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(71) Applicants: **ARGENX BV** [BE/BE]; Industriepark Zwijnaarde 7, 9052 Ghent (BE). **UNIVERSITÉ DE MONTRÉAL** [CA/CA]; 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **NEW YORK UNIVERISTY** [US/US]; 70 Washington Square South, New York, Massachusetts 10012 (US).

(72) Inventors: **VANHAUWAERT, Roeland**; c/o argenx IIP BV, Zwijnaarde 7, 9052 Ghent (BE). **SILENCE, Karen**; c/o argenx IIP BV, Zwijnaarde 7, 9052 Ghent (BE). **ROBITAILLE, Richard**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **ARBOUR, Danielle**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **RENAUD, Laurence**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **BURDEN, Steven J.**; 295 Millerton Road, Sharon, Massachusetts 06069 (US).

(74) Agent: **PEDANOU, Victoria** et al.; c/o Dechert LLP, One International Place, 40th Floor, 100 Oliver Street, Boston, Massachusetts 02110-2605 (US).

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(54) Title: ANTI-MUSK ANTIBODIES FOR USE IN TREATING NEUROMUSCULAR DISORDERS

(57) Abstract: The present invention relates to an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject. In an embodiment, this antibody or antigen binding fragment thereof is combined with an anticholinergic compound.

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Anti-MuSK Antibodies for Use in Treating Neuromuscular Disorders

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 63/364,685, filed May 13, 2022, and EP Application No. 22154118.8 , filed January 28, 2022, the entire disclosure of which is hereby incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING

This application contains a sequence listing which has been submitted electronically in ST.26 format and is hereby incorporated by reference in its entirety (said ST.26 copy, created January 27, 2023, is named "196198_SL.XML" and is 364,704 bytes in size).

FIELD OF THE INVENTION

The present invention relates to an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder such as ALS (amyotrophic lateral sclerosis) in a human subject. In an embodiment, this antibody or antigen binding fragment thereof is combined with an anticholinergic compound.

BACKGROUND

ALS is an adult-onset non-cell autonomous neuromuscular/neurodegenerative disorder which causes the progressive loss of upper and lower motor neurons (MN), leading to gradual paralysis and death in 2 to 5 years. Neuromuscular junction (NMJ) denervation is a hallmark of ALS [1] and is present in several disease models of ALS [2-6], even preceding the death of MN [1, 3].

The currently approved ALS treatment (Riluzole) benefits only 20% of ALS patients by extending their life for approximately three months. The effect of Riluzole on muscle function is very limited. Moreover, ALS is considered a genetically heterogenous disease likely representing several subgroups with differing underlying pathology. There is currently no cure available, nor will patient-tailored therapies likely be able to aid all ALS patients because of the different underlying disease mechanisms

Therefore, there is still a need for a new therapy for ALS and other diseases that mimic ALS ('ALS-like diseases').

LEGEND TO THE DRAWINGS

Figure 1 *Combo treatment ameliorates locomotor functions in SOD1G37R mice.* ARGX-119 treatment was started at P400 (disease pre-onset or asymptomatic) and darifenacin treatment was started at ~P425 (disease onset) and continued until sacrifice (~P520). A) Diagram depicting the Rotarod apparatus used to

assess motor function, coordination and balance following an acceleration of the rotating wheel. B) Latency to fall (sec) on the Rotarod of ARGX-119 antibody (big gray circle), Combo treatment of ARGX-119+darifenacin treated mice (black triangle), darifenacin-treated mice (small gray circle) versus double placebo-treated mice (gray square) between ~400 until 520 days of age. C) Grip strength meter used to monitor the overall strength of mice forelimbs and hindlimbs. D) Evolution of grip strength measurements during the course of the treatment, from ~P400 to P520 for the groups. E) Evolution of body weight measurements during the course of the treatment, from ~P400 to P520 for the groups. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$. One-way ANOVA and multiple t-test.

Figure 2 *Treatment improves the contractile properties of the EDL muscle.* A) Picture of the set up of the muscle force transducer and the nerve and muscle stimulating electrodes used to evoke muscle contractions. Examples of raw data show a muscle contraction elicited by nerve or muscle stimulation, used to calculate the contractile capacity ratio. B-C) Peak twitch force of the EDL muscle generated by nerve stimulations (B) or muscle stimulation at different frequencies (5Hz-300Hz) (C) from ARGX-119 antibody (gray circle) and Combo treatment of ARGX-119+darifenacin treated mice (black triangle) versus double placebo-treated mice (gray square). D) Histogram showing the mean ± SEM of the contractile capacity ratio expressed in percentage for the EDL muscle, representing the proportion of the peak force generated by nerve stimulation over muscle stimulation (stimulation frequencies between 5Hz-100Hz). E) Histogram showing the mean ± SEM of EDL muscle weight from ARGX-119 antibody, combo-treated and placebo-treated mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Repeated one-way ANOVA and multiple t-test.

Figure 3 *Combo-treatment improves contractile properties of the Soleus muscle. A*-B) Peak twitch force of the Soleus (SOL) muscle generated by nerve stimulations (A) or muscle stimulation at different frequencies (5Hz-300Hz) (B) ARGX-119 antibody (gray circle) and Combo treatment of ARGX-119+darifenacin treated mice (black triangle) versus double placebo-treated mice (gray square). C) Histogram showing the mean ± SEM of the contractile capacity ratio expressed in percentage for the SOL muscle, representing the proportion of the peak force generated by nerve stimulation over muscle stimulation (stimulation frequencies between 5Hz-100Hz). D) Histogram showing the mean ± SEM of EDL muscle weight from ARGX-119 antibody, combo-treated and placebo-treated mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Repeated one-way ANOVA and multiple t-test.

Figure 4 *Combo-treatment preserves muscle fatigue properties.* A) Diagram illustrating the EDL fatigue protocol, which consists of 18 trains of 10 stimulations elicited at 120Hz for 300 ms (1 train per second). Nerve stimulations alone are used 9 out of 10 bouts and muscle stimulation is superimposed to the nerve stimulation every 10 stimulations. The fatigue protocol is followed by a 30-minute recovery period. B-C) Peak contractile force during the fatigue protocol and the recovery period expressed as the percentage of the initial baseline force generated before the fatigue protocol, for nerve stimulation (B) and nerve+muscle (C) of the EDL muscle. Note the higher resistance to fatigue in placebo-treated (gray square) compared to double-treated ARGX-119+darifenacin animals (black triangle) and ARGX-119 antibody-treated (gray circle). This illustrates a significant alteration of the normal fast-twitch muscle properties that is normally highly fatigable. D-E) Peak contractile force during the fatigue protocol and the recovery period expressed

as the percentage of the initial baseline force generated before the fatigue protocol, for nerve stimulation (B) and nerve+muscle (C) of the SOL muscle. *, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Repeated one-way ANOVA and multiple t-test.

DETAILED DESCRIPTION OF THE INVENTION

General Definitions

The following terms or definitions are provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainsview, New York (1989); and Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks, to the general background art referred to above and to the further references cited therein.

As used herein, the singular forms “a”, “an”, and “the”' include both singular and plural referents unless the context clearly dictates otherwise.

The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with 'including', 'includes' or 'containing', 'contains', and are inclusive or open-ended and do not exclude additional, non-recited members, compounds, products, elements or method steps. The expression “essentially consists of” used in the context of a product or a composition (“a product essentially consisting of” or “a composition essentially consisting of”) means that additional molecules may be present but that such molecule does not change/alter the characteristic/activity/functionality of said product or composition. For example, a composition may essentially consist of an antibody or an antibody fragment if the composition as such would exhibit similar characteristic/activity/functionality as one of the antibody or as the one of the antibody fragments.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

The term “about” as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/-5% or

less, more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

The terms 'disorder' and 'disease' are used herein interchangeably.

As used herein, amino acid residues will be indicated either by their full name or according to the standard three-letter or one-letter amino acid code.

As used herein, the terms "polypeptide" or "protein" are used interchangeably, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. A "peptide" is also a polymer of amino acids with a length which is usually of up to 50 amino acids. A polypeptide or peptide is represented by an amino acid sequence.

As used herein, the terms "nucleic acid molecule", "polynucleotide", "polynucleic acid", "nucleic acid" are used interchangeably and refer to polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. A nucleic acid molecule is represented by a nucleic acid sequence, which is primarily characterized by its base sequence. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. Non-limiting examples of polynucleotides include a gene, a gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, control regions, isolated RNA of any sequence, nucleic acid probes, and primers. The nucleic acid molecule may be linear or circular.

As used herein, the term "homology" denotes at least secondary structural identity or similarity between two macromolecules, particularly between two polypeptides or polynucleotides, from same or different taxons, wherein said similarity is due to shared ancestry. Hence, the term 'homologues' denotes so-related macromolecules having said secondary and optionally tertiary structural similarity. For comparing two or more nucleotide sequences, the '(percentage of) sequence identity' between a first nucleotide sequence and a second nucleotide sequence may be calculated using methods known by the person skilled in the art, e.g. by dividing the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence by the total number of nucleotides in the first nucleotide sequence and multiplying by 100% or by using a known computer algorithm for sequence alignment such as NCBI Blast. In determining the degree of sequence similarity between two amino acid sequences, the skilled person may take into account so-called 'conservative' amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Possible

conservative amino acid substitutions have been already exemplified herein. Amino acid sequences and nucleic acid sequences are said to be 'exactly the same' if they have 100% sequence identity over their entire length.

Throughout this application, each time one refers to a specific amino acid sequence SEQ ID NO (take SEQ ID NO: Y as example), one may replace it by: a polypeptide comprising an amino acid sequence that has at least 80% sequence identity or similarity with amino acid sequence SEQ ID NO: Y. Throughout this application, the wording “a sequence is at least X% identical with another sequence” may be replaced by “a sequence has at least X% sequence identity with another sequence”.

Each amino acid sequence described herein by virtue of its identity percentage (at least 80%) with a given amino acid sequence respectively has in a further preferred embodiment an identity of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity with the given amino acid sequence respectively. In a preferred embodiment, sequence identity is determined by comparing the whole length of the sequences as identified herein. Each amino acid sequence described herein by virtue of its similarity percentage (at least 80%) with a given amino acid sequence respectively has in a further preferred embodiment a similarity of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more similarity with the given amino acid sequence respectively. In a preferred embodiment, sequence similarity is determined by comparing the whole length of the sequences as identified herein. Unless otherwise indicated herein, identity or similarity with a given SEQ ID NO means identity or similarity based on the full length of said sequence (i.e. over its whole length or as a whole).

“Sequence identity” is herein defined as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. The identity between two amino acid sequences is preferably defined by assessing their identity within a whole SEQ ID NO as identified herein or part thereof. Part thereof may mean at least 50% of the length of the SEQ ID NO, or at least 60%, or at least 70%, or at least 80%, or at least 90%.

In the art, “identity” also means the degree of sequence relatedness between amino acid sequences, as the case may be, as determined by the match between strings of such sequences. “Similarity” between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. “Identity” and “similarity” can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heine, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48:1073 (1988).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

Preferred computer program methods to determine identity and similarity between two sequences include e.g. the GCG program package (Devereux, J., et al., Nucleic Acids Research 12 (1): 387 (1984)), BestFit, FASTA, BLASTN, and BLASTP (Altschul, S. F. et al., J. Mol. Biol. 215:403-410 (1990)), EMBOSS Needle (Madeira, F., et al., Nucleic Acids Research 47(W1): W636-W641 (2019)). The BLAST program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990)). The EMBOSS program is publicly available from EMBL-EBI. The well-known Smith Waterman algorithm may also be used to determine identity. The EMBOSS Needle program is the preferred program used.

Preferred parameters for polypeptide sequence comparison include the following: Algorithm: Needleman and Wunsch, J. Mol. Biol. 48 (3):443-453 (1970); Comparison matrix: BLOSUM62 from Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992); Gap Open Penalty: 10; and Gap Extend Penalty: 0.5. A program useful with these parameters is publicly available as the EMBOSS Needle program from EMBL-EBI. The aforementioned parameters are the default parameters for a Global Pairwise Sequence alignment of proteins (along with no penalty for end gaps).

Preferred parameters for nucleic acid comparison include the following: Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970); Comparison matrix: DNAfull; Gap Open Penalty: 10; Gap Extend Penalty: 0.5. A program useful with these parameters is publicly available as the EMBOSS Needle program from EMBL-EBI. The aforementioned parameters are the default parameters for a Global Pairwise Sequence alignment of nucleotide sequences (along with no penalty for end gaps).

As used herein, the terms “disorder” and “disease” are used interchangeably.

Also provided herein are embodiments wherein any embodiment described herein may be combined with any one or more other embodiments, provided the combination is not mutually exclusive.

Anti-MuSK Antibody or antigen-binding fragment thereof

All antibodies or antigen-binding fragments thereof defined herein are encompassed as such in the present invention. The antibodies or antigen-binding fragments thereof are also for use in the treatment of a neuromuscular disorder in a human subject.

The present invention relates to anti-MuSK antibody-based molecules, including anti-MuSK antibodies, epitope-binding domains thereof, antigen binding fragments thereof and antibody derivatives that are for treating a neuromuscular disease or condition. In an embodiment, the wording “antibody-based molecule” may be replaced by the word “antibody” or by the expression “antibody or a functional fragment thereof” or by the expression “antibody or antigen binding fragment”.

The term “anti-MuSK antibody” may be replaced by the term “MuSK antibody”.

Any anti-MuSK antibody-based molecule including anti-MuSK antibodies, epitope-binding domains thereof, antigen binding fragments thereof and antibody derivatives that is capable of binding muscle-specific

tyrosine protein kinase (MuSK) is encompassed within the present invention. In an embodiment, such anti-MuSK antibody is also able to activate the signaling and/or phosphorylation of MuSK. The invention provides the insight that such antibody-based molecules are useful for the treatment of conditions where a subject is in need of increased MuSK signaling or MuSK phosphorylation, such as neuromuscular disease or conditions. Therefore in a first aspect, there is provided an anti-MuSK antibody or antigen-binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject.

MuSK is a receptor tyrosine kinase that is expressed in skeletal muscle and has a crucial, master role in forming and maintaining neuromuscular synapses (Burden et al., "The Role of MuSK in Synapse Formation and Neuromuscular Disease," Cold Spring Harb. Perspect. Biol. 5:a009167 (2013), which is hereby incorporated by reference in its entirety). MuSK is a single pass, 120kDa transmembrane protein, composed of an extracellular region containing three Ig-like domains and a Frizzled (Fz)-like domain, and an intracellular region containing a juxtamembrane region, a kinase domain and a short cytoplasmic tail (Jennings et al., "Muscle-Specific trk-Related Receptor with a Kringle Domain Defines a Distinct Class of Receptor Tyrosine Kinases," Proc. Natl. Acad. Sci. USA 90:2895-2899 (1993) and Valenzuela et al., "Receptor Tyrosine Kinase Specific for the Skeletal Muscle Lineage: Expression in Embryonicmuscle, at the Neuromuscular Junction, and After Injury," Neuron 15: 573-584 (1995), which are hereby incorporated by reference in their entirety). MuSK phosphorylation is stimulated by agrin, a signal provided by motor neurons. Once activated, MuSK stimulates pathways that (1) cluster and anchor AChRs and additional muscle proteins critical for synaptic transmission, (2) enhance transcription of genes encoding synaptic proteins in muscle 'synaptic nuclei' and (3) promote the production of retrograde signals that promote presynaptic differentiation and attachment of motor nerve terminals to muscle. In the absence of MuSK, neuromuscular synapses fail to form (Burden et al., "The Role of MuSK in Synapse Formation and Neuromuscular Disease," Cold Spring Harb. Perspect. Biol. 5:a009167 (2013), which is hereby incorporated by reference in its entirety). In addition to its role during synapse formation, MuSK is also required to maintain adult synapses, as inhibition of MuSK expression in adult muscle leads to profound defects in presynaptic and postsynaptic differentiation (Kong et al., "Inhibition of Synapse Assembly in Mammalian Muscle in vivo by RNA Interference,"
EMBO Rep 5:183-188 (2004) and Hesser et al., "Synapse Disassembly and Formation of New Synapses in Postnatal Muscle Upon Conditional Inactivation of MuSK," Mol. Cell. Neurosci. 31:470-480 (2006), which are hereby incorporated by reference in their entirety). Consistent with these findings in mice, mutations that impair MuSK kinase activity or inhibit signaling steps downstream from MuSK cause myasthenia (CM), characterized by structurally and functionally defective synapses, leading to muscle weakness and fatigue (Beeson et al., "Dok-7 Mutations Underlie a Neuromuscular Junction Synaptopathy," Science 313:1975-1978 (2006); Muller et al., "Phenotypical Spectrum of DOK7 Mutations in Congenital Myasthenic Syndromes," Brain 130:1497-1506 (2007); and Selcen et al., "A Compensatory Subpopulation of Motor Neurons in a Mouse Model of Amyotrophic Lateral Sclerosis," J. Comp. Neurol. 490:209-219 (2008), which are hereby incorporated by reference in their entirely).

The amino acid sequence of human MuSK has the amino acid sequence of SEQ ID NO: 129 below.
MRELVNIPLVHILTLVAFSGTEKLPKAPVITTPLETVDALVEEVATFMCAVESYPQPEISWTRNKILIKLFDTR
YSIRENGQLLTILSVEDSDDGIYCCTANNGVGGAVESCGALQVKMKPKITRPPINVKIIEGLKAVLPCTTMG
NPKPSVSWIKGDSPLRENSRIAVLESGSLRIHNVQKEDAGQYRCVAKNSLGTAYSKVVKLEVEVFARILRA
PESHNVTFGSFVTLHCTATGIPVPTITWIENGNAVSSGSIQESVKDRVIDSRLQLFITKPGLYTCIATNKHGE
KFSTAKAAATISIAEWSKPQKDNKGYCAQYRGEVCNAVLAKDALVFLNTSYADPEEAQELLVHTAWNELK
VVSPVCRPAAEALLCNHIFQECSPGVVPTPIPICREYCLAVKELFCAKEWLVMEEKTHRGLYRSEMHLLS
VPECSKLPSMHWDPTACARLPHLDYNKENLKTFPPMTSSKPSVDIPNLPSSSSSSFSVSPTYSMTVIISIM
SSFAIFVLLTITTLYCCRRRKQWKNKKRESAAVTLTTLPSELLLDRLHPNPMYQRMPLLLNPKLLSLEYPR
NNIEYVRDIGEGAFGRVFQARAPGLLPYEPFTMVAVKMLKEEASADMQADFQREAALMAEFDNPNIVKLL
GVCAVGKPMCLLFEYMAYGDLNEFLRSMSPHTVCSLSHSDLSMRAQVSSPGPPPLSCAEQLCIARQVAA
GMAYLSERKFVHRDLATRNCLVGENMVVKIADFGLSRNIYSADYYKANENDAIPIRWMPPESIFYNRYTTE
SDVWAYGVVLWEIFSYGLQPYYGMAHEEVIYYVRDGNILSCPENCPVELYNLMRLCWS
KLPADRPSFTSIHRILERMCERAEGTVSV (SEQ ID NO: 129)

In accordance with the present invention, the MuSK antibody-based molecules described herein bind to an epitope within the Frizzled (Fz)-like domain of the MuSK protein. The Fz-like domain of MuSK has the amino acid sequence of SEQ ID NO: 130 as shown below.
DNKGYCAQYRGEVCNAVLAKDALVFLNTSYADPEEAQELLVHTAWNELKVVSPVCRPAAEALLCNHIFQ
ECSPGVVPTPIPICREYCLAVKELFCAKEWLVMEEKTHRGLYRSEMHLLSVPECSKLPSMHWDPTACAR
L (SEQ ID NO: 130)

The term “epitope” as used herein refers to an antigenic determinant capable of being bound to an antibody. Epitopes usually comprise surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former, but not the latter, is lost in the presence of denaturing solvents. An epitope may comprise amino acid residues directly involved in the binding (also called the immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues that are effectively blocked by the specific antigen-binding peptide (in other words, the amino acid residue is within the footprint of the specific antigen-binding peptide). An epitope typically includes at least 3, and more usually, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids in a unique spatial conformation.

In an embodiment, the MuSK antibody or antigen binding fragment, for use according to the invention binds the MuSK Frizzled (Fz) like domain. In an embodiment, the MuSK antibody or antigen binding fragment immunospecifically bind an epitope within the MuSK Fz-like domain sequence of SEQ ID NO: 130 more frequently, more rapidly, with greater duration and/or with greater affinity or avidity than an alternative epitope. In an embodiment, the MuSK antibody-based molecules described herein bind immunospecifically to any 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid residues of SEQ ID NO: 130. The term "affinity", "specific

binding", "binding", "immunospecific binding", "binding activity" or "specific binding activity", as used herein, refers to the degree to which an antibody or an antibody fragment as defined herein binds to an epitope within the MuSK-Fz-like domain sequence of SEQ ID NO:130.

In an embodiment, the MuSK antibody-based molecules as disclosed herein bind to the MuSK Fz-like domain with an affinity corresponding to a KD of about 10-7 M or less. For example, the MuSK antibody-based molecules disclosed herein bind to the MuSK Fz-like domain with an affinity corresponding to a KD of about 10-8 M, of about 10-9 M, of about 10-10 M, of about 10-11 M, of about 10-12 M or less when determined by, for instance, surface plasmon resonance (SPR) technology in a Biacore 3000 instrument (preferably using the antibody as the ligand and MuSK as the analyte). The MuSK antibody-based molecules as disclosed herein bind to the MuSK Fz-like domain with an affinity corresponding to a KD that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., bovine serum albumin, casein, etc.). The amount with which the affinity is lower is dependent on the KD of the antibody, so that when the KD of the antibody is very low (that is, the antibody is highly specific), then the amount with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold. The term “kd” (sec -1 or 1/s), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. The value is also referred to as the koff value. The term “ka” (M-1 x sec-1 or 1/M), as used herein, refers to the association rate constant of a particular antibody-antigen interaction. The term “KD” (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the kd by the ka. The term “KA” (M-1 or 1/M), as used herein, refers to the association equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the ka by the kd.

In an embodiment, the MuSK antibody-based molecules described herein have a pH-dependent binding affinity for MuSK that allows for antibody recycling to enhance antigen binding. For example, in an embodiment, the association rate constant or dissociation rate constant may differ under acidic vs. neutral vs. basic pH conditions. In one embodiment, the MuSK antibody-based molecules described herein have a higher dissociation rate constant under acidic pH conditions, e.g., pH of <7.0, compared to neutral pH conditions, e.g., pH of ~7.0-7.9. In some embodiments, the MuSK antibody-based molecules described herein have a 2-fold to 3-fold higher dissociation rate constant (i.e., decreased binding affinity) at an acidic pH (e.g., pH ~5.5) as compared to a neutral pH. (pH ~7.4). In an embodiment, the MuSK antibody-based molecules bind the MuSK Fz-like domain with a higher affinity at neutral pH conditions than at acidic pH conditions. In other words, in an embodiment, the MuSK antibody-based molecules binds the MuSK Fz-like domain with a higher dissociation rate at acidic pH conditions than under neutral pH conditions. Neutral pH conditions may be defined as being a pH comprised from 7.0 to 7.9. Acidic pH conditions may be defined as being a pH being less than 7.0. Higher may mean at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300% higher. Antibodies having this pH dependent dissociation characteristic dissociate from the antigen after binding and activation but before lysosomal degradation.

Once dissociated, the antibody is transported via the neonatal Fc receptor back into circulation and is released to bind more antigen.

In some embodiments, binding of the MuSK antibodies of the present invention to their respective epitopes within the Fz-like domain activates MuSK signaling. In particular, when the MuSK antibodies of the present invention bind their respective epitope of the MuSK Fz-like domain, this binding induces MuSK phosphorylation and activation. The MuSK antibodies of the present invention induce MuSK phosphorylation by about 50% to about 100% relative to MuSK phosphorylation induced by agrin activation (as measured, e.g., in a C2C12 phosphorylation assay). In an embodiment, the MuSK antibodies of the present invention induce about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% 95% MuSK phosphorylation (relative to MuSK phosphorylation induced by agrin activation). In an embodiment, the MuSK antibody-based molecules of the present invention induce about 90% to about 100% MuSK phosphorylation (relative to MuSK phosphorylation induced by agrin activation), upon MuSK binding. Phosphorylation of MuSK may be assessed using techniques known to the skilled person such as western blotting or a C2C12 myotube phosphorylation assay. Such antibodies activating MuSK signalling (i.e. induction of the dimerization of MuSK, induction of the tyrosine phosphorylation of MuSK) are agonist antibodies.

In some embodiments, the MuSK antibodies of the present invention, i.e., MuSK antibodies that bind to the Fz-domain of MuSK, do not interfere (i.e., do not block, impede, inhibit, or reduce) with natural ligand binding and stimulation of MuSK. In some embodiments, the MuSK antibodies co-stimulate MuSK activation with its natural ligand, i.e., agrin, to produce an additive effect of activation, e.g, MuSK phosphorylation. Thus, in some embodiments, the MuSK antibodies of the present invention potentiate natural MuSK activation, i.e., phosphorylation, induced by natural ligand binding. Such MuSK antibodies are agonist antibodies. In some embodiments, the antibodies of the invention, in combination with the natural ligand, activate MuSK (i.e., MuSK phosphorylation) to >100% of endogenous activation levels such as at least 110%, 130%, 150%, 200% of endogenous activation levels.

Accordingly, in an embodiment, activities of the MuSK antibody-based molecules of the invention include: (i) binding to an epitope of human muscle-specific tyrosine-protein kinase (MuSK), said epitope present in the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 130, wherein said antibody-based molecule induces MuSK phosphorylation upon binding to its epitope, and/or (ii) binding to the MuSK Fz-like domain does not block, impede, or inhibit natural or endogenous MuSK ligand induced phosphorylation, and may potentiate said natural or endogenous MuSK ligand induced phosphorylation, and (iii) binding to the MuSK Fz-like domain occurs with a higher affinity at neutral pH conditions than at acidic pH conditions.

All these features have been further defined herein.

Antibody-based molecules include, without limitation antibodies, full antibodies, epitope binding fragments of whole antibodies, antigen binding fragment of whole antibodies and antibody derivatives. An epitope binding fragment of an antibody can be obtained through the actual fragmenting of a parental antibody (for example, a Fab or (Fab)2 fragment). Alternatively, the epitope binding fragment is an amino acid sequence

that comprises a portion of the amino acid sequence of such parental antibody. As used herein, a molecule is said to be a "derivative" of an antibody (or relevant portion thereof) if it is obtained through the actual chemical modification of a parent antibody or portion thereof, or if it comprises an amino acid sequence that is substantially similar to the amino acid sequence of such parental antibody or relevant portion thereof (for example, differing by less than 30%, less than 20%, less than 10%, or less than 5% from such parental molecule or such relevant portion thereof, or by 10 amino acid residues, or by fewer than 10, 9, 8, 7, 6, 5, 4, 3 or 2 amino acid residues from such parental molecule or relevant portion thereof).

In an embodiment, an antibody-based molecule of the present invention is an intact immunoglobulin or a molecule having an epitope-binding 333 acids encoding such fragments in recombinant cells (see e.g., Evans et al. "Rapid Expression Of An Anti-Human C5 Chimeric Fab Utilizing A Vector That Replicates In COS And 293 Cells," J. Immunol. Meth. 184:123-38 (1995), which is hereby incorporated by reference in its entirety). For example, a chimeric gene encoding a portion of a F(ab')2 fragment could include DNA sequences encoding the CH1 domain and hinge region of the heavy chain, followed by a translational stop codon to yield such a truncated antibody fragment molecule. Suitable fragments capable of binding to a desired epitope may be readily screened for utility in the same manner as an intact antibody.

Antibody derivatives include those molecules that contain at least one epitope-binding domain of an antibody, and are typically formed using recombinant techniques. One exemplary antibody derivative includes a single chain Fv (scFv). A scFv is formed from the two domains of the Fv fragment, the VL and the VH, which may be encoded by separate genes. Such gene sequences or their encoding cDNA are joined, using recombinant methods, by a flexible linker (typically of about 10, 12, 15 or more amino acid residues) that enables them to be made as a single protein chain in which the VL and VH associate to form monovalent epitope-binding molecules (see e.g., Bird et al. "Single-Chain Antigen-Binding Proteins," Science 242:423-426 (1988); and Huston et al. "Protein Engineering Of Antibody Binding Sites: Recovery Of Specific Activity In An Anti-Digoxin Single-Chain Fv Analogue Produced In Escherichia coli," Proc. Natl. Acad. Sci. (U.S.A.) 85:5879-5883 (1988), which are hereby incorporated by reference in their entirety). Alternatively, by employing a flexible linker that is not too short (e.g., not less than about 9 residues) to enable the VL and VH of a different single polypeptide chains to associate together, one can form a bispecific antibody, having binding specificity for two different epitopes.

In another embodiment, the antibody derivative is a divalent or bivalent single-chain variable fragment, engineered by linking two scFvs together either in tandem (i.e., tandem scFv), or such that they dimerize to form a diabody (Holliger et al. "'Diabodies': Small Bivalent And Bispecific Antibody Fragments," Proc. Natl. Acad. Sci. (U.S.A.) 90(14), 6444-8 (1993), which is hereby incorporated by reference in its entirety). In yet another embodiment, the antibody is a triabody, i.e., a trivalent single chain variable fragment, engineered by linking three scFvs together, either in tandem or in a trimer formation to form a triabody. In another embodiment, the antibody is a tetrabody of four single chain variable fragments. In another embodiment, the antibody is a "linear antibody" which is an antibody comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) that form a pair of antigen binding regions (see Zapata et al. Protein Eng.

8(10):1057-1062 (1995), which is hereby incorporated by reference in its entirety). In another embodiment, the antibody derivative is a minibody, consisting of the single-chain Fv regions coupled to the CH3 region (i.e., scFv-CH3).

These and other useful antibody fragments and derivatives in the context of the present invention are discussed further herein. It also should be understood that the term antibody-based molecule, unless specified otherwise, also includes antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, antigen binding fragments and antibody fragments retaining the ability to specifically bind to the antigen (epitope-binding fragments, antigen binding fragments or functional fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques.

An antibody as generated herein may be of any isotype. As used herein, "isotype" refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) that is encoded by heavy chain constant region genes. The choice of isotype typically will be guided by the desired effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) induction. Exemplary isotypes are IgG1, IgG2, IgG3, and IgG4. Particularly useful isotypes of the MuSK antibodies disclosed herein include IgG1 and IgG2.

Either of the human light chain constant regions, kappa or lambda, may be used. If desired, the class of a MuSK antibody of the present invention may be switched by known methods. For example, an antibody of the present invention that was originally IgM may be class switched to an IgG antibody of the present invention. Further, class switching techniques may be used to convert one IgG subclass to another, for instance from IgG1 to IgG2. Thus, the effector function of the antibodies of the present invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses.

In an embodiment, one, two, or more amino acid substitutions are introduced into an IgG constant region Fc region to alter the effector function(s) of the antibody-based molecule. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 238, 239, 243, 265, 267, 268, 292, 297, 300, 318, 320, 322, 327, 328, 329, 330, 331, 332, and 396, numbered according to the EU numbering system (https://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html#notes, and Edelman, G.M. et al., Proc. Natl. Acad. USA, 63, 78-85 (1969). PMID: 5257969), can be replaced with a different amino acid residue such that the antibody-based molecule has an altered affinity for an effector ligand but retains the antigen-binding ability. In an embodiment, the amino acid 234 or 235 has been replaced. In another embodiment, the amino acids 234 and 235 have been replaced. In this context, a preferred amino acid sequence of a human IgG constant Fc region comprises SEQ ID NO:266 or 267. In this context, for example, the amino acids 234 and 235 numbered according to the EU numbering system correspond to amino acids 7 and 8 in SEQ ID NO:266 and 267 (i.e. a human IgG constant Fc region of an antibody-based molecule disclosed herein), or the amino acids 234 and 235 numbered according the EU numbering system correspond to amino acids 238 and 239 in SEQ ID NO:268 and 270 (i.e. a human full length heavy chain of an antibody-based molecule disclosed herein). The positions typically differ, because variable regions

vary in length, which introduces a “delta” between the numberings. In the case depicted above, that delta is 4. Accordingly, the same holds for other amino acid positions identified above (i.e. 236, 237, 238, 239, 243, 265, 267, 268, 292, 297, 300, 318, 320, 322, 327, 328, 329, 330, 331, 332, and 396) numbered according to the EU numbering system when identifying the corresponding positions in SEQ ID NO: 266 or 267 or 268 or 270. Within the application as filed, one can either refer to the position of an amino acid using the EU numbering system or using the actual position in a given Fc region (for example SEQ ID NO: 266 or 267) or in a full length heavy chain (for example SEQ ID NO: 268 or 270).

Accordingly, in an embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 amino acid substitutions are introduced into SEQ ID NO: 266 or 267. In an embodiment, 1, 2, 3, 4 amino acid substitutions are introduced into SEQ ID NO:266 or 267. In an embodiment, 1 or 2 amino acid substitutions are introduced into SEQ ID NO:266 or 267.Accordingly, in an embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 amino acid substitutions are introduced into SEQ ID NO: 266 or 267 and said substitutions are introduced at amino acid positions selected from amino acid residues 234, 235, 236, 237, 239, 243, 267, 292, 297, 300, 318, 320, 322, 328, 330, 332, and 396 numbered according the EU numbering system of said sequence. In an embodiment, 1 or 2 amino acid substitutions are introduced into SEQ ID NO:266 or 267. In an embodiment, the amino acid 234 or 235 numbered according to the EU numbering system of SEQ ID NO: 266 or 267 has been replaced. In another embodiment, the amino acids 234 and 235 numbered according to the EU numbering system of SEQ ID NO: 266 or 267 have been replaced.

The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, each of which is herein incorporated by reference in its entirety. In an embodiment, one or more amino acid substitutions may be introduced into the Fc region of the antibody-based molecule described herein to remove potential glycosylation sites on the Fc region, which may reduce Fc receptor binding (see, e.g., Shields RL et al., (2001) J Biol Chem 276: 6591-604, which is herein incorporated by reference in its entirety). In an embodiment, the binding to an effector ligand is reduced of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or is no longer detectable compared to the binding to the same ligand by the antibody not having any amino acid substitutions into its human IgG constant Fc region.

In a first embodiment, one or more of the following mutations have been introduced into the constant region of the antibody-based molecule described herein (all numbered according to the EU numbering system): an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L

substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; or a P396L substitution.

In a second embodiment, one or more of the following mutations have been introduced into the constant region of the antibody-based molecule described herein (all numbered according to the EU numbering system): an L234A and/or an L235A substitution; an L234A and an L235A substitution; an L234A, an L235A and a P329G substitution; an L234A, an L235A and a G236K substitution; an L234A, an L235A and a G236E substitution; an L234A, an L235A and a G236R substitution; an L234A and a G236R substitution; an L234A, L235S and a G236R substitution; an L234A, L235T and a G236R substitution; an L234D, L235H and a G236R substitution; an L234D, L235K and a G236R substitution; an L234D and a G236R substitution; an L234D, L235Q and a G236R substitution; an L234D, L235S and a G236R substitution; an L234E, L235D and a G236R substitution; an L234E, L235H and a G236R substitution; an L234E, L235I and a G236R substitution; an L234G, L235H and a G236R substitution; an L234G, L235Q and a G236R substitution; an L234G, L235S and a G236R substitution; an L234H, L235I and a G236R substitution; an L234H, L235S and a G236R substitution; an L234K, L235Q and a G236R substitution; an L234K, L235R and a G236R substitution; an L234K, L235S and a G236R substitution; an L234K, L235T and a G236R substitution; an L234K, L235V and a G236R substitution; an L234Q, L235A and a G236R substitution; an L234Q, L235D and a G236R substitution; an L234Q, L235H and a G236R substitution; an L234Q and a G236R substitution; an L234Q, L235Q and a G236R substitution; an L234Q, L235R and a G236R substitution; an L234Q, L235S and a G236R substitution; an L234Q, L235T and a G236R substitution; an L234Q, L235V and a G236R substitution; an L234R, L235D and a G236R substitution; an L234R, L235E and a G236R substitution; an L234R, L235H and a G236R substitution; an L234R, L235I and a G236R substitution; an L234R, L235K and a G236R substitution; an L234R and a G236R substitution; an L234R, L235Q and a G236R substitution; an L234R, L235R and a G236R substitution; an L234R, L235T and a G236R substitution; an L234S, L235E and a G236R substitution; an L234S, L235G and a G236R substitution; an L234S, L235H and a G236R substitution; an L234S, L235I and a G236R substitution; an L234S and a G236R substitution; an L234S, L235R and a G236R substitution; L234S, L235T and a G236R substitution; L234S, L235V and a G236R substitution; an L234T, L235A and a G236R substitution; an L234T, L235D and a G236R, an L234T, L235H and a G236R substitution; an L234T, L235I and a G236R substitution; an L234T, L235K and a G236R substitution; an L234T, L235Q and a G236R substitution; an L234T, L235R and a G236R substitution; an L234T, L235S and a G236R substitution; an L234T, L235T and a G236R substitution; an L234T, L235V and a G236R substitution; a G236R and an L328R substitution; an L234A, an L235A, a G237A, a P238S, an H268A, an A330S and a P331S substitution; an E233P, an L234V, an L235A, a G326 deletion, an A327G, an A330S and a P331S substitution; an L235A and a G236R substitution; an L235S and a G236R substitution.

In a third embodiment, one or more of the following mutations have been introduced into the Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein (all numbered according

to the EU numbering system): an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; or a P396L substitution.

In a fourth embodiment, one or more of the following mutations have been introduced into the Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein (all numbered according to the EU numbering system): an L234A and/or an L235A substitution; an L234A and an L235A substitution; an L234A, an L235A and a P329G substitution; an L234A, an L235A and a G236K substitution; an L234A, an L235A and a G236E substitution; an L234A, an L235A and a G236R substitution; an L234A and a G236R substitution; an L234A, L235S and a G236R substitution; an L234A, L235T and a G236R substitution; an L234D, L235H and a G236R substitution; an L234D, L235K and a G236R substitution; an L234D and a G236R substitution; an L234D, L235Q and a G236R substitution; an L234D, L235S and a G236R substitution; an L234E, L235D and a G236R substitution; an L234E, L235H and a G236R substitution; an L234E, L235I and a G236R substitution; an L234G, L235H and a G236R substitution; an L234G, L235Q and a G236R substitution; an L234G, L235S and a G236R substitution; an L234H, L235I and a G236R substitution; an L234H, L235S and a G236R substitution; an L234K, L235Q and a G236R substitution; an L234K, L235R and a G236R substitution; an L234K, L235S and a G236R substitution; an L234K, L235T and a G236R substitution; an L234K, L235V and a G236R substitution; an L234Q, L235A and a G236R substitution; an L234Q, L235D and a G236R substitution; an L234Q, L235H and a G236R substitution; an L234Q and a G236R substitution; an L234Q, L235Q and a G236R substitution; an L234Q, L235R and a G236R substitution; an L234Q, L235S and a G236R substitution; an L234Q, L235T and a G236R substitution; an L234Q, L235V and a G236R substitution; an L234R, L235D and a G236R substitution; an L234R, L235E and a G236R substitution; an L234R, L235H and a G236R substitution; an L234R, L235I and a G236R substitution; an L234R, L235K and a G236R substitution; an L234R and a G236R substitution; an L234R, L235Q and a G236R substitution; an L234R, L235R and a G236R substitution; an L234R, L235T and a G236R substitution; an L234S, L235E and a G236R substitution; an L234S, L235G and a G236R substitution; an L234S, L235H and a G236R substitution; an L234S, L235I and a G236R substitution; an L234S and a G236R substitution; an L234S, L235R and a G236R substitution; L234S, L235T and a G236R substitution; L234S, L235V and a G236R substitution; an L234T, L235A and

a G236R substitution; an L234T, L235D and a G236R, an L234T, L235H and a G236R substitution; an L234T, L235I and a G236R substitution; an L234T, L235K and a G236R substitution; an L234T, L235Q and a G236R substitution; an L234T, L235R and a G236R substitution; an L234T, L235S and a G236R substitution; an L234T, L235T and a G236R substitution; an L234T, L235V and a G236R substitution; a G236R and an L328R substitution; an L234A, an L235A, a G237A, a P238S, an H268A, an A330S and a P331S substitution; an E233P, an L234V, an L235A, a G326 deletion, an A327G, an A330S and a P331S substitution; an L235A and a G236R substitution; an L235S and a G236R substitution.

