilizumab treatment in AQP4+ NMOSD subjects with a history of immunosuppressant therapy as compared to those without.

Methods

[0204] Subjects previously completed a 28-week randomized phase 2/3 trial of inebilizumab vs placebo, with an optional Open-Label Extension (OLE) (>2 years). Immunosuppressant medication for the prevention or treatment of NMOSD relapses was allowed prior to dosing on Day 1. In this post hoc analysis, AQP4+ subjects who received inebilizumab (through the OLE) were grouped by no history of immunosuppression therapy beyond treatment of acute NMOSD attacks (naïve), or prior azathioprine (AZA) and/or mycophenolate mofetil (MMF) therapy. Outcomes compared for these two groups included annualized relapse and hospitalization rates, as well as safety assessments.

Results

[0205] Among subjects who received inebilizumab during the study, 94 received prior AZA/MMF and 103 were immunosuppressant naïve. The total patient-years of inebilizumab treatment in the prior AZA/MMF group was 300.35 and for immunosuppressant naïve subjects, 335.7. The annualized relapse rate (95% confidence interval [CI]) for subjects with prior AZA/MMF was 0.11 (0.07, 0.17), compared to 0.08 (0.05, 0.14) for naïve. The annualized NMOSD-related inpatient hospitalization rate (annualized rate [95% CI]) for prior AZA/MMF was 0.15 (0.08, 0.27), and 0.12 (0.06, 0.22) for naïve. The percentage of subjects with ≥1 study drug-related treatment-emergent adverse event (TEAE) was 30.9% (29/94) in prior AZA/MMF and 47.6% (49/103) of naïve; 4.3% (4) of prior AZA/MMF and 5.8% (6) of immunosuppressant-naïve reported ≥1 study drug-related serious adverse event. Most adverse events were infection-related for both groups; (72.3% (68/94) for prior AZA/MMF and 77.7% (80/94) for naïve).

[0206] In conclusion, the post hoc analysis evaluating long-term outcomes of inebilizumab in AQP4+ NMOSD subjects treated with prior AZA/MMF therapy demonstrated a similar efficacy and safety profile as subjects without prior immunosuppressant therapy.

CLAIMS

1. A method of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, thereby treating the NMOSD.
2. The method of claim 1, wherein the subject in need has a Fc Gamma Receptor IIIa (*FCGR3A*) gene that comprises a rs396991 polymorphism.
3. The method of claim 2, wherein the subject in need comprises a V allele as determined by a V/F or V/V genotype at amino acid position 158 of the *FCGR3A* gene.
4. A method of treatment, the method comprising determining the presence of a rs396991 polymorphism in a *FCGR3A* gene of a subject in need; and administering Inebilizumab to the subject in need if the determination is positive for the rs396991 polymorphism.
5. The method of claim 4, wherein the subject in need has an autoimmune disease.
6. The method of claim 5, wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
7. The method of claim 6, wherein the autoimmune disease is NMOSD.
8. The method of claim 6, wherein the autoimmune disease is MG.
9. The method of claim 6, wherein the autoimmune disease is IgG4RD.
10. The method of claim 4, wherein the subject in need has at least two autoimmune diseases.
11. The method of claim 10, wherein the at least two autoimmune diseases are selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
12. A method of treating Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering Inebilizumab to a subject in need that comprises a rs396991 polymorphism in the *FCGR3A* gene, wherein the administering is more effective in reducing B-cell or plasma-cell levels in the subject in need that comprises the rs396991 polymorphism of the *FCGR3A* gene as compared to an otherwise comparable subject lacking the rs396991 polymorphism.
13. The method of claim 12, wherein levels of B-cells or plasma-cells are determined at least 1, 2, 4, 8, 12, 16, 22, 28, and/or 32 weeks after the administering.
14. The method of any one of claims 1-13, wherein the administering is effective in reducing a criteria selected from the group consisting of: Annualized Attack Rate

(AAR), incidence of disability as assessed by Expanded Disability Status Scale (EDSS) score, incidence of hospitalization, a number of lesions as detected by magnetic resonance imaging, and combinations thereof.

15. The method of claim 14, wherein the administering is effective in reducing the AAR score, wherein the reduction is at least about 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100%.
16. The method of claim 14, wherein the administering is effective in reducing the number of MRI lesions.
17. The method of claim 16, wherein the reduction is of at least about 20%, 30%, 40%, 50%, 60%, 70%, 73%, 75%, 80%, 85%, 90%, 95%, 97%, or up to about 100% as compared to a comparable un-treated subject.
18. The method of claim 14, wherein the administering is effective in reducing incidence of hospitalization as compared to a comparable un-treated subject.
19. The method of any one of claims 1-18, wherein the administering is repeated.
20. The method of claim 19, wherein the administering is repeated daily, monthly, or yearly.
21. The method of claim 20, wherein the administering is repeated monthly.
22. The method of claim 21, wherein the administering is repeated every 6 months.
23. The method of any one of claims 19-22, wherein the administering is continued for at least about 1 year, 2 years, 3 years, 4 years, 5 years, or up to about the lifetime of the subject in need.
24. The method of any one of claims 1-23, wherein the subject in need was previously administered Inebilizumab.
25. The method of any one of claims 1-24, wherein the administering is intravenous.
26. The method of claim 25, wherein the administering is by intravenous infusion.
27. The method of any one of claims 1-26, wherein the Inebilizumab is administered at a dose of about 300 mg.
28. A method of treating or preventing an autoimmune disease, the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months.
29. The method of claim 28, wherein the administering is repeated every 6 months for at least about 1 year.

30. The method of any one of claims 28-29, wherein the autoimmune disease is selected from the group consisting of: Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
31. The method of claim 30, wherein the autoimmune disease is NMOSD.
- 5 32. The method of claim 30, wherein the autoimmune disease is MG.
33. The method of claim 30, wherein the autoimmune disease is IgG4RD.
34. A method of treating or preventing Neuromyelitis Optica Spectrum Disorder (NMOSD), the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months.
- 10 35. A method of treating or preventing an autoimmune disease, the method comprising administering 300 mg of Inebilizumab to a subject in need that has a level of B-cells of less than about 4 cells/ μ L of blood, wherein the administering is repeated every 6 months, and wherein the autoimmune disease is selected from the group consisting of:
- 15 Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia Gravis (MG), and IgG4RD.
36. The method of any one of claims 34-35, wherein the administering is continued for at least about 2.5 years.
37. A method of treating neuromyelitis optica spectrum disorder, the method comprising administering Inebilizumab to a subject in need, wherein the subject has no anti-aquaporin 4 immunoglobulin G antibodies (AQP4-IgG⁻) and has immunoglobulin G antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG⁺).
- 20 38. The method of claim 37, wherein about 300 mg of the Inebilizumab is administered.
39. The method of any one of claims 37-38, wherein the administration is continued for at least 6 months, 1 year, 2 years, or 3 years.
- 25 40. The method of claim 39, wherein the administration is continued for at least 2 years.
41. The method of any one of claims 1-40, wherein the subject in need has increased serum autoreactivity to an autoantibody that is not related to NMOSD as compared to a healthy control subject.

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