In an embodiment, one or more of the following mutations are introduced into the Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein: an L234A and/or an L235A substitution (numbered according to the EU numbering system). In an embodiment, the following mutations are introduced into the Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein: an L234A and an L235A substitutions numbered according to the EU numbering system. This embodiment results in an antibody-based molecule with a heavy chain represented by SEQ ID NO:268 or 270.
Such an antibody with altered, diminished even abolished effector function is attractive in the context of the invention.

In an embodiment, an anti-MuSK antibody or antigen binding fragment thereof is provided which:
- is an agonist MuSK antibody and/or
- has reduced or eliminated effector function.
This antibody or antigen-binding fragment thereof is preferably for use in the treatment of a neuromuscular disorder in a human subject.

In an embodiment, an anti-MuSK antibody or antigen binding fragment thereof is provided which:
- binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129,
- is an agonist MuSK antibody and
- has reduced or eliminated effector function.
This antibody or antigen-binding fragment thereof is preferably for use in the treatment of a neuromuscular disorder in a human subject.
Reduced or eliminated effector function may be obtained as earlier described herein by introducing mutation in the human IgG constant Fc region. Preferably, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 amino acid substitutions are introduced into said Fc region. Preferably, at least 1, 2, 3, 4, amino acid substitutions are introduced into said Fc region.
Said Fc region may comprise SEQ ID NO: 266 or 267 and said substitutions are introduced at amino acid positions selected from amino acid residues 234, 235, 236, 237, 238, 239, 243, 265, 267, 268, 292, 297, 300, 318, 320, 322, 327, 328, 329, 330, 331, 332, and 396 numbered according to the EU numbering system of said sequence.

In an embodiment, said Fc region may comprise SEQ ID NO: 266 or 267 and said substitutions are introduced at amino acid positions selected from amino acid residues 234 or 235 of said sequence numbered according to the EU numbering system.

In an embodiment, said Fc region may comprise SEQ ID NO: 266 or 267 and said substitutions are introduced at amino acid positions selected from amino acid residues 234 and 235 numbered according to the EU numbering system of said sequence.

In an embodiment, one or more of the following mutations (all numbered according to the EU numbering system) are introduced into the human IgG constant Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; or a P396L substitution.

In one embodiment, each of the combinations of mutations described earlier in the fourth embodiment of this application in the human IgG constant Fc region of the antibody-based molecule described herein may be made.

In a preferred embodiment, L234A or L235A substitution is introduced into the human IgG constant Fc region of the antibody-based molecule described herein. In a more preferred embodiment, L234A and L235A substitutions are introduced into the human IgG constant Fc region of the antibody-based molecule described herein. This embodiment results in an antibody-based molecule with a heavy chain represented by SEQ ID NO:268 or 270.

In an even more preferred embodiment, said anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

b) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

Within this context, the identity or similarity is of at least 81%, 82%, 83%, 84%, 85%, 86%, 87%. 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

Preferred anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269.

Preferred anti-MuSK antibody or antigen binding fragment thereof, comprises:

c) A full length heavy chain comprising SEQ ID NO: 270 and
d) A full length light chain comprising SEQ ID NO: 271.

In an embodiment, the antibody-based molecules of the present invention are "humanized," particularly if they are to be employed for therapeutic purposes. The term "humanized" refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and a remaining immunoglobulin structure based upon the structure and /or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete non-human antibody variable domains fused to human constant domains, or only the complementarity determining regions (CDRs) of such variable domains grafted to appropriate human framework regions of human variable domains. The framework residues of such humanized molecules may be wild-type (e.g., fully human) or they may be modified to contain one or more amino acid substitutions not found in the human antibody whose sequence has served as the basis for humanization. Humanization lessens or eliminates the likelihood that a constant region of the molecule will act as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A.F. et al. "Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response," Proc. Natl. Acad. Sci. USA 86:4220-4224 (1989), which is hereby incorporated by reference in its entirety). Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions so as to reshape them as closely as possible to human form. The variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the antigens in question and determine binding capability. The CDRs are flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from non-human antibody onto the FRs present in the human antibody to be modified. Suitable methods for humanizing the non-human antibody described herein are known in the art see e.g., Sato, K. et al., Cancer Res 53:851-856 (1993); Riechmann, L. et al., "Reshaping Human Antibodies for Therapy," Nature 332:323-327 (1988); Verhoeyen, M. et al., "Reshaping Human Antibodies: Grafting An Antilysozyme Activity," Science 239:1534-1536 (1988); Kettleborough, C. A. et al., "Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation," Protein Engineering 4:773-3783 (1991); Maeda, H. et al., "Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity," Human

Antibodies Hybridoma 2:124-134 (1991); Gorman, S. D. et al., "Reshaping A Therapeutic CD4 Antibody," Proc. Natl. Acad. Sci. USA 88:4181-4185 (1991); Tempest, P.R. et al., "Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection In Vivo," Bio/Technology 9:266-271 (1991); Co, M. S. et al., "Humanized Antibodies For Antiviral Therapy," Proc. Natl. Acad. Sci. USA 88:2869-2873 (1991); Carter, P. et al., "Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy," Proc. Natl. Acad. Sci. USA 89:4285-4289 (1992); and Co, M.S. et al., "Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen," J. Immunol. 148:1149-1154 (1992), which are hereby incorporated by reference in their entirety. In some embodiments, humanized MuSK antibodies of the present invention preserve all CDR sequences (for example, a humanized antibody containing all six CDRs from the llama or mouse antibody). In other embodiments, humanized MuSK antibodies of the present invention have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody. Methods of humanizing an antibody are well-known in the art and suitable for humanizing the antibodies disclosed herein (see, e.g., U.S. Patent No. 5,225,539 to Winter; U.S. Patent Nos. 5,530,101 and 5,585,089 to Queen and Selick; U.S. Patent No. 5,859,205 to Robert et al.; U.S. Patent No. 6,407,213 to Carter; and U.S. Patent No. 6,881,557 to Foote, which are hereby incorporated by reference in their entirety).

In some antibodies only part of a CDR, namely the subset of CDR residues required for binding termed the "specificity determining residues" ("SDRs"), are needed to retain binding of the antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies from regions of Kabat CDRs lying outside Chothia hypervariable loops (see, Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242 (1992); Chothia, C. et al., "Canonical Structures For The Hypervariable Regions Of Immunoglobulins," J. Mol. Biol. 196:901-917 (1987), which are hereby incorporated by reference in their entirety), by molecular modeling and/or empirically, or as described in Gonzales, N.R. et al., "SDR Grafting Of A Murine Antibody Using Multiple Human Germline Templates To Minimize Its Immunogenicity," Mol. Immunol. 41:863-872 (2004), which is hereby incorporated by reference in its entirety. In such humanized antibodies, at positions in which one or more donor CDR residues is absent or in which an entire donor CDR is omitted, the amino acid residue occupying the position can be an amino acid residue occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of non-human amino acids in a humanized antibody and consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Substitutions may also cause changes of activity. Such substitutions causing a significant reduction in activity are also preferably avoided. In this context, the antibody or antibody fragment should still exhibit a detectable activity of the antibody as earlier defined herein or an activity of the antibody at least to some extent. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

Phage display technology can alternatively be used to increase (or decrease) CDR affinity of the antibody-based molecules of the present invention. This technology, referred to as affinity maturation, employs mutagenesis or "CDR walking" and re-selection using the target antigen or an antigenic fragment thereof

to identify antibodies having CDRs that bind with higher (or lower) affinity to the antigen when compared with the initial or parental antibody (see, e.g. Glaser et al., "Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System," J. Immunology 149:3903-3913 (1992), which is hereby incorporated by reference in its entirety). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR from another member of such library and which contain variants potentially representing each possible amino acid substitution for each CDR residue. Mutants with increased (or decreased) binding affinity for the antigen can be screened by contacting the immobilized mutants with labeled antigen. Any screening method known in the art can be used to identify variant antibody-based binding molecules with increased or decreased affinity to the antigen (e.g., ELISA) (See Wu, H. et al., "Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized mAb," Proc. Natl. Acad. Sci. USA 95:6037-6042 (1998); Yelton et al., "Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis," J. Immunology 155:1994 (1995), which are hereby incorporated by reference in their entirety). CDR walking, which randomizes the light chain may be used (see, Schier, R. et al., "Isolation Of Picomolar Affinity Anti-c-erbB-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site," J. Mol. Biol. 263:551-567 (1996), which is hereby incorporated by reference in its entirety).

Methods for affinity maturation of the MuSK antibody molecule are described herein and disclosed for example, in Krause, J.C. et al., "An Insertion Mutation That Distorts Antibody Binding Site Architecture Enhances Function of a Human Antibody," MBio. 2(1): e00345-10 (2011); Kuan, C.T. et al., "Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas," Int. J. Cancer 10.1002/ijc.25645 (2010); Hackel, B.J. et al., "Stability And CDR Composition Biases Enrich Binder Functionality Landscapes," J. Mol. Biol. 401(1):84-96 (2010); Montgomery, D.L. et al., "Affinity Maturation And Characterization Of A Human Monoclonal Antibody Against HIV-1 gp41," MAbs 1(5):462-474 (2009); Gustchina, E. et al., "Affinity Maturation By Targeted Diversification Of The CDR-H2 Loop Of A Monoclonal Fab Derived From A Synthetic Naïve Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1 Neutralization Potency And Breadth," Virology 393(1):112-119 (2009); Finlay, W.J. et al., "Affinity Maturation Of A Humanized Rat Antibody For Anti-RAGE Therapy: Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions," J. Mol. Biol. 388(3):541-558 (2009); Bostrom, J. et al., "Improving Antibody Binding Affinity And Specificity For Therapeutic Development," Methods Mol. Biol. 525:353-376 (2009); Steidl, S. et al., "In Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification," Mol. Immunol. 46(1):135-144 (2008); and Barderas, R. et al., "Affinity Maturation Of Antibodies Assisted By In Silico Modeling," Proc. Natl. Acad. Sci. USA 105(26):9029-9034 (2008), which are hereby incorporated by reference in their entirety.

In the context of this application, an amino acid alteration (change or modification) may be an amino acid substitution, addition, deletion or chemical modification.

In an embodiment, the MuSK-antibody based molecule as described herein comprises the amino acid sequence of any one, any two, any three, any four, any five, or any six CDRs as provided in Tables 1 and 2 herein.

In one embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises:

(i) a complementarity-determining region 1 (CDR-H1) comprising an amino acid sequence of any one of SEQ ID NOs: 1-16, 135, 136, 147-149 or a modified amino acid sequence of any one of SEQ ID NOs: 1-16, 135, 136, or 147-149 said modified sequence having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NOs: 1-16, 135, 136 or 147-149;

(ii) a complementarity-determining region 2 (CDR-H2) comprising an amino acid sequence of any one of SEQ ID NOs: 17-32, 137, 138, 150-155 or a modified amino acid sequence of any one of SEQ ID NOs: 17-32, 137, 138, or 150-155 said modified sequences having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NOs: 17-32, 137, 138, or 150-155; and

(iii) a complementarity-determining region 3 (CDR-H3) comprising an amino acid sequence of any one of SEQ ID NOs: 33-48, 139, 140, 156-158, 240-251, or a modified amino acid sequence of any one of SEQ ID NO: 33-48, 139, 140, 156-158, or 240-251, said modified sequence having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NOs: 33-48, 139, 140, 156-158, or 240-251.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (i) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 1 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 1, the CDR-H2 of SEQ ID NO: 17 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:17, and the CDR-H3 of SEQ ID NO: 33 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:33; (ii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 34 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:34; (iii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 3 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:3, the CDR-H2 of SEQ ID NO: 19 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:19, and the CDR-H3 of SEQ ID NO: 35 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:35; (iv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 4 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:4, the CDR-H2 of SEQ ID NO: 20 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:20, and the CDR-H3 of SEQ ID NO: 36 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:36; (v) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 5 or having 1, 2, 3, 4 or 5 amino acid

alterations relative to SEQ ID NO:5, the CDR-H2 of SEQ ID NO: 21 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:21, and the CDR-H3 of SEQ ID NO: 37 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:37; (vi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 6 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:6, the CDR-H2 of SEQ ID NO: 22 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:22, and the CDR-H3 of SEQ ID NO: 38 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:38; (vii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 7 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:7, the CDR-H2 of SEQ ID NO: 23 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:23, and the CDR-H3 of SEQ ID NO: 39 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:39; (viii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 8 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:8, the CDR-H2 of SEQ ID NO: 24 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:24, and the CDR-H3 of SEQ ID NO: 40 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:40; (ix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 9 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:9, the CDR-H2 of SEQ ID NO: 25 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:25, and the CDR-H3 of SEQ ID NO: 41 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:41; (x) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 10 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:10, the CDR-H2 of SEQ ID NO: 26 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:26, and the CDR-H3 of SEQ ID NO: 42 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:42; (xi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 11 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:11, the CDR-H2 of SEQ ID NO: 27 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:27, and the CDR-H3 of SEQ ID NO: 43 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:43; (xii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 12 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:12, the CDR-H2 of SEQ ID NO: 28 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:28, and the CDR-H3 of SEQ ID NO: 44 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:44; (xiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 13 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:13, the CDR-H2 of SEQ ID NO: 29 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:29, and the CDR-H3 of SEQ ID NO: 45 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:45; (xiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 14 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:14, the CDR-H2 of SEQ ID NO: 30 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:30, and the CDR-H3 of SEQ ID NO: 46 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:46; (xv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 15 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:15, the CDR-H2 of SEQ ID NO: 31 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:31, and the CDR-H3 of SEQ ID NO: 47 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:47; (xvi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 16 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:16, the CDR-H2 of SEQ ID NO: 32 or having 1, 2, 3, 4 or 5 amino

acid alterations relative to SEQ ID NO:32, and the CDR-H3 of SEQ ID NO: 48 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:48; (xvii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:135, the CDR-H2 of SEQ ID NO: 137 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:137, and the CDR-H3 of SEQ ID NO: 139 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:139; and (xviii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 136 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:136, the CDR-H2 of SEQ ID NO: 138 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:138, and the CDR-H3 of SEQ ID NO: 140 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:140. The sequences of the heavy chain CDRs are provided in Table 1.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (ii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 240 (X2m1) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:240; (ii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 241 (X2m2) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:241; (ii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 242 (X2m3) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:242; (ii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 243 (X2m4) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:243; (ii.e) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 244 (X2m5) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:244; (ii.f) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 245 (X2m6) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:245; (ii.g) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 246 (X2m7) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:246; (ii.h) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:2, the CDR-H2 of SEQ ID NO: 18 or having 1, 2, 3, 4 or

5 amino acid alterations relative to SEQ ID NO:18, and the CDR-H3 of SEQ ID NO: 247 (X2m8) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:247.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (xvii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:135, the CDR-H2 of SEQ ID NO: 137 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:137, and the CDR-H3 of SEQ ID NO: 248 (X17m1) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:248; (xvii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:135, the CDR-H2 of SEQ ID NO: 137 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:137, and the CDR-H3 of SEQ ID NO: 249(X17m2) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:249; (xvii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:135, the CDR-H2 of SEQ ID NO: 137 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:137, and the CDR-H3 of SEQ ID NO: 250 (X17m3) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:250; (xvii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:135, the CDR-H2 of SEQ ID NO: 137 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:137, and the CDR-H3 of SEQ ID NO: 251 (X17m6) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:251.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises: (xix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:147, the CDR-H2 of SEQ ID NO: 150 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:150, and the CDR-H3 of SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156; (xx) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:148, the CDR-H2 of SEQ ID NO: 151 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:151 and the CDR-H3 of SEQ ID NO: 157 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:157; (xxi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:149, the CDR-H2 of SEQ ID NO: 152 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:152, and the CDR-H3 of SEQ ID NO: 158 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:158.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises (xxii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:147, the CDR-H2 of SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:153, and the CDR-H3 of SEQ ID NO:156

(3B2g1m1/3B2g2m1) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156; (xxiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:147, the CDR-H2 of SEQ ID NO: 154 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:154, and the CDR-H3 of SEQ ID NO: 156 (3B2g1m2/3B2g2m2) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156; (xxiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:147, the CDR-H2 of SEQ ID NO: 155 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:155, and the CDR-H3 of SEQ ID NO: 156 (3B2g1m4/3B2g2m4) or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156. The sequences of the heavy chain CDRs are provided in Table 1.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (i) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 1, the CDR-H2 of SEQ ID NO: 17, and the CDR-H3 of SEQ ID NO: 33; (ii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 34; (iii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 3, the CDR-H2 of SEQ ID NO: 19, and the CDR-H3 of SEQ ID NO: 35; (iv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 4, the CDR-H2 of SEQ ID NO: 20, and the CDR-H3 of SEQ ID NO: 36; (v) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 5, the CDR-H2 of SEQ ID NO: 21, and the CDR-H3 of SEQ ID NO: 37; (vi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 6, the CDR-H2 of SEQ ID NO: 22, and the CDR-H3 of SEQ ID NO: 38; (vii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 7, the CDR-H2 of SEQ ID NO: 23, and the CDR-H3 of SEQ ID NO: 39; (viii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 8, the CDR-H2 of SEQ ID NO: 24, and the CDR-H3 of SEQ ID NO: 40; (ix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 9, the CDR-H2 of SEQ ID NO: 25, and the CDR-H3 of SEQ ID NO: 41; (x) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 10, the CDR-H2 of SEQ ID NO: 26, and the CDR-H3 of SEQ ID NO: 42; (xi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 11, the CDR-H2 of SEQ ID NO: 27, and the CDR-H3 of SEQ ID NO: 43; (xii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 12, the CDR-H2 of SEQ ID NO: 28, and the CDR-H3 of SEQ ID NO: 44; (xiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 13, the CDR-H2 of SEQ ID NO: 29, and the CDR-H3 of SEQ ID NO: 45; (xiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 14, the CDR-H2 of SEQ ID NO: 30, and the CDR-H3 of SEQ ID NO: 46; (xv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 15, the CDR-H2 of SEQ ID NO: 31, and the CDR-H3 of SEQ ID NO: 47; (xvi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 16, the CDR-H2 of SEQ ID NO: 32, and the CDR-H3 of SEQ ID NO: 48; (xvii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 139; and (xviii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 136, the CDR-H2 of SEQ ID NO: 138, and the CDR-H3 of SEQ ID NO: 140. The sequences of the heavy chain CDR sequences are provided in Table 1 below.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (ii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 240 (X2m1); (ii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 241 (X2m2); (ii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 242 (X2m3); (ii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 243 (X2m4); (ii.e) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 244 (X2m5); (ii.f) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 245 (X2m6); (ii.g) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 246 (X2m7); (ii.h) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 247 (X2m8).

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises: (xvii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 248 (X17m1); (xvii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 249(X17m2); (xvii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 250 (X17m3); (xvii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 251 (X17m6).

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises: (xix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156; (xx) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157; (xxi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158;

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises (xxii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO:156 (3B2g1m1/3B2g2m1); (xxiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the CDR-H3 of SEQ ID NO: 156 (3B2g1m2/3B2g2m2); (xxiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 155, and the CDR-H3 of SEQ ID NO: 156 (3B2g1m4/3B2g2m4). The sequences of the heavy chain CDR sequences are provided in Table 1 below.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises the CDR-H1 of SEQ ID NO: 147, CDR-H2 of SEQ ID NO: 153 or a CDR-H2 amino acid sequence having at least 0,1,2,3,4, or 5 alterations relative to SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO:156 (3B2g2m1). In an embodiment, the CDR-H2 amino acid sequence has at least 0,1,2,3,4, or 5 alterations relative to SEQ ID NO: 153. In accordance with this embodiment, the CDR-H2 amino acid sequence has at least 0,1,2,3,4, or 5 alterations relative to SEQ ID NO: 153, wherein said alterations are present at residues 1, 2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or any combination thereof.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO:156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1).

In an embodiment, the CDR-H2 of the antibody comprises a proline (P) at position 3, a tryptophan (W) at position 4, and a serine (S) or asparagine (N) at position 5.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain, where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1).

The sequences of the heavy chain CDR sequences are provided in Table 1 below.

mAb/Fab name	HCDR1		HCDR2		HCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
X1	SSSIH	1	SISSSSGSTSYADSVKG	17	KYWSQYYWAHYYGGLDY	33
X2	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGMDY	34
X2m1	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGFDY	240
X2m2	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGLDY	241
X2m3	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGFDY	242

mAb/Fab name	HCDR1		HCDR2		HCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
X2m4	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGLDY	243
X2m5	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGMDY	244
X2m6	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGMDY	245
X2m7	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGFDY	246
X2m8	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGLDY	247
X3	SSSIH	3	SISSSSGYTYYADSVKG	19	SWYEMWMSGYFGFDY	35
X4	SSSIH	4	SISSSSGSTYYADSVKG	20	GEHDYYVFGYLGMDY	36
X5	SSSIH	5	SISSSSGSTSYADSVKG	21	SYTMFYYGGWYGSGYFGMDY	37
X6	SSSIH	6	SISSYSGYTYYADSVKG	22	TYGSYYVSSYTGMDY	38
X7	SSSIH	7	SISSSYSSTYYADSVKG	23	LAGLYHYPGYLGLDY	39
X8	SSSIH	8	SISSSSGSTSYADSVKG	24	SWSYHPWYYHVGWYTGLDY	40
X9	SSSIH	9	SIYSSSGSTYYADSVKG	25	SGGEFYITSYYGMDY	41
X10	SSSIH	10	SISSSYSSTSYADSVKG	26	KYYRWRHNKYQGFDY	42
X11	SSSIH	11	SISSYSGSTYYADSVKG	27	SWGSYYVSGFVGFDY	43
X12	SSSIH	12	YISPSSGYTSYADSVKG	28	QYWVPQWWITQYFGMDY	44
X13	SSSIH	13	SISSSSGSTSYADSVKG	29	SSEHWYTIGYYGIDY	45
X14	SSSIH	14	SISSSSGYTYYADSVKG	30	GSHHWFLWIYSGLDY	46
X15	SSSIH	15	SISSSYGSTSYADSVKG	31	SEGDRYVSGYMGMDY	47
X16	SSSIH	16	SIYSSYGYTSYADSVKG	32	NWGYYMYWGWYYALDY	48
X17	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVFGYLGMDY	139
X17m1	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVSGYLGMDY	248
X17m2	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVYGYLGMDY	249
X17m3	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVSGYLGFDY	250
X17m6	YSSIH	135	SIYSSSGSTYYADSVKG	137	GEHGYYVSGYLGFDY	251
X18	SSSIH	136	SISSSSGYTSYADSVKG	138	KYSKRAYPDYYWRGLDY	140
14D10	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
7G4	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3C4	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3B2	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3G3	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
31G2	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
31B7	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
17H10	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157

mAb/Fab name	HCDR1		HCDR2		HCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
23B6	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
30E1	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
30A11	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
16F11	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
4C11	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7A12	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7G12	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7B8	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
3B2g1m1	DYGMS	147	AIPWSGGSTYYKESVKG	153	RSGRIAFGALDA	156
3B2g1m2	DYGMS	147	AIPGSGGSTYYKESVKG	154	RSGRIAFGALDA	156
3B2g1m4	DYGMS	147	AIPWQGGSTYYKESVKG	155	RSGRIAFGALDA	156
3B2g2m1	DYGMS	147	AIPWSGGSTYYKESVKG	153	RSGRIAFGALDA	156
3B2g2m2	DYGMS	147	AIPGSGGSTYYKESVKG	154	RSGRIAFGALDA	156
3B2g2m4	DYGMS	147	AIPWQGGSTYYKESVKG	155	RSGRIAFGALDA	156

In some embodiments, the MuSK antibody-based molecules as disclosed herein further comprise a light chain variable domain. The light chain variable domain comprises

(i) a complementarity-determining region 1 (CDR-L1) having an amino acid sequence of any one of SEQ ID NOs: 49-64, 141, 142, 159-169, or a modified amino acid sequence of any one of SEQ ID NO: 49-64, 141, 142, or 159-169, said modified sequence having at least 80% sequence identity to any one of SEQ ID NO: 49-64, 141, 142, or 159-169;

(ii) a complementarity-determining region 2 (CDR-L2) having an amino acid sequence of any one of SEQ ID NOs: 65-80, 143, 144, 170-179, or a modified amino acid sequence of any one of SEQ ID NO: 65-80, 143, 144 or 170-179, said modified sequence having at least 80% sequence identity to any one of SEQ ID NO: 65-80, 143, 144 or 170-179; and

(iii) a complementarity-determining region 3 (CDR-L3) having an amino acid sequence of any one of SEQ ID NOs: 81-96, 145, 146, 180-195, or a modified amino acid sequence of any one of SEQ ID NO: 81-96, 145, 146, or 180-195, said modified sequence having at least 80% sequence identity to any one of SEQ ID NO: 81-96, 145, 146 or 180-195.

In some embodiments, the MuSK antibody-based molecules as disclosed herein further comprise a light chain variable domain. The light chain variable domain comprises

(iv) a complementarity-determining region 1 (CDR-L1) having an amino acid sequence of any one of SEQ ID NOs: 49-64, 141, 142, 159-169, or a modified amino acid sequence of any one of SEQ ID NO: 49-64, 141, 142, or 159-169, said modified sequence having 1, 2, 3, 4 or 5 amino acid alterations relative to to any one of SEQ ID NO: 49-64, 141, 142, or 159-169;

(v) a complementarity-determining region 2 (CDR-L2) having an amino acid sequence of any one of SEQ ID NOs: 65-80, 143, 144, 170-179, or a modified amino acid sequence of any one of SEQ ID NO: 65-80, 143, 144 or 170-179, said modified sequence having 1, 2, 3, 4 or 5 amino acid alterations relative to any one of SEQ ID NO: 65-80, 143, 144 or 170-179; and

(vi) a complementarity-determining region 3 (CDR-L3) having an amino acid sequence of any one of SEQ ID NOs: 81-96, 145, 146, 180-195, or a modified amino acid sequence of any one of SEQ ID NO: 81-96, 145, 146, or 180-195, said modified sequence having 1, 2, 3, 4 or 5 amino acid alterations relative to any one of SEQ ID NO: 81-96, 145, 146 or 180-195.

In an embodiment, the light chain variable domain of the MuSK antibody based molecule disclosed herein comprises (i) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 49 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:49, the CDR-L2 of SEQ ID NO: 65 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:65, and the CDR-L3 of SEQ ID NO: 81 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:81; (ii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:50, the CDR-L2 of SEQ ID NO: 66 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:66, and the CDR-L3 of SEQ ID NO: 82 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:82; (iii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 51 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:51, the CDR-L2 of SEQ ID NO: 67 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:67, and the CDR-L3 of SEQ ID NO: 83 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:83; (iv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 52 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:52, the CDR-L2 of SEQ ID NO: 68 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:68, and the CDR-L3 of SEQ ID NO: 84 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:84; (v) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 53 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:53, the CDR-L2 of SEQ ID NO: 69 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:69, and the CDR-L3 of SEQ ID NO: 85 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:85; (vi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 54 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:54, the CDR-L2 of SEQ ID NO: 70 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:70, and the CDR-L3 of SEQ ID NO: 86 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:86; (vii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 55 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:55, the CDR-L2 of SEQ ID NO: 71 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:71, and the CDR-L3 of SEQ ID NO: 87 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:87; (viii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 56 or having 1, 2, 3, 4 or 5 amino

acid alterations relative to SEQ ID NO:56, the CDR-L2 of SEQ ID NO: 72 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:72, and the CDR-L3 of SEQ ID NO: 88 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:88; (ix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 57 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:57, the CDR-L2 of SEQ ID NO: 73 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:73, and the CDR-L3 of SEQ ID NO: 89 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:89; (x) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 58 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:58, the CDR-L2 of SEQ ID NO: 74 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:74, and the CDR-L3 of SEQ ID NO: 90 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:90; (xi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 59 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:59, the CDR-L2 of SEQ ID NO: 75 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:75, and the CDR-L3 of SEQ ID NO: 91 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:91; (xii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 60 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:60, the CDR-L2 of SEQ ID NO: 76 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:76, and the CDR-L3 of SEQ ID NO: 92 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:92; (xiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 61 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:61, the CDR-L2 of SEQ ID NO: 77 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:77, and the CDR-L3 of SEQ ID NO: 93 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:93; (xiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 62 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:62, the CDR-L2 of SEQ ID NO: 78 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:78, and the CDR-L3 of SEQ ID NO: 94 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:94; (xv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 63 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:63, the CDR-L2 of SEQ ID NO: 79 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:79, and the CDR-L3 of SEQ ID NO: 95 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:95; (xvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 64 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:64, the CDR-L2 of SEQ ID NO: 80 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:80, and the CDR-L3 of SEQ ID NO: 96 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:96; (xvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:141, the CDR-L2 of SEQ ID NO: 143 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:143, and the CDR-L3 of SEQ ID NO: 145 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:145; (xviii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 142 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:142, the CDR-L2 of SEQ ID NO: 144 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:144, and the CDR-L3 of SEQ ID NO: 146 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:146. The sequences of the light chain CDRs are provided in Table 2 below.

In an embodiment, the light chain variable domain of the MuSK antibody based molecule disclosed herein comprises (xix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 170 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:170, and the CDR-L3 of SEQ ID NO: 180 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:180; (xx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 171 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:171, and the CDR-L3 of SEQ ID NO: 181 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:181; (xxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 160 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:160, the CDR-L2 of SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:172, and the CDR-L3 of SEQ ID NO: 182 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:182; (xxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:172, and the CDR-L3 of SEQ ID NO: 183 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:183; (xxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 171 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:171, and the CDR-L3 of SEQ ID NO: 184 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:184; (xxiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 173 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:173, and the CDR-L3 of SEQ ID NO: 185 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:185; (xxv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:159, the CDR-L2 of SEQ ID NO: 173 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:173, and the CDR-L3 of SEQ ID NO: 186 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:186; (xxvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 161 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:161, the CDR-L2 of SEQ ID NO: 174 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:174, and the CDR-L3 of SEQ ID NO: 187 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:187; (xxvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 162 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:162, the CDR-L2 of SEQ ID NO: 174 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:174, and the CDR-L3 of SEQ ID NO: 188 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:188; (xxviii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 163 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:163, the CDR-L2 of SEQ ID NO: 174 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:174, and the CDR-L3 of SEQ ID NO: 188 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:188; (xxix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 164 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:164, the CDR-L2 of SEQ ID NO: 174 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:174, and the CDR-L3 of SEQ ID NO: 189 or

having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:189; (xxx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 165 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:165, the CDR-L2 of SEQ ID NO: 175 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:175, and the CDR-L3 of SEQ ID NO: 190 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:190; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 166 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:166, the CDR-L2 of SEQ ID NO: 176 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:176, and the CDR-L3 of SEQ ID NO: 191 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:191; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 167 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:167, the CDR-L2 of SEQ ID NO: 177 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:177, and the CDR-L3 of SEQ ID NO: 192 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:192; (xxxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 168 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:168, the CDR-L2 of SEQ ID NO: 178 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:178, and the CDR-L3 of SEQ ID NO: 193 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:193; (xxxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 169 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:169, the CDR-L2 of SEQ ID NO: 179 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:179, and the CDR-L3 of SEQ ID NO: 194 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:194.

In an embodiment, the light chain variable domain of the MuSK antibody based molecule disclosed herein comprises (i) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 49, the CDR-L2 of SEQ ID NO: 65, and the CDR-L3 of SEQ ID NO: 81; (ii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82; (iii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 51, the CDR-L2 of SEQ ID NO: 67, and the CDR-L3 of SEQ ID NO: 83; (iv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 52, the CDR-L2 of SEQ ID NO: 68, and the CDR-L3 of SEQ ID NO: 84; (v) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 53, the CDR-L2 of SEQ ID NO: 69, and the CDR-L3 of SEQ ID NO: 85; (vi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 54, the CDR-L2 of SEQ ID NO: 70, and the CDR-L3 of SEQ ID NO: 86; (vii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 55, the CDR-L2 of SEQ ID NO: 71, and the CDR-L3 of SEQ ID NO: 87; (viii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 56, the CDR-L2 of SEQ ID NO: 72, and the CDR-L3 of SEQ ID NO: 88; (ix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 57, the CDR-L2 of SEQ ID NO: 73, and the CDR-L3 of SEQ ID NO: 89; (x) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 58, the CDR-L2 of SEQ ID NO: 74, and the CDR-L3 of SEQ ID NO: 90; (xi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 59, the CDR-L2 of SEQ ID NO: 75, and the CDR-L3 of SEQ ID NO: 91; (xii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 60, the CDR-L2 of SEQ ID NO: 76, and the CDR-L3 of SEQ ID NO: 92; (xiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 61, the CDR-L2 of SEQ ID NO: 77, and the CDR-L3 of SEQ ID NO:

93; (xiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 62, the CDR-L2 of SEQ ID NO: 78, and the CDR-L3 of SEQ ID NO: 94; (xv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 63, the CDR-L2 of SEQ ID NO: 79, and the CDR-L3 of SEQ ID NO: 95; (xvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 64, the CDR-L2 of SEQ ID NO: 80, and the CDR-L3 of SEQ ID NO: 96; (xvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145; (xviii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 142, the CDR-L2 of SEQ ID NO: 144, and the CDR-L3 of SEQ ID NO: 146. The sequences of the light chain CDRs are provided in Table 2 below.

In an embodiment, the light chain variable domain of the MuSK antibody based molecule disclosed herein comprises (xix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 170, and the CDR-L3 of SEQ ID NO: 180; (xx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 181; (xxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 160, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 182; (xxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183; (xxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 184; (xxiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 185; (xxv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 186; (xxvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 161, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 187; (xxvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 162, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188; (xxviii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 163, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188; (xxix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 164, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 189; (xxx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 165, the CDR-L2 of SEQ ID NO: 175, and the CDR-L3 of SEQ ID NO: 190; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 166, the CDR-L2 of SEQ ID NO: 176, and the CDR-L3 of SEQ ID NO: 191; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 167, the CDR-L2 of SEQ ID NO: 177, and the CDR-L3 of SEQ ID NO: 192; (xxxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 168, the CDR-L2 of SEQ ID NO: 178, and the CDR-L3 of SEQ ID NO: 193; (xxxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 169, the CDR-L2 of SEQ ID NO: 179, and the CDR-L3 of SEQ ID NO: 194.

In an embodiment, the light chain variable domain of the MuSK antibody based molecule disclosed herein comprises the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 or a CDR-L3 having 1, 2, 3, 4 or 5 amino acid alterations relative to the amino acid sequence of SEQ ID NO: 195, wherein said alteration is present at residue 1, 2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or any combination thereof.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a light chain variable domain, where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO:159 or having 1, 2, 3, 4 or 5 amino acid alternations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alternations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alternations relative SEQ ID NO:195 (3B2g2m1).

In an embodiment, the CDR-L1, CDR-L2, CDR-L3 amino acid sequence has at least 0, 1, 2, 3, 4 or 5 amino acid alternations relative to SEQ ID NO: 159, 172 or 195 (respectively).

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a light chain variable domain, where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and

a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

The sequences of the light chain CDR sequences are provided in Table 2 below.

mAb/Fab name	LCDR1		LCDR2		LCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
X1	RASQSVSSAVA	49	SASSLYS	65	QQSSSSLIT	81
X2	RASQSVSSAVA	50	SASSLYS	66	QQSGVWLIT	82
X3	RASQSVSSAVA	51	SASSLYS	67	QQSSSSLIT	83
X4	RASQSVSSAVA	52	SASSLYS	68	QQSYKPGALIT	84
X5	RASQSVSSAVA	53	SASSLYS	69	QQSSSSLIT	85
X6	RASQSVSSAVA	54	SASSLYS	70	QQSSSSLIT	86
X7	RASQSVSSAVA	55	SASSLYS	71	QQSSRSSLLT	87
X8	RASQSVSSAVA	56	SASSLYS	72	QQSSSSLIT	88
X9	RASQSVSSAVA	57	SASSLYS	73	QQSSSSLIT	89
X10	RASQSVSSAVA	58	SASSLYS	74	QQSLWYPVT	90
X11	RASQSVSSAVA	59	SASSLYS	75	QQNSYYLIT	91
X12	RASQSVSSAVA	60	SASSLYS	76	QQSSSSLIT	92
X13	RASQSVSSAVA	61	SASSLYS	77	QQSYGSFSLIT	93
X14	RASQSVSSAVA	62	SASSLYS	78	QQGSYHLIT	94
X15	RASQSVSSAVA	63	SASSLYS	79	QQSGVWLIT	95
X16	RASQSVSSAVA	64	SASSLYS	80	QQWSSAQALIT	96
X17	RASQSVSSAVA	141	SASSLYS	143	QQSYKPGALIT	145
X18	RASQSVSSAVA	142	SASSLYS	144	QQSYWWPIT	146

mAb/Fab name	LCDR1		LCDR2		LCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
14D10	GLSSGSVTSSNYPD	159	TTNSRHS	170	ALYMGGGSNVYV	180
7G4	GLSSGSVTSSNYPD	159	STNSRHS	171	ALYMGRGSNKDYV	181
3C4	GLSSGSVTASNYPD	160	STDSRHS	172	ALYMYSDSKLYV	182
3B2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3G3	GLSSGSVTSSNYPD	159	STNSRHS	171	ALYMGSDIRNYV	184
31G2	GLSSGSVTSSNYPD	159	STNSRLS	173	ALYMGSGSRNYV	185
31B7	GLSSGSVTSSNYPD	159	STNSRLS	173	ALYMGSESRNYV	186
17H10	GGNRIGGKSVQ	161	ADSRRPS	174	HVWGSTASAD	187
23B6	GGDNIGSKNAQ	162	ADSRRPS	174	HVWDSSTNAW	188
30E1	GGDNIGSKNTQ	163	ADSRRPS	174	HVWDSSTNAW	188
30A11	GGDNIASKNVQ	164	ADSRRPS	174	QVWDSSTNVAV	189
16F11	KSSQSVVFGSNQKSYLN	165	YASTQES	175	QQAYSAPT	190
4C11	RSSQSVLYSSNQKNYLN	166	WASARES	176	QQSYKPPYG	191
7A12	ESSQSVLYNQKNYLN	167	WASTRQS	177	QQAYNAPLT	192
7G12	KSSQRVQLGSNQKSYLN	168	YASTQQS	178	QQGYSAPFT	193
7B8	KSSQSVLYNQKNYLA	169	WASTRES	179	QQGYSVPYT	194
3B2g1m1	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g1m2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g1m4	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g2m1	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195
3B2g2m2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195
3B2g2m4	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195

Suitable amino acid modifications to the heavy chain CDR sequences and/or the light chain CDR sequences of the MuSK antibody-based molecule disclosed herein include, for example, conservative substitutions or functionally equivalent amino acid residue substitutions that result in variant CDR sequences having similar or enhanced binding characteristics to those of the CDR sequences disclosed herein as described above. Encompassed by the present invention are CDRs of Tables 1 and 2 containing 1, 2, 3, 4, 5, or more amino acid alterations (depending on the length of the CDR) that maintain or enhance MuSK binding of the antibody. Suitable amino acid modifications to the heavy chain CDR sequences of Table 1 and/or the light chain CDR sequences of Tables 1 and 2 include, for example, conservative substitutions or functionally equivalent amino acid residue substitutions that result in variant CDR sequences having similar or enhanced binding characteristics to those of the CDR sequences of Table 1 and Table 2. Conservative substitutions are those that take place within a family of amino acids that are

related in their side chains. Genetically encoded amino acids can be divided into four families: (1) acidic (aspartate, glutamate); (2) basic (lysine, arginine, histidine); (3) nonpolar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); and (4) uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. Alternatively, the amino acid repertoire can be grouped as (1) acidic (aspartate, glutamate); (2) basic (lysine, arginine histidine), (3) aliphatic (glycine, alanine, valine, leucine, isoleucine, serine, threonine), with serine and threonine optionally grouped separately as aliphatic-hydroxyl; (4) aromatic (phenylalanine, tyrosine, tryptophan); (5) amide (asparagine, glutamine); and (6) sulfur-containing (cysteine and methionine) (Stryer (ed.), Biochemistry, 2nd ed, WH Freeman and Co., 1981, which is hereby incorporated by reference in its entirety). Non-conservative substitutions can also be made to the heavy chain CDR sequences of Table 1 and the light chain CDR sequences of Table 2. Non-conservative substitutions involve substituting one or more amino acid residues of the CDR with one or more amino acid residues from a different class of amino acids to improve or enhance the binding properties of CDR. The amino acid sequences of the heavy chain CDRs of Table 1 and/or the light chain CDRs of Table 2 may further comprise one or more internal neutral amino acid insertions or deletions that maintain or enhance MuSK binding.

In an embodiment, the MuSK antibody-based molecule comprises:

(i) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 1, the CDR-H2 of SEQ ID NO: 17, and the CDR-H3 of SEQ ID NO: 33, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 49, the CDR-L2 of SEQ ID NO: 65, and the CDR-L3 of SEQ ID NO: 81;

(ii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 34, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82;

(iii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 3, the CDR-H2 of SEQ ID NO: 19, and the CDR-H3 of SEQ ID NO: 35, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 51, the CDR-L2 of SEQ ID NO: 67, and the CDR-L3 of SEQ ID NO: 83;

(iv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 4, the CDR-H2 of SEQ ID NO: 20, and the CDR-H3 of SEQ ID NO: 36, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 52, the CDR-L2 of SEQ ID NO: 68, and the CDR-L3 of SEQ ID NO: 84;

(v) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 5, the CDR-H2 of SEQ ID NO: 21, and the CDR-H3 of SEQ ID NO: 37, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 53, the CDR-L2 of SEQ ID NO: 69, and the CDR-L3 of SEQ ID NO: 85;

(vi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 6, the CDR-H2 of SEQ ID NO: 22, and the CDR-H3 of SEQ ID NO: 38, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 54, the CDR-L2 of SEQ ID NO: 70, and the CDR-L3 of SEQ ID NO: 86;

(vii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 7, the CDR-H2 of SEQ ID NO: 23, and the CDR-H3 of SEQ ID NO: 39, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 55, the CDR-L2 of SEQ ID NO:71, and the CDR-L3 of SEQ ID NO: 87;

(viii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 8, the CDR-H2 of SEQ ID NO: 24, and the CDR-H3 of SEQ ID NO: 40, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 56, the CDR-L2 of SEQ ID NO: 72, and the CDR-L3 of SEQ ID NO: 88;

(ix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 9, the CDR-H2 of SEQ ID NO: 25, and the CDR-H3 of SEQ ID NO: 41, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 57, the CDR-L2 of SEQ ID NO: 73, and the CDR-L3 of SEQ ID NO: 89;

(x) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 10, the CDR-H2 of SEQ ID NO: 26, and the CDR-H3 of SEQ ID NO: 42, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 58, the CDR-L2 of SEQ ID NO: 74, and the CDR-L3 of SEQ ID NO: 90;

(xi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 11, the CDR-H2 of SEQ ID NO: 27, and the CDR-H3 of SEQ ID NO: 43, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 59, the CDR-L2 of SEQ ID NO: 75, and the CDR-L3 of SEQ ID NO: 91;

(xii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 12, the CDR-H2 of SEQ ID NO: 28, and the CDR-H3 of SEQ ID NO: 44, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 60, the CDR-L2 of SEQ ID NO: 76, and the CDR-L3 of SEQ ID NO: 92;

(xiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 13, the CDR-H2 of SEQ ID NO: 29, and the CDR-H3 of SEQ ID NO: 45, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 61, the CDR-L2 of SEQ ID NO: 77, and the CDR-L3 of SEQ ID NO: 93;

(xiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 14, the CDR-H2 of SEQ ID NO: 30, and the CDR-H3 of SEQ ID NO: 46, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 62, the CDR-L2 of SEQ ID NO: 78, and the CDR-L3 of SEQ ID NO: 94;

(xv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 15, the CDR-H2 of SEQ ID NO: 31, and the CDR-H3 of SEQ ID NO: 47, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 63, the CDR-L2 of SEQ ID NO: 79, and the CDR-L3 of SEQ ID NO: 95;

(xvi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 16, the CDR-H2 of SEQ ID NO: 32, and the CDR-H3 of SEQ ID NO: 48, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 64, the CDR-L2 of SEQ ID NO: 80, and the CDR-L3 of SEQ ID NO: 96;

(xvii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 139, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145; and

(xviii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 136, the CDR-H2 of SEQ ID NO: 138, and the CDR-H3 of SEQ ID NO: 140, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 142, the CDR-L2 of SEQ ID NO: 144, and the CDR-L3 of SEQ ID NO: 146.

In an embodiment, the MuSK antibody-based molecule comprises:

(ii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 240, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m1);

(ii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 241, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m2);

(ii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 242, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m3);

(ii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 243, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m4);

(ii.e) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 244, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m5);

(ii.f) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 245, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m6);

(ii.g) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 246, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m7);

(ii.f) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 247, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82 (X2m8).

In an embodiment, the MuSK antibody-based molecule comprises:

(xvii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 248, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145 (X17m1);

(xvii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 249, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145 (X17m2);

(xvii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 250, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145 (X17m3);

(xvii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 251, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145 (X17m6).

In an embodiment, the MuSK antibody-based molecule comprises:

(i) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 170, and the CDR-L3 of SEQ ID NO: 180 (14D10);

(ii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 181 (7G4);

(iii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 160, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 182 (3C4);

(iv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2);

(v) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 184 (3G3);

(vi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 185 (31G2);

(vii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 186 (31B7);

(viii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 161, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 187 (17H10);

(ix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 162, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188 (23B6);

(x) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 163, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188 (30E1);

(xi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 164, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 189 (30A11);

(xii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 165, the CDR-L2 of SEQ ID NO: 175, and the CDR-L3 of SEQ ID NO: 190 (16F11);

(xiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 166, the CDR-L2 of SEQ ID NO: 176, and the CDR-L3 of SEQ ID NO: 191 (4C11);

(xiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 167, the CDR-L2 of SEQ ID NO: 177, and the CDR-L3 of SEQ ID NO: 192 (7A12);

(xv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 168, the CDR-L2 of SEQ ID NO: 178, and the CDR-L3 of SEQ ID NO: 193 (7G12);

(xvi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 169, the CDR-L2 of SEQ ID NO: 179, and the CDR-L3 of SEQ ID NO: 194 (7B8);

(xvii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2g1m1);

(xviii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2g1m2);

(xvix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 155, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2g1m4);

(xx) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 (3B2g2m1);

(xxi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 (3B2g2m2); and

(xxii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 155, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 (3B2g2m4)

In a preferred embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain and a light chain variable domain, where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO:156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the CDR-H2 of the antibody comprises a proline (P) at position 3, a tryptophan (W) at position 4, and a serine (S) or asparagine (N) at position 5.

In a more preferred embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain and a light chain variable domain, where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

The MuSK antibody-based molecule as described herein may comprise a variable light (VL) chain, a variable heavy (VH) chain, or a combination of VL and VH chains. In some embodiments, the VH chain of the MuSK antibody-based molecule comprises any one of the VH amino acid sequences provided in Table 3 below, or an amino acid sequence that is at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, identical or similar to any one of the VH amino acid sequences listed in Table 3. In some embodiments, the VL chain of the MuSK antibody-based molecule comprises any one of the VL amino acid sequences provided in Table 3 below, or an amino acid sequence that is at least 60%, identical or similar to any one of the VL amino acid sequences listed in Table

3. In an embodiment, the identity or similarity is at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%.

mAb/Fab name	Domain	Sequence	SEQ ID NO:
X1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKY WSQYYWAHYYGGLDYWGQGTLVTVSS	97
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	98
X2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGMDYWGQGTLVTVSS	99
X2m1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGFDYWGQGTLVTVSS	252
X2m2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGLDYWGQGTLVTVSS	253
X2m3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGFDYWGQGTLVTVSS	254
X2m4	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGLDYWGQGTLVTVSS	255
X2m5	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGMDYWGQGTLVTVSS	256
X2m6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGMDYWGQGTLVTVSS	257
X2m7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGFDYWGQGTLVTVSS	258
X2m8	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGLDYWGQGTLVTVSS	259

mAb/Fab name	Domain	Sequence	SEQ ID NO:
X2	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSGVWLITFGQGTKVEIK	100
X3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVASISSSSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSWYEMWMSGYFGFDYWGQGTLVTVSS	101
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGTKVEIK	102
X4	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVASISSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGEHDYYVFGYLGMDYWGQGTLVTVSS	103
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYKPGALITFGQGTKVEIK	104
X5	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFYSSSIHWVRQAPGKGLEWVASISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSYTMFYYGGWYGSGYFGMDYWGQGTLVTVSS	105
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGTKVEIK	106
X6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVASISSYSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGSYYVSSYTGMDYWGQGTLVTVSS	107
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGTKVEIK	108
X7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTLYSSSIHWVRQAPGKGLEWVASISSSYSSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARLAGLYHYPGYLGLDYWGQGTLVTVSS	109
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSRSSLLTFGQGTKVEIK	110
X8	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVASISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSWSYHPWYYHVGWYTGLDYWGQGTLVTVSS	111
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGTKVEIK	112

mAb/Fab name	Domain	Sequence	SEQ ID NO:
X9	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSG GEFYITSYYGMDYWGQGTLVTVSS	113
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	114
X10	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSYSSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKY YRWRHNKYQGFDYWGQGTLVTVSS	115
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSLWYPVTFGQG TKVEIK	116
X11	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSWG SYYVSGFVGFDYWGQGTLVTVSS	117
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQNSYYLITFGQGT KVEIK	118
X12	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAY ISPSSGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARQYW VPQWWITQYFGMDYWGQGTLVTVSS	119
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	120
X13	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSSE HWYTIGYYGIDYWGQGTLVTVSS	121
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYGSFSLITFGQ GTKVEIK	122
X14	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGS HHWFLWIYSGLDYWGQGTLVTVSS	123
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQGSYHLITFGQGT KVEIK	124
X15	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGMDYWGQGTLVTVSS	125

mAb/Fab name	Domain	Sequence	SEQ ID NO:
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSGVWLITFGQGTKVEIK	126
X16	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVASIYSSYGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARNWGYYMYWGWYYALDYWGQGTLVTVSS	127
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQWSSAQALITFGQGTKVEIK	128
X17	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVASIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDHGYYVFGYLGMDYWGQGTLVTVSS	131
X17m1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVASIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDHGYYVSGYLGMDYWGQGTLVTVSS	260
X17m2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVASIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDHGYYVYGYLGMDYWGQGTLVTVSS	261
X17m3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVASIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDHGYYVSGYLGFDYWGQGTLVTVSS	262
X17m6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVASIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGEHGYYVSGYLGFDYWGQGTLVTVSS	263
X17	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYKPGALITFGQGTKVEIK	132
X18	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVASISSSSGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKYSKRAYPDYYWRGLDYWGQGTLVTVSS	133
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYWWPITFGQGTKVEIK	134
14D10	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKRSGRIAFGALDAWGQGTLVTVSS	196
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLIYTTNSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMGGGSNVYVFGGGTKLTVL	197

mAb/Fab name	Domain	Sequence	SEQ ID NO:
7G4	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	198
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMGRGSNK DYVFGGGTKLTVL	199
3C4	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	200
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTASNYPDWYQQTPGQAPRGLI YSTDSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMYSDSKLY VFGGGTKLTVL	201
3B2	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	202
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRGLI YSTDSRHSGVPSRFSGSISGNKAALTITGAQSEDEADYYCGLYMYSGSKN YVFGGGTKLTVL	203
3G3	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	204
	VL	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRHSGVPSRFSGSTSGNKAALTITGAQPEDEADYYCALYMGSDIRN YVFGGGTKLTVL	205
31G2	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	206
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRLSGVPSRFSGSFSGNKAALTITGAQPEDEADYYCALYMGSGSRN YVFGGGTKLTVL	207
31B7	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	208
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRLSGVPSRFSGSFSGNKAALTITGAQPEDEADYYCALYMGSESRN YVFGGGTKLTVL	209
17H10	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	210

mAb/Fab name	Domain	Sequence	SEQ ID NO:
	VL	SYELTQSPSVSVALRQTAKITCGGNRIGGKSVQWYQQKPGQAPMLVIYAD SRRPSGIPERFTGSNSGNTATLTITGAQAEDEADYYCHVWGSTASADFGG GTHLTVL	211
23B6	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	212
	VL	SYELTQSPSVSVALRQTAKITCGGDNIGSKNAQWYQQKPGQAPVMVLYAD SRRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCHVWDSSTNAWFGG GTHLTVL	213
30E1	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	214
	VL	SYELTQSPSVSVALRRTAKITCGGDNIGSKNTQWYQQKPGQAPVLVIYADS RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCHVWDSSTNAWFGGG THLTVL	215
30A11	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	216
	VL	SYELTQSPSVTVALRQTAKITCGGDNIASKNVQWYQQKPGQAPSLVIWAD SRRPSGIPVRFSGSNFGNTATLTISGAQAEDEADYYCQVWDSSTNVAVFG GGTHLTVL	217
16F11	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	218
	VL	DIVMTQSPSSVTASVGEKVTINCKSSQSVVFGSNQKSYLNWYQQRPGQSP RLLIYYASTQESGIPDRFSGSGSTTDFTLTISSVQPEDAAVYYCQQAYSAPT FGSGTRLEIK	219
4C11	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	220
	VL	DIVMTQSPSSVTASAGERVTINCRSSQSVLYSSNQKNYLNWYQQRLGQSP RLLIYWASARESGVPDRFSGSGSTTNFTLTISSFQPEDAAVYYCQQSYKPP YGFGSGTRLEIK	221
7A12	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	222
	VL	EIVLTQSPSSVTASIGEKVTINCESSQSVLYNQKNYLNWYQQRPGQSPRLLI YWASTRQSGVPDRFSGSGSGSTTDFTLTISSFQPEDVAVYYCQQAYNAPL TFGPGTKVELK	223

mAb/Fab name	Domain	Sequence	SEQ ID NO:
7G12	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	224
	VL	EIVLTQSPNSVTASVGEKVTINCKSSQRVQLGSNQKSYLNWYQQRPGQSP RLLIYYASTQQSGIPDRFSGSGSATDFTLTINSVQPEDAAVYYCQQGYSAP FTFGQGTKVELK	225
7B8	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	226
	VL	EIVLTQSPSSVTASAGEKVTINCKSSQSVLYNQKNYLAWYQQRPGQSPRLL IYWASTRESGVPDRFSGSGSTTDFTLTISSFQPEDVAVYYCQQGYSVPYTF GSGTRLEIK	227
3B2g1m1	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	228
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	229
3B2g1m2	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPGSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	230
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	231
3B2g1m4	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWQGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	232
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	233
3B2g2m1	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	234
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	235
3B2g2m2	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPGSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	236

mAb/Fab name	Domain	Sequence	SEQ ID NO:
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	237
3B2g2m4	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWQGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	238
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	239

In an embodiment, the MuSK antibody-based molecule disclosed herein comprises: a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 97 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 98; (ii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to any one of SEQ ID NOs: 99 and 252-259 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 100; (iii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 101 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 102; (iv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 103 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 104; (v) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 105 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 106; (vi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 107 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 108; or (vii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 109 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 110; (viii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 111 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 112; (ix) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 113 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 114; (x) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 115 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 116; (xi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 117 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 118; (xii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 119 and a light chain variable domain comprising an amino acid sequence

that is at least 80% identical to SEQ ID NO: 120; (xiii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 121 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 122; (xiv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 123 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 124; (xv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 125 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 126; (xvi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 127 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 128; (xvii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to any one of SEQ ID NOs: 131 and 260-263 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 132; and (xviii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 133 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 134.

In some embodiments, the MuSK antibody-based molecule disclosed herein comprises: (i) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 196 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 197; (ii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 198 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 199; (iii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 200 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 201; (iv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 202 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 203; (v) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 204 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 205; (vi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 206 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 207; (vii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 208 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 209; (viii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 210 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 211; (vix) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 212 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 213; (x) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 214 and a light chain variable domain comprising an amino

acid sequence that is at least 80% identical to SEQ ID NO: 215; (xi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 216 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 217; (xii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 218 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 219; (xiii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 220 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 221; (xiv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 222 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 223; (xv) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 224 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 225; (xvi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 226 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 227; (xvii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 228 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 229; (xviii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 230 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 231; (xix) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 232 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 233; (xx) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 234 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 235; (xxi) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 236 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 237; (xxii) a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 238 and a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 239.

In a preferred embodiment, the MuSK antibody-based molecule (or the anti-MuSK antibody or antigen binding fragment thereof) disclosed herein comprises

a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the MuSK antibody-based molecule disclosed herein comprises

a heavy chain variable domain comprising amino acid sequence SEQ ID NO: 234 and a light chain variable domain comprising amino acid sequence SEQ ID NO: 235.

In a preferred embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In a more preferred embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a

light chain variable domain, where the wild-type human IgG constant Fc region comprising at least 80% sequence identity to SEQ ID NO: 266 or 267, where the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the CDR-H2 of the antibody comprises a proline (P) at position 3, a tryptophan (W) at position 4, and a serine (S) or asparagine (N) at position 5.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, where the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the CDR-H2 of the antibody comprises a proline (P) at position 3, a tryptophan (W) at position 4, and a serine (S) or asparagine (N) at position 5.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, where the heavy chain variable domain comprising SEQ ID NO: 234 and the light chain variable domain comprising SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the CDR-H2 of the antibody comprises a proline (P) at position 3, a tryptophan (W) at position 4, and a serine (S) or asparagine (N) at position 5.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO:

266 or 267, where the heavy chain variable domain comprising SEQ ID NO: 234 and the light chain variable domain comprising SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain,

where the wild-type human IgG constant Fc region comprising at least 80% sequence identity to SEQ ID NO: 266 or 267, wherein a), wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A, and

where the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain,

where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, wherein L234A and/or L235A substitution(s) is(are) numbered according the EU numbering system introduced into said Fc region, and

where the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and

- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, wherein L234A and L235A substitutions numbered according the EU numbering system are introduced into said Fc region, and

where the heavy chain variable domain comprising SEQ ID NO: 234 and the light chain variable domain comprising SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 268 and
b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 269, and
c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q

substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 270 and
b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 271, and
c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution;

a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof, comprises:

a) A full length heavy chain comprising SEQ ID NO: 270 and
b) A full length light chain comprising SEQ ID NO: 271, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

Polynucleotides

Another aspect of the present invention is directed to isolated polynucleotides encoding the MuSK antibody-based molecules described herein. In one embodiment, the polynucleotide encoding the MuSK antibody of the present invention comprises a nucleotide sequence encoding any one, any two, any three, any four, any five, or any six of the CDRs described supra, including the heavy chain CDRs of SEQ ID NOs: 1-48, 135-140, 147-158, 240-251 and the light chain CDRs of SEQ ID NOs: 49-96, 141-146, and 159-195. Accordingly, the invention provides a polynucleotide for use in the treatment of a neuromuscular disease in a human subject, which polynucleotide comprises a nucleotide sequence encoding the anti-MuSK antibody or antigen binding fragment or VH, VL or CDRs domain thereof.

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VH domain, where the VH domain comprises (i) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 1, the CDR-H2 of SEQ ID NO: 17, and the CDR-H3 of SEQ ID NO: 33; (ii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 34; (iii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 3, the CDR-H2 of SEQ ID NO: 19, and the CDR-H3 of SEQ ID NO: 35; (iv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 4, the CDR-H2 of SEQ ID NO: 20, and the CDR-H3 of SEQ ID NO: 36; (v) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 5, the CDR-H2 of SEQ ID NO: 21, and the CDR-H3 of SEQ ID NO: 37; (vi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 6, the CDR-H2 of SEQ ID NO: 22, and the CDR-H3 of SEQ ID NO: 38; (vii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 7, the CDR-H2 of SEQ ID NO: 23, and the CDR-H3 of SEQ ID NO: 39; (viii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 8, the CDR-H2 of SEQ ID NO: 24, and the CDR-H3 of SEQ ID NO: 40; (ix) a heavy chain variable domain comprising the CDR-H1 of

SEQ ID NO: 9, the CDR-H2 of SEQ ID NO: 25, and the CDR-H3 of SEQ ID NO: 41; (x) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 10, the CDR-H2 of SEQ ID NO: 26, and the CDR-H3 of SEQ ID NO: 42; (xi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 11, the CDR-H2 of SEQ ID NO: 27, and the CDR-H3 of SEQ ID NO: 43; (xii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 12, the CDR-H2 of SEQ ID NO: 28, and the CDR-H3 of SEQ ID NO: 44; (xiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 13, the CDR-H2 of SEQ ID NO: 29, and the CDR-H3 of SEQ ID NO: 45; (xiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 14, the CDR-H2 of SEQ ID NO: 30, and the CDR-H3 of SEQ ID NO: 46; (xv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 15, the CDR-H2 of SEQ ID NO: 31, and the CDR-H3 of SEQ ID NO: 47; (xvi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 16, the CDR-H2 of SEQ ID NO: 32, and the CDR-H3 of SEQ ID NO: 48; (xvii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 139; and (xviii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 136, the CDR-H2 of SEQ ID NO: 138, and the CDR-H3 of SEQ ID NO: 140.

In some embodiments, the polynucleotide comprises a nucleotide sequence encoding a VH domain, where the VH domain comprises (ii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 240 (X2m1); (ii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 241 (X2m2); (ii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 242 (X2m3); (ii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 243 (X2m4); (ii.e) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 244 (X2m5); (ii.f) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 245 (X2m6); (ii.g) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 246 (X2m7); (ii.h) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 2, the CDR-H2 of SEQ ID NO: 18, and the CDR-H3 of SEQ ID NO: 247 (X2m8).

In some embodiments, the polynucleotide comprises a nucleotide sequence encoding a VH domain, where the VH domain comprises (xvii.a) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 248 (X17m1); (xvii.b) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 249(X17m2); (xvii.c) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 250 (X17m3); (xvii.d) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 135, the CDR-H2 of SEQ ID NO: 137, and the CDR-H3 of SEQ ID NO: 251 (X17m6).

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VH domain, where the VH domain comprises: (xix) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156; (xx) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 148, the CDR-H2 of SEQ ID NO: 151, and the CDR-H3 of SEQ ID NO: 157; (xxi) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 149, the CDR-H2 of SEQ ID NO: 152, and the CDR-H3 of SEQ ID NO: 158.

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VH domain, where the VH domain comprises: (xxii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO:156; (xxiii) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the CDR-H3 of SEQ ID NO: 156; (xxiv) a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 155, and the CDR-H3 of SEQ ID NO: 156.

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VL domain, where the VL domain comprises (i) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 49, the CDR-L2 of SEQ ID NO: 65, and the CDR-L3 of SEQ ID NO: 81; (ii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 50, the CDR-L2 of SEQ ID NO: 66, and the CDR-L3 of SEQ ID NO: 82; (iii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 51, the CDR-L2 of SEQ ID NO: 67, and the CDR-L3 of SEQ ID NO: 83; (iv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 52, the CDR-L2 of SEQ ID NO: 68, and the CDR-L3 of SEQ ID NO: 84; (v) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 53, the CDR-L2 of SEQ ID NO: 69, and the CDR-L3 of SEQ ID NO: 85; (vi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 54, the CDR-L2 of SEQ ID NO: 70, and the CDR-L3 of SEQ ID NO: 86; (vii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 55, the CDR-L2 of SEQ ID NO:71, and the CDR-L3 of SEQ ID NO: 87; (viii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 56, the CDR-L2 of SEQ ID NO: 72, and the CDR-L3 of SEQ ID NO: 88; (ix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 57, the CDR-L2 of SEQ ID NO: 73, and the CDR-L3 of SEQ ID NO: 89; (x) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 58, the CDR-L2 of SEQ ID NO: 74, and the CDR-L3 of SEQ ID NO: 90; (xi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 59, the CDR-L2 of SEQ ID NO: 75, and the CDR-L3 of SEQ ID NO: 91; (xii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 60, the CDR-L2 of SEQ ID NO: 76, and the CDR-L3 of SEQ ID NO: 92; (xiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 61, the CDR-L2 of SEQ ID NO: 77, and the CDR-L3 of SEQ ID NO: 93; (xiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 62, the CDR-L2 of SEQ ID NO: 78, and the CDR-L3 of SEQ ID NO: 94; (xv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 63, the CDR-L2 of SEQ ID NO: 79, and the CDR-L3 of SEQ ID NO: 95; (xvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 64, the CDR-L2 of SEQ ID NO: 80, and the CDR-L3 of SEQ ID NO: 96; (xvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 141, the CDR-L2 of SEQ ID NO: 143, and the CDR-L3 of SEQ ID NO: 145; and (xviii) a light chain variable domain

comprising the CDR-L1 of SEQ ID NO: 142, the CDR-L2 of SEQ ID NO: 144, and the CDR-L3 of SEQ ID NO: 146.

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VL domain, where the VL domain comprises (xix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 170, and the CDR-L3 of SEQ ID NO: 180; (xx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 181; (xxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 160, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 182; (xxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183; (xxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 171, and the CDR-L3 of SEQ ID NO: 184; (xxiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 185; (xxv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 173, and the CDR-L3 of SEQ ID NO: 186; (xxvi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 161, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 187; (xxvii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 162, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188; (xxviii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 163, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 188; (xxix) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 164, the CDR-L2 of SEQ ID NO: 174, and the CDR-L3 of SEQ ID NO: 189; (xxx) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 165, the CDR-L2 of SEQ ID NO: 175, and the CDR-L3 of SEQ ID NO: 190; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 166, the CDR-L2 of SEQ ID NO: 176, and the CDR-L3 of SEQ ID NO: 191; (xxxi) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 167, the CDR-L2 of SEQ ID NO: 177, and the CDR-L3 of SEQ ID NO: 192; (xxxii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 168, the CDR-L2 of SEQ ID NO: 178, and the CDR-L3 of SEQ ID NO: 193; (xxxiii) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 169, the CDR-L2 of SEQ ID NO: 179, and the CDR-L3 of SEQ ID NO: 194.

In an embodiment, the polynucleotide comprises a nucleotide sequence encoding a VL domain, where the VL domain comprises (xxxiv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183; (xxxv) a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195.

In an embodiment, the isolated polynucleotide encoding the MuSK antibody based molecule encodes any one of the VH and/or VL domain sequences as provided in Table 3 infra. The nucleic acid molecules described herein include isolated polynucleotides, portions of expression vectors or portions of linear DNA sequences, including linear DNA sequences used for in vitro transcription/translation, and vectors

compatible with prokaryotic, eukaryotic or filamentous phage expression, secretion, and/or display of the antibodies or binding fragments thereof described herein.

In a preferred embodiment, the polynucleotide comprises a nucleotide sequence encoding a VH that comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234. In another preferred embodiment, the polynucleotide comprises a nucleotide sequence encoding a VL that comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235

In a preferred embodiment, the polynucleotide comprises a nucleotide sequence encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), said molecule comprising a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the polynucleotide comprises a nucleotide sequence encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the polynucleotide comprises or consists of a nucleotide sequence that is at least 80% 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 264. In an even more preferred embodiment, the polynucleotide comprises or consists of SEQ ID NO:264.

In another more preferred embodiment, the polynucleotide comprises or consists of a nucleotide sequence that is at least 80% 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 265. In an even more preferred embodiment, the polynucleotide comprises or consists of SEQ ID NO:265.

In a more preferred embodiment, the polynucleotide comprises a nucleotide sequence encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain is encoded by a nucleotide sequence that is at least 80% identical to SEQ ID NO: 264 and the light chain variable domain is encoded by a nucleotide sequence that is at least 80% identical to SEQ ID NO: 265. In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the polynucleotide comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the polynucleotide comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:277 encoding the heavy chain variable domain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the polynucleotide comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO: 277 encoding the heavy chain variable domain, and

b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the polynucleotide comprises:

a) a nucleotide sequence SEQ ID NO:276 and

b) a nucleotide sequence SEQ ID NO:278.

The polynucleotides of the invention may be produced by chemical synthesis such as solid phase polynucleotide synthesis on an automated polynucleotide synthesizer and assembled into complete single or double stranded molecules. Alternatively, the polynucleotides may be produced by other techniques such a PCR followed by routine cloning. Techniques for producing or obtaining polynucleotides of a given sequence are well known in the art.

The polynucleotides may comprise at least one non-coding sequence, such as a promoter or enhancer sequence, intron, polyadenylation signal, a cis sequence facilitating RepA binding, and the like. The polynucleotide sequences may also comprise additional sequences encoding for example a linker sequence, a marker or a tag sequence, such as a histidine tag or an HA tag to facilitate purification or detection of the protein, a signal sequence, a fusion protein partner such as RepA, Fc portion, or bacteriophage coat protein such as pIX or pIII.

Vector

In another aspect, there is provided a vector (preferably an expression vector) for use in the treatment of a neuromuscular disorder in a human subject comprising the polynucleotide encoding the MuSK antibody-based molecule (or Anti-MuSK antibody or antigen binding fragment thereof) as described herein.

Such vectors include, without limitation, plasmid vectors, viral vectors, including without limitation, vaccina vector, lentiviral vector, adenoviral vector, adeno-associated viral vector, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotides described herein into a given organism or genetic background by any means to facilitate expression of the encoded antibody polypeptide. In one embodiment, the polynucleotide encoding the heavy chain variable

domain, alone or together with the polynucleotide encoding the light chain variable domain as described herein, are combined with sequences of a promoter, a translation initiation segment (e.g., a ribosomal binding sequence and start codon), a 3′ untranslated region, polyadenylation signal, a termination codon, and transcription termination to form one or more expression vector constructs.

In one embodiment, the vector is an adenoviral-associated viral (AAV) vector. A number of therapeutic AAV vectors suitable for delivery of the polynucleotides encoding antibodies described herein to the central nervous system are known in the art. See e.g., Deverman et al., "Gene Therapy for Neurological Disorders: Progress and Prospects," Nature Rev. 17:641-659 (2018), which in hereby incorporated by reference in its entirety. Suitable AAV vectors include serotypes AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or AAV11 in their native form or engineered for enhanced tropism. AAV vectors known to have tropism for the CNS that are particularly suited for therapeutic expression of the MuSK antibodies described herein include, AAV1, AAV2, AAV4, AAV5, AAV8 and AAV9 in their native form or engineered for enhanced tropism. In one embodiment, the AAV vector is an AAV2 vector. In another embodiment, the AAV vector is an AAV5 vector (Vitale et al., "Anti-tau Conformational scFv MC1 Antibody Efficiently Reduces Pathological Tau Species in Adult JNPL3 Mice," Acta Neuropathol. Commun. 6:82 (2018), which is hereby incorporate by reference in its entirety). In another embodiment, the AAV vector is an AAV9 vector (Haiyan et al., "Targeting Root Cause by Systemic scAAV9-hIDS Gene Delivery: Functional Correction and Reversal of Severe MPSII in Mice," Mol. Ther. Methods Clin. Dev. 10:327-340 (2018), which is hereby incorporated by reference in its entirety). In another embodiment, the AAV vector is an AAVrh10 vector (Liu et al., "Vectored Intracerebral Immunizations with the Anti-Tau Monoclonal Antibody PHF1 Markedly Reduces Tau Pathology in Mutant Transgenic Mice," J. Neurosci. 36(49): 12425-35 (2016), which is hereby incorporated by reference in its entirety).

In another embodiment the AAV vector is a hybrid vector comprising the genome of one serotype, e.g., AAV2, and the capsid protein of another serotype, e.g., AAV1 or AAV3-9 to control tropism. See e.g., Broekman et al., "Adeno-associated Virus Vectors Serotyped with AAV8 Capsid are More Efficient than AAV-1 or -2 Serotypes for Widespread Gene Delivery to the Neonatal Mouse Brain," Neuroscience 138:501-510 (2006), which is hereby incorporated by reference in its entirety. In one embodiment, the AAV vector is an AAV2/8 hybrid vector (Ising et al., "AAV-mediated Expression of Anti-Tau ScFv Decreases Tau Accumulation in a Mouse Model of Tauopathy," J. Exp. Med. 214(5):1227 (2017), which is hereby incorporated by reference in its entirety). In another embodiment the AAV vector is an AAV2/9 hybrid vector (Simon et al., "A Rapid Gene Delivery-Based Mouse Model for Early-Stage Alzheimer Disease-Type Tauopathy," J. Neuropath. Exp. Neurol. 72(11): 1062-71 (2013), which is hereby incorporated by reference in its entirety).

In another embodiment, the AAV vector is one that has been engineered or selected for its enhanced CNS transduction after intraparenchymal administration, e.g., AAV-DJ (Grimm et al., J. Viol. 82:5887-5911 (2008), which is hereby incorporated by reference in its entirety); increased transduction of neural stem and

progenitor cells, e.g., SCH9 and AAV4.18 (Murlidharan et al., J. Virol. 89: 3976-3987 (2015) and Ojala et al., Mol. Ther. 26:304-319 (2018), which are hereby incorporated by reference in their entirety); enhanced retrograde transduction, e.g., rAAV2-retro (Muller et al., Nat. Biotechnol. 21:1040-1046 (2003), which is hereby incorporated by reference in its entirety); selective transduction into brain endothelial cells, e.g., AAV-BR1 (Korbelin et al., EMBO Mol. Med. 8: 609-625 (2016), which is hereby incorporated by reference in its entirety); or enhanced transduction of the adult CNS after IV administration, e.g., AAV-PHP.B and AAVPHP.eB (Deverman et al., Nat. Biotechnol. 34: 204-209 (2016) and Chan et al., Nat. Neurosci. 20: 1172-1179 (2017), which are hereby incorporated by reference in their entirety.

In accordance with this embodiment, the expression vector construct encoding the MuSK antibody-based molecule includes the polynucleotide encoding the heavy chain polypeptide, a functional fragment thereof, a variant thereof, or combinations thereof. The expression construct can alternatively include a nucleic acid sequence encoding the light chain polypeptide, a functional fragment thereof, a variant thereof, or combinations thereof. In an embodiment, the expression vector construct includes a nucleic acid sequence encoding the heavy chain polypeptide, a functional fragment thereof, or a variant thereof, and the light chain polypeptide, a functional fragment thereof, or a variant thereof.

In an embodiment, the expression construct further comprises a promoter sequence suitable for driving expression of the MuSK antibody-based molecule. Suitable promoter sequences include, without limitation, the elongation factor 1-alpha promoter (EF1a) promoter, a phosphoglycerate kinase-1 promoter (PGK) promoter, a cytomegalovirus immediate early gene promoter (CMV), a chimeric liver-specific promoter (LSP), a cytomegalovirus enhancer/chicken beta-actin promoter (CAG), a tetracycline responsive promoter (TRE), a transthyretin promoter (TTR), a simian virus 40 promoter (SV40) and a CK6 promoter. Other promoters suitable for driving gene expression in mammalian cells that are known in the art are also suitable for incorporation into the expression constructs disclosed herein.

In an embodiment, the expression construct (or expression vector) further encodes a linker sequence. The linker sequence can encode an amino acid sequence that spatially separates and/or links the one or more components of the expression construct (heavy chain and light chain components of the encoded antibody).

In a preferred embodiment, the expression vector comprises a polynucleotide that encodes an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the expression vector comprises a nucleotide encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and comprising a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the expression vector comprises a polynucleotide encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and comprising:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the expression vector comprises a polynucleotide encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and comprising:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:277 encoding the heavy chain variable domain, and

b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the expression vector comprises a polynucleotide encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and comprising:

c) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO: 277 encoding the heavy chain variable domain, and

d) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the expression vector comprises a polynucleotide encoding an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) and comprising:

a) a nucleotide sequence SEQ ID NO:276 and

b) a nucleotide sequence SEQ ID NO:278.

Host cell

Another aspect of the present invention is a host cell or cell-free expression system for use in the treatment of a neuromuscular disease in a human subject, wherein the cell contains the expression vector encoding the MuSK antibodies (or antigen binding fragment thereof) and optionally producing said MuSK antibodies as described herein.

The MuSK antibody-based molecules described herein can optionally be produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art (see e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2001); Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), which are hereby incorporated by reference in their entirety).

In some embodiments, the host cell chosen for expression may be of mammalian origin. Suitable mammalian host cells include, without limitation, COS-1 cells, COS-7 cells, HEK293 cells, BHK21 cells, CHO cells, BSC-1 cells, HeG2 cells, SP2/0 cells, HeLa cells, mammalian myeloma cells, mammalian lymphoma cells, or any derivative, immortalized or transformed cell thereof. Other suitable host cells include, without limitation, yeast cells, insect cells, and plant cells. Alternatively, the host cell may be selected from a species or organism incapable of glycosylating polypeptides, e.g., a prokaryotic cell or organism, such as BL21, BL21(DE3), BL21-GOLD(DE3), XL1-Blue, JM109, HMS174, HMS174(DE3), and any of the natural or engineered E. coli spp, Klebsiella spp., or Pseudomonas spp strains.

The MuSK antibody-based molecules described herein can be prepared by any of a variety of techniques using the isolated polynucleotides, vectors, and host cells described supra. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies via conventional techniques, or via transfection of antibody genes, heavy chains and/or light chains into suitable bacterial or mammalian cell hosts, in order to allow for the production of antibodies, wherein the antibodies may be recombinant. In an embodiment, the MuSK antibody-based molecule described herein is a monoclonal antibody or functional binding fragment thereof. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Transfecting the host cell can be carried out using a variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., by electroporation, calcium- phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies described herein in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, in particular mammalian cells is sometimes preferable, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

As noted above, exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216-4220 (1980), which is hereby incorporated by reference in its entirety). Other suitable mammalian host cells include, without limitation, NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody described herein. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and

heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies described herein.

The antibodies and antibody binding fragments are recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

In a preferred embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:277 encoding the heavy chain variable domain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises:

a) a nucleotide sequence that is at least 80% identical to SEQ ID NO:276 encoding the full length heavy chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO: 277 encoding the heavy chain variable domain, and
b) a nucleotide sequence that is at least 80% identical to SEQ ID NO:278 encoding the full length light chain, wherein said nucleotide sequence comprising a nucleotide sequence that is at least 80% identical to SEQ ID NO:279 encoding the light chain variable domain.

In an embodiment, the identity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the host cell expresses an antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises:

a) a nucleotide sequence SEQ ID NO:276 and

b) a nucleotide sequence SEQ ID NO:278.

Compositions Comprising MuSK Antibody-Based Molecules

The MuSK antibody-based molecules or polynucleotide encoding the MuSK antibody-based molecules of the present invention may be advantageously administered as compositions.

Therefore in a further aspect, there is provided a composition for use in the treatment of a neuromuscular disorder in a human subject, said composition comprising an antibody or antigen binding fragment, a polynucleotide, an expression vector or a host cell or cell free expression system as defined herein.

In an embodiment, said composition is a pharmaceutical composition. In an embodiment, said pharmaceutical composition comprising at least one pharmaceutically acceptable carrier or excipients.

In an embodiment, such compositions are pharmaceutical compositions comprising an active therapeutic agent (i.e., the MuSK antibody) and one or more of a variety of other pharmaceutically acceptable components. See REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (21st Edition) (2005) (Troy, D.B. et al. (Eds.) Lippincott Williams & Wilkins (Publs.), Baltimore MD), which is hereby incorporated by reference in its entirety. The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically acceptable, non-toxic carriers, excipients, diluents, fillers, salts, buffers, detergents (e.g., a nonionic detergent, such as Tween-20 or Tween- 80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition, and which are vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected to not affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, or non-toxic, nontherapeutic, non-immunogenic stabilizers and the like. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate-buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well-known in the pharmaceutical arts.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated.

The compositions may also include large, slowly metabolized macromolecules, such as proteins, polysaccharides like chitosan, polylactic acids, polyglycolic acids and copolymers (e.g., latex functionalized sepharose, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (e.g., oil droplets or liposomes). Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the active antibody-based molecule of the present invention (e.g., less than a substantial impact (e.g., 10% or less relative inhibition, 5% or less relative inhibition, etc.) on antigen binding).

The pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha- tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

The pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The antibodies of the present invention may be prepared with carriers that will protect the antibodies against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid alone or with a wax, or other materials well-known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In one embodiment, the antibodies of the present invention may be formulated to ensure proper distribution in vivo. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to achieve high drug concentration. The carrier may be an aqueous or non-aqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the

composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For parenteral administration, agents of the present invention are typically formulated as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water, oil, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin. Peanut oil, soybean oil, and mineral oil are all examples of useful materials. In general, glycols, such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Agents of the invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained release of the active ingredient.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles, such as polylactide, polyglycolide, or copolymer, for enhanced adjuvant effect (Langer, et al., Science 249:1527 (1990); Hanes, et al., Advanced Drug Delivery Reviews 28:97-119 (1997), which are hereby incorporated by reference in their entirety). Additional formulations suitable for other modes of administration include oral, intranasal, and pulmonary formulations, suppositories, and transdermal applications.

In an embodiment, the composition comprises the anti-MuSK antibody (or antigen-binding fragment) which comprises a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO: 156, and a light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 (3B2g2m1). In an embodiment, the composition comprises the anti-MuSK antibody (or antigen-binding fragment) which comprises a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 153, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2g1m1). In an embodiment, the anti-MuSK antibody (or antigen-binding fragment) which comprises a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the

CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2g1m2).

In an embodiment, the anti-MuSK antibody (or antigen-binding fragment) which comprises a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 154, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 195 (3B2g2m2).

In an embodiment, the anti-MuSK antibody (or antigen-binding fragment) which comprises a heavy chain variable domain comprising the CDR-H1 of SEQ ID NO: 147, the CDR-H2 of SEQ ID NO: 150, and the CDR-H3 of SEQ ID NO: 156, and the light chain variable domain comprising the CDR-L1 of SEQ ID NO: 159, the CDR-L2 of SEQ ID NO: 172, and the CDR-L3 of SEQ ID NO: 183 (3B2).

In a preferred embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO:156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprises SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK), that comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence

that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) numbered according the EU numbering system is(are) introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) numbered according the EU numbering system is(are) introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 268 and

b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 269, and

c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A

substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the binding to an effector ligand is reduced of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or is no longer detectable compared to the binding to the same ligand by the antibody not having any amino acid substitutions into its human IgG constant Fc region.

In an embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 270 and
b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 271, and
c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the

combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the composition comprises an antibody or antigen-binding fragment that binds to human muscle-specific tyrosine-protein kinase (MuSK) that comprises:

a) A full length heavy chain comprising SEQ ID NO: 270 and
b) A full length light chain comprising SEQ ID NO: 271, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the binding to an effector ligand is reduced of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or is no longer detectable compared to the binding to the same ligand by the antibody not having any amino acid substitutions into its human IgG constant Fc region.

The MuSK antibody based molecules of the present invention can be administered by parenteral, topical, oral or intranasal means for therapeutic treatment. Intramuscular injection (for example, into the arm or leg muscles) and intravenous infusion are preferred methods of administration of the molecules of the present invention. In some methods, such molecules are administered as a sustained release composition or device, such as a Medipad™ device (Elan Pharm. Technologies, Dublin, Ireland). In some methods, the antibodies disclosed herein are injected directly into a particular tissue, for example intracranial injection.
In one embodiment, a pharmaceutical composition of the present invention is administered parenterally. The phrases “parenteral administration” and “administered parenterally” as used herein denote modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intracranial, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection, subcutaneous and infusion. In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

In an embodiment, an anti-MuSK antibody or antigen binding fragment thereof (or a polynucleotide, an expression vector, a host cell, or a composition) for use according to the invention is administered in combination with an anticholinergic compound. An anticholinergic compound is a compound that is able to inhibit the effect of the neurotransmitter acetylcholine at synapses or at neuroeffector junctions such as neuromuscular junctions. Preferably, an anticholinergic compound is a compound that is able to dampen muscarinic acetylcholine receptor activity.
The anticholinergic compound may also be formulated in a composition as the anti-MuSK antibody or antigen-binding fragment. The type of compositions disclosed herein for the anti-MuSK antibody or antigen-binding fragment may also be used for a composition comprising the anticholinergic compound. The two

compounds may be present in a single composition. Alternatively, they may be formulated in separate compositions.

The use of a compound results in activating, inducing a mechanism that promotes NMJ (neuromuscular junction) stability and/or repair is attractive for the treatment of any neuromuscular disease, especially wherein such NMJ is affected. In a preferred embodiment, the use of two different compounds, each activating, inducing a mechanism that promotes NMJ stability and/or repair is even more attractive as it is demonstrated that such combined treatments is synergistic. Therefore, said combination is highly beneficial for the treatment of a neuromuscular disease, especially a neuromuscular disease or disorder with affected NMJ such as ALS.

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof, polynucleotide, expression vector, host cell, or composition for use according to any of the preceding claims, wherein the neuromuscular disorder is characterized by an impaired neuromuscular transmission and/or an NMJ denervation.

An impaired neuromuscular transmission may be characterized by at least one of:

a. muscarinic overexcitability,
b. motor neuron death,
c. NMJ denervation and
d. impaired synaptic transmission

In an embodiment, an impaired neuromuscular transmission or impaired synaptic transmission may be characterized by a deficient MuSK signaling, deficient MuSK dimerization, deficient MuSK phosphorylation, deficient MuSK signaling and/or deficient acetylcholine receptor clustering.

In an embodiment, an impaired neuromuscular transmission or impaired synaptic transmission may be characterized by a poor motor performance, a decreased grip strength, the poor contractile properties of a muscle at the NMJ, the poor resistance to fatigue of the muscle, a decreased muscle weight.

In an embodiment, a neuromuscular disorder is analyzed or assessed or diagnosed via electrophysiological assessment; pharmacodynamic assessment; the level of neurofilaments (e.g. neurofilament light chain (NFL)) in blood serum, plasma and/or cerebrospinal fluid (CSF); or NMJ biopsies.

A neuromuscular disorder may be selected from the group consisting of: amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), myasthenia gravis (MG), congenital myasthenia, Lambert-Eaton myasthenic syndrome (LEMS), Lyme disease, poliomyelitis, post-poliomyelitis, heavy metal intoxication, Kennedy syndrome, adult-onset Tay-Sachs disease, hereditary spastic paraplegia, multifocal neuropathy, cervical spondylosis, extramedullary tumor with compressive radiculopathy and myelopathy, inclusion body myositis, progressive bulbar palsy, progressive muscular atrophy, motor neuron syndrome and thyrotoxic myopathy. A preferred neuromuscular disorder is ALS.

In an embodiment, an anti-MuSK antibody or antigen binding fragment thereof as defined herein may be administrated to an asymptomatic ALS subject. It means that such antibody or antigen binding fragment may be administrated prior to the onset of ALS in said subject. The same applies to other neuromuscular disorders.

In this context, an asymptomatic ALS subject may be a subject which has been diagnosed as being predisposed to develop a neuromuscular disorder or disease as ALS. Identifying an individual (or subject) with a neuromuscular disorder may mean that the identification is carried out using a diagnostic method. Such subject may be a symptomatic subject diagnosed at disease onset or after disease onset, or predisposed to develop a neuromuscular disorder or disease (i.e. an asymptomatic subject diagnosed prior to disease onset which is synonymous with disease pre-onset).

A neuromuscular disorder may be caused by a genetic defect. A genetic defect is caused in whole or in part by a change in the genomic DNA sequence relative to the genomic DNA sequence of a corresponding individual or subject not suffering from said genetic defect. A genetic defect can be caused by a mutation in one gene (monogenic disorder), by mutations in multiple genes (multifactorial inheritance disorder), by a combination of gene mutations and environmental factors, or by damage to chromosomes (changes in the number or structure of entire chromosomes, the structures that carry genes). Types of genetic mutation include base substitutions, deletions and insertions.

In an embodiment, the human subject is identified as having (or as being predisposed to develop) a neuromuscular disease caused by genetic defect. In one embodiment, the neuromuscular disease is ALS, and the genetic defect is in the SOD1 gene. Individuals or human subjects predisposed to develop ALS include those having one or more risk factors for developing ALS, including, growing older, having a personal or family history, or a genetic predisposition of one or more SOD-1 associated diseases. One underlying genetic cause or predisposition for ALS is a mutation(s) in the human SOD1 gene. Accordingly, identification of a subject suffering from or susceptible to or predisposed to develop ALS can be performed by genetic testing of the subject's SOD1 gene using assays known in the art, such as e.g., genetic sequencing. At least 180 mutations in human SOD1 are known in the art to be linked to ALS. In an embodiment, the SOD1 mutation is one or more of the mutations selected from the group consisting of: A4V, H46R, G93S, A4T, G141X, D133A, V148G, N139K, G85R, G93A, V14G, C6S, I113T, D49K, G37R, A89V, E100G, D90A, T137A, E100K, G41A, G41D, G41S, G13R, G72S, L8V, F20C, Q22L, H48R, T54R, S591, V87A, T88deltaTAD, A89T, V97M, S105deltaSL, VI 18L, D124G, LI 14F, D90A, G12R G147R and G37R. In one embodiment, the mutation in the SOD1 gene is G37R.

Accordingly an asymptomatic individual or subject may be identified (prior to disease onset) when said subject has one SOD1 mutation selected from the group consisting of: A4V, H46R, G93S, A4T, G141X, D133A, V148G, N139K, G85R, G93A, V14G, C6S, I113T, D49K, G37R, A89V, E100G, D90A, T137A,

E100K, G41A, G41D, G41S, G13R, G72S, L8V, F20C, Q22L, H48R, T54R, S591, V87A, T88deltaTAD, A89T, V97M, S105deltaSL, VI 18L, D124G, LI 14F, D90A, G12R G147R and G37R.

Analysis of a subject's susceptibility to ALS disease (i.e. asymptomatic subject susceptible to develop ALS) may be performed by analyzing the family history of the subject for ALS. Analysis of the family history may include a three-generation pedigree documenting ALS, a review of medical records and autopsy studies of family members, and identification of an autosomal dominant pattern of SOD1 mutation.

Identification of an individual or subject asymptomatic for ALS (but predisposed to develop such disease) may also be analyzed by an ALS marker. For example, an ALS specific marker may be circulating micro-RNAs, circular RNAs (circRNAs) or messenger RNAs (mRNAs), TDP-42 aggregates, 8-oxo-deoxyguanosine (8-oxodG), 15-F2t-isoprostane (IsoP), plasma TNF-a, IL-10, TRAIL, plasma IL-1b , CSF TRAIL, pro-inflammatory T-helper (Th)17 cells, Th1 cells, anti-inflammatory Th2, regulatory T cells (Treg), pro-inflammatory IL-1b, IL-6, IFN-g, anti-inflammatory IL-10, cholesterol, LDL-cholesterol, apolipoprotein B, HDL-cholesterol, apolipoprotein-AI, plasma creatinine (PCr), plasma ferritin, transferrin, hepcidin, chitotriosidase-1 (CHIT1), chitinase-3-like protein 2 (CHI3L2/YKL39), total tau (tTau), phosphorylated tau (pTau), amyloidb (Ab), novel INHAT repressor (NIR), ubiquitin C-terminal hydrolase-L1 (UCHL1), microtubule-associated protein 2, capping actin protein, gelsolin-like (CAPG), or glycoprotein nonmetastatic melanoma protein B (GPNMB). The human subject may be considered susceptible to ALS disease, when at least one of such marker measurements deviates at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% from a normal human subject at the same age but without ALS. Analysis of a subject's susceptibility to ALS disease may also be analyzed by imaging. For example, such imaging analysis may be an MRI assessment of skeletal muscle, imaging-derived functional muscle scores, or tongue ultrasound predicted bulbar progression combined with or without MRI.

In an embodiment, the anticholinergic compound is administered separately, sequentially, or concurrently with the anti-MuSK antibody or antigen binding fragment thereof, polynucleotide, expression vector, host cell, cell-free expression system or composition.

In an embodiment, the anticholinergic compound is administered at disease onset or within 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days or within 1, 2, 3, 4, 5, 6, 7, weeks; or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months following disease onset. In an embodiment, the anticholinergic compound is administered at disease onset or within one week after disease onset. Surprisingly, attractive results were obtained when the anticholinergic compound was administered at disease onset or as soon as possible after disease onset. It is to be understood by the skilled person that the anticholinergic compound is preferably not used to reduce, diminish a symptom associated with the neuromuscular disorder (such as ALS). In an embodiment, the anticholinergic compound is not used to reduce, diminish urinary urgency. In an embodiment, the anticholinergic compound is not used to reduce or diminish urinary urgency in a neuromuscular disease such as ALS.

In an embodiment, the anticholinergic compound is administered at disease onset, or within 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days; within 1, 2, 3, 4, 5, 6, or 7 weeks; or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months following disease onset, but prior to a diagnosis of the disease. In preferred embodiments, the administration is between diagnosis and 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days; within 1, 2, 3, 4, 5, 6, or 7 weeks; or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months prior to diagnosis.

In an embodiment the anti-MuSK antibody or antigen binding fragment thereof, polynucleotide, expression vector, host cell, or composition is administered pre-onset of the disease or at disease onset. Pre-onset of the disease may mean 1, 2, 3 ,4 ,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days pre-onset of the disease or 1, 2, 3, 4, 5, 6, 7, 8 weeks pre-disease onset or 1, 2, 3 ,4 ,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 months pre-disease onset. In this context, a human subject at pre-onset may mean the human subject is asymptomatic for said neuromuscular disorder such as ALS.

Accordingly, in another aspect of this invention, there is provided an anti-MuSK antibody or antigen binding fragment thereof (polynucleotide, expression vector, host cell, cell-free expression system or composition) for use in the treatment of ALS in a human subject wherein said antibody or antigen binding fragment is administered at pre-onset of the disease, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease. In this context, a human subject at pre-onset may mean the human subject is asymptomatic for ALS. In an embodiment, the subject is diagnosed as being predisposed to develop a neuromuscular disorder or disease, such as ALS.

In an embodiment, the antibody or antigen binding fragment binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129.

In an embodiment, the antibody or antigen binding fragment thereof comprises wild-type human IgG constant Fc region comprising at least 80% sequence identity to SEQ ID NO: 266 or 267.

In an embodiment, the antibody or antigen binding fragment is an agonist MuSK antibody and/or has reduced or eliminated effector function.

In an embodiment, the reduced or eliminated effector function is obtained by introducing one or more of the following mutations (all numbered according to the EU numbering system) into the human IgG constant Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an

L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; or a P396L substitution.

In a preferred embodiment, L234A or L235A substitution is introduced into the human IgG constant Fc region of the antibody-based molecule described herein. In a more preferred embodiment, L234A and L235A substitutions are introduced into the human IgG constant Fc region of the antibody-based molecule described herein. This embodiment results in an antibody-based molecule with a heavy chain represented by SEQ ID NO:268 or 270.

In an embodiment, the antibody or antigen binding fragment comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

b) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

In an embodiment, the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

wherein the VH comprises:

- a CDR-H1 amino acid sequence which comprises SEQ ID NO:147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

wherein the VL comprises:

- a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

- wherein the VH comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the VL comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and
- wherein the VH comprises:
  - a CDR-H1 amino acid sequence which comprises SEQ ID NO: 147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
  - a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
  - a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and
- wherein the VL comprises:
  - a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
  - a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
  - a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody or antigen binding fragment comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and
b) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

In an embodiment, the antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

Surprisingly, attractive results were obtained when the anti-MuSK antibody (or antigen binding fragment or polynucleotide or expression vector of host cell) was administered pre-disease onset. In an embodiment, the human subject is therefore asymptomatic for said neuromuscular disorder such as ALS. In such embodiment, the human subject has been diagnosed as susceptible to develop a neuromuscular disorder as ALS in view of his/her familial history, genetic background or in view of an increased level of neurofilaments (e.g. neurofilament light chain (NFL)) as determined in his/her blood serum or in his/her cerebrospinal fluid (CSF), or in view of a positive genetic test for ALS associated genetic mutation(s), or in view of a change in the level of biomarkers for ALS, or combinations thereof. However he/she does not yet have developed any visible symptoms; he/she is asymptomatic. In a preferred embodiment, the human

subject is administered as early as possible after being diagnosed with the genetic defect or ALS associated genetic mutation(s) but not yet having developed any visible symptoms (i.e. asymptomatic subject). In a more preferred embodiment, the human subject is administered as early as possible after being diagnosed with the genetic defect or ALS associated genetic mutation(s) but not yet having developed any visible symptoms, and said human subject has a familial history of ALS. "Immediately" in this context may mean within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours or within 1, 2, 3, 4, 5, 6, or 7 days, or within 1, 2, 3, or 4 weeks. In this context, such treatment shows limited or no toxicity and/or side effects, or any deleterious effects in the eventuality that the ALS diagnosis is not confirmed.

In another aspect of the invention, there is provided a combination comprising an anti-MuSK antibody or antigen binding fragment thereof described herein and an anticholinergic compound. Said combination is preferably for use in the treatment of a neuromuscular disease in a human subject.

Said neuromuscular disorder is characterized by an impaired neuromuscular transmission and/or an denervation at the NMJ (neuromuscular junction). the neuromuscular disorder is characterized by at least one of:

a. muscarinic overexcitability,
b. motor neuron death,
c. neuromuscular junction (NMJ) denervation and
d. impaired synaptic transmission.

In an embodiment, the neuromuscular disorder is selected from the group consisting of: amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), myasthenia gravis (MG), congenital myasthenia, Lambert-Eaton myasthenic syndrome (LEMS), Lyme disease, poliomyelitis, post-poliomyelitis, heavy metal intoxication, Kennedy syndrome, adult-onset Tay-Sachs disease, hereditary spastic paraplegia, multifocal neuropathy, cervical spondylosis, extramedullary tumor with compressive radiculopathy and myelopathy, inclusion body myositis, progressive bulbar palsy, progressive muscular atrophy, motor neuron syndrome and thyrotoxic myopathy. In preferred embodiment, the neuromuscular disease is ALS.

In this context, a combination does not require that an anti-MuSK antibody or antigen binding fragment thereof described herein, and an anticholinergic compound are physically present together in one composition.

In an embodiment, the anticholinergic compound is administered separately, sequentially, or concurrently. In an embodiment, the antibody or antigen binding fragment is administered at pre-onset of the disease, or within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease. In an embodiment, said antibody or antigen binding fragment is administered at pre-onset of the disease, or preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease and/or wherein the anticholinergic compound is administered at disease onset or within 1, 2, 3, 4, 5, 6, or 7 weeks following disease onset. In this context, a human subject at pre-

onset may mean the human subject is asymptomatic for said neuromuscular disorder. In an embodiment, the subject treated with the antibody had been first diagnosed as being predisposed to develop a neuromuscular disorder or disease.

In an embodiment, the anti-MuSK antibody or antigen binding fragment thereof binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129.

In an embodiment, the antibody or antigen binding fragment thereof comprises wild-type human IgG constant Fc region comprising at least 80% sequence identity to SEQ ID NO: 266 or 267.

In an embodiment, the antibody or antigen binding fragment is an agonist MuSK antibody and/or has reduced or eliminated effector function.

In an embodiment, the reduced or eliminated effector function is obtained by introducing one or more of the following mutations (all numbered according to the EU numbering system) into the human IgG constant Fc region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule described herein: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; or a P396L substitution.

In a preferred embodiment, L234A or L235A substitution is introduced into the human IgG constant Fc region of the antibody-based molecule described herein. In a more preferred embodiment, L234A and L235A substitutions are introduced into the human IgG constant Fc region of the antibody-based molecule described herein. This embodiment results in an antibody-based molecule with a heavy chain represented by SEQ ID NO:268 or 270.

In an embodiment, the antibody or antigen binding fragment comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

b) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

In an embodiment, the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

wherein the VH comprises:

- a CDR-H1 amino acid sequence which comprises SEQ ID NO:147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

wherein the VL comprises:

- a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

- wherein the VH comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the VL comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and
- wherein the VH comprises:
  - a CDR-H1 amino acid sequence which comprises SEQ ID NO: 147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
  - a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
  - a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and
- wherein the VL comprises:
  - a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
  - a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
  - a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and

b) A full length light chain comprising SEQ ID NO: 269, and

c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 270 and

b) A full length light chain comprising SEQ ID NO: 271, and

c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the anti-MuSK antibody or antigen fragment thereof (polynucleotide, expression vector, host cell, cell-free expression system or composition) is administered pre-onset of the disease(such as 1, 2, 3 ,4 ,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days pre-onset of the disease or 1, 2, 3, 4, 5, 6, or 7 weeks pre-onset of the disease), and the anticholinergic compound is administered at disease onset (such as within 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days or within 1, 2, 3, 4, 5, 6, or 7 weeks following disease onset). In this context, a human subject at pre-onset may mean the human subject is asymptomatic for said neuromuscular disorder such as ALS.

In a preferred embodiment, disease onset includes at least one of the symptoms selected from the group consisting of: muscle twitches, muscle cramps, spasticity, muscle weakness, slurred and/or nasal speech, difficulty chewing or swallowing, dysphagia, dysarthria and dyspnea. In a more preferred embodiment, the disease is ALS and disease onset includes at least one of the symptoms selected from the group consisting of: muscle twitches, muscle cramps, spasticity, muscle weakness, slurred and/or nasal speech, difficulty chewing or swallowing, dysphagia, dysarthria and dyspnea.

Disease onset may be assessed by a physician or veterinarian. In an embodiment, beginning of weight loss is considered as disease onset.

In a preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,

- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) numbered according the EU numbering system is(are) introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant

Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) is(are) numbered according the EU numbering system introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 268 and

b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 269, and

c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and

b) A full length light chain comprising SEQ ID NO: 269, and

c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 270 and

b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 271, and

c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 270 and

b) A full length light chain comprising SEQ ID NO: 271, and

c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In embodiments, the anticholinergic compound is a muscarinic receptor antagonist. A muscarinic receptor, also known as a muscarinic acetylcholine receptor or mAchR, is an acetylcholine receptor that forms a G-protein receptor complex in in the cell membrane of certain neurons and other cells. Muscarinic receptors play several roles in mediating the effect of the neurotransmitter acetylcholine. For example, muscarinic receptor are comprised in pre-synaptic membranes of somatic neurons in neuromuscular junctions, where they are involved in the regulation of acetylcholine release.

Five subtypes of muscarinic receptors, M1-M5, are commonly recognized. This classification originates from their different selectivity towards certain agonists and antagonists. M1, M3 and M5 receptors are coupled with Gq proteins in the cell membrane, while M2 and M4 receptors are coupled with Gi/o proteins in the cell membrane. Without being bound to this theory, genes *CHRM1-5* encode for M1-M5 receptors, respectively.

The basal or constitutive activity of a muscarinic receptor is defined as the physical, biological and/or chemical activity of the receptor in the absence of acetylcholine, muscarinic receptor agonists and muscarinic receptor antagonists.

An agonist of a muscarinic receptor, also called a muscarinic receptor agonist, is defined as a compound that increases the physical, biological and/or chemical activity of the receptor when it contacts the receptor. An increased activity means an activity similar to the activity caused by contacting the receptor with acetylcholine.

An antagonist of a muscarinic receptor, also called a muscarinic receptor antagonist, is defined as a muscarinic receptor neutral antagonist or muscarinic receptor negative antagonist.

A muscarinic receptor neutral antagonist is a compound that competes with a muscarinic receptor neutral agonist or with a muscarinic receptor negative antagonist for binding to the receptor, thereby blocking the action of the agonist or the negative antagonist (i.e. increasing or decreasing the activity), while the neutral antagonist does not significantly alter the basal activity of the receptor upon binding alone.

In embodiments, the anticholinergic compound is a muscarinic receptor neutral antagonist.

A muscarinic receptor negative antagonist is a compound that decreases the physical, biological and/or chemical activity of the receptor when it contacts the receptor, even in the absence of a muscarinic receptor agonist. A decreased activity means an activity opposite to the activity caused by contacting the receptor with acetylcholine.

In embodiments, the anticholinergic compound is a muscarinic receptor negative antagonist.

A muscarinic receptor antagonist is defined as selective for one or more muscarinic receptor subtypes M1, M2, M3, M4 and/or M5 if the effect of the antagonist (blocking an agonist, blocking a negative antagonist or decreasing the activity) is only significant upon contacting a muscarinic receptor of the one or more subtypes, while there is significantly less or no effect upon contacting a muscarinic receptor of another subtype. Hence, is a muscarinic receptor antagonist is said to be selective for muscarinic receptor M3, it is understood that significantly less or no effect is obtained upon contacting the antagonist with a muscarinic receptor of subtype M1, M2, M4 or M5. In this context, significantly less may be at least 10-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-, 200-, 300-, 400-, 500-, 600-, 700-, 800-, 900-, 1000-, 10000-, 100000- or 1000000-fold, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 120%, 140%, 160%, 180%, 200%, 220%, 240%, 260%, 280%, 300%, 320%, 340%, 360%, 380%, 400%, 420%, 440%, 460%, 480%, 500%, 600%, 700%, 800%, 900%, 1000%, 1500%, 2000%, 2500%, 3000%, 3500%, 4000%, 4500%, 5000%, 5500%, 6000%, 6500%, 7000%, 7500%, 8000%, 8500%, 9000%, 9500%, 10000%, 20000%, 30000%, 40000%, 50000%, 60000%, 70000%, 80000%, 90000%, 100000%, 1000000%, 10000000% or 100000000% less.

The activity of a muscarinic receptors, preferably of subtype M1, M3 and M5, may be measured using dynamic Ca2+ imaging. These receptors regulate the level of IP3 which then control the release of Ca2+ from internal stores [7].

In embodiments, the anticholinergic compound is a muscarinic receptor antagonist which is:

- selective for muscarinic receptor M1, or
- selective for muscarinic receptor M3, or
- selective for muscarinic receptor M5, or
- selective for muscarinic receptor M1 and muscarinic receptor M3, or
- selective for muscarinic receptor M1 and muscarinic receptor M5, or
- selective for muscarinic receptor M3 and muscarinic receptor M5, or
- selective for muscarinic receptor M1, muscarinic receptor M3, and muscarinic receptor M5.

In embodiments, the anticholinergic compound is a muscarinic receptor antagonist which is:

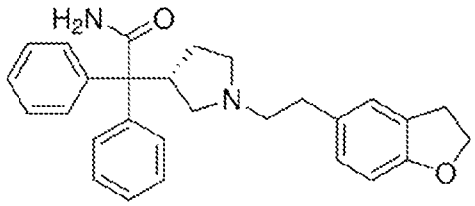
- selective for muscarinic receptor M3, or
- selective for muscarinic receptor M1 and muscarinic receptor M3, or
- selective for muscarinic receptor M3 and muscarinic receptor M5, or
- selective for muscarinic receptor M1, muscarinic receptor M3, and muscarinic receptor M5.

In embodiments, the anticholinergic compound is darifenacin, ipratropium bromide, tiotropium bromide, trospium, glycopyrronium, aclidinium, umeclidinium, solifenacin, dicylomine, fesoterodine, flavoxate, glycopyrrolate, propantheline, 1R,2R,4S,5S,7S)-7-[({4-fluoro-2-(thiophen-2-yl)phenyl}carbamoyl)oxy]-9,9-dimethyl-3-oxa-9-azatricyclo[3.3.1.02,4]nonan-9-ium formate (BS46 in [38]), N-(2-[3-([3R]-1-(cyclohexylmethyl)-3-piperidinyl]methylamino)-3-oxopropyl]amino-2-oxoethyl)-3,3,3-triphenyl-propioamide (J-115311 in [39]), 3,3,3-triphenylpropionamide derivatives with one or two amino acid residues between the triphenylpropionic acid moiety and the piperidinylmethylamine moiety ([40]), OrM3 ([41]) or (3R)-3-[[[(3-fluorophenyl)[(3,4,5-trifluorophenyl)methyl]amino]carbonyl]oxy]-1-[2-oxo-2-(2-thienyl)ethyl]-1-azoniabicyclo[2.2.2]octane bromide (CHF 5407 in [41]). Without being bound to this theory, these compounds can be considered muscarinic receptor antagonists.

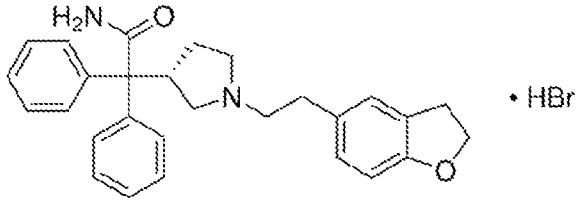
In embodiments, the anticholinergic compound is darifenacin, ipratropium bromide, tiotropium bromide, trospium, glycopyrronium, aclidinium, umeclidinium, solifenacin, dicylomine, fesoterodine, flavoxate, glycopyrrolate, or propantheline. Without being bound to this theory, darifenacin, ipratropium bromide and tiotropium bromide can be considered muscarinic receptor antagonists.

In embodiments, the anticholinergic compound is darifenacin, ipratropium bromide, tiotropium bromide or trospium. Without being bound to this theory, darifenacin, ipratropium bromide and tiotropium bromide can be considered muscarinic receptor antagonists.

In a preferred embodiment, the anticholinergic compound is darifenacin. Darifenacin may be represented by the following structure:



Preferably, darifenacin is darifenacin hydrobromide. Darifenacin hydrobromide may be represented by the following structure:



In embodiments, any of the anticholinergic compounds disclosed in the embodiments above may be present as a pharmaceutically acceptable salt thereof. In particular, the anticholinergic compound is darifenacin or a pharmaceutically acceptable salt thereof.

Examples of pharmaceutically acceptable salts include, without limitation, alkali metal (for example, sodium, potassium or lithium) or alkaline earth metals (for example, calcium) salts; however, any salt that is generally non-toxic and effective when administered to the subject being treated is acceptable. Further salts may include, without limitation: (1) acid addition salts, which can be obtained by reaction of the free base of the parent compound with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perchloric acid and the like, or with organic acids such as acetic acid, oxalic acid, (D) or (L) malic acid, maleic acid, urethane sulfonic acid, ethanesuifonic acid, p-toluenesulfonic acid salicylic acid, tartaric acid citric acid, succinic acid or malonic acid and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion, or coordinates with an organic base such as ethanol amine, diethanolamine, triethanolamine, trimethamine, N-methylglucamine, and the like. Pharmaceutically acceptable salts are well known to those skilled in the art, and any such pharmaceutically acceptable salts may be contemplated in connection with the embodiments described herein.

Acceptable salts may be obtained using standard procedures known in the art, including (without limitation) reacting a sufficiently acidic compound with a suitable base affording a physiologically acceptable anion. Suitable acid addition salts are formed from acids that form non-toxic salts. Illustrative, albeit nonlimiting, examples include the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, lactate, maiate, maleate, malooate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinale, nitrate, orotate, oxalate, palniitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosyiate and trifluoroacetate salts. Suitable base salts of the compounds

described herein are formed from bases that form non-toxic salts illustrative, albeit nonlimiting, examples include the arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

The anticholinergic compounds disclosed in the embodiments above may be administered as a composition, preferably a therapeutical composition. In embodiments, the composition is formulated as a once-a-day extended release tablet for oral use comprising darifenacin, preferably as darifenacin hydrobromide. Preferably, the compositions comprise one or more of the following excipients: dibasic calcium phosphate anhydrous, hypromellose, magnesium stearate, titanium dioxide, iron oxide yellow, iron oxide red, PEG 400 and/or talc. In an embodiment, the composition is known as ENABLEX™. ENABLEX™ is formulated as a 7.5 mg or 15 mg darifenacin (as darifenacin hydrobromide).

In a preferred embodiment, the neuromuscular disorder is ALS, the anti-MuSK antibody or antigen binding fragment comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1)

and an anticholinergic compound is used.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a more preferred embodiment, the neuromuscular disorder is ALS, the anti-MuSK antibody or antigen binding fragment comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to

SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1)

and an anticholinergic compound is used.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In a more preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO:

266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In a preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) is(are) numbered according the EU numbering system introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising SEQ ID NO: 147 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising SEQ ID NO: 153 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising SEQ ID NO: 156 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising SEQ ID NO: 159 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising SEQ ID NO: 172 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising SEQ ID NO: 195 or having 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

In a more preferred embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises wild-type human IgG constant Fc region wherein L234A and/or L235A substitution(s) numbered according the EU numbering system is(are) introduced into said Fc region, a heavy chain variable domain and a light chain variable domain, where the wild-type human IgG constant Fc region comprising SEQ ID NO: 266 or 267, a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the light chain variable domain comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and

where the heavy chain variable domain comprises:

- a CDR-H1 amino acid sequence comprising or consisting of SEQ ID NO: 147,
- a CDR-H2 amino acid sequence comprising or consisting of SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence comprising or consisting of SEQ ID NO:156 (3B2g2m1) and

where the light chain variable domain comprises:

- a CDR-L1 amino acid sequence comprising or consisting of SEQ ID NO: 159,
- a CDR-L2 amino acid sequence comprising or consisting of SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence comprising or consisting of SEQ ID NO:195 (3B2g2m1).

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 268 and

b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 269, and

c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R

substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 270 and
b) A full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 271, and
c) Wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

In an embodiment, the identity or similarity is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

In an embodiment, the neuromuscular disorder is ALS and the anti-MuSK antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 270 and
b) A full length light chain comprising SEQ ID NO: 271, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

In an embodiment a human subject or a patient who has been diagnosed with a neuromuscular disorder such as one of those disclosed above, the MuSK antibody of the present invention optionally combined with the anticholinergic compound are administered to such patient in an amount sufficient to cure, treat, or at least partially arrest the symptoms of the disease (as adduced by biochemical, histologic and/or behavioral assessment), including its complications and intermediate pathological phenotypes in development of the disease. In some embodiments, the administration of the therapeutic molecules of the present invention reduces or eliminates the neuromuscular disorder.

Effective doses of the provided therapeutic molecules of the present invention (i.e. anti-MuSK antibody or antigen binding fragment thereof and anticholinergic compound)), for the treatment of the above-described conditions may vary depending upon many different factors, including means of administration, target site, physiological state of the patient, other medications administered. Treatment dosages are typically titrated to optimize their safety and efficacy. On any given day that a dosage is given, the dosage of the MuSK antibody based molecules as described herein may range from about 0.0001 to about 100 mg/kg, and more usually from about 0.01 to about 20 mg/kg, of the patient's body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg body weight. Exemplary dosages thus include: from about 0.1 to about 10 mg/kg body weight, from about 0.1 to about 5 mg/kg body weight, from about 0.1 to about 2 mg/kg body weight, from about 0.1 to about 1 mg/kg body weight, for instance about 0.15 mg/kg body weight, about 0.2 mg/kg body weight, about 0.5 mg/kg body weight, about 1 mg/kg body weight, about 1.5 mg/kg body weight, about 2 mg/kg body weight, about 5 mg/kg body weight, or about 10 mg/kg body weight

A physician or veterinarian having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of antibody-based molecule in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. If desired, the effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day,

optionally, in unit dosage forms. While it is possible the antibody-based molecule of the present invention to be administered alone, it is preferable to administer the antibody-based molecule as a pharmaceutical composition as described above.

For therapeutic purposes, the MuSK antibody-based molecules (and optionally the anticholinergic compound) of the present invention are usually administered on multiple occasions. Intervals between single dosages (e.g., a bolus or infusion) can be weekly, monthly, or yearly. In some embodiments, the MuSK antibody-based molecules (and optionally the anticholinergic compound) of the present invention are administered to the human subject at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 times over the course of four months.

In certain embodiments, the human subject is administered a loading dose or loading doses of the pharmaceutical composition followed by a maintenance dose or maintenance doses. In some instances, three loading doses are administered, wherein the loading doses are separated by two weeks for e.g., on day 1, day 15, and day 29. In some instances, the maintenance doses are administered every 4 weeks beginning 4 weeks after the third loading dose (e.g., for 1 month, 2 months, three months, four months, five months, six months, seven months, eight months, nine months, ten months).

In certain embodiments, the human subject is administered three loading doses of the pharmaceutical composition followed by at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) maintenance dose. In some instances, the three loading doses are administered two weeks apart. In some instances, the three loading doses are administered 14 days apart. In some instances, the maintenance dose/doses are administered every 4 weeks beginning 4 weeks after the third loading dose. In some instances, the maintenance dose/doses are administered every month beginning one month after the third loading dose. In some instances, the maintenance dose/doses are administered every 28 days beginning 28 days after the third loading dose.

In some methods, dosage is adjusted to achieve a plasma concentration of 1 ng/mL to 1000 µg/ml, preferably 1-1000 µg/mL, more preferably 25-300 µg/mL. Alternatively, the therapeutic molecules of the present invention can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and non-human antibodies. scFv molecules generally have short serum half-lives.

In another embodiment, a pharmaceutical composition comprising a recombinant nucleic acid sequence encoding the MuSK antibody-based molecule as described herein (and optionally in combination with an anticholinergic compound), is administered to a subject to facilitate in vivo expression and formation of the antibody-based molecule for the treatment of conditions mediated by reduced signaling and/or

phosphorylation of MuSK. Expression vector constructs suitable for use in this embodiment of the invention are described supra.

The polynucleotide compositions can result in the generation of the MuSK antibody-based molecule in the subject within at least about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, 45 hours, 50 hours, or 60 hours of administration of the composition to the subject. The composition can result in generation of the antibody-based molecule in the subject within at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days of administration of the composition to the subject. The composition can result in generation of the antibody-based molecule in the subject within about 1 hour to about 6 days, about 1 hour to about 5 days, about 1 hour to about 4 days, about 1 hour to about 3 days, about 1 hour to about 2 days, about 1 hour to about 1 day, about 1 hour to about 72 hours, about 1 hour to about 60 hours, about 1 hour to about 48 hours, about 1 hour to about 36 hours, about 1 hour to about 24 hours, about 1 hour to about 12 hours, or about 1 hour to about 6 hours of administration of the composition to the subject.

The composition, when administered to the subject in need thereof, can result in the persistent generation of the antibody-based molecule in the subject. The composition can result in the generation of the antibody-based molecule in the subject for at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, 42 days, 43 days, 44 days, 45 days, 46 days, 47 days, 48 days, 49 days, 50 days, 51 days, 52 days, 53 days, 54 days, 55 days, 56 days, 57 days, 58 days, 59 days, or 60 days.

The term “treatment” or “treating” as used herein means ameliorating, slowing or reversing the progress or severity of a disease or disorder, or ameliorating, slowing or reversing one or more symptoms or side effects of such disease or disorder. For purposes of this invention, “treatment” or “treating” further means an approach for obtaining beneficial or desired clinical results, where “beneficial or desired clinical results” include, without limitation, alleviation of a symptom, diminishment of the extent of a disorder or disease, stabilized (i.e., not worsening) disease or disorder state, delay or slowing of the progression a disease or disorder state, amelioration or palliation of a disease or disorder state, and remission of a disease or disorder, whether partial or total, detectable or undetectable.

Accordingly, in an embodiment, anti-MuSK antibody or antigen binding fragment according to the invention or a composition according to the invention is for use in the treatment of a neuromuscular disorder in a human subject, wherein said treatment results in a stabilization of said disorder. The stabilization may be for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 months or at least 1, 2 or 3 years. Each of the therapeutic effects further characterized herein could be seen as a stabilization of the disorder.

In an embodiment, the use of an anti-MuSK antibody or antigen-binding fragment (or polynucleotice, expression vector, host cell, composition) exhibits a therapeutic effect on the treated human subject defined herein.

Such a therapeutic effect may be at least one of the effects disclosed below.

By binding to an epitope of MuSK, the anti-MuSK antibody or antigen binding fragment of the invention are able to elicit an agonistic MuSK activity. Within the context of the application "elicit an agonistic MuSK activity" may be replaced by "activate MuSK". An agonistic MuSK activity or an activation of MuSK may be triggered at the molecular and/or at the cellular level and/or in a more biological complex system as a NMJ, a synapse, a living organism. In the context of the application, an agonistic MuSK activity may be replaced by the triggering of a MuSK-induced signal or by the induction of MuSK activation in a muscle cell at the NMJ. A MuSK-induced signal (or MuSK activation or MuSK activity) may be at least one of the induction of MuSK dimerization, the induction of MuSK tyrosine phosphorylation, the induction or increase of induction of AChRs clustering at the NMJ (or clustering in vitro in myotubes AChR patches), the increase of the number or percentage of fully innervated NMJ, the decrease of the number or percentage of fully denervated NMJ, maintenance of the number or percentage of fully innervated NMJ (disease stabilization / disease progression stabilization), an improvement of the reliability of synaptic transmission, an improvement of motor performance, a prevention/stabilization or even a reduction/decrease of motor neuron death, an extension of the lifespan of a treated subject.

A MuSK-induced signal by the anti-MuSK antibody of the invention may be the induction of MuSK dimerization, which may be assessed by western blotting. In the context of the invention, an agonistic activity of MuSK may have been assessed when the induction of MuSK dimerization is increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% or more in an experiment using the antibody of the invention by comparison with the same experimental setting without any antibody or with a negative control or with a negative control antibody. Alternatively, in the context of the invention, an agonistic activity of MuSK antibody may have been assessed when the induction of MuSK dimerization is the same or about the same (20% less, 10% less or the same or 10% more or 20% more) in an experiment using the antibody of the invention by comparison with the same experimental setting without a positive control antibody. Such a MuSK dimerization may be assessed without agrin. A positive control in the assessment of MuSK dimerization is agrin.

A MuSK-induced signal by the anti-MuSK antibody of the invention may be the induction of MuSK tyrosine phosphorylation and such phosphorylation may be assessed by western blotting using an antibody specific for tyrosine phosphorylation. In the context of the invention, an agonistic activity of MuSK may have been assessed when the induction of MuSK tyrosine phosphorylation is increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 120%, 150%, 180%, 200% or more in an experiment using the antibody of the invention by comparison with the same experimental setting without any antibody. Alternatively, in the context of the invention, an agonistic activity of MuSK may have been assessed when the induction of MuSK tyrosine phosphorylation is the same or about the same (20% less, 10% less or the

same or 10% more or 20% more) in an experiment using the antibody of the invention by comparison with the same experimental setting without a positive control antibody. Such a MuSK tyrosine phosphorylation may be assessed without agrin. A positive control in the assessment of MuSK tyrosine phosphorylation is agrin.

A MuSK-induced signal by the annti-MuSK antibody of the invention may be the induction of acetylcholine receptor (AChR) clustering at the NMJ and such clustering may be assessed by staining of AChR using an antibody specifically binding to AChR and visualising such staining in fluorescent microscopy using techniques known to the skilled person. Alternatively, the clustering may be assessed in vitro in myotubes AChR patches. A preferred antibody used to visualise AChR clustering is an antibody specific for AChR. More preferred antibody is AlexaFluor488 conjugated α-bungarotoxin (B13422, ThermoFisher). Usually the region to be analysed in fixed in paraformaldehyde and incubated at room temperature with the relevant antibody of the invention or with a positive or negative control and subsequently each region is washed with PBS and observed under an epi-fluorescent microscopy. In the context of the invention, an agonistic activity of MuSK may have been assessed when the induction of AChR clustering at the NMJ is the same or about the same (i.e. 20% less, 10% less or the same or 10% more or 20% more) or is increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the antibody of the invention by comparison with the same experimental setting without any antibody. Such a AchR clustering may be assessed without agrin. A positive control in the assessment of AchR clustering is agrin.
In a preferred embodiment, the anti-MuSK antibody of the invention exhibits an induction or increase of induction of acetylcholine receptor clustering at the NMJ and such clustering may be assessed by visualizing a staining or an increased staining for AchRs at the NMJ of diaphragms of mice compared to the staining obtained without MuSK agonist antibody. In an embodiment, this induction or increase of clustering of AchRs at the NMJ results in a more normal/physiological NMJ morphology maintaining synaptic innervation and/or pre- and post-synaptic alignment.

A MuSK-induced signal by the anti-MuSK antibody of the invention in a muscle cell at the NMJ may be the increase of the number or percentage of fully innervated NMJ, the decrease of the number or percentage of fully denervated NMJ, maintenance of the number or percentage of fully innervated NMJ (disease stabilization / disease progression stabilization), an improvement of the reliability of synaptic transmission, a prevention/stabilization or even a reduction/decrease of motor neuron death. Each of these features could be assessed using techniques known to the skilled person such as staining of AchR using the a-bungarotoxin antibody as earlier defined herein, presynaptic labelling and quantifying innervation by fluorescent confocal microscopy, EMG single fibre EMG, electrophysiology of single synapses, staining of motor neuron cell bodies in bone marrow specific regions. All these assays have been described in Cantor S et al 2018 (Elife, 2018;7:e34375).

An anti-MuSK antibody or antigen-binding fragment may improve the motor performance and/or grip strength of the treated subject. The motor performance and grip strength of a treated subject may have

been considered to have been improved when such motor perfromance or grip strength may have been increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anti-MuSK antibody of the invention by comparison with the same experimental setting without any antibody. The motor performance (or grip strength) of a treated subject may be assessed using assays known to the skilled person. The experimental part discloses some exemplary methods.

An anti-MuSK antibody or antigen-binding fragment may improve the contractile properties of a muscle at the NMJ of the treated subject. The contractile properties of a muscle of a treated subject may have been considered to have been improved when such contractile properties may have been increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anti-MuSK antibody of the invention by comparison with the same experimental setting without any antibody. The contractile properties of the muscle of a treated subject (at the NMJ) may be assessed using assays known to the skilled person. The experimental part discloses some exemplary methods. In this context, the subject may be an animal.

An anti-MuSK antibody or antigen-binding fragment may improve the resistance to fatigue of a muscle at the NMJ of the treated subject. The resistance to fatigure of a muscle of a treated subject may have been considered to have been improved when such fatigue properties may have been improved of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anti-MuSK antibody of the invention by comparison with the same experimental setting without any antibody. The fatigure properties of the muscle of a treated subject (at the NMJ) may be assessed using assays known to the skilled person. The experimental part discloses some exemplary methods. In this context, the subject may be an animal.

An anti-MuSK antibody or antigen-binding fragment may induce an increase of the muscle weight at the NMJ of the treated subject. The muscle weight of a treated subject may have been considered to have been improved when such weight may have been increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anti-MuSK antibody of the invention by comparison with the same experimental setting without any antibody. The experimental part discloses some exemplary methods. In this context, the subject may be an animal.

A MuSK-induced signal or effect by the anti-MuSK antibody of the invention may be characterized by the improvement of the quality of life or the delay in the apparition of the deterioration of the quality of life of a treated subject. The quality of life may be quantify by the weight of the subject. The improvement of the quality of life or the delay in the apparition of the deterioration of the quality of life may be of at least 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year or more. This is assessed in comparison with the expected quality of life (or the expected apparition of the deterioration of the quality of life) of a subject

suffering from the same condition and having not been treated with an antibody of the invention. In this context, the subject may be an animal.

A MuSK-induced signal or effect by the anti-MuSK antibody of the invention may be characterized by the lifespan of a treated subject. The extension may be of at least 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year or more. This is assessed in comparison with the expected lifespan of a subject suffering from the same condition and having not been treated with an antibody of the invention. In this context, the subject may be an animal.

The properties of the anti-MuSK antibody described herein may be measured in accordance with the assays described herein. An activating activity of a MuSK agonist antibody may be measured relative to a control, for example a negative control antibody (such as an isotype control) that may not bind MuSK. A preferred control antibody not binding to MuSK is Motavizumab which targets RSV (Review, MAbs, 1(5), 439-442, Sept-Octo 2009, DOI: 10.4161/mabs.1.5.9496). A preferred positive control agonist MuSK antibody is mAb#13 from Genentech. Another preferred positive control molecule for evidencing an activating MuSK activity is agrin (rat agrin from R&D systems, 550-AG).

In another embodiment, the anti-MuSK antibody or antigen-binding fragment thereof (or polynucleotide, expression vector, host cell, composition) combined with an anticholinergic compound as defined earlier herein exhibit a therapeutic effect in the treated human subject defined herein. In a preferred embodiment, additional and more preferably synergistic therapeutic effects are elicited when both compounds are used compared to the use of the anti-MuSK antibody or antigen-binding fragment (or polynucleotide, expression vector, host cell, composition) as stand alone therapy.
Additional therapeutic effects may be the reduction ('dampening') of the muscarinic activity of perisynaptic Schwann cells (PSC), the NMJ repair. Such additional therapeutic effects may be the specific reduction ('dampening') of the muscarinic activity of PSC. Such additional therapeutic effects may be the reduction ('dampening') of the hyperexcitability of PSC in the context of a neuromuscular discorder. The compound or combination of the present invention specifically acts on the muscarinic receptor. The compound or combination of the present invention does not seem to have any effect on the purigenic receptor expressed on PSC.

NMJ repair may be the induction or increase of nerve sprouting and/or the increase of the innervation status of the NMJ. Each of these effects may be assessed using techniques known to the skilled person. Also, dampening the muscarinic activity of PSCs may help to maintain NMJ innervation.
In the context of the invention, an induction or increase of nerve sprouting (or of the innervation status of the NMJ) may have been assessed when the induction of nerve sprouting at the NMJ (or of the innervation status of the NMJ) is increased of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anticholinergic compound by comparison with the same experimental setting

without said compound. Nerve sprouting or innervation status may be assessed using immunohistochemistry on nerve-muscle preparations. The experimental part discloses how to obtain such nerve-muscle preparations.

In the context of the invention, the reduction of the muscarinic activity of PSC (or the reduction of the muscarinic hyperexcitability or overexcitability) may have been assessed when such activity (or such hyperexcitability or overexcitability) has been reduced of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in an experiment using the anticholinergic compound by comparison with the same experimental setting without said compound.

Accordingly, in an embodiment, the use of an anti-MuSK antibody or antigen-binding fragment (or polynucleotide, expression vector, host cell, composition), preferably combined with an anticholinergic compound as defined earlier herein, exhibit one or more of the following therapeutic effects:

- an increase of the number or percentage of fully innervated NMJ in the subject, maintenance of the number or percentage of fully innervated NMJ in the subject, the decrease of the number or percentage of fully denervated NMJ in the subject, an improvement of the reliability of synaptic transmission, a prevention, stabilization or reduction of motor neuron death in the subject; and/or
- an improvement of the motor performance and/or grip strength of the subject; and/or
- an improvement of the contractile properties of a muscle at the NMJ of the subject; and/or
- an improvement of the resistance to fatigue of a muscle at the NMJ of the subject; and/or
- an induction of an increase of the muscle weight at the NMJ of the subject; and/or
- an improvement of the quality of life or the delay in the apparition of the deterioration of the quality of life of the subject; and/or
- a reduction of the muscarinic activity (or the reduction of the muscarinic hyperexcitability or overexcitability) of perisynaptic Schwann cells (PSC) in the subject, or of the NMJ repair in the subject.

As demonstrated in the experimental part, synergistic therapeutic effects are obtained when both compounds are used. These synergistic effects include the improvement/increase of the following parameters/symptoms: locomotor function and grip strength, the contractile properties of a muscle at the NMJ, resistance to fatigue of the muscle, muscle weight, impact on the general condition of life such as body weight.

An “effective amount,” of the antibody-based molecule refers to an amount sufficient, at dosages and for periods of time necessary, to achieve an intended biological effect or a desired therapeutic result including, without limitation, clinical results. The phrase “therapeutically effective amount” when applied to an antibody-based molecule of the invention is intended to denote an amount of the antibody that is sufficient to ameliorate, palliate, stabilize, reverse, slow or delay the progression of a disorder or disease state, or of a symptom of the disorder or disease. In an embodiment, the method of the present invention provides for

administration of the antibody-based molecule in combinations with other compounds. In such instances, the “effective amount” is the amount of the combination sufficient to cause the intended biological effect.

In a further aspect, there is provided a method for the prevention and/or treatment of a neuromuscular disease and/or disorder and/or condition comprising administering to a subject in need thereof, an anti-MuSK antibody or antigen-binding fragment thereof (a polynucleotide, an expression vector, host cell, or composition all as earlier defined herein) and preferably an anticholinergic compound. All features of this method have been defined earlier herein.

In a further aspect, there is provided a use of an anti-MuSK antibody or antigen-binding fragment thereof (a polynucleotide, an expression vector, host cell, or composition all as earlier defined herein) and preferably an anticholinergic compound for the manufacture of a medicament for the prevention and/or treatment of a neuromuscular disease and/or disorder and/or condition. All features of this use have been defined earlier herein.

All documents cited in the present specification are hereby incorporated by reference in their entirety. Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention. Each embodiment described herein may be combined together with any other embodiment described herein, unless otherwise indicated.

The present invention is further described by the following examples which should not be construed as limiting the scope of the invention.

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5

EXAMPLES

Example 1: Methods

Animals

Mice overexpressing the human mutated *SOD1*^{G37R} transgene, line 29, were obtained from The Jackson Laboratory and bred at Université de Montréal animal facilities on a C57BL/6 background. This mouse model is a late onset, slowly progressing model of ALS that recapitulates the human phenotype of the disease. Characterization of this strain phenotype has been previously published in several ALS studies (*5, 7, 20, 31, 32*). All experiments were performed in accordance with the guidelines of the Canadian Council of Animal Care and the Comité de déontologie animale of Université de Montréal.

Preclinical trial design

The pre-clinical trial design was made according to guidelines for preclinical animal research in ALS/MND (*33*). The study was conducted in a double-blind manner. Fifteen male mice from the SOD1^{G37R} background were randomly assigned to three groups.

1. ARGX-119 (3B2g2m1-hIgG1LALAdelk: full length heavy chain with reduced effector function SEQ ID NO: 268 and full length light chain with reduced effector function SEQ ID: 269) :and darifenacin
2. ARGX-119 (3B2g2m1-hIgG1LALAdelk) and Vehicle (darifenacin control)
3. Isotype control mAb + Vehicle (darifenacin control)

ARGX-119 treatment was started at pre-onset (before symptoms or asymptomatic) and darifenacin treatment at disease onset (appearance of symptoms) and continued until sacrifice. A set of neurological scores (level 1 to 5, Appendix 1) was used to determine the onset of symptoms and the progression and severity of symptoms during disease progression. Onset of disease was assessed by the beginning of weight loss (*34*) and appearance of tremor, representing a neurological score of 1 while the endpoint of this study was at the late symptomatic stage, representing a neurological score of between 3 and 5.

Intra-peritoneal injections of ARGX-119 MuSK antibody (3B2g2m1-hIgG1LALAdelk) or the placebo (Motavizumab-hIgG1LALAdelk: full length heavy chain with reduced effector function SEQ ID NO: 272 and full length light chain with reduced effector function SEQ ID: 273) were initiated at P400 at an initial dose of 20 mg/kg and then weekly at a dose of 10 mg/kg until sacrifice. Darifenacin was given orally (10 mg/kg diluted in DMSO, 5 days/week) initiated at disease onset (~P425). Placebo for darifenacin was DMSO alone. Mice received both treatments for about 4 months, until the age of ~520 days, the median age at which they normally reach critical disease endpoints.

Motavizumab-hIgG1LALAdelk: SEQ ID NO: 272 is derived from SEQ ID NO: 274 and SEQ ID: 273 from SEQ ID:275.

Treatment, behavior monitoring, experiments and results analysis were done blindly. Standard ALS behavioral measurements were performed weekly to measure disease progression in the various study groups. This includes rotarod test, grip strength measurements, weight measurements and tail suspension test to assess hindlimb extension reflex. At the time of the sacrifice, *Extensor Digitorum Longus* (EDL) and *Soleus* (SOL) muscles and their innervation were dissected and placed in a physiological chamber. Two sets of measurements were acquired. First, the functional properties of the muscles (strength and fatigue) were determined using a force transducer. Second, muscles were fixed, and the muscle mass were determined.

Rotarod acceleration protocol

Motor coordination, strength and balance were assessed using a rotarod (TSE Rotarod, TSE Systems Gmbh, Germany). Animals were placed onto a rotating wheel at a starting speed of 4 rpm, increasing to 40 rpm in 300 seconds. Mice had two attempts per block and 2 blocks per session (with a rest time between block) to remain on the rotarod during the acceleration protocol, and the two longest latencies to fall were averaged.

Grip strength

To measure the overall strength of the limbs of mice, a grip strength meter was used (Fig 1C). Mice had nine attempts per session (3 blocks of 3 attempts; each block was separated by a 1 min rest period) and the best three values of each block were averaged.

Nerve–muscle preparations

Preparations of EDL and SOL muscles and their innervating nerve were dissected in oxygenated Ree's solution (in mM), as follows: 110 NaCl, 5 KCl, 1 $MgCl_2$, 25 $NaHCO_3$, 2 $CaCl_2$, 11 glucose, 0.3 glutamic acid, 0.4 glutamine, 5 BES (N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid sodium salt), 0.036 choline chloride, and 4.34×10^{-7} cocarboxylase. After dissection, nerve muscle preparations were constantly perfused with oxygenated Ree's solution (95% O_2, 5% CO_2).

Measurements of neuromuscular properties

The EDL and SOL nerve-muscle preparations were attached vertically to a fixed force transducer (model 402A-500mN, Aurora Scientific Inc.) using surgical threads. The preparations were attached at the tendons level to the transducer at one extremity and to an adaptable hook at the opposite extremity (Fig. 2A). A platinum reference electrode was then juxtaposed to the muscle, positioned near an extremity of the muscle, close to a tendon. To stimulate the muscle, a second platinum electrode was juxtaposed at the other extremity of the muscle. To elicit muscle contractions from motor nerve and neuromuscular activity, the tibial nerve (SOL) or the deep peroneal nerve (EDL) was suctioned into an electrode made of PE tubing and filled with physiological solution. Hence, this system was designed to elicit muscle contractions from both muscle and/or nerve stimulations. Neuromuscular contractile basal force responses were elicited by a single supra-maximal square-wave of 500 mV, 0.1ms pulse imposed on the motor nerve. Muscle contractile basal force responses were elicited by square pulse stimulation of 15 V, 1 ms. Optimal muscle

length was determined by gradually stretching the muscle until maximal contractile force output was attained.

Force-frequency curve: Nerve and muscle stimulations were performed to generate a standard force-frequency curve. Alternate nerve and muscle stimulations were performed at various frequencies for 500 ms (5Hz, 10Hz, 20Hz, 30Hz, 40Hz, 50Hz, 60Hz, 70Hz, 80Hz, 90Hz, 100Hz, 120Hz, 140Hz, 160Hz, 180Hz, 200Hz, 250Hz and 300Hz) and the force generated monitored. There was a 2-minute rest period between each stimulation. The proportion of the muscle capacity that is used by the neuromuscular system upon nerve stimulation was expressed as the contractile capacity ratio and calculated as follow for each frequency: $\frac{Force_{Nerve}}{Force_{Muscle}} X\ 100$

Maximal force: The maximal force generated by the alternated nerve and muscle stimulation was obtained at a frequency of 50 Hz and 80 Hz for 2 sec, each separated by either 2 (SOL) or 5 (EDL) minutes.

Muscle fatigue: The fatigue protocol is illustrated in Figure 4A. The fatigue protocol was adapted to each muscle owing to the differences in their intrinsic properties. For the EDL, fatigue was tested using a bout of 180 nerve stimulations for a duration of 300 ms, elicited at a frequency of 120Hz. The rest period between each stimulation was 700 ms, for a total protocol duration of 3 min. Muscular stimulations were super-imposed to nerve stimulations every 10 stimulations (18 simultaneous nerve-muscle stimulations), to evaluate muscular reserve. The fatigue protocol for the SOL consisted of a bout of 300 nerve stimulations for 500 ms at 50Hz, with a rest period of 600 ms between stimulations, for a total duration of 5 min 30. Muscular stimulations were super-imposed to nerve stimulations every 10 stimulations (30 simultaneous nerve-muscle stimulations).

Muscle recovery: Each fatigue protocol was followed by a 30 min recovery period during which neuromuscular and neuromuscular + muscular contractile force (120Hz - 300ms for the EDL and 50Hz – 500ms for the SOL) were measured after the fatigue protocol at 5 s, 10 s, 15 s, 30 s, 45 sec, 1 min, 1.5 min, 2 min, 2.5 min, 5min, 10 min, 20 min and 30 min.

Muscle weight

After each experiment, muscles were fixed (10 min, PFA) and washed (3 washes of 5 min each, PBS 1X). Then, both tendons were cut and muscles were weighted and stored at 4°C for further processing.

Statistics

Results are represented as the mean ± SEM where the number of animals is identified as *N (number of replicates)* and the number of muscles is represented by *n (number of observations)*. One-way ANOVA Kruskal-Wallis test and multiple t-test were used in most cases where three or four different groups were compared. Repeated one-way ANOVA with *post hoc* Bonferroni multiple comparison test was used to compare the values obtained at various frequencies or over time from the same animals in the different groups. The confidence level used in the study was 95% ($\alpha = 0.05$). All analyses were made with GraphPad 8 software (Prism).

Example 2: Results

ARGX-119 antibody combined with darifenacin improves locomotor function and grip strength

The locomotor function and general strength of the animal were tested to investigate if the combination of treatment with ARGX-119 antibody and darifenacin could improve muscle function.

First, the motor performance, balance and coordination, were measured using a standard acceleration protocol on the Rotarod (Figure 1A), which is known to reveal ALS motor deficits as disease progresses (*34*). Figure 1B shows the progressive decline of motor performance of mice of the ARGX-119 alone, darifenacin alone or placebo-treated group as revealed by a shorter latency to fall of the Rotarod, showing the expected progression of ALS motor phenotype. However, the motor performance of mice combo-treated with ARGX-119 antibody and darifenacin mice was much less pronounced, resulting in a significantly improved motor performance compared to the ARGX-119+DMSO ($p<0.001$), PBS+darifenacin ($p<0.0001$) and placebo-treated mice ($p<0.0001$) (Figure 1B; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, PBS+darifenacin N=5, Placebo N=5, One-way ANOVA, Kruskal-Wallis's test and multiple t-test). This is particularly evident when approaching the end stage, where a significantly higher score obtained at age P475 until P525 was observed for the combo-treated mice compared to the placebo group. Indeed, at this late symptomatic stage, most of the placebo mice were no longer able to run onto the rotating wheel while more than half of the darifenacin-treated group were still able to run. Interestingly, there was a tendency of improved motor behavior at the Rotarod for the ARGX-119+DMSO treated mice from the ages of P510 to P525 ($p=0.07$, $p=0.06$ and $p=0.053$ respectively). These results demonstrated the beneficial impact of the combo-treatment compared to other mono-treatments and the placebo group.

Second, the grip strength measured to assess if the combined treatment ameliorated the general strength of the animals (Figure 1C). Mice of all groups began the trial with a similar grip strength force. However, combo-treated mice performed better than the mice in the placebo group as shown by the larger grip strength generated at P460 until the end of the preclinical trial (Figure 1D; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, PBS+darifenacin N=5, Placebo N=5, One-way ANOVA, $p<0.05$, Tukey's test and multiple t-test). Interestingly, at P507, PBS+darifenacin treated mice had a significant increase of grip strength compared to the placebo group.

Third, it was tested whether the combined-treatment of ARGX-119 antibody with darifenacin had an impact on the general condition of the mice. To this end, the changes in the body weight of the animals were monitored, a metric that is directly related to the progression of the disease and survival whereby animals present an important gradual body weight loss after symptoms onset. However, no difference was observed between the groups (ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, PBS+darifenacin N=5, Placebo N=5, $p>0.05$, One-way ANOVA, Tukey's test and multiple t-test).

Example 2.1: Combo-treatment improves neuromuscular contractile muscle force and NMJ efficacy

Improved contractile muscle properties are strong indicators that the muscle and NMJ functions should also be improved by the combined treatment. Two muscles with different properties and resistance to the disease were investigated. The EDL was used as a fast-twitch fatigable muscle that is vulnerable to the disease and the SOL as a slow-twitch fatigue resistant muscle that is also more resistant to the disease. A muscle force transducer was used to measure the force generated by the muscles upon stimulation of the motor nerve and/or direct muscle stimulation (see figure 2A). With this system, stimulation of the motor nerve at various frequencies elicits muscle contraction through NMJ efficacy, reflecting the strength of contractile fibers associated with innervated NMJs only. Muscle stimulation, on the contrary, depolarizes all muscle fibers and reflect maximal twitch force of all the muscle, independent of the innervation status. This method is especially useful to characterize diseases like ALS presenting NMJ and muscular deficits (*35*, *36*).

Fast-fatigable EDL muscle:

First, a standard protocol of stimulation was performed to generate a force frequency curve (5Hz-300Hz) to characterize NMJ efficacy following the ARGX-119 and darifenacin chronic treatments. Muscle force generated by the contractions elicited by the stimulation of the motor nerve and NMJ activation was significantly higher than EDL from the placebo group ($p<0.001$) or the ARGX-119+DMSO group ($p<0.05$) during the protocol (Figure 2B; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5; Repeated One-way ANOVA, Bonferroni *post* hoc test). Indeed, the generated twitch force were 66.3 ± 15.7 mN for the combo-treatment, 47.2 ± 12.4 mN for the placebo group and 53.6 ± 14.9 mN for the ARGX-119+DMSO group. There was no significant difference with the group ARGX-119 antibody group compared to placebo treated mice.

For the direct muscle stimulation, significant differences between the combo-treated mice and the other groups were observed at higher frequencies (Figure 2C). The combo-treated group showed significant high peak force (mN) compared to the ARGX-119 and DMSO group ($p<0.05$) as well as the placebo group ($p<0.001$). This is indicative of the preservation of the fast-twitch properties of the EDL (*37, 38*) (Figure 2C, ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, Repeated One-way ANOVA, Bonferroni *post* hoc test and multiple t-test). Interestingly, this increase in contractile force was also observed with a better preservation of the EDL muscle weight in the combo-treated animals compared to the placebo group as shown in Figure 2E.

Next, the proportion of the muscle capacity that is used by the neuromuscular system upon nerve stimulation was determined. This was expressed as the *contractile capacity ratio*. This ratio is at 100% in WT mice, indicating that the neuronal control of the muscle recruits 100% of its contractile capacity. Hence, if treatments improve NMJ innervation, resulting in an increase in force generated, it is posited that this ratio should be higher in EDL muscles from combined-treated animals compared to the other groups. As shown in Figure 2D, the ratio was significantly higher for the EDL of ARGX-119+DMSO mice, with 61.1 ±

1.0% compared to 52.3 ± 1.8% ($p<0.001$) for combined-treated mice and to 53.3 ± 3.6% ($p<0.0001$) for the placebo group (ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, One-way ANOVA, Kruskal-Wallis's test).

Slow twitch SOL muscle:

Then, the same protocol was performed, but for the SOL muscle (Figure 3). Combined-treated group demonstrated significantly higher twitch forces than from the ARGX-119+DMSO treated group ($p<0.0001$) and placebo group ($p<0.0001$) during the protocol for the nerve stimulation (Figure 3A; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, Repeated One-way ANOVA and multiple t-test). However, the ARGX-119+DMSO treated group generated smaller contraction force forces in comparison to placebo ($p<0.05$). The generated twitch force was 114.5 ± 3.5 mN for the combo-treatment, 83.1 ± 4.5 mN for the placebo group and 64.6 ± 5.1 mN for the ARGX-119+DMSO group. In the case of the direct muscle stimulation, combined-treated group demonstrated once again significantly higher twitch forces (140.6 ± 2.1 mN) than from the ARGX-119+DMSO treated group (98.3 ± 2.1 mN; $p<0.01$) and placebo group (119.5 ± 1.6 mN; $p<0.0001$) during the protocol for the nerve stimulation (Figure 3B; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, Repeated One-way ANOVA). There was also a significant difference between the ARGX-119+DMSO treated group and placebo group ($p<0.05$).

The contractile capacity ratio (Figure 3C) was significantly higher in combined-treated mice, with 82.0 ± 2.8% compared to 68.4 ± 7.8% for the ARGX-119-treated group ($p<0.0001$) and 56.1 ± for the control mice ($p<0.0001$; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, Repeated One-way ANOVA). Interestingly, this increase in contractile force and contractile capacity ratio was observed with a better preservation of the SOL muscle weight in treated animals. Indeed, the ARGX-119+darifenacin treated mice had better preserved muscle weight compared to the Placebo group ($p<0.05$; ARGX-119+DMSO N=5, ARGX-119+darifenacin N=4, Placebo N=5, One-way ANOVA). Interestingly, there was also a significant difference between the ARGX-119+DMSO treated group and placebo group ($p<0.001$), where the SOL from the ARGX-119 generated the better contractile capacity ratio than the placebo ones.

Overall, these results suggest that the combo treatment improves muscle and neuromuscular contractile forces as well as muscle weight for both muscles, but the contractile capacity ratio was increased for EDL and SOL only for the ARGX-119+DMSO group.

Example 2.2: ARGX-119 antibody combined with darifenacin preserves muscle fatigue properties

In addition to the force generated, a muscle is also characterised by its resistance to fatigue. For instance, fast-twitch muscles composed mainly of fast fatigable motor units like the EDL show higher fatigue in comparison to slow twitch muscle like the SOL (*37*). In ALS, alteration at the type of innervation (from fast to slow twitch) and in the properties of muscles themselves alter the fatigue properties, rendering them more resistant. Since the treatment by the combined ARGX-119+darifenacin significantly preserved muscle strength, next investigated the resistance to fatigue of the EDL and the SOL muscles.

A fatigue stimulation protocol was used, followed by a 30 min recovery period (see Fig 4A). EDL muscles from the placebo-treated group showed an atypical resistance to fatigue when the nerve is directly stimulated. However, as revealed by a delayed recovery, EDL muscles from the combined ARGX-119+darifenacin-treated mice showed a level of fatigue that is more typical for this type of fast twitch muscle (Figure 4B; ARGX-119+darifenacin N=4, ARGX-119+DMSO N=5, Placebo N=5; $p<0.05$, one-way ANOVA, Kruskal-Wallis's test and multiple t-test). Interestingly, during the recovery, there was a significant difference between the ARGX-119 treatment and the placebo group ($p<0.05$), where the ARGX-119+DMSO treated muscle showed a more typical fatigue. This suggests that the used of a combined treatment as well as a mono-treatment like ARGX-119 antibody improved muscle properties.

However, no difference was found in the rate of fatigue and recovery of nerve + muscle stimulation, when all muscle fibers are recruited (Figure 4C; ARGX-119+darifenacin N=4, ARGX-119+DMSO N=5, Placebo N=5; $p>0.05$; one-way ANOVA, Kruskal-Wallis's test).

SOL is a slow-twitch and resistant muscle that is expected to be also more resistant from the denervation seen in degenerative diseases compared to the fast-twitch muscle EDL and to recover faster. For the nerve stimulation of the SOL muscle, the placebo group showed a more pronounced fatigue as revealed by a slower fatigue recovery in comparison to the ARGX-119+darifenacin treated-group, which is atypical for this fatigue-resistant muscle. Hence, the combo treatment restored the fatigue-resistant properties of the SOL muscle. (Figure 4D-E; ARGX-119+darifenacin N=4, ARGX-119+DMSO N=5, Placebo N=5; $p<0.01$; one-way ANOVA, Kruskal-Wallis's test and multiple t-test). For the nerve + muscle stimulation, there is a significant difference between the ARGX-119+darifenacin and the ARGX-119+DMSO groups ($p<0.001$) and the ARGX-119+DMSO and the placebo groups ($p<0.0001$).

CLAIMS

1. An anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject.

2. An anti-MuSK antibody or antigen binding fragment thereof, for use according to claim 1, wherein the antibody or antigen binding fragment binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129.

3. An anti-MuSK antibody or antigen binding fragment thereof, for use according to claim 1 or 2, wherein the antibody or antigen binding fragment thereof comprises wild-type human IgG constant Fc region comprising at least 80% sequence identity to SEQ ID NO: 266 or 267.

4. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any of the preceding claims, which is an agonist MuSK antibody and/or has reduced or eliminated effector function.

5. An anti-MuSK antibody or antigen binding fragment thereof, preferably for use according to any of the preceding claims, wherein the reduced or eliminated effector function is obtained by introducing one or more of the following mutations (all numbered according to the EU numbering system) into the constant region SEQ ID NO: 266 or SEQ ID NO: 267 of the antibody-based molecule: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

6. An anti-MuSK antibody or antigen binding fragment thereof, preferably for use according to any of the preceding claims, wherein the antibody or antigen binding fragment thereof comprises wild-type human IgG constant Fc region SEQ ID NO: 266 or 267, and wherein L234A and L235A mutations numbered according to the EU numbering system are introduced to said Fc region.

7. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any one of the preceding claims, wherein the antibody or antigen binding fragment comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

b) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

8. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any one of the preceding claims, wherein the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

wherein the VH comprises:

- a CDR-H1 amino acid sequence which comprises SEQ ID NO:147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

wherein the VL comprises:

- a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

9. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any one of the preceding claims, wherein the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL):

- wherein the VH comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and the VL comprises an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235, and
- wherein the VH comprises:

- a CDR-H1 amino acid sequence which comprises SEQ ID NO: 147 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 147,
- a CDR-H2 amino acid sequence which comprises SEQ ID NO: 153 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 153, and
- a CDR-H3 amino acid sequence which comprises SEQ ID NO: 156 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:156 (3B2g2m1) and

– wherein the VL comprises:

- a CDR-L1 amino acid sequence which comprises SEQ ID NO: 159 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 159,
- a CDR-L2 amino acid sequence which comprises SEQ ID NO: 172 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO: 172, and
- a CDR-L3 amino acid sequence which comprises SEQ ID NO: 195 or has 1, 2, 3, 4 or 5 amino acid alterations relative to SEQ ID NO:195 (3B2g2m1).

10. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any one of the preceding claims, wherein the antibody or antigen binding fragment comprises:
– a heavy chain variable domain (VH) comprising SEQ ID NO: 234, and
– a light chain variable domain (VL) comprising SEQ ID NO: 235.

11. An anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject wherein the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) as identified in table 3 and/or a CDR as identified in table 1 or 2.

12. An anti-MuSK antibody or antigen binding fragment thereof, preferably for use in the treatment of a neuromuscular disorder in a human subject wherein the antibody or antigen binding fragment comprises:
– a full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 270 and a full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 271, or
– a full length heavy chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 268 and a full length light chain comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 269,
– wherein one or more of the following mutations (all numbered according to the EU numbering system) have been introduced into the full length heavy chain: an N297A substitution; an N297Q substitution; an L234A substitution; an L234D substitution; an L234E substitution; an L234G substitution; an L234H substitution; an L234F substitution; an L234K substitution; an L234Q substitution; an L234R substitution; an L234S substitution; an L234T substitution; an L235A substitution; an L235D substitution; an

L235E substitution; an L235F substitution; an L235G substitution; an L235V substitution; an L235H substitution; an L235I substitution; an L235K substitution; an L235R substitution; an L235S substitution; L235T substitution; an L235Q substitution; an L237A substitution; an S239D substitution; an E233P substitution; an L234V substitution; a C236 deletion; a G236E substitution; a G236R substitution; a G236K substitution; a G237A substitution; a P238A substitution; an F243L substitution; a D265A substitution; an S267E substitution; an H268A substitution; an R292P substitution; a Y300L substitution; a K322A substitution; a K322Q substitution; an A327Q substitution; an L328F substitution; an L328R substitution; a P329A substitution; a P329G substitution; an A330L substitution; an A330S substitution; a P331S substitution; an I332E substitution; a P396L substitution; or each of the combinations of mutations described earlier in the fourth embodiment of this application, preferably the mutations is L234A or L235A, more preferably the mutations are L234A and L235A.

13. An anti-MuSK antibody or antigen binding fragment thereof, preferably for use according to claim 12, wherein the antibody or antigen binding fragment comprises:
- A full length heavy chain comprising SEQ ID NO: 270 and
- A full length light chain comprising SEQ ID NO: 271, and
- Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

14. An anti-MuSK antibody or antigen binding fragment thereof, preferably for use according to claim 12, wherein the antibody or antigen binding fragment comprises:
a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

15. A polynucleotide for use in the treatment of a neuromuscular disorder in a human subject, said polynucleotide comprising a nucleotide sequence which encodes the antibody or antigen binding fragment thereof of any of claims 1 to 14 or a VH or VL or CDR thereof.

16. An expression vector for use in the treatment of a neuromuscular disorder in a human subject, comprising the polynucleotide of claim 15, preferably operably linked to a regulatory region which allows expression of the antibody or antigen binding fragment thereof or VH or VL or CDR thereof in a host cell or cell-free expression system.

17. A host cell or cell-free expression system for use in the treatment of a neuromuscular disorder in a human subject containing the expression vector of claim 16.

18. A composition for use in the treatment of a neuromuscular disorder in a human subject comprising an antibody or antigen binding fragment thereof as defined in any one of claims 1 to 14, a polynucleotide as defined in claim 15, an expression vector as defined in claim 16 or a host cell or cell-free expression system as defined in claim 17.

19. A composition for use in the treatment of a neuromuscular disorder in a human subject according to claim 18, which is a pharmaceutical composition comprising at least one pharmaceutically acceptable carrier or excipient.

20. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any of the preceding claims, wherein the antibody or antigen binding fragment, the polynucleotide, the expression vector, the host cell, the cell-free expression system or the composition is administered in combination with an anticholinergic compound.

21. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to claim 20, wherein the anticholinergic compound is administered separately, sequentially, or concurrently.

22. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any claim 20 or 21, wherein the anticholinergic compound is a muscarinic receptor antagonist, preferably a muscarinic receptor antagonist selective for muscarinic receptor M1 and/or muscarinic receptor M3 and/or muscarinic receptor M5.

23. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any one of claims 22, wherein the muscarinic receptor antagonist is selective for muscarinic receptor M3, preferably wherein the anticholinergic compound is darifenacin, ipratropium bromide, tiotropium bromide or trospium.

24. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any of the preceding claims, wherein the neuromuscular disorder is characterized by an impaired neuromuscular transmission and/or an denervation at the NMJ (neuromuscular junction).

25. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any of the preceding claims, wherein the neuromuscular disorder is characterized by at least one of:
    a. muscarinic overexcitability,
    b. motor neuron death,
    c. neuromuscular junction (NMJ) denervation and
    d. impaired synaptic transmission.

26. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any of the preceding claims, wherein the neuromuscular disorder is selected from the group consisting of: amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), myasthenia gravis (MG), congenital myasthenia, Lambert-Eaton myasthenic syndrome (LEMS), Lyme disease, poliomyelitis, post-poliomyelitis, heavy metal intoxication, Kennedy syndrome, adult-onset Tay-Sachs disease, hereditary spastic paraplegia, multifocal neuropathy, cervical spondylosis, extramedullary tumor with compressive radiculopathy and myelopathy, inclusion body myositis, progressive bulbar palsy, progressive muscular atrophy, motor neuron syndrome and thyrotoxic myopathy.

27. An anti-MuSK antibody or antigen binding fragment for use according to any one of claims 1 to 14, or any of claims 15 to 26 when referring back to any of claims 1 to 14, wherein the disorder is ALS.

28. An anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of ALS in a human subject wherein said antibody or antigen binding fragment is administered to an asymptomatic human subject, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease.

29. An anti-MuSK antibody or antigen binding fragment thereof, according to claim 28, wherein the asymptomatic human subject is diagnosed as being predisposed to develop a neuromuscular disorder or disease.

30. An anti-MuSK antibody or antigen binding fragment thereof, for use according to claim 28 or 29, wherein the antibody or antigen binding fragment binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129.

31. An anti-MuSK antibody or antigen binding fragment thereof, for use according to claim 28 to 30, wherein the antibody or antigen binding fragment comprises:

c) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

d) a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

32. An anti-MuSK antibody or antigen binding fragment thereof, for use according to any one of claim 28 to 31 wherein the antibody or antigen binding fragment comprises:

a) A full length heavy chain comprising SEQ ID NO: 268 and

b) A full length light chain comprising SEQ ID NO: 269, and

c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

33. An anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition, for use according to any one of claims 20 to 27, wherein the anticholinergic compound is administered at disease onset or within 1, 2, 3, 4, 5, 6, or 7 weeks following disease onset.

34. A combination comprising an anti-MuSK antibody or antigen binding fragment thereof and an anticholinergic compound preferably for use in the treatment of ALS in a human subject.

35. A combination according to claim 34, wherein said antibody or antigen binding fragment is administered to an asymptomatic human subject, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease and/or wherein the anticholinergic compound is administered at disease onset or within 1, 2, 3, 4, 5, 6, or 7 weeks following disease onset.

36. A combination according to claim 35 wherein the asymptomatic human subject treated with the antibody had been first diagnosed as being predisposed to develop a neuromuscular disorder or disease.

37. A combination according to claim 34 to 36, wherein the anti-MuSK antibody or antigen binding fragment thereof binds the MuSK Frizzled (Fz)-like domain sequence of SEQ ID NO: 129.

38. A combination according to any one of claims 34 to 37, wherein the anti-MuSK antibody or antigen binding fragment thereof comprises:

a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 234 and

a light chain variable domain (VL) comprising an amino acid sequence that is at least 80% identical or similar to SEQ ID NO: 235.

39. A combination according to any one of claim 34 to 38, wherein the antibody or antigen binding fragment comprises:
a) A full length heavy chain comprising SEQ ID NO: 268 and
b) A full length light chain comprising SEQ ID NO: 269, and
c) Wherein the full length heavy chain comprises L234A and L235A mutations numbered according the EU numbering system.

40. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition, for use according to any one of claims 1 to 39, wherein the anti-MuSK antibody or antigen binding fragment thereof, the combination, the polynucleotide, the expression vector, the host cell, the cell-free expression system or the composition is administered at disease onset, to an asymptomatic human subject, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease.

41. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to claim 40 wherein disease onset includes at least one of the symptoms selected from the group consisting of: muscle twitches, muscle cramps, spasticity, muscle weakness, slurred and/or nasal speech, difficulty chewing or swallowing, dysphagia, dysarthria and dyspnea.

42. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any one of the preceding claims, wherein the neuromuscular disorder is analyzed via electrophysiological assessment or pharmacodynamic assessment: in neurofilaments (e.g. neurofilament light chain (NFL)) in blood serum, plasma and/or cerebrospinal fluid (CSF); or NMJ biopsies.

43. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any one of the preceding claims, wherein the administration of said anti-MuSK antibody or antigen binding fragment thereof, said combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or composition to said human subject results in one or more of the following therapeutic effects:
- an increase of the number or percentage of fully innervated NMJ in the subject, maintenance of the number or percentage of fully innervated NMJ in the subject, the decrease of the number or percentage of fully denervated NMJ in the subject, an

improvement of the reliability of synaptic transmission, a prevention, stabilization or reduction of motor neuron death in the subject; and/or

- an improvement of the motor performance and/or grip strength of the subject; and/or
- an improvement of the contractile properties of a muscle at the NMJ of the subject; and/or
- an improvement of the resistance to fatigue of a muscle at the NMJ of the subject; and/or
- an induction of an increase of the muscle weight at the NMJ of the subject; and/or
- an improvement of the quality of life or the delay in the apparition of the deterioration of the quality of life of the subject; and/or
- a reduction of the muscarinic activity (or a reduction of the muscarinic hyperexcitability) of perisynaptic Schwann cells (PSC) in the subject, or of the NMJ repair in the subject.

44. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any one of the preceding claims, wherein said treatment results in a stabilization of said disorder.

45. An anti-MuSK antibody or antigen binding fragment thereof, a combination, a polynucleotide, an expression vector, a host cell, a cell-free expression system or a composition for use according to any one of the preceding claims, wherein treatment of the neuromuscular disorder results in an improvement, relative to a human subject not being treated with the anti-MuSK antibody or antigen binding fragment thereof, a polynucleotide, an expression vector, a host cell, a cell-free expression system or the composition via electrophysiological assessment or pharmacodynamic assessment; in neurofilaments (e.g. neurofilament light chain (NFL)) in blood serum, plasma and/or cerebrospinal fluid (CSF); or in NMJ biopsies of the treated human subject.

FIG. 1A

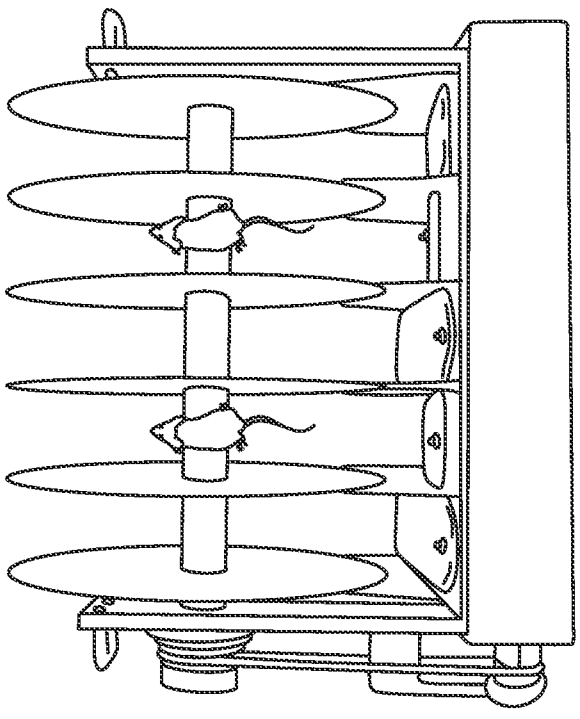


FIG. 1B

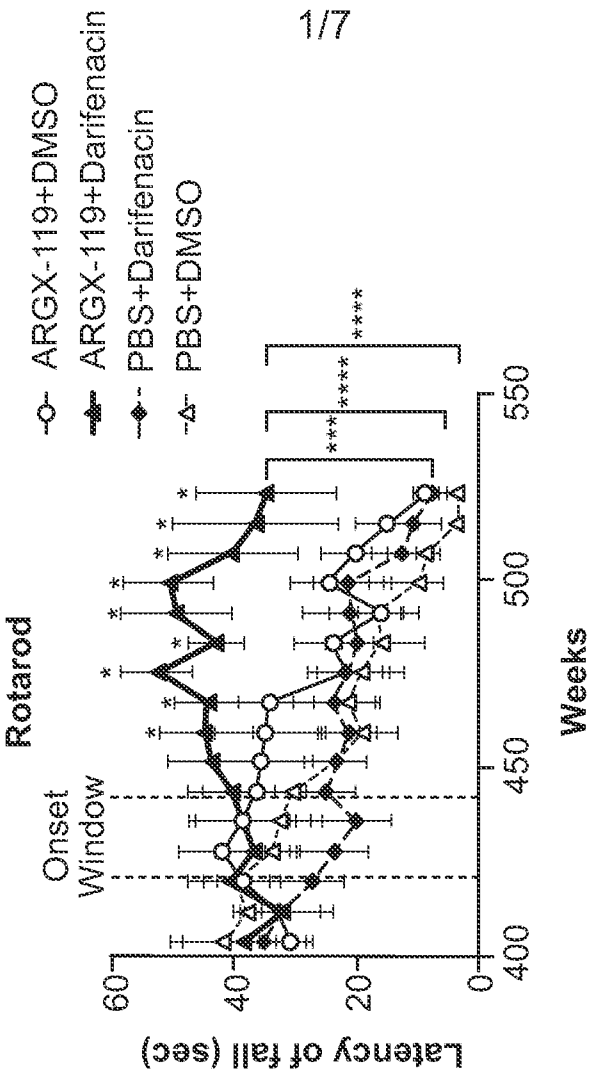

ARGX-119+DMSO
ARGX-119+Darifenacin
PBS+Darifenacin
PBS+DMSO
Rotarod
Onset Window
Latency of fall (sec)
Weeks
0
20
40
60
400
450
500
550
*
***
****

FIG. 1C

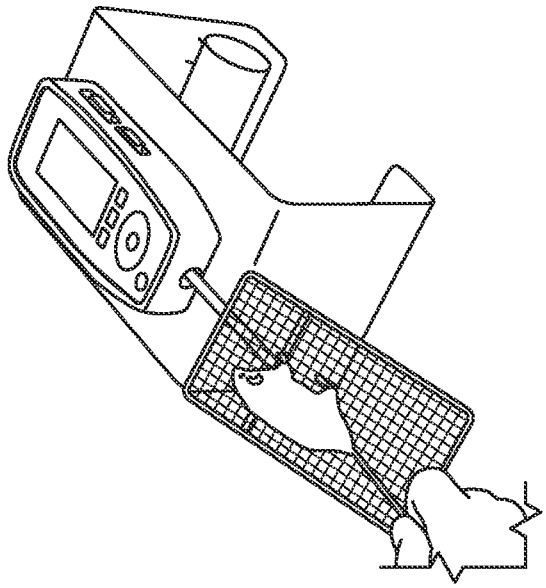


FIG. 1D

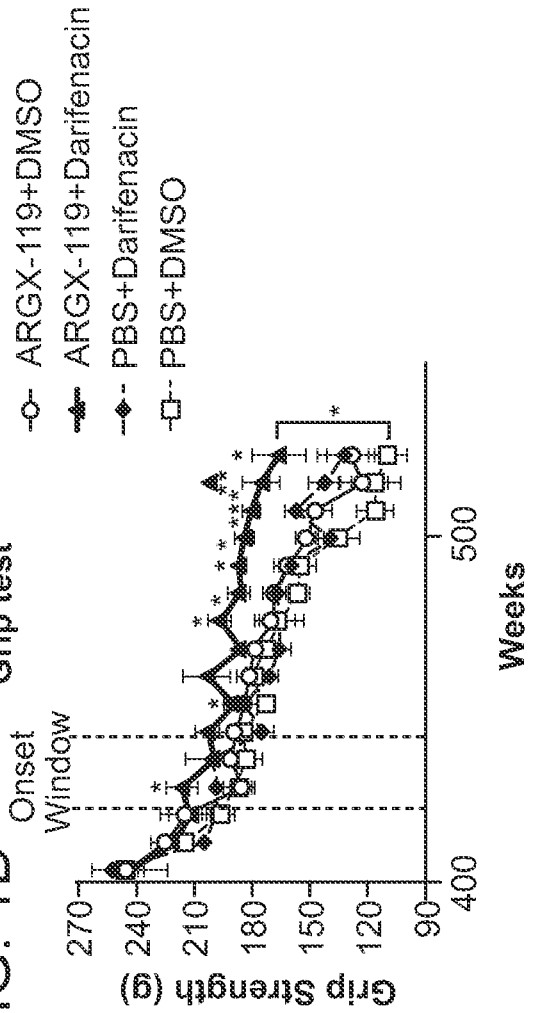
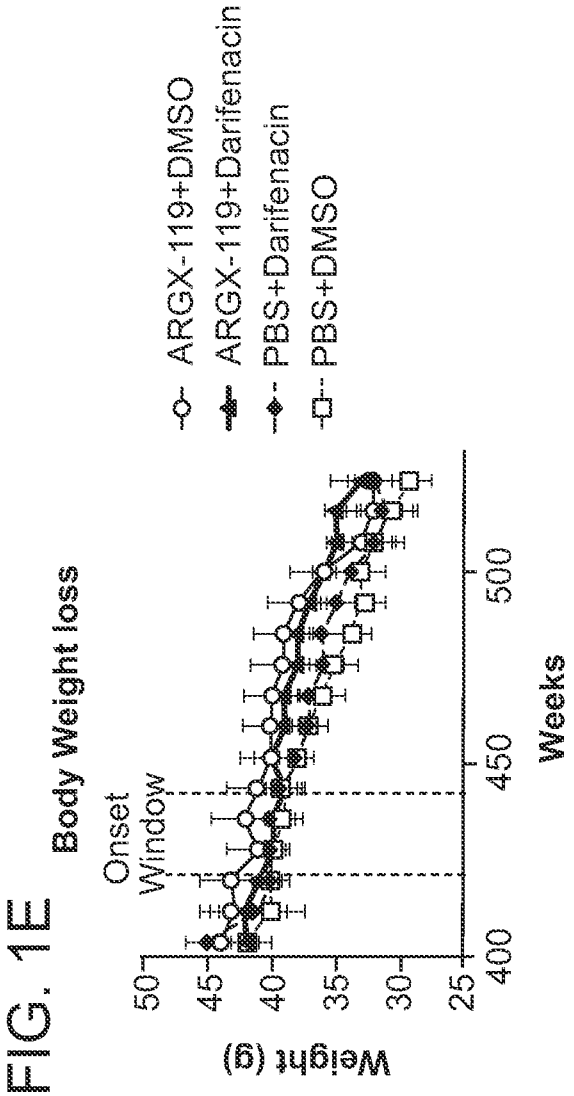


FIG. 1E



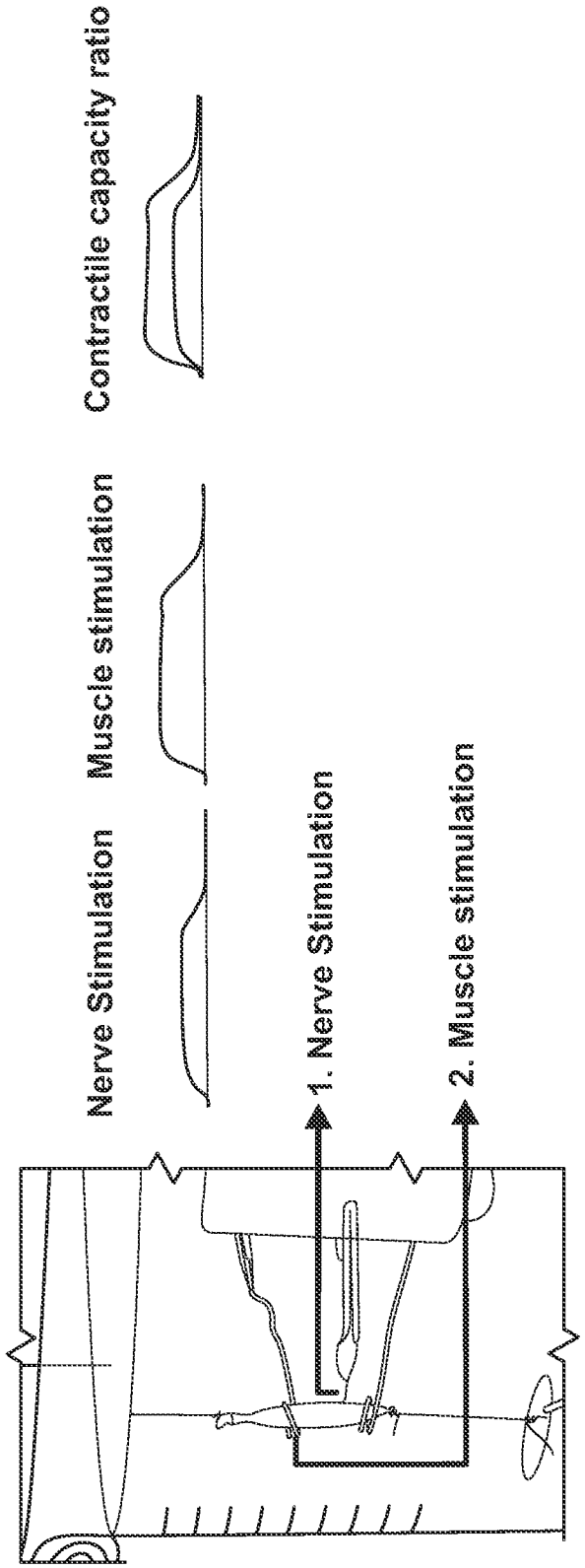

Contractile capacity ratio
Muscle stimulation
Nerve Stimulation
1. Nerve Stimulation
2. Muscle stimulation

FIG. 2A

FIG. 2B

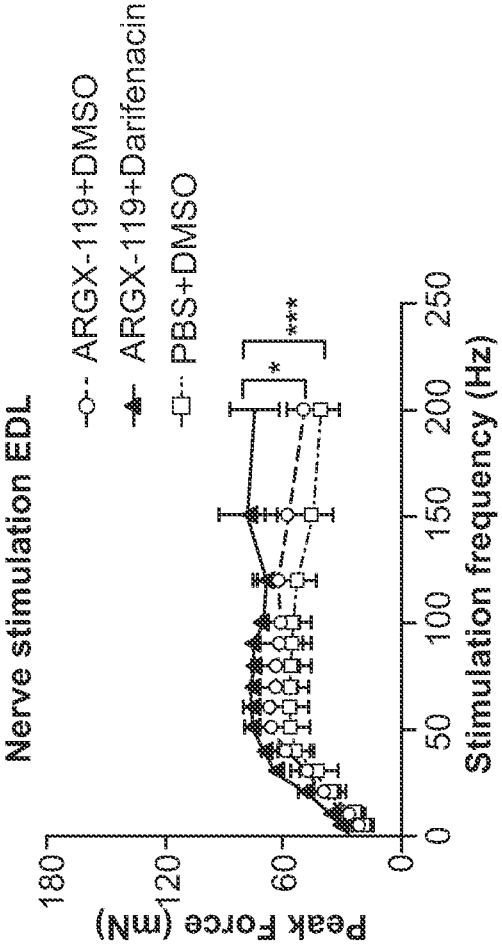


FIG. 2C

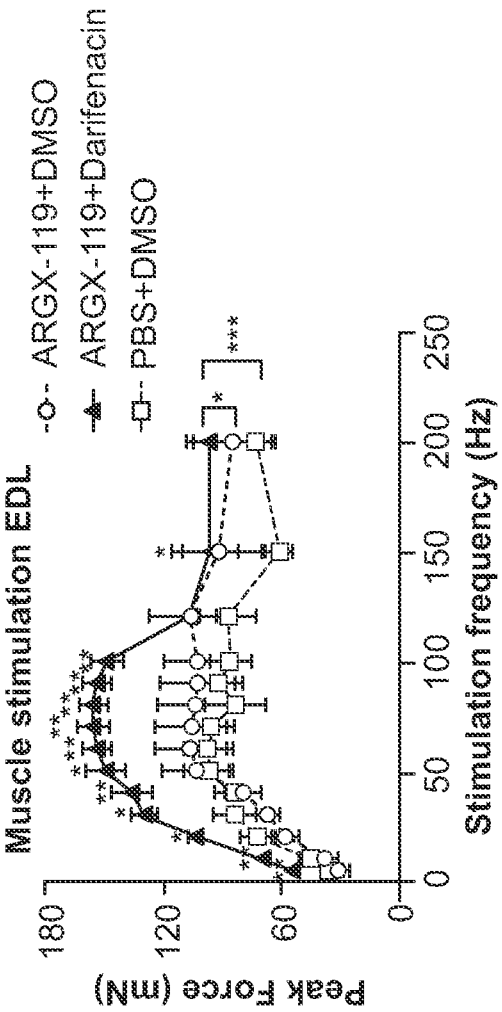


FIG. 2D

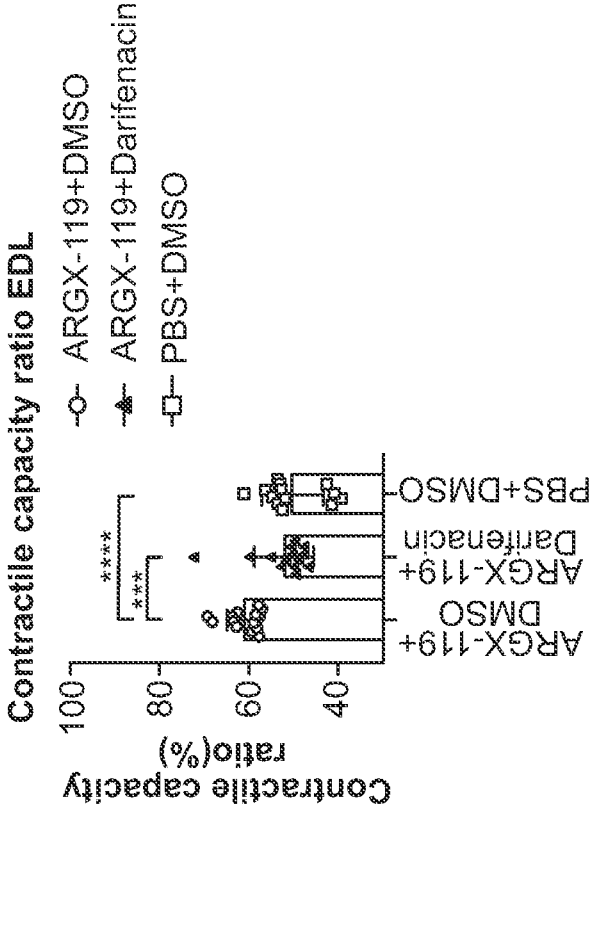


FIG. 2E

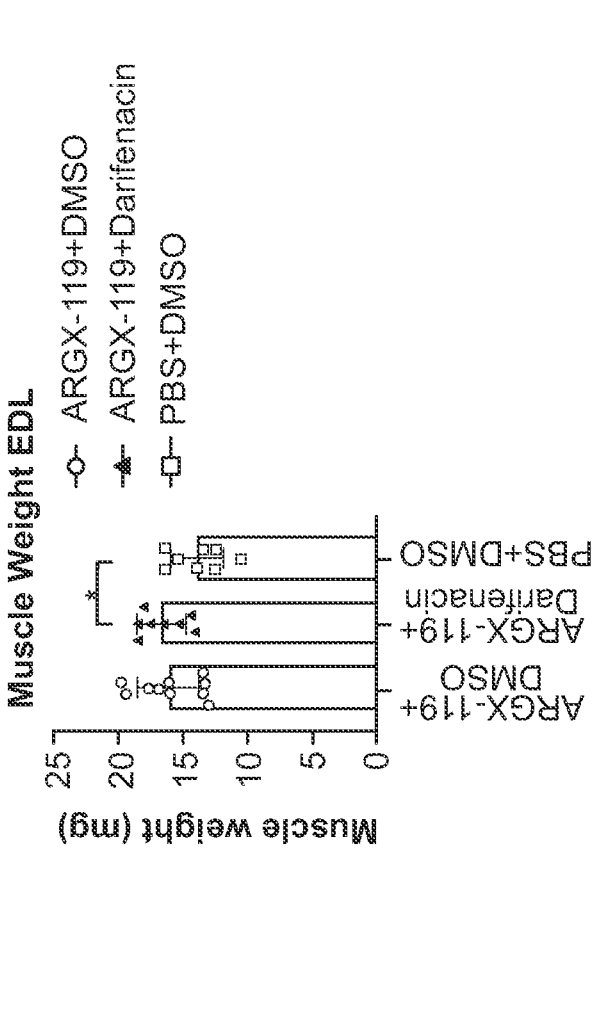


FIG. 3A

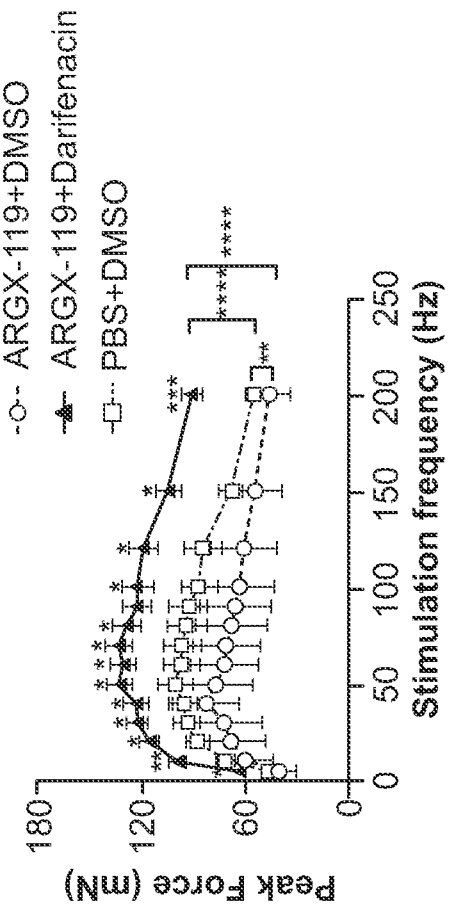


FIG. 3B

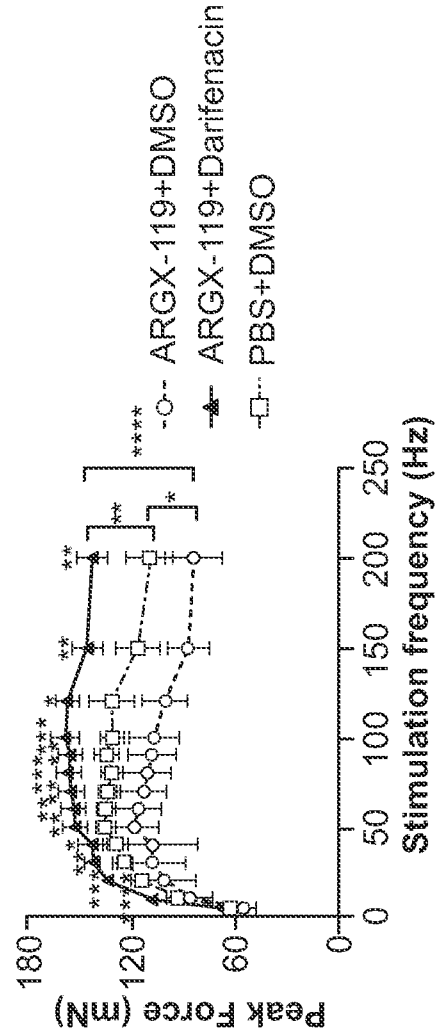


FIG. 3C

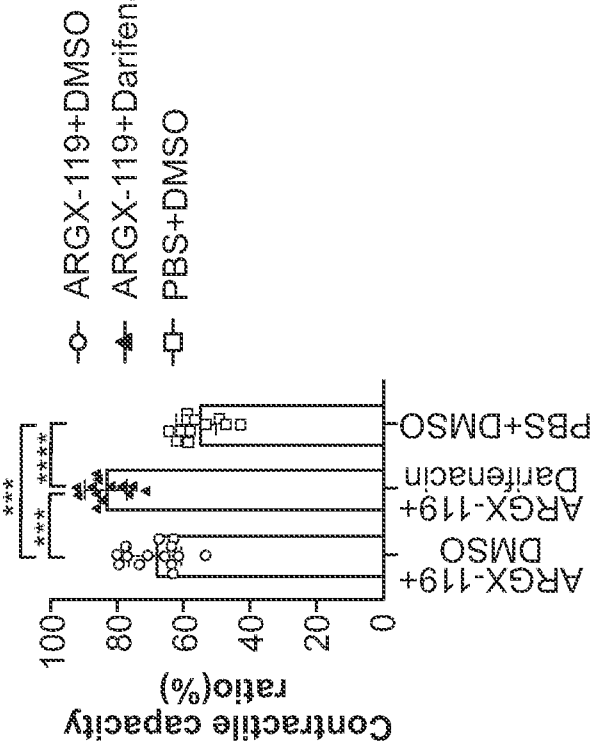
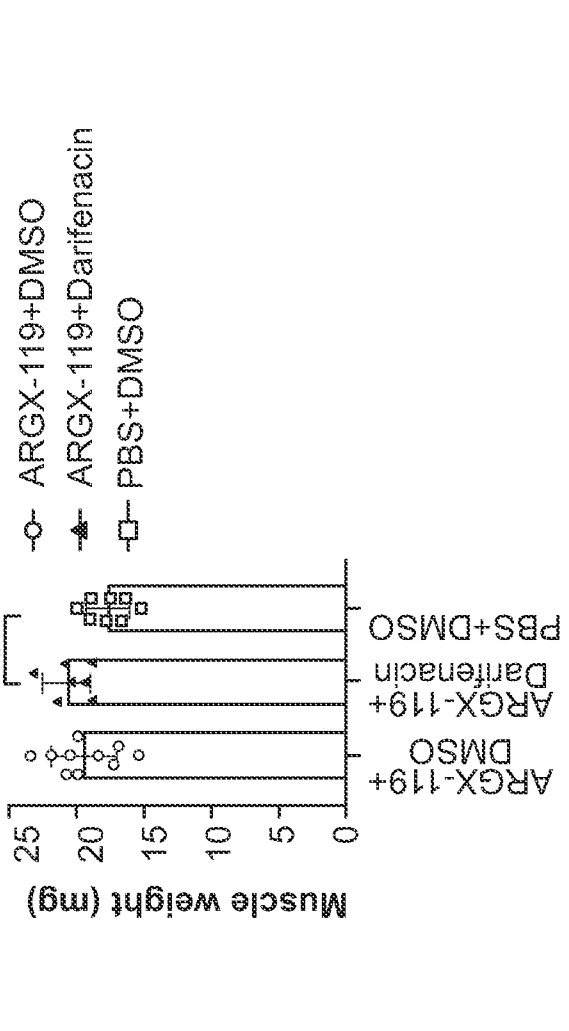


FIG. 3D



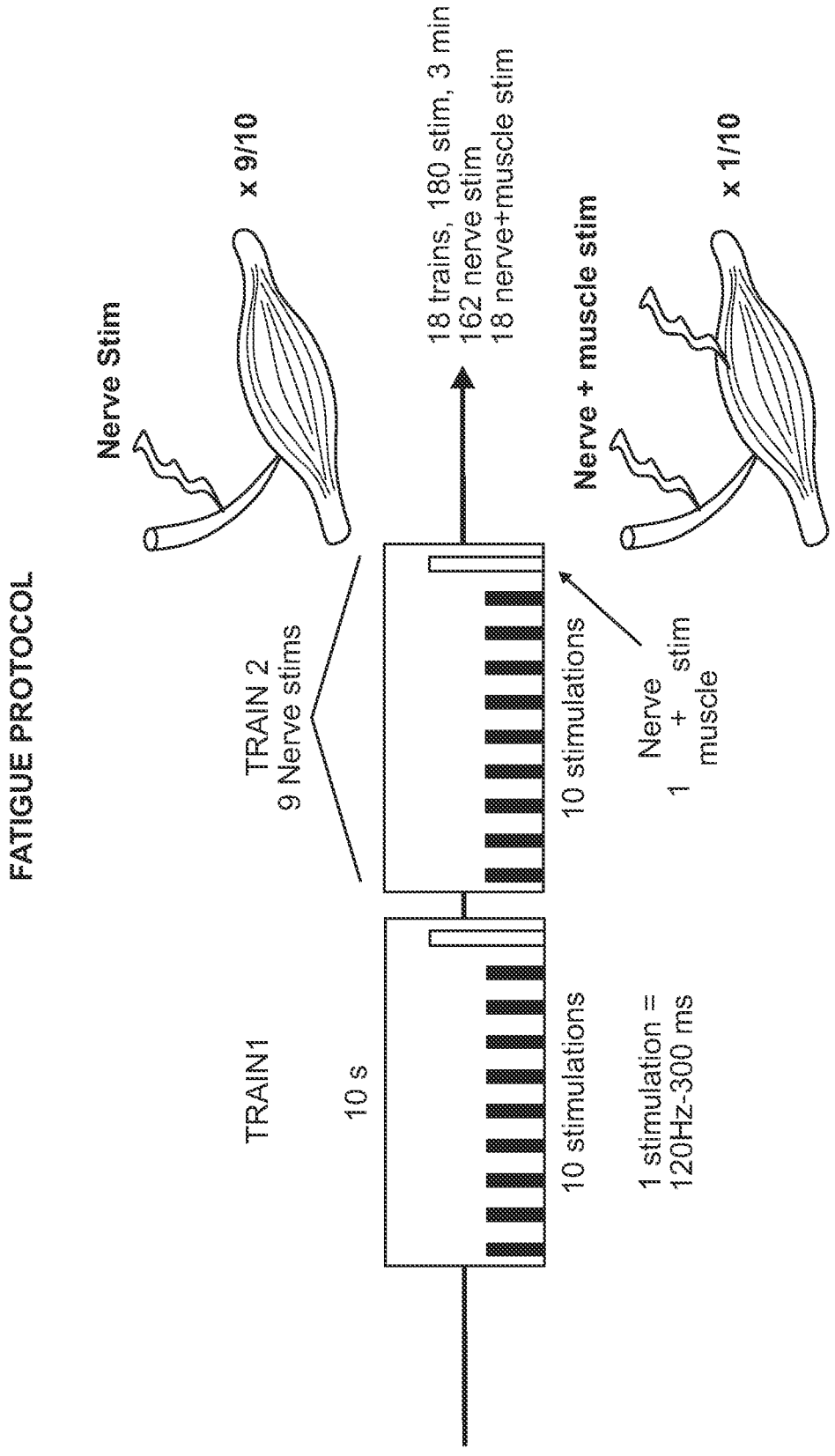

FATIGUE PROTOCOL
Nerve Stim
x 9/10
18 trains, 180 stim, 3 min
162 nerve stim
18 nerve+muscle stim
Nerve + muscle stim
x 1/10
TRAIN 2
9 Nerve stims
10 stimulations
Nerve + muscle
1 stim
TRAIN1
10 s
10 stimulations
1 stimulation = 120Hz-300 ms

FIG. 4A

FIG. 4B

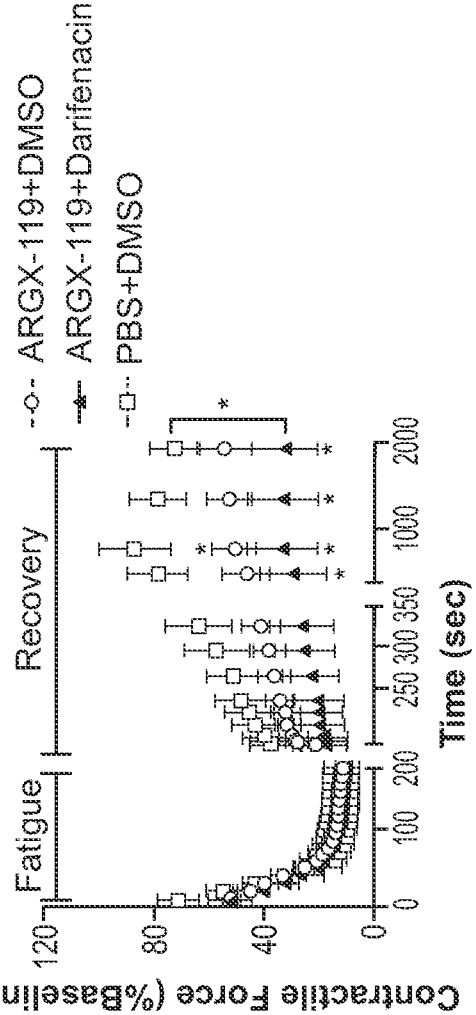


FIG. 4C

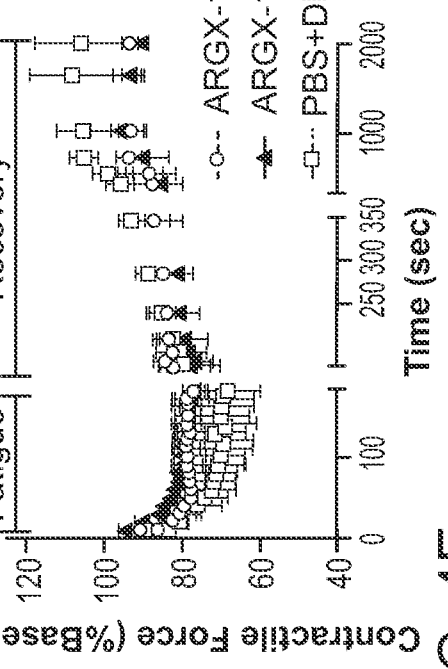


FIG. 4D

FIG. 4E

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

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(71) Applicants: **ARGENX BV** [BE/BE]; Industriepark Zwijnaarde 7, 9052 Ghent (BE). **UNIVERSITÉ DE MONTRÉAL** [CA/CA]; 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **NEW YORK UNIVERISTY** [US/US]; 70 Washington Square South, New York, Massachusetts 10012 (US).

(72) Inventors: **VANHAUWAERT, Roeland**; c/o argenx IIP BV, Zwijnaarde 7, 9052 Ghent (BE). **SILENCE, Karen**; c/o argenx IIP BV, Zwijnaarde 7, 9052 Ghent (BE). **ROBITAILLE, Richard**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **ARBOUR, Danielle**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **RENAUD, Laurence**; c/o Université de Montréal, 2900, boul. Edouard-Monpetit, Montreal, Québec H3T 1J4 (CA). **BURDEN, Steven J.**; 295 Millerton Road, Sharon, Massachusetts 06069 (US).

(74) Agent: **PEDANOU, Victoria** et al.; c/o Dechert LLP, One International Place, 40th Floor, 100 Oliver Street, Boston, Massachusetts 02110-2605 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
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21 September 2023 (21.09.2023)

WO 2023/147489 A3

(54) Title: ANTI-MUSK ANTIBODIES FOR USE IN TREATING NEUROMUSCULAR DISORDERS

(57) Abstract: The present invention relates to an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject. In an embodiment, this antibody or antigen binding fragment thereof is combined with an anticholinergic compound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/61476

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 39/395, C07K 16/40, C07K 16/28, A61K 39/00 (2023.01)
ADD. C07K 16/00, A61P 21/00 (2023.01)

CPC - INV. C07K 16/40, C07K 16/286, C07K 16/28

ADD. C07K 16/00, A61P 21/00, A61K 2039/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2021/212053 A2 (NEWYORK UNIVERSITY et al.) 21 October 2021 (21.10.2021) abstract; para [0084]-[0085]; [0128]; [0155]; [0167]-[0181]; SEQ ID NOs: 97, 98, 129	1, 2, 11 --- 3, 28-30, 34-36
Y	WO 2021/126320 A1 (AMYLYX PHARMACEUTICALS INC.) 24 June 2021 (24.06.2021) p. 18, para 2; p. 37, para 2; p. 46, para 5 to p. 47, para 1; claim 37	28-30, 34-36
Y	US 2007/0041967 A1 (JUNG et al.) 22 February 2007 (22.02.2007) abstract; para [0059]; [0079]; [0100]; SEQ ID NO: 15	3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2023 (22.06.2023)	JUL 20 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/61476

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/61476

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-10, 15-27, 31-33, 37-45
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

--continued on extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-3, 11, 28-30, 34-36

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

--continued from: Box No. III Observations where unity of invention is lacking--

Group I+, claims 1-3, 11-14, directed to an anti-MuSK antibody or antigen binding fragment thereof with a specified one or more sequences. The anti-MuSK antibody or fragment thereof will be searched to the extent that the anti-MuSK antibody encompasses variable heavy chain (VH) sequence SEQ ID NO: 97, variable light chain (VL) sequence SEQ ID NO: 98 (first VH and VL sequences in Table 3), and constant Fc region SEQ ID NO: 266. The first named invention was determined based on these being the first listed variable sequences (claim 11, Table 3), and first listed constant Fc region sequence (claim 3). This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-3, 11 encompass this first named invention, and thus these claims will be searched without fee to the extent that the anti-MuSK antibody comprises VH SEQ ID NO: 97, VL SEQ ID NO: 98, and constant Fc region SEQ ID NO: 266. Additional anti-MuSK antibody sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected anti-MuSK antibody sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be where the anti-MuSK antibody comprises VH SEQ ID NO: 99, VL SEQ ID NO: 100, and constant Fc region SEQ ID NO: 266, (claims 1-3, 11).

[Note, none of the applicant supplied SEQ ID NOs for full length heavy or light chain sequences comprise any of SEQ ID NOs: 97, 98, 99 or 100. Thus, claims to specific full length heavy chain sequences and/or full-length light chain sequences are excluded from the first embodiment and exemplary election of Group I+]

Group II, claims 28-30, 34-36, directed to an anti-MuSK antibody or antigen binding fragment thereof administered to an asymptomatic human subject, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease, or a combination comprising an anti-MuSK antibody or antigen binding fragment thereof and an anti-cholinergic compound.

The inventions listed as Groups I+ and II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ requires the special technical feature of wherein the antibody or antigen binding fragment comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) as identified in table 3 and/or a CDR as identified in table 1 or 2, which is not required by Group II.

Group II requires the special technical feature of administration to an asymptomatic human subject, preferably within 1, 2, 3, 4, 5, or 6 months prior to the onset of the disease, or a combination comprising an anti-MuSK antibody or antigen binding fragment thereof and an anti-cholinergic compound, which is not required by Group I+.

The inventions of Group I+ each include the special technical feature of a unique anti-MuSK antibody amino acid sequence(s), and is considered a distinct technical feature.

Common Technical Features

Groups I+ and II share the common technical feature of an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of ALS in a human subject.

No technical features are shared between the peptide amino acid sequences of Group I+ and accordingly these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to further share the technical feature of including: an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject wherein the antibody or antigen binding fragment comprises specific heavy chain variable domain (VH) and light chain variable domain (VL) sequences and/or CDR sequences, these shared technical features are previously disclosed by WO 2021/212053 A1 to New York University (hereinafter "NYU").

NYU teaches an anti-MuSK antibody or antigen binding fragment thereof for use in the treatment of a neuromuscular disorder in a human subject (abstract "The present invention further discloses methods of treating neuromuscular conditions using the aforementioned MuSK antibodies"; para [00167] "In therapeutic applications (i.e., in applications involving a patient who has been diagnosed with a neuromuscular disorder such as amyotrophic lateral sclerosis (ALS), myasthenia gravis, or congenital myasthenia) the MuSK antibody based molecules of the present invention are administered to such patient in an amount sufficient to cure, treat, or at least partially arrest the symptoms of the disease"; [0155] "for animal or human administration"), wherein the antibody or antigen binding fragment comprises specific heavy chain variable domain (VH) and light chain variable domain (VL) sequences and/or CDR sequences (para [0107] "the antibody-based molecule that binds to human muscle-specific tyrosine-protein kinase (MuSK) comprises a heavy chain variable region, where the heavy chain variable region comprises: (i) a complementarity-determining region 1...").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I+ and II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

**Continuation of item 4 above: claims 4-10, 15-27, 31-33, 37-45 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

(19)国家知识产权局

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(71)申请人 阿根思有限公司
地址 比利时
申请人 蒙特利尔大学 纽约大学

(72)发明人 鲁兰·瓦努瓦特 卡伦·西伦斯
理查德·罗比泰尔
丹妮尔·阿布尔 劳伦斯·雷诺
史蒂文·J·布尔登

(74)专利代理机构 北京集佳知识产权代理有限公司 11227
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A61K 39/00(2006.01)
C07K 16/00(2006.01)
A61P 21/00(2006.01)

权利要求书6页 说明书101页
序列表（电子公布） 附图11页

(54)发明名称

用于治疗神经肌肉病症的抗MuSK抗体

(57)摘要

本发明涉及用于在人对象中治疗神经肌肉病症的抗MuSK抗体或其抗原结合片段。在一个实施方案中,将该抗体或其抗原结合片段与抗胆碱能化合物组合。

CN 119013308 A

1.抗MuSK抗体或其抗原结合片段,其用于在人对象中治疗神经肌肉病症。

2.根据权利要求1所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列。

3.根据权利要求1或2所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或其抗原结合片段包含野生型人IgG恒定Fc区,所述野生型人IgG恒定Fc区与SEQ ID NO:266或SEQ ID NO:267包含至少80%序列同一性。

4.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其为激动剂MuSK抗体并且/或者效应物功能已被降低或消除。

5.优选地根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中效应物功能的降低或消除是通过将以下突变(均根据EU编号系统编号)的一个或更多个引入基于抗体的分子的恒定区SEQ ID NO:266或SEQ ID NO:267中获得的:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地所述突变是L234A或L235A,更优选地所述突变是L234A和L235A。

6.优选地根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或其抗原结合片段包含野生型人IgG恒定Fc区SEQ ID NO:266或SEQ ID NO:267,并且其中将根据EU编号系统编号的L234A和L235A突变引入所述Fc区。

7.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:

a)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,和

b)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

8.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

其中所述VH含有:

-包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

-包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

-包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

其中所述VL含有:

-包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

-包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

-包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

9.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

-其中所述VH包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,并且所述VL包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,以及

-其中所述VH含有:

o包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

o包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

o包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

-其中所述VL含有:

o包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

o包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

o包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

10.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:

-重链可变结构域(VH),其包含SEQ ID NO:234,和

-轻链可变结构域(VL),其包含SEQ ID NO:235。

11.抗MuSK抗体或其抗原结合片段,其用于在人对象中治疗神经肌肉病症,其中所述抗体或抗原结合片段包含如表3中所确定的重链可变结构域(VH)和轻链可变结构域(VL)以及/或者如表1或2中所确定的CDR。

12.抗MuSK抗体或其抗原结合片段,其优选地用于在人对象中治疗神经肌肉病症,其中所述抗体或抗原结合片段含有:

-包含与SEQ ID NO:270具有至少80%同一性或相似性的氨基酸序列的全长重链和包含与SEQ ID NO:271具有至少80%同一性或相似性的氨基酸序列的全长轻链,或者

-包含与SEQ ID NO:268具有至少80%同一性或相似性的氨基酸序列的全长重链和包含与SEQ ID NO:269具有至少80%同一性或相似性的氨基酸序列的全长轻链,

-其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入所述全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替

换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地所述突变是L234A或L235A,更优选地所述突变是L234A和L235A。

13.抗MuSK抗体或其抗原结合片段,其优选地用于根据权利要求12所述应用,其中所述抗体或抗原结合片段包含:

-全长重链,其包含SEQ ID NO:270,和

-全长轻链,其包含SEQ ID NO:271,并且

-其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

14.抗MuSK抗体或其抗原结合片段,其优选地用于根据权利要求12所述应用,其中所述抗体或抗原结合片段包含:

a)全长重链,其包含SEQ ID NO:268,和

b)全长轻链,其包含SEQ ID NO:269,并且

c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

15.多核苷酸,其用于在人对象中治疗神经肌肉病症,所述多核苷酸包含编码权利要求1至14中任一项所述的抗体或其抗原结合片段或者其VH或VL或CDR的核苷酸序列。

16.表达载体,其用于在人对象中治疗神经肌肉病症,其包含权利要求15所述的多核苷酸,优选地与允许抗体或其抗原结合片段或者其VH或VL或CDR在宿主细胞或无细胞表达系统中表达的调节区可操作地连接。

17.宿主细胞或无细胞表达系统,其用于在人对象中治疗神经肌肉病症,其含有权利要求16所述的表达载体。

18.组合物,其用于在人对象中治疗神经肌肉病症,其包含如权利要求1至14中任一项所限定的抗体或其抗原结合片段、如权利要求15中所限定的多核苷酸、如权利要求中16所限定的表达载体或者如权利要求17中所限定的宿主细胞或无细胞表达系统。

19.根据权利要求18所述的组合物,其用于在人对象中治疗神经肌肉病症,其为包含至少一种可药用载体或赋形剂的药物组合物。

20.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述抗体或抗原结合片段,多核苷酸、表达载体、宿主细胞、无细胞表达体系或者组合物与抗胆碱能化合物组合施用。

21.根据权利要求20所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述抗胆碱能化合物单独、顺序或同时施用。

22.根据权利要求20或21中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述抗胆碱能化合物是毒蕈碱受体拮抗剂,优选地是对毒蕈碱受体M1和/或毒蕈碱受体M3和/或毒蕈碱受体M5具有选择性的毒蕈碱受体拮抗剂。

23.根据权利要求22中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述毒蕈碱受体拮抗剂对毒蕈碱受体M3具有选择性,优选地其中所述抗胆碱能化合物是达非那新、异丙托溴铵、噻托溴铵或曲司氯铵。

24.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述神经肌肉病症的特征在于神经肌肉传递受损和/或NMJ(神经肌肉接头)处的去神经。

25.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述神经肌肉病症的特征在于以下至少一种:

a.毒蕈碱过度兴奋,

b.运动神经元死亡,

c.神经肌肉接头(NMJ)去神经以及

d.突触传递受损。

26.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述神经肌肉病症选自以下:肌萎缩侧索硬化(ALS)、脊髓性肌萎缩(SMA)、重症肌无力(MG)、先天性肌无力、兰伯特-伊顿肌无力综合征(LEMS)、莱姆病、脊髓灰质炎、脊髓灰质炎后、重金属中毒、肯尼迪综合征、成年发作的泰-萨克斯病、遗传性痉挛性截瘫、多灶性神经病、颈椎病、髓外肿瘤伴压迫性神经根病和脊髓病、包涵体肌炎、进行性延髓麻痹、进行性肌萎缩、运动神经元综合征和甲状腺毒性肌病。

27.根据权利要求1至14中任一项或当回引权利要求1至14中任一项时权利要求15至26中任一项所述应用的抗MuSK抗体或抗原结合片段,其中所述病症为ALS。

28.抗MuSK抗体或其抗原结合片段,其用于在人对象中治疗ALS,其中所述抗体或抗原结合片段施用于无症状人对象,优选地在疾病发作之前1、2、3、4、5或6个月内施用于无症状人对象。

29.根据权利要求28所述的抗MuSK抗体或其抗原结合片段,其中所述无症状人对象被诊断为易于发生神经肌肉病症或疾病。

30.根据权利要求28或29所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列。

31.根据权利要求28至30所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:

c)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,和

d)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

32.根据权利要求28至31中任一项所述应用的抗MuSK抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:

a)全长重链,其包含SEQ ID NO:268,和

b)全长轻链,其包含SEQ ID NO:269,并且

c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

33.根据权利要求20至27中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述抗胆碱能化合物在疾病发作时施用或者在疾病发作之后1、2、3、4、5、6或7周内施用。

34.组合,其包含抗MuSK抗体或其抗原结合片段和抗胆碱能化合物,其优选地用于在人对象中治疗ALS。

35.根据权利要求34所述的组合,其中所述抗体或抗原结合片段施用于无症状人对象,优选地在疾病发作之前1、2、3、4、5或6个月内施用于无症状人对象,以及/或者其中所述抗胆碱能化合物在疾病发作时施用或在疾病发作之后1、2、3、4、5、6或7周内施用。

36.根据权利要求35所述的组合,其中用所述抗体治疗的无症状人对象已首先被诊断为易于发生神经肌肉病症或疾病。

37.根据权利要求34至36所述的组合,其中所述抗MuSK抗体或其抗原结合片段结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列。

38.根据权利要求34至37中任一项所述的组合,其中所述抗MuSK抗体或其抗原结合片段包含:

重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,和

轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

39.根据权利要求34至38中任一项所述的组合,其中所述抗体或抗原结合片段包含:

a)全长重链,其包含SEQ ID NO:268,和

b)全长轻链,其包含SEQ ID NO:269,并且

c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

40.根据权利要求1至39中任一项所述应用的抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中在疾病发作时,将所述抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物施用于无症状人对象,优选地在疾病发作之前1、2、3、4、5或6个月内将所述抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物施用于无症状人对象。

41.根据权利要求40所述应用的抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中疾病发作包括至少一种选自以下的症状:肌肉颤搐、肌肉痉挛、痉挛、肌无力、口齿不清和/或鼻音、咀嚼困难或吞咽困难、吞咽困难、构音障碍和呼吸困难。

42.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中在以下中通过电生理学评估或药效学评估来分析神经肌肉病症:在血清、血浆和/或脑脊液(CSF)中的神经丝(例如神经丝轻链(NFL));或者NMJ活检。

43.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、组合、多核

苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中向所述人对象施用所述抗MuSK抗体或其抗原结合片段、所述组合、多核苷酸、表达载体,宿主细胞、无细胞表达系统或者组合物导致以下治疗作用的一种或更多种:

-所述对象中完全受神经支配的NMJ的数目或百分比的提高,所述对象中完全受神经支配NMJ的数目或百分比的维持,所述对象中完全去神经的NMJ的数目或百分比的降低,突触传递的可靠性的改善,所述对象中运动神经元死亡的预防、稳定或降低;以及/或者

-所述对象的运动表现和/或握力的改善;以及/或者

-所述对象的NMJ处的肌肉的收缩特性的改善;以及/或者

-所述对象的NMJ处的肌肉的对疲劳的抗性的改善;以及/或者

-诱导所述对象的NMJ处的肌肉重量增加;以及/或者

-所述对象生活质量的改善或生活质量恶化出现的延迟;以及/或者

-所述对象中的突触周围施万细胞(PSC)的毒蕈碱活性的降低(或毒蕈碱过度兴奋的降低)或所述对象中的NMJ修复。

44.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中所述治疗导致所述病症的稳定。

45.根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、组合、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物,其中相对于没有用所述抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物治疗的人对象,所述神经肌肉病症的治疗导致受治人对象通过电生理学评估或药效学评估中的改善;在血清、血浆和/或脑脊液(CSF)中的神经丝(例如神经丝轻链(NFL))中的改善;或者在NMJ活检中的改善。

用于治疗神经肌肉病症的抗MuSK抗体

[0001] 相关申请

[0002] 本申请要求2022年5月13日提交的美国临时专利申请序列No.63/364,685和2022年1月28日提交的EP申请No.22154118.8的优先权,其全部公开内容在此通过引用并入本文。

[0003] 序列表的引用

[0004] 本申请包含以ST.26格式以电子方式提交并且在此通过引用整体并入的序列表(所述ST.26副本创建于2023年1月27日,命名为“196198_SL.XML”并且大小为364,704字节)。

技术领域

[0005] 本发明涉及抗MuSK抗体或其抗原结合片段,其用于在人对象中治疗神经肌肉病症,例如ALS(amyotrophic lateral sclerosis)(肌萎缩侧索硬化)。在一个实施方案中,将该抗体或其抗原结合片段与抗胆碱能化合物组合。

背景技术

[0006] ALS是成年发作的非细胞自主性神经肌肉/神经退行性病症,其导致上和下运动神经元(motor neuron,MN)的进行性丧失,导致2至5年内逐渐瘫痪和死亡。神经肌肉接头(Neuromuscular junction,NMJ)去神经是ALS的标志[1],并且存在于ALS的数种疾病模型[2-6]中,甚至在MN死亡之前[1,3]。

[0007] 目前批准的ALS治疗(利鲁唑(Riluzole))通过将寿命延长约三个月仅使20%的ALS患者受益。利鲁唑对肌肉功能的作用非常有限。此外,ALS被认为是遗传异质性疾病,可能代表具有不同潜在病理状态的数个亚组。由于潜在的疾病机制不同,目前没有可用的治疗,患者定制的治疗也不可能帮助所有ALS患者。

[0008] 因此,仍然需要用于ALS和其他类似ALS的疾病(“ALS样疾病”)的新的治疗。

附图说明

[0009] 图1组合处理在SOD1G37R小鼠中改善运动功能。ARGX-119处理在P400(疾病发作前或无症状)开始,并且达非那新处理在约P425(疾病发作)开始,并持续至处死(约P520)。A)描绘转轮加速之后用于评估运动功能、协调和平衡的转棒仪装置的图。B)约400日龄至520日龄的经ARGX-119抗体(大灰色圆圈)、经ARGX-119+达非那新组合处理来处理的小鼠(黑色三角形)、经达非那新处理的小鼠(小灰色圆圈)与经双安慰剂处理的小鼠(灰色方块)落在旋转棒上的等待时间(秒)。C)用于监测小鼠前肢和后肢的整体力量的握力计。D)处理过程期间握力测量值的演变,各组从约P400至P520。E)处理过程期间体重测量值的演变,各组从约P400至P520。*$p<0.05$,***$p<0.001$,****$p<0.0001$。单因素ANOVA和多重t检验。

[0010] 图2处理改善EDL肌肉的收缩特性。A)用于引起肌肉收缩的神经和肌肉刺激电极和肌力传感器设置(set up)的图。原始数据的实例示出了由神经或肌肉刺激引发的肌肉收

缩,用于计算收缩能力比率。B至C)由来自经ARGX-119抗体(灰色圆圈)和经ARGX-119+达非那新组合处理来处理的小鼠(黑色三角形)与经双安慰剂处理的小鼠(灰色正方形)在不同频率(5Hz至300Hz)的神经刺激(B)或肌肉刺激(C)产生的EDL肌肉的峰值抽搐力(twitch force)。D)示出以百分比表示的EDL肌肉收缩能力比率的平均值±SEM的柱状图,表示神经刺激与肌肉刺激(刺激频率为5Hz至100Hz)产生的峰值力的比例。E)示出来自经ARGX-119抗体、经组合处理和经安慰剂处理小鼠的EDL肌肉重量的平均值±SEM的直方图。*p<0.05,**p<0.01,***p<0.001,****p<0.0001。重复单因素ANOVA和多重t检验。

[0011] 图3组合处理改善比目鱼肌的收缩特性。A至B)由经ARGX-119抗体(灰色圆圈)和经ARGX-119+达非那新组合处理来处理的小鼠(黑色三角形)与经双安慰剂处理的小鼠(灰色正方形)在不同频率(5Hz至300Hz)的神经刺激(A)或肌肉刺激(B)产生的比目鱼(Soleus,SOL)肌的峰值抽搐力。C)示出以百分比表示的SOL肌肉收缩能力比率的平均值±SEM的柱状图,表示神经刺激与肌肉刺激(刺激频率为5Hz至100Hz)产生的峰值力的比例。D)示出来自经ARGX-119抗体、经组合处理和经安慰剂处理小鼠的EDL肌肉重量的平均值±SEM的直方图。*p<0.05,**p<0.01,***p<0.001,****p<0.0001。重复单因素ANOVA和多重t检验。

[0012] 图4组合处理保持了肌肉疲劳特性。A)示出EDL疲劳方案的图,该方案由在120Hz下引发300ms(每秒1轮)的10个刺激的18次训练(train)组成。10回中有9回使用单独的神经刺激,并且每10次刺激将肌肉刺激叠加到神经刺激上。疲劳方案之后是30分钟的恢复期。B至C)对于EDL肌肉的神经刺激(B)和神经+肌肉(C),疲劳方案和恢复期期间的峰值收缩力表示为疲劳方案之前产生的初始基线力的百分比。注意,与经双处理ARGX-119+达非那新的动物(黑色三角形)和经ARGX-119抗体处理(灰色圆圈)相比,经安慰剂处理(灰色正方形)的对疲劳的抗性更高。这说明了正常快速颤搐肌肉特性的显著改变,该特性通常是高度易疲劳的。D至E)对于SOL肌肉的神经刺激(D)和神经+肌肉(E),疲劳方案和恢复期期间的峰值收缩力表示为疲劳方案之前产生的初始基线力的百分比。*p<0.05,**p<0.01,***p<0.001,****p<0.0001。重复单因素ANOVA和多重t检验。

具体实施方式

[0013] 一般定义

[0014] 仅提供以下术语或定义来帮助理解本发明。除非本文中明确定义,否则本文中使用的所有术语具有与其对于本发明领域的技术人员来说相同的含义。对于本领域的定义和术语,实践者特别地参考Sambrook et al.,Molecular Cloning:A Laboratory Manual,第二版,Cold Spring Harbor Press,Plainsview,New York(1989);和Ausubel et al.,Current Protocols in Molecular Biology(Supplement 47),John Wiley&Sons,New York(1999)。本文中提供的定义不应被解释为具有小于本领域普通技术人员所理解的范围。

[0015] 除非另外指明,否则对技术人员将明显的是,可以以本身已知的方式进行并且已经进行了未具体详细描述的所有方法、步骤、技术和操作。例如,再次参考标准手册、上面提及的一般背景技术以及其中引用的另一些参考文献。

[0016] 除非上下文另外明确指出,否则本文中使用的没有数量词修饰的名词表示一个/种和更多个/种。

[0017] 本文中使用的术语“包含”与“包括”或“含有”同义,并且是包括性的或开放式的,而不排除另外的非记载成员、化合物、产品、要素或方法步骤。在产品或组合物(“基本上由.....组成的产品”或“基本上由.....组成的组合物”)的上下文中使用的表述“基本上由.....组成”意指可存在另外的分子,但这样的分子不改变所述产品或组合物的特征/活性/功能。例如,如果组合物本身将表现出与抗体之一或抗体片段之一相似的特征/活性/功能,则组合物可基本上由抗体或抗体片段组成。

[0018] 通过端点对数值范围的列举包括在相应范围内纳入的所有数字和分数,以及所列举的端点。

[0019] 本文中涉及例如参数、量、时距(temporal duration)等可测量值时使用的术语“约/大约”意在涵盖指定值的或者相对于指定值的+/-10%或更小、优选+/-5%或更小、更优选+/-1%或更小、并且还更优选+/-0.1%或更小的变化,在此范围内这样的变化适合于在所公开的发明中实施。应理解,修饰语“约”所指的值本身也被具体地且优选地公开。

[0020] 术语‘病症’和‘疾病’在本文中可互换使用。

[0021] 本文中使用的氨基酸残基将通过它们的全名或根据标准的三字母或单字母氨基酸代码来表示。

[0022] 本文中使用的术语“多肽”或“蛋白质”可互换使用,并且是指任何长度的氨基酸(其可包括编码和非编码氨基酸、经化学或生物化学修饰或衍生的氨基酸)的聚合物形式和具有经修饰肽骨架的多肽。“肽”也是氨基酸的聚合物,其长度通常多至50个氨基酸。多肽或肽由氨基酸序列表示。

[0023] 本文中使用的术语“核酸分子”、“多核苷酸”、“多核酸”、“核酸”可互换使用,并且是指任何长度的核苷酸(脱氧核糖核苷酸或核糖核苷酸或其类似物)的聚合物形式。核酸分子由核酸序列表示,其主要特征在于其碱基序列。多核苷酸可具有任何三维结构,并且可以进行任何已知或未知的功能。多核苷酸的非限制性实例包括基因、基因片段、外显子、内含子、信使RNA(mRNA)、转移RNA、核糖体RNA、核酶、cDNA、重组多核苷酸、分支多核苷酸、质粒、载体、任何序列的分离的DNA、控制区、任何序列的分离RNA、核酸探针和引物。核酸分子可以是线性的或环状的。

[0024] 本文中使用的术语“同源性”表示来自相同或不同分类单元的两个大分子之间,特别是两个多肽或多核苷酸之间的至少二级结构同一性或相似性,其中所述相似性归因于共同祖先。因此,术语“同源物”表示具有所述二级和任选三级结构相似性的如此相关的大分子。为了比较两个或更多个核苷酸序列,第一核苷酸序列与第二核苷酸序列之间的“序列同一性(百分比)”可以使用本领域技术人员已知的方法来计算,例如通过将第一核苷酸序列中与第二核苷酸序列中对应位置处的核苷酸相同的核苷酸数量除以第一核苷酸序列中核苷酸的总数并乘以100%,或者通过使用用于序列比对的已知的计算机算法,例如NCBI Blast。在确定两个氨基酸序列之间的序列相似性的程度时,本领域技术人员可以考虑所谓的“保守”氨基酸替换,其通常可以描述为其中一个氨基酸残基被具有相似化学结构的另一氨基酸残基替换并且对多肽的功能、活性或其他生物学特性影响很小或基本没有影响的的氨基酸替换。可能的保守氨基酸替换已在本文中例示。如果氨基酸序列和核酸序列在其整个长度上具有100%序列同一性,则称它们为“完全相同”。

[0025] 在本申请通篇,每次提到特定的氨基酸序列SEQ ID NO(以SEQ ID NO:Y为例),可

以将其替换为:包含与氨基酸序列SEQ ID NO:Y具有至少80％序列同一性或相似性的氨基酸序列的多肽。在本申请通篇,措辞“与另一序列至少X％相同的序列”可以替换为“与另一序列具有至少X％序列同一性的序列”。

[0026] 在另一些优选的实施方案中,本文中所述的每个氨基酸序列凭借其分别与给定氨基酸序列的同一性百分比(至少80％)而分别与该给定的氨基酸序列具有至少80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％的同一性或更高的同一性。在一个优选的实施方案中,序列同一性通过比较本文中所标识的序列的全长来确定。在另一些优选的实施方案中,本文中所述的每个氨基酸序列凭借其分别与给定氨基酸序列的相似性百分比(至少80％)而分别与该给定的氨基酸序列具有至少80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％的相似性或更高的相似性。在一个优选的实施方案中,序列相似性通过比较本文中所标识的序列的全长来确定。除非本文中另有说明,否则与给定SEQ ID NO的同一性或相似性意指基于所述序列的全长(即在其全长上或作为整体)的同一性或相似性。

[0027] “序列同一性”在本文中定义为两个或更多个氨基酸(多肽或蛋白质)序列或者两个或更多个核酸(多核苷酸)序列之间的关系,如通过比较序列所确定的。两个氨基酸序列之间的同一性优选地通过评估它们在如本文中所标识的整个SEQ ID NO或其部分内的同一性来确定。其部分可以意指SEQ ID NO长度的至少50％、或至少60％、或至少70％、或至少80％、或至少90％。

[0028] 在本领域中,“同一性”还意指氨基酸序列之间的序列关联性程度,这视情况而定,如由这样的序列的串之间的匹配所确定。两个氨基酸序列之间的“相似性”通过比较一个多肽的氨基酸序列及其保守氨基酸代替物与第二多肽的序列来确定。“同一性”和“相似性”可以很容易地通过已知方法计算,包括但不限于以下中所描述的那些:

[0029] ComputationalMolecular Biology,Lesk,A.M.,ed.,Oxford University Press,New York,1988;Biocomputing:lnfo rmatics and Genome Projects,Smith,D.W.,ed,Academic Press,New York,1993;Computer Analysis of Sequence Data,Part 1,Griffin,A.M.,and Griffin,H.G.,eds,Humana Press,New Jersey,1994;Sequence Analysis in Molecular Biology,von Heine,G.,Academic Press,1987;和Sequence Analysis Primer,Gribskov,M.and Devereux,J.,eds,M Stockton Press,New York,1991;和Carillo,H.,and Lipman,D.,SIAM J.Applied Math.,48:1073(1988).

[0030] 确定同一性的优选方法被设计为在测试的序列之间给出最大匹配。确定同一性和相似性的方法被编入公开可用的计算机程序中。确定两个序列之间的同一性和相似性的优选计算机程序方法包括例如GCG程序包(Devereux,J.,et al.,Nucleic Acids Research 12(1):387(1984))、BestFit、FASTA、BLASTN和BLASTP(Altschul,S.F.et al.,J.Mol.Biol.215:403-410(1990))、EMBOSS Needle(Madeira,F.,et al.,NucleicAcids Research 47(W1):W636-W641(2019))。BLAST程序可从NCBI和其他来源公开获得(BLAST Manual,Altschul,S.,et al.,NCBINLM NIH Bethesda,MD 20894;Altschul,S.,et al.,J.Mol.Biol.215:403-410(1990))。EMBOSS程序可从EMBL-EBI公开获得。公知的Smith Waterman算法也可用于确定同一性。EMBOSS Needle程序是优选使用的程序。

[0031] 用于多肽序列比较的优选参数包括以下:算法:Needleman and Wunsch,J.Mol.Biol.48(3):443-453(1970);比较矩阵:BLOSUM62,其来自Henikoff and Henikoff,Proc.Natl.Acad.Sci.USA.89:10915-10919(1992);空位开放罚分:10,和空位延伸罚分:0.5。可用于这些参数的程序作为来自EMBL-EBI的EMBOSS Needle程序可公开获得。前述参数是蛋白质全局配对序列比对的默认参数(以及对末端空位没有罚分)。

[0032] 用于核酸比较的优选参数包括以下:算法:Needleman and Wunsch,J.Mol.Biol.48:443-453(1970);比较矩阵:DNAfull;空位开放罚分:10;空位延伸罚分:0.5。可用于这些参数的程序作为来自EMBL-EBI的EMBOSS Needle程序可公开获得。前述参数是核苷酸序列的全局配对序列比对的默认参数(以及对末端空位没有罚分)。

[0033] 本文中使用的术语'病症'和'疾病'可互换使用。

[0034] 本文中还提供了这样的一些实施方案,其中本文中所述的任何实施方案可以与任何一个或更多个另外的实施方案组合,只要该组合不是相互排斥的。

[0035] 抗MuSK抗体及其抗原结合片段

[0036] 本文中限定的所有抗体或其抗原结合片段均就其本身而言涵盖在本发明中。抗体或其抗原结合片段也用于在人对象中治疗神经肌肉病症。

[0037] 本发明涉及基于抗MuSK抗体的分子,包括抗MuSK抗体、其表位结合结构域、其抗原结合片段和抗体衍生物(用于治疗神经肌肉疾病或病症)。在一个实施方案中,措辞"基于抗体的分子"可以由词语"抗体"或由表述"抗体或其功能片段"或由表述"抗体或抗原结合片段"替代。

[0038] 术语"抗MuSK抗体"可由术语"MuSK抗体"替换。

[0039] 任何基于抗MuSK抗体的分子,包括抗MuSK抗体、其表位结合结构域、其抗原结合片段和能够结合肌肉特异性酪氨酸蛋白激酶(MuSK)的抗体衍生物,均涵盖在本发明内。在一个实施方案中,这样的抗MuSK抗体也能够激活MuSK的信号传导和/或磷酸化。本发明提供了这样的见解:这样的基于抗体的分子可用于治疗其中对象需要提高的MuSK信号传导或MuSK磷酸化的病症,例如神经肌肉疾病或病症。因此,在第一方面中,提供了用于在人对象中治疗神经肌肉病症的抗MuSK抗体或其抗原结合片段。

[0040] MuSK是受体酪氨酸激酶,其在骨骼肌中表达,并在形成和维持神经肌肉突触中具有关键的主要作用(Burden et al.,"The Role of MuSK in Synapse Formation and Neuromuscular Disease,"Cold Spring Harb.Perspect.Biol.5:a009167(2013),其在此通过引用整体并入)。MuSK是单通道、120kDa跨膜蛋白,由包含三个Ig样结构域和一个卷曲(Fz)样结构域的胞外区域和包含近膜区域、激酶结构域和短胞质尾区的胞内区域构成(Jennings et al.,"Muscle-Specific trk-Related Receptor with a Kringle Domain Defines a Distinct Class of Receptor Tyrosine Kinases,"Proc.Natl.Acad.Sci.USA 90:2895-2899(1993)和Valenzuela et al.,"Receptor Tyrosine Kinase Specific for the Skeletal Muscle Lineage:Expression in Embryonicmuscle,at the Neuromuscular Junction,and After Injury,"Neuron15:573-584(1995),其在此通过引用整体并入)。MuSK磷酸化受到突触蛋白聚糖的刺激,突触蛋白聚糖是运动神经元提供的信号。一旦被激活,MuSK会刺激以下途径:(1)聚集和锚定AChR和对突触传递至关重要的另外的肌肉蛋白,(2)增强编码突触蛋白的基因在肌肉"突触核"中的转录,以及(3)促进逆行信

号的产生,所逆行信号促进突触前分化和运动神经末梢与肌肉的附着。在不存在MuSK的情况下,神经肌肉突触无法形成(Burden et al.,“The Role ofMuSK in Synapse Formation and Neuromuscular Disease,”Cold Spring Harb.Perspect.Biol.5:a009167(2013),其在此通过引用整体并入)。除了其在突触形成期间发挥的作用之外,还需要MuSK来维持成体突触(adult synapse),因为成体肌肉中MuSK表达的抑制导致突触前和突触后分化的严重缺陷(Kong et al.,“Inhibition of Synapse Assembly in Mammalian Muscle in vivo by RNA Interference,”EMBO Rep 5:183-188(2004)和Hesser et al.,“Synapse Disassembly and Formation of New Synapses in Postnatal Muscle Upon Conditional Inactivation of MuSK,”Mol.Cell.Neurosci.31:470-480(2006),其在此通过引用整体并入)。与小鼠中的这些发现一致,损害MuSK激酶活性或抑制MuSK下游信号传导步骤的突变会导致肌无力(CM),其特征在于突触结构和功能缺陷,导致肌无力和疲劳(Beeson et al.,“Dok-7 Mutations Underlie a Neuromuscular Junction Synaptopathy,”Science 313:1975-1978(2006);Muller et al.,“Phenotypical Spectrum of DOK7 Mutations in Congenital Myasthenic Syndromes,”Brain 130:1497-1506(2007);和Selcen et al.,“A Compensatory Subpopulation of Motor Neurons in a Mouse Model of Amyotrophic Lateral Sclerosis,”J.Comp.Neurol.490:209-219(2008),其在此通过引用整体并入)。

[0041] 人MuSK的氨基酸序列具有以下SEQ ID NO:129的氨基酸序列。

[0042] MRELVNIPLVHI LTLVAFSGTEKLPKAPVITTPLETVDALVEEVATFMCAVESYPQPEISWTRNKILIKLFDTR

[0043] YSI RENGQLLTILSVEDSDDGIYCCTANNGVGGAVESCGALQVKMKPKITRPPINVKIIEGLKAVLPCTTMG

[0044] NPKPSVSWIKGDSPLRENSRIAVLESGSLRIH NVQKEDAGQYRCVAKNSLGTAYSKVVKLEVEVFARILRA

[0045] PESH NVTFGSFVTLHCTATGIPVPTITWI ENGNAVSSGSIQESVKDRVIDSRLQLFITKPGLYTCIATN KHGE

[0046] KFSTAKAAATISIAEWSKPQKDNKGYCAQYRGEVC NAVLAKDALVFLNTSYADPEEAQELLVHTAWNELK

[0047] VVSPVCRPAAEALLCNHIFQECSPGVVPTPIPICREYCLAVKELFCAKEWLVMEEKTHRGLYRSEMHLLS

[0048] VPECSKLPSM HWD PTACARLPH LDYN KEN LKTFPPMTSSKPSVDIPN LPSSSSSSFSVSPTYSMTVI ISIM

[0049] SSFAIFVLLTITTLYCCRRRKQWKNKKRESAAVTLTTLPSELLLDRLHPN PMYQRMPLLLNPKLLSLEYPR

[0050] NNIEYVRDIGEGAFGRVFQARAPGLLPYEPFTMVAVKMLKEEASADMQADFQREAALMAEFDNPNIVKLL

[0051] GVCAVGKPMCLLFEYMAYGDLN EFLRSMSPHTVCSLSHSDLSMRAQVSSPGPPPLSCAEQLCIARQVAA

[0052] GMAYLSERKFVHRDLATRNCLVGENMVVKIADFGLSRNIYSADYYKANENDAIPIRWMPPESIFYNRYT

TE

[0053] SDVWAYGVVLWEIFSYGLQPYYGMAHEEVIYYVRDGNILSCPENCPVELYNLMRLCWS

[0054] KLPADRPSFTSIHRILERMCERAEGTVSV(SEQ ID NO:129)

[0055] 根据本发明,本文中所述的基于MuSK抗体的分子与MuSK蛋白的卷曲(Fz)样结构域内的表位结合。MuSK的Fz样结构域具有如下所示的SEQ ID NO:130的氨基酸序列。

[0056] DNKGYCAQYRGEVCNAVLAKDALVFLNTSYADPEEAQELLVHTAWNELKVVSPVCRPAAEALLCNHIFQ

[0057] ECSPGVVPTPIPICREYCLAVKELFCAKEWLVMEEKTHRGLYRSEMHLLSVPECSKLPSMHWDPTACARL(SEQ ID NO:130)

[0058] 本文中使用的术语“表位”是指能够与抗体结合的抗原决定簇。表位通常包含分子的表面基团例如氨基酸或糖侧链,并且通常具有特定的三维结构特征,以及特定的电荷特征。构象和非构象表位的区别在于与前者(而非后者)的结合在变性溶剂的存在下会丢失。表位可包含直接参与结合的氨基酸残基(也称为表位的免疫显性组分)和不直接参与结合的其他氨基酸残基,例如被特定抗原结合肽有效阻断的氨基酸残基(换句话说,氨基酸残基在特定抗原结合肽的“覆盖区(footprint)”内)。表位通常包含至少3个,并且更通常是至少5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20个或更多个空间构象独特的氨基酸。

[0059] 在一个实施方案中,根据本发明应用的MuSK抗体或抗原结合片段结合MuSK卷曲(Fz)样结构域。在一个实施方案中,相比于替代表位,MuSK抗体或抗原结合片段更频繁、更快速、更持久和/或以更强的亲和力或亲合力免疫特异性地结合SEQ ID NO:130的MuSK Fz样结构域序列中的表位。在一个实施方案中,本文中所述的基于MuSK抗体的分子与SEQ ID NO:130的任何2、3、4、5、6、7、8、9、10或更多个氨基酸残基免疫特异性地结合。本文中使用的术语“亲和力”、“特异性结合”、“结合”、“免疫特异性结合”、“结合活性”或“特异性结合活性”是指本文中所定义的抗体或抗体片段与SEQ ID NO:130的MuSK-Fz样结构域序列内的表位结合的程度。

[0060] 在一个实施方案中,本文中公开的基于MuSK抗体的分子以对应于约10-7M或更小的KD的亲和力与MuSK Fz样结构域结合。例如,本文中公开的基于MuSK抗体的分子以对应于以下KD的亲和力与MuSK Fz样结构域结合,所述K_D为约10-8M、约10-9M、约10-10M、约10-11M、约10-12M或更低,当通过例如表面等离子体共振(surface plasmon resonance,SPR)技术在Biacore 3000仪器中(优选使用抗体作为配体和MuSK作为分析物)所确定的。本文中所公开的基于MuSK抗体的分子以对应于以下K_D的亲和力与MuSK Fz样结构域结合,所述K_D是所述基于MuSK抗体的分子与非特异性抗原(例如,牛血清白蛋白、酪蛋白等)结合的亲和力至少10倍低,例如至少100倍低、例如至少1,000倍低、例如至少10,000倍低,例如至少100,000倍低。亲和力降低的量取决于抗体的KD,所以当抗体的KD非常低时(即抗体是高度特异性的),那么对抗原的亲和力的量比对非特异性抗原的亲和力低,其可以是至少10,000倍低。本文中使用的术语“kd”(秒$^{-1}$或1/秒)是指特定抗体-抗原相互作用的解离速率常数。该值也称为koff值。本文中使用的术语“ka”(M-1×秒-1或1/M)是指特定抗体-抗原相互作用的结合速率常数。本文中使用的术语“KD”(M)是指特定抗体-抗原相互作用的解离平衡常数并且通过将kd除以ka获得。本文中使用的术语“K_A”(M-1或1/M)是指特定抗体-抗原相互作用的缔合平衡常数并且通过将ka除以kd获得。

[0061] 在一个实施方案中,本文中所述的基于MuSK抗体的分子对MuSK具有pH依赖性结合亲和力,这允许抗体再循环以增强抗原结合。例如,在一个实施方案中,缔合速率常数或解离速率常数在酸性与中性与碱性pH条件下可以不同。在一个实施方案中,与中性pH条件(例如约7.0至7.9的pH)相比,本文中所述的基于MuSK抗体的分子在酸性pH条件(例如<7.0的pH)下具有更高的解离速率常数。在一些实施方案中,与中性pH(约7.4的pH)相比,本文中所述的基于MuSK抗体的分子在酸性pH(例如,约5.5的pH)下具有2倍至3倍高的解离速率常数(即,降低的结合亲和力)。在一个实施方案中,基于MuSK抗体的分子与MuSK Fz样结构域结合,其亲和力在中性pH条件下比在酸性pH条件下更高。换言之,在一个实施方案中,基于MuSK抗体的分子与MuSK Fz样结构域结合,其解离速率在酸性pH条件下比在中性pH条件下更高。中性pH条件可以定义为包含7.0到7.9的pH。酸性pH条件可定义为小于7.0的pH。更高可意味着至少高10%、20%、30%、40%、50%、60%、70%、80%、90%、100%、150%、200%、250%、300%。具有这种pH依赖性解离特征的抗体在结合和激活之后但在溶酶体降解之前与抗原解离。一旦解离,抗体就会通过新生Fc受体被转运回循环并被释放以结合更多抗原。

[0062] 在一些实施方案中,本发明的MuSK抗体与Fz样结构域内它们各自的表位的结合激活了MuSK信号传导。特别地,当本发明的MuSK抗体结合MuSK Fz样结构域中它们各自的表位时,这种结合诱导了MuSK磷酸化和激活。相对于突触蛋白聚糖激活诱导的MuSK磷酸化,本发明的MuSK抗体诱导约50%至约100%的MuSK磷酸化(如在例如C2C12磷酸化测定中测量的)。在一个实施方案中,本发明的MuSK抗体诱导约55%、60%、65%、70%、75%、80%、85%、90%、95%或100%95%的MuSK磷酸化(相对于突触蛋白聚糖激活诱导的MuSK磷酸化)。在一个实施方案中,本发明的基于MuSK抗体的分子在MuSK结合时诱导约90%至约100%的MuSK磷酸化(相对于由突触蛋白聚糖激活诱导的MuSK磷酸化)。MuSK的磷酸化可以使用技术人员已知的技术例如western印迹或C2C12肌管磷酸化测定来评估。这样的激活MuSK信号传导(即诱导MuSK的二聚化,诱导MuSK酪氨酸磷酸化)的抗体是激动剂抗体。

[0063] 在一些实施方案中,本发明的MuSK抗体,即与MuSK的Fz-结构域结合的MuSK抗体,不干扰(即,不阻断、阻碍、抑制或减少)天然配体结合和MuSK的刺激。在一些实施方案中,MuSK抗体与其天然配体(即突触蛋白聚糖)共刺激MuSK激活,以产生累加的激活效应,例如MuSK磷酸化。因此,在一些实施方案中,本发明的MuSK抗体增强由天然配体结合诱导的天然MuSK激活,即磷酸化。这样的MuSK抗体是激动剂抗体。在一些实施方案中,本发明的抗体与天然配体组合,将MuSK激活(即MuSK磷酸化)至>100%的内源性激活水平,例如至少110%、130%、150%、200%的内源性激活水平。

[0064] 因此,在一个实施方案中,本发明的基于MuSK抗体的分子的活性包括:(i)与人肌肉特异性酪氨酸蛋白激酶(MuSK)的表位结合,所述表位存在于SEQ ID NO:130的MuSK卷曲(Fz)样结构域序列中,其中所述基于抗体的分子在与其表位结合之后诱导MuSK磷酸化,和/或(ii)与MuSK Fz样结构域的结合不阻断、阻碍或抑制天然或内源性MuSK配体诱导的磷酸化,并且可以增强所述天然或内源性MuSK配体诱导的磷酸化,以及(iii)与MuSK Fz样结构域的结合以在中性pH条件下比在酸性pH条件下更高的亲和力发生。所有这些特征已在本文中进一步定义。

[0065] 基于抗体的分子包括但不限于抗体、完整抗体、完整抗体的表位结合片段、完整抗体的抗原结合片段和抗体衍生物。抗体的表位结合片段可以通过亲本抗体的实际片段化获

得(例如,Fab或(Fab)2片段)。或者,表位结合片段是包含这样的亲本抗体的氨基酸序列的一部分的氨基酸序列。如本文中使用的,如果分子通过亲本抗体或其部分的实际化学修饰获得,或者如果其包含的氨基酸序列为与这样的亲本抗体或其相关部分的氨基酸序列基本相似(例如,与这样的亲本分子或这样的其相关部分的差异小于30%、小于20%、小于10%或小于5%,或与这样的亲本分子或其相关部分的差异在于10个氨基酸残基或少于10、9、8、7、6、5、4、3或2个氨基酸残基),则称该分子为抗体(或其相关部分)的"衍生物"。

[0066] 在一些实施方案中,本发明的基于抗体的分子是完整的免疫球蛋白或在重组细胞中具有编码这样的片段的表位结合333个酸的分子(参见例如,Evans et al."Rapid Expression Of An Anti-Human C5 Chimeric Fab Utilizing AVector That Replicates In COS And 293Cells,"J.Immunol.Meth.184:123-38(1995),其在此通过引用整体并入)。例如,编码F(ab')2片段的一部分的嵌合基因可以包括编码CH1结构域和重链铰链区的DNA序列,随后是翻译终止密码子以产生这样的截短的抗体片段分子。可以以与完整抗体相同的方式容易地筛选能够与所期望表位结合的合适片段以供使用。

[0067] 抗体衍生物包括含有抗体的至少一个表位结合结构域的那些分子,并且通常使用重组技术形成。一种示例性抗体衍生物包括单链Fv(scFv)。scFv由Fv片段的两个结构域VL和VH形成,它们可以由不同的基因编码。使用重组方法,通过灵活的接头(通常具有约10、12、15或更多个氨基酸残基)将这样的基因序列或其编码cDNA连接起来,这使得它们能够制成单个蛋白质链,其中VL和VH缔合以形成单价表位结合分子(参见例如,Bird et al."Single-Chain Antigen-Binding Proteins,"Science242:423-426(1988);和Huston et al."Protein Engineering Of Antibody Binding Sites:Recovery Of Specific Activity In An Anti-Digoxin Single-Chain Fv Analogue Produced In Escherichia coli,"Proc.Natl.Acad.Sci.(U.S.A.)85:5879-5883(1988),其在此通过引用整体并入)。或者,通过使用不太短(例如,不少于约9个残基)的柔性接头以使不同单条多肽链的VL和VH能够缔合在一起,其可以形成对两个不同的表位具有结合特异性的双特异性抗体。

[0068] 在另一个实施方案中,抗体衍生物是二价(divalent)或二价(bivalent)单链可变片段,其通过将两个scFv串联(即串联scFv)连接在一起或使它们二聚化形成双抗体来改造(Holliger et al.""Diabodies':Small Bivalent And Bispecific Antibody Fragments,"Proc.Natl.Acad.Sci.(U.S.A.)90(14),6444-8(1993),其在此通过引用整体并入)。在又一个实施方案中,抗体是三抗体,即三价单链可变片段,其通过将三个scFv串联或以三聚体形式连接在一起以形成三抗体来改造。在另一个实施方案中,抗体是四个单链可变片段的四抗体。在另一个实施方案中,抗体是"线性抗体",其是包含形成抗原结合区对的串联Fd区段对(VH-CH1-VH-CH1)的抗体(参见Zapata et al.Protein Eng.8(10):1057-1062(1995),其在此通过引用整体并入)。在另一个实施方案中,抗体衍生物是微抗体,其由与CH3区偶联的单链Fv区(即,scFv-CH3)组成。

[0069] 本文中还讨论了这些和本发明背景下的另一些有用抗体片段和衍生物。还应理解,除非另有说明,否则术语基于抗体的分子还包括抗体样多肽,例如嵌合抗体和人源化抗体,抗原结合片段以及保留与抗原特异性结合能力的抗体片段(表位结合片段、抗原结合片段或功能片段),其由任何已知技术,例如酶促切割、肽合成和重组技术提供。

[0070] 如本文中产生的抗体可以是任何同种型。本文中使用的"同种型"是指由重链恒定

区基因编码的免疫球蛋白类别(例如IgG1、IgG2、IgG3、IgG4、IgD、IgA、IgE或IgM)。同种型的选择通常由所期望的效应物功能指导,例如抗体依赖性细胞毒性(antibody-dependent cellular cytotoxicity,ADCC)诱导。示例性同种型是IgG1、IgG2、IgG3和IgG4。本文中公开的MuSK抗体的特别有用的同种型包括IgG1和IgG2。

[0071] 可以使用人轻链恒定区κ或λ。如果期望的话,可以通过已知方法转换本发明的MuSK抗体的类别。例如,原本是IgM的本发明的抗体可以被类别转换为本发明的IgG抗体。此外,类别转换技术可用于将一种IgG亚类转化为另一种,例如从IgG1转化为IgG2。因此,本发明的抗体的效应物功能可以通过同种型转换而改变为,例如IgG1、IgG2、IgG3、IgG4、IgD、IgA、IgE或IgM抗体以用于多种治疗用途。

[0072] 在一个实施方案中,将一个、两个或更多个氨基酸替换引入到IgG恒定区Fc区中以改变基于抗体的分子的效应物功能。例如,可将选自根据EU编号系统(https://www.imgt.org/IMGTScientificChart/Hu IGHGnber.html#notes和Edelman,G.M.et al.,Proc.Natl.Acad.USA,63,78-85(1969).PMID:5257969)编号的氨基酸残基234、235、236、237、238、239、243、265、267、268、292、297、300、318、320、322、327、328、329、330、331、332和396的一个或更多个氨基酸替换为不同的氨基酸残基,使得基于抗体的分子对效应物配体具有改变的亲和力但保留抗原结合能力。在一个实施方案中,氨基酸234或235已经被替换。在另一个实施方案中,氨基酸234和235已经被替换。在本上下文中,人IgG恒定Fc区的优选氨基酸序列包含SEQ ID NO:266或SEQ ID NO:267。在本上下文中,例如,根据EU编号系统编号的氨基酸234和235对应于SEQ ID NO:266和267中的氨基酸7和8(即本文中公开的基于抗体的分子的人IgG恒定Fc区),或者根据EU编号系统编号的氨基酸234和235对应于SEQ ID NO:268和270中的氨基酸238和239(即本文中公开的基于抗体的分子的人全长重链)。位置通常不同,因为可变区的长度不同,其在编号中引入了“δ”。在上面描述的情况下,该δ是4。因此,当鉴定SEQ ID NO:266或SEQ ID NO:267或SEQ ID NO:268或SEQ ID NO:270中的相应位置时,以上鉴定的根据EU编号系统编号的其他氨基酸位置(即236、237、238、239、243、265、267、268、292、297、300、318、320、322、327、328、329、330、331、332和396)也是如此。在所提交的申请中,可使用EU编号系统或使用给定Fc区(例如SEQ ID NO:266或SEQ ID NO:267)中或全长重链(例如SEQ ID NO:268或270)中的实际位置来指代氨基酸的位置。

[0073] 因此,在一个实施方案中,将1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16或17个氨基酸替换引入到SEQ ID NO:266或SEQ ID NO:267中。在一个实施方案中,将1、2、3、4个氨基酸替换引入到SEQ ID NO:266或SEQ ID NO:267中。在一个实施方案中,将1或2个氨基酸替换引入到SEQ ID NO:266或SEQ ID NO:267中。因此,在一个实施方案中,将1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16或17个氨基酸替换引入到SEQ ID NO:266或SEQ ID NO:267中,并且所述替换在选自所述序列的根据EU编号系统编号的氨基酸残基234、235、236、237、239、243、267、292、297、300、318、320、322、328、330、332和396的氨基酸位置处引入。在一个实施方案中,将1或2个氨基酸替换引入到SEQ ID NO:266或SEQ ID NO:267中。在一个实施方案中,SEQ ID NO:266或SEQ ID NO:267的根据EU编号系统编号的氨基酸234或235已被替换。在另一个实施方案中,SEQ ID NO:266和267的根据EU编号系统编号的氨基酸234或235已被替换。

[0074] 与之亲和力改变的效应配体可以是例如Fc受体或补体的C1组分。该方法在以下中

进行了更详细的描述:美国专利No.5,624,821和5,648,260,其各自通过引用整体并入本文。在一个实施方案中,可将一个或更多个氨基酸替换引入到本文中所述基于抗体的分子的Fc区中以去除Fc区上潜在的糖基化位点,其可降低Fc受体结合(参见例如,Shields RL et al.,(2001)J Biol Chem 276:6591-604,其通过引用整体并入本文)。在一个实施方案中,与在其人IgG恒定Fc区中不具有任何氨基酸替换的抗体与效应物配体的结合相比,与相同配体的结合降低至少10%、20%、30%、40%、50%、60%、70%、80%、90%,或不再可检测。

[0075] 在第一实施方案中,以下突变中的一个或更多个已被引入到本文中所述的基于抗体的分子的恒定区中(均根据EU编号系统编号):N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换或P396L替换。

[0076] 在第二实施方案中,以下突变中的一个或更多个已被引入到本文中所述的基于抗体的分子的恒定区中(均根据EU编号系统编号):L234A和/或L235A替换;L234A和L235A替换;L234A、L235A和P329G替换;L234A、L235A和G236K替换;L234A、L235A和G236E替换;L234A、L235A和G236R替换;L234A和G236R替换;L234A、L235S和G236R替换;L234A、L235T和G236R替换;L234D、L235H和G236R替换;L234D、L235K和G236R替换;L234D和G236R替换;L234D、L235Q和G236R替换;L234D、L235S和G236R替换;L234E、L235D和G236R替换;L234E、L235H和G236R替换;L234E、L235I和G236R替换;L234G、L235H和G236R替换;L234G、L235Q和G236R替换;L234G、L235S和G236R替换;L234H、L235I和G236R替换;L234H、L235S和G236R替换;L234K、L235Q和G236R替换;L234K、L235R和G236R替换;L234K、L235S和G236R替换;L234K、L235T和G236R替换;L234K、L235V和G236R替换;L234Q、L235A和G236R替换;L234Q、L235D和G236R替换;L234Q、L235H和G236R替换;L234Q和G236R替换;L234Q、L235Q和G236R替换;L234Q、L235R和G236R替换;L234Q、L235S和G236R替换;L234Q、L235T和G236R替换;L234Q、L235V和G236R替换;L234R、L235D和G236R替换;L234R、L235E和G236R替换;L234R、L235H和G236R替换;L234R、L235I和G236R替换;L234R、L235K和G236R替换;L234R和G236R替换;L234R、L235Q和G236R替换;L234R、L235R和G236R替换;L234R、L235T和G236R替换;L234S、L235E和G236R替换;L234S、L235G和G236R替换;L234S、L235H和G236R替换;L234S、L235I和G236R替换;L234S和G236R替换;L234S、L235R和G236R替换;L234S、L235T和G236R替换;L234S、L235V和G236R替换;L234T、L235A和G236R替换;L234T、L235D和G236R、L234T、L235H和G236R替换;L234T、L235I和G236R替换;L234T、L235K和G236R替换;L234T、L235Q和G236R替换;L234T、L235R和G236R替换;L234T、L235S和G236R替换;L234T、L235T和G236R替换;L234T、L235V和G236R替换;G236R和L328R替换;L234A、L235A、G237A、P238S、H268A、A330S和P331S替换;E233P、L234V、L235A、G326缺失、A327G、A330S和P331S替换;L235A和G236R替换;L235S和G236R替换。

[0077] 在第三实施方案中,以下突变中的一个或更多个已被引入到本文中所述的基于抗体的分子的Fc区SEQ ID NO:266或SEQ ID NO:267中(均根据EU编号系统编号):N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换或P396L替换。

[0078] 在第四实施方案中,以下突变中的一个或更多个已被引入到本文中所述的基于抗体的分子的Fc区SEQ ID NO:266或者SEQ ID NO:267中(均根据EU编号系统编号):L234A和/或L235A替换;L234A和L235A替换;L234A、L235A和P329G替换;L234A、L235A和G236K替换;L234A、L235A和G236E替换;L234A、L235A和G236R替换;L234A和G236R替换;L234A、L235S和G236R替换;L234A、L235T和G236R替换;L234D、L235H和G236R替换;L234D、L235K和G236R替换;L234D和G236R替换;L234D、L235Q和G236R替换;L234D、L235S和G236R替换;L234E、L235D和G236R替换;L234E、L235H和G236R替换;L234E、L235I和G236R替换;L234G、L235H和G236R替换;L234G、L235Q和G236R替换;L234G、L235S和G236R替换;L234H、L235I和G236R替换;L234H、L235S和G236R替换;L234K、L235Q和G236R替换;L234K、L235R和G236R替换;L234K、L235S和G236R替换;L234K、L235T和G236R替换;L234K、L235V和G236R替换;L234Q、L235A和G236R替换;L234Q、L235D和G236R替换;L234Q、L235H和G236R替换;L234Q和G236R替换;L234Q、L235Q和G236R替换;L234Q、L235R和G236R替换;L234Q、L235S和G236R替换;L234Q、L235T和G236R替换;L234Q、L235V和G236R替换;L234R、L235D和G236R替换;L234R、L235E和G236R替换;L234R、L235H和G236R替换;L234R、L235I和G236R替换;L234R、L235K和G236R替换;L234R和G236R替换;L234R、L235Q和G236R替换;L234R、L235R和G236R替换;L234R、L235T和G236R替换;L234S、L235E和G236R替换;L234S、L235G和G236R替换;L234S、L235H和G236R替换;L234S、L235I和G236R替换;L234S和G236R替换;L234S、L235R和G236R替换;L234S、L235T和G236R替换;L234S、L235V和G236R替换;L234T、L235A和G236R替换;L234T、L235D和G236R、L234T、L235H和G236R替换;L234T、L235I和G236R替换;L234T、L235K和G236R替换;L234T、L235Q和G236R替换;L234T、L235R和G236R替换;L234T、L235S和G236R替换;L234T、L235T和G236R替换;L234T、L235V和G236R替换;G236R和L328R替换;L234A、L235A、G237A、P238S、H268A、A330S和P331S替换;E233P、L234V、L235A、G326缺失、A327G、A330S和P331S替换;L235A和G236R替换;L235S和G236R替换。

[0079] 在一个实施方案中,以下突变中的一个或更多个被引入到本文中所述的基于抗体的分子的Fc区SEQ ID NO:266或者SEQ ID NO:267中:L234A和/或L235A替换(均根据EU编号系统编号)。在一个实施方案中,以下突变被引入到本文中所述的基于抗体的分子的Fc区SEQ ID NO:266或SEQ ID NO:267中:均根据EU编号系统编号的L234A和L235A替换。该实施方案产生具有由SEQ ID NO:268或SEQ ID NO:270表示的重链的基于抗体的分子。

[0080] 在本发明的背景下,这样的具有改变的、减弱的甚至消除的效应物功能的抗体是具有吸引力的。

[0081] 在一个实施方案中,提供了抗MuSK抗体或其抗原结合片段,其:-为激动剂MuSK抗体并且/或者

[0082] -效应物功能已被降低或消除。

[0083] 该抗体或其抗原结合片段优选地用于在人对象中治疗神经肌肉病症。

[0084] 在一个实施方案中,提供了抗MuSK抗体或其抗原结合片段,其:-结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列,

[0085] -为激动剂MuSK抗体并且/或者

[0086] -效应物功能已被降低或消除。

[0087] 该抗体或其抗原结合片段优选地用于在人对象中治疗神经肌肉病症。

[0088] 如本文中先前所述,通过将突变引入人IgG恒定Fc区中可获得降低或消除的效应物功能。优选地,将至少1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16或17个氨基酸替换引入到所述Fc区中。优选地,将至少1、2、3、4个氨基酸替换引入到所述Fc区中。

[0089] 所述Fc区可包含SEQ ID NO:266或SEQ ID NO:267并且所述替换在选自所述序列的根据EU编号系统编号的氨基酸残基234、235、236、237、238、239、243、265、267、268、292、297、300、318、320、322、327、328、329、330、331、332和396的氨基酸位置被引入。

[0090] 在一个实施方案中,所述Fc区可包含SEQ ID NO:266或SEQ ID NO:267并且所述替换在选自所述序列的根据EU编号系统编号的氨基酸残基234或235的氨基酸位置被引入。

[0091] 在一个实施方案中,所述Fc区可包含SEQ ID NO:266或SEQ ID NO:267并且所述替换在选自所述序列的根据EU编号系统编号的氨基酸残基234和235的氨基酸位置被引入。

[0092] 在一个实施方案中,以下突变(均根据EU编号系统编号)中的一个或更多个已被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区SEQ ID NO:266或SEQ ID NO:267中:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换或P396L替换。

[0093] 在一个实施方案中,可制备本文中所述的基于抗体的分子的人IgG恒定Fc区中的在本申请的第四实施方案中前面描述的突变的组合的每一者。

[0094] 在一个优选的实施方案中,L234A或L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。在一个更优选的实施方案中,L234A和L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。该实施方案产生具有由SEQ ID NO:268或SEQ ID NO:270表示的重链的基于抗体的分子。

[0095] 在一个甚至更优选的实施方案中,所述抗MuSK抗体或其抗原结合片段,包含:

[0096] a)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列和

[0097] b)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性

的氨基酸序列。

[0098] 在该上下文中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%。

[0099] 优选的抗MuSK抗体或其抗原结合片段,包含:

[0100] a)全长重链,其包含SEQ ID NO:268和

[0101] b)全长轻链,其包含SEQ ID NO:269。

[0102] 优选的抗MuSK抗体或其抗原结合片段,包含:

[0103] a)全长重链,其包含SEQ ID NO:270和

[0104] b)全长轻链,其包含SEQ ID NO:271。

[0105] 在一个实施方案中,本发明的基于抗体的分子是“人源化的”,特别是如果它们要用于治疗目的时。术语“人源化”是指通常使用重组技术制备的嵌合分子,其具有来源于来自非人物种的免疫球蛋白的抗原结合位点和基于人免疫球蛋白的结构和/或序列的剩余免疫球蛋白结构。抗原结合位点可以包含与人恒定结构域融合的完整非人抗体可变结构域,或者包含接枝到人可变结构域的适当人框架区的这样的可变结构域的仅互补决定区(complementarity determining region,CDR)。这样的人源化分子的框架残基可以是野生型的(例如,完全人的),或者它们可以经修饰以包含人抗体(人抗体的序列已作为人源化的基础)中不存在的一个或更多个氨基酸替换。人源化减少了或消除了分子的恒定区将在人个体中充当免疫原的可能性,但对外源可变区的免疫应答的可能性保持不变(LoBuglio,A.F.et al.“Mouse/Human Chimeric Monoclonal Antibody In Man:Kinetics And Immune Response,”Proc.Natl.Acad.Sci.USA 86:4220-4224(1989),其在此通过引用整体并入)。另一种方法不仅侧重于提供人来源的恒定区,而且修饰可变区以重构它们使其尽可能接近人形式。重链和轻链的可变区都包含三个互补决定区(CDR),它们响应于所讨论的抗原而变化并决定结合能力。CDR侧接四个框架区(FR),它们在给定的物种中相对保守,并且假定为CDR提供了支架。当针对特定抗原制备非人抗体时,可通过将来源于非人抗体的CDR接枝到待修饰的人抗体中存在的FR上来“重构”或“人源化”可变区。用于人源化本文中所述的非人抗体的合适方法是本领域已知的,参见例如

[0106] Sato,K.et al.,Cancer Res 53:851-856(1993);Riechmann,L et al.,“Reshaping Human Antibodies for Therapy,”Nature 332:323-327(1988);Verhoeyen,M.et al.,“Reshaping Human Antibodies:Grafting An Antilysozyme Activity,”Science 239:1534-1536(1988);Kettleborough,C.A.et al.,“Humanization OfA Mouse Monoclonal Antibody By CDR-Grafting:The Importance Of Framework Residues On Loop Conformation,”Protein Engineering 4:773-3783(1991);Maeda,H.et al.,“Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity,”Human Antibodies Hybridoma 2:124-134(1991);Gorman,S.D.et al.,“Reshaping A Therapeutic CD4 Antibody,”Proc.Natl.Acad.Sci.USA 88:4181-4185(1991);Tempest,P.R.et al.,“Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection In Vivo,”Bio/Technology 9:266-271(1991);Co,M.S.et al.,“Humanized Antibodies For Antiviral Therapy,”Proc.Natl.Acad.Sci.USA 88:2869-2873(1991);Ca rter,P.et al.,“Humanization OfAn

Anti-p185her2Antibody For Human CancerTherapy,"Proc Natl.Acad.Sci.USA 89:4285-4289(1992);和Co,M.S.et al.,"Chimeric And Humanized Antibodies With Specificity For The CD33Antigen,"J.Immunol.148:1149-1154(1992)

[0107] ,其在此通过引用整体并入。在一些实施方案中,本发明的人源化MuSK抗体保留所有CDR序列(例如,包含来自美洲驼或小鼠抗体的所有六个CDR的人源化抗体)。在另一些实施方案中,本发明的人源化MuSK抗体具有相对于原始抗体改变的一个或更多个CDR(一、二、三、四、五、六个)。将抗体人源化的方法是本领域公知的并且适合于将本文中公开的抗体人源化(参见,例如,Winter的美国专利号5,225,539;Queen和Selick的美国专利号5,530,101和5,585,089;Robert等的美国专利号5,859,205;Carter的美国专利号6,407,213;以及Foote的美国专利号6,881,557,其在此通过引用整体并入)。

[0108] 在一些抗体中,仅需要CDR的一部分,即结合所需的称为"特异性决定残基"("specificity determining residue,SDR")的CDR残基的子集来保持抗体的结合。不接触抗原且不在SDR中的CDR残基可以基于先前对位于Chothia高变环之外的Kabat CDR区域的研究(参见Kabat et al.,Sequences of Proteins of Immunological Interest.National Institutes of Health Publication No.91-3242(1992);Chothia,C.et al.,"Canonical Structures For The Hypervariable Regions Of Immunoglobulins,"J.Mol.Biol.196:901-917(1987),其在此通过引用整体并入)通过分子建模和/或以经验为主地或如Gonzales,N.R.et al.,"SDR Grafting Of A Murine Antibody Using Multiple Human Germline Templates To Minimize Its Immunogenicity,"MOl.Immunol.41:863-872(2004)(其在此通过引用整体并入)中所述进行鉴定。在这样的人源化抗体中,在其中一个或更多个供体CDR残基不存在或其中整个供体CDR被省略的位置处,占据所述位置的氨基酸残基可以是占据接受体抗体序列中相应位置的氨基酸残基(通过Kabat编号)。所包括的CDR中供体氨基酸的接受体的这样的替换的数量反映了竞争考虑的平衡。这样的替换可有利于减少人源化抗体中非人氨基酸的数量,并从而降低潜在的免疫原性。然而,替换也可引起亲和力的变化,并且优选避免亲和力的显著降低。替换也可导致活性变化。还优选避免这样的导致活性显著降低的替换。在这种情况下,抗体或抗体片段仍应表现出如本文中早先定义的抗体的可检测活性或至少在一定程度上表现出抗体的活性。也可以凭经验选择CDR内的替换位置和要替换的氨基酸。

[0109] 噬菌体展示技术可替代地用于提高(或降低)本发明的基于抗体的分子的CDR亲和力。该技术(称为亲和力成熟)采用诱变或"CDR步移(CDR walking)"和使用靶抗原或其抗原片段进行重新选择,以鉴定当与初始或亲本抗体比较时具有以更高(或更低)亲和力与抗原结合的CDR的抗体(参见例如Glaser et al.,"Antibody Engineering By Codon-Based Mutagenesis In AFilamentous Phage Vector System,"J.Immunology149:3903-3913(1992),其在此通过引用整体并入)。诱变整个密码子而不是单个核苷酸导致氨基酸突变的半随机库。文库可以由变体克隆库组成构成,每个变体克隆与这样的文库的另一个成员的不同之处在于单个CDR中的单个氨基酸改变,并且其包含可能代表每个CDR残基的每个可能氨基酸替换的变体。可以通过将固定化的突变体与经标记的抗原接触来筛选对抗原具有提高(或降低)的结合亲和力的突变体。本领域已知的任何筛选方法可用于鉴定对抗原具有提高或降低的亲和力的基于变体抗体的结合分子(例如,ELISA)(参见Wu,H.et al.,

"Stepwise In Vitro Affinity Maturation Of Vitaxin,An Alphav Beta3-Specific Humanized mAb,"Proc.Natl.Acad.Sci.USA 95:6037-6042(1998);Yelton et al.,"Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis,"J.Immunology 155:1994(1995),其在此通过引用整体并入)。可以使用使轻链随机化的"CDR步移"(参见Schier,R.et al.,"Isolation OfPicomolar Affinity Anti-c-erbB-2Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site,"J.Mol.Biol.263:551-567(1996),其在此通过引用整体并入)。

[0110] 用于MuSK抗体分子亲和力成熟的方法在本文中描述并公开于例如

[0111] Krause,J.C.et al.,"An Insertion Mutation T hat Distorts Antibody Binding Site Arcnitecture Enhances Function of a Human Antibody,"MBio.2(1):e00345-10(2011);Kuan,C.T.et al.,"Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas,"Int.J.Cancer 10.1002/ijc.25645(2010);Hackel,B.J.et al.,"Stability And CDR Composition Biases Enrich Binder Functionality Landscapes,"J.Mol.Biol.401(1):84-96(2010);Montgomery,D.L.et al.,"Affinity Maturation And Characterization Of A Human Monoclonal Antibody Against HlV-1gp41."MAbs1(5):462-474(2009);Gustchina,E.et al.,"Affinity Maturation By Targeted Diversification Of The CDR-H2Loop Of A Monoclonal Fab Derived From A Synthetic Naïve Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1Neutralization Potency And Breadth,"Virology 393(1):112-119(2009);Finlay,WJ.et al.,"Affinity Maturation Of A Humanized Rat Antibody For Anti-RAGE Therapy:Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions,"J.Mol.Biol.388(3):541-558(2009);Bostrom,J.et al.,"Improving Antibody Binding Affinity And Specificity For Therapeutic Development,"Methods Mol.Biol.525:353-376(2009);Steidl,S.et al.,"ln Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification,"Mol.Immunol.46(1):135-144(2008);和Barderas,R.et al.,"Affinity Maturation Of Antibodies Assisted By ln Silico Modeling,"Proc.Natl.Acad.Sci.USA 105(26):9029-9034(2008),

[0112] 其在此通过引用整体并入。

[0113] 在本申请的上下文中,氨基酸改变(变化或修饰)可以是氨基酸替换、添加、缺失或化学修饰。

[0114] 在一个实施方案中,如本文中所述的基于MuSK抗体的分子包含任意一个、任意两个、任意三个、任意四个、任意五个或任意六个如本文中表1和表2中提供的CDR的氨基酸序列。

[0115] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含:

[0116] (i)互补决定区1(CDR-H1),其包含SEQ ID NO:1至16、135、136、147至149中任一个的氨基酸序列,或者SEQ ID NO:1至16、135、136、147至149中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:1至16、135、136、147至149具有1、2、3、4或5个氨基酸改变;(ii)互补决定区2(CDR-H2),其包含SEQ ID NO:17至32、137、138、150至155中任一个的氨基酸序列,或者SEQ ID NO:17至32、137、138、150至155中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:17至32、137、138、150至155具有1、2、3、4或5个氨基酸改变;和

[0117] (iii)互补决定区3(CDR-H3),其包含SEQ ID NO:33至48、139、140、156至158、240至251中任一个的氨基酸序列,或者SEQ ID NO:33至48、139、140、156至158、或240至251中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:33至48、139、140、156至158、或240至251具有1、2、3、4或5个氨基酸改变。

[0118] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(i)重链可变结构域,其包含:SEQ ID NO:1的CDR-H1或者相对于SEQ ID NO:1具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:17的CDR-H2或者相对于SEQ ID NO:17具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:33的CDR-H3或者相对于SEQ ID NO:33具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:34的CDR-H3或者相对于SEQ ID NO:34具有1、2、3、4或5个氨基酸改变的CDR-H3;(iii)重链可变结构域,其包含:SEQ ID NO:3的CDR-H1或者相对于SEQ ID NO:3具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:19的CDR-H2或者相对于SEQ ID NO:19具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:35的CDR-H3或者相对于SEQ ID NO:35具有1、2、3、4或5个氨基酸改变的CDR-H3;(iv)重链可变结构域,其包含:SEQ ID NO:4的CDR-H1或者相对于SEQ ID NO:4具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:20的CDR-H2或者相对于SEQ ID NO:20具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:36的CDR-H3或者相对于SEQ ID NO:36具有1、2、3、4或5个氨基酸改变的CDR-H3;(v)重链可变结构域,其包含:SEQ ID NO:5的CDR-H1或者相对于SEQ ID NO:5具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:21的CDR-H2或者相对于SEQ ID NO:21具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:37的CDR-H3或者相对于SEQ ID NO:37具有1、2、3、4或5个氨基酸改变的CDR-H3;(vi)重链可变结构域,其包含:SEQ ID NO:6的CDR-H1或者相对于SEQ ID NO:6具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:22的CDR-H2或者相对于SEQ ID NO:22具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:38的CDR-H3或者相对于SEQ ID NO:38具有1、2、3、4或5个氨基酸改变的CDR-H3;(vii)重链可变结构域,其包含:SEQ ID NO:7的CDR-H1或者相对于SEQ ID NO:7具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:23的CDR-H2或者相对于SEQ ID NO:23具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:39的CDR-H3或者相对于SEQ ID NO:39具有1、2、3、4或5个氨基酸改变的CDR-H3;(viii)重链可变结构域,其包含:SEQ ID NO:8的CDR-H1或者相对于SEQ ID NO:8具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:24的CDR-H2或者相对于SEQ ID NO:24具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:40的CDR-H3或者相对于SEQ ID NO:40具有1、2、3、4或5个氨基酸改变的

CDR-H3;(ix)重链可变结构域,其包含:SEQ ID NO:9的CDR-H1或者相对于SEQ ID NO:9具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:25的CDR-H2或者相对于SEQ ID NO:25具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:41的CDR-H3或者相对于SEQ ID NO:41具有1、2、3、4或5个氨基酸改变的CDR-H3;(x)重链可变结构域,其包含:SEQ ID NO:10的CDR-H1或者相对于SEQ ID NO:10具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:26的CDR-H2或者相对于SEQ ID NO:26具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:42的CDR-H3或者相对于SEQ ID NO:42具有1、2、3、4或5个氨基酸改变的CDR-H3;(xi)重链可变结构域,其包含:SEQ ID NO:11的CDR-H1或者相对于SEQ ID NO:11具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:27的CDR-H2或者相对于SEQ ID NO:27具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:43的CDR-H3或者相对于SEQ ID NO:43具有1、2、3、4或5个氨基酸改变的CDR-H3;(xii)重链可变结构域,其包含:SEQ ID NO:12的CDR-H1或者相对于SEQ ID NO:12具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:28的CDR-H2或者相对于SEQ ID NO:28具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:44的CDR-H3或者相对于SEQ ID NO:44具有1、2、3、4或5个氨基酸改变的CDR-H3;(xiii)重链可变结构域,其包含:SEQ ID NO:13的CDR-H1或者相对于SEQ ID NO:13具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:29的CDR-H2或者相对于SEQ ID NO:29具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:45的CDR-H3或者相对于SEQ ID NO:45具有1、2、3、4或5个氨基酸改变的CDR-H3;(xiv)重链可变结构域,其包含:SEQ ID NO:14的CDR-H1或者相对于SEQ ID NO:14具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:30的CDR-H2或者相对于SEQ ID NO:30具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:46的CDR-H3或者相对于SEQ ID NO:46具有1、2、3、4或5个氨基酸改变的CDR-H3;(xv)重链可变结构域,其包含:SEQ ID NO:15的CDR-H1或者相对于SEQ ID NO:15具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:31的CDR-H2或者相对于SEQ ID NO:31具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:47的CDR-H3或者相对于SEQ ID NO:47具有1、2、3、4或5个氨基酸改变的CDR-H3;(xvi)重链可变结构域,其包含:SEQ ID NO:16的CDR-H1或者相对于SEQ ID NO:16具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:32的CDR-H2或者相对于SEQ ID NO:32具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:48的CDR-H3或者相对于SEQ ID NO:48具有1、2、3、4或5个氨基酸改变的CDR-H3;(xvii)重链可变结构域,其包含:SEQ ID NO:135的CDR-H1或者相对于SEQ ID NO:135具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:137的CDR-H2或者相对于SEQ ID NO:137具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:139的CDR-H3或者相对于SEQ ID NO:139具有1、2、3、4或5个氨基酸改变的CDR-H3;和(xviii)重链可变结构域,其包含:SEQ ID NO:136的CDR-H1或者相对于SEQ ID NO:136具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:138的CDR-H2或者相对于SEQ ID NO:138具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:140的CDR-H3或者相对于SEQ ID NO:140具有1、2、3、4或5个氨基酸改变的CDR-H3。重链CDR的序列在表1中提供。

[0119] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(ii.a)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:240的CDR-H3(X2m1)或者相对于SEQ

ID NO:240具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.b)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:241的CDR-H3(X2m2)或者相对于SEQ ID NO:241具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.c)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:242的CDR-H3(X2m3)或者相对于SEQ ID NO:242具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.d)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:243的CDR-H3(X2m4)或者相对于SEQ ID NO:243具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.e)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:244的CDR-H3(X2m5)或者相对于SEQ ID NO:244具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.f)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:245的CDR-H3(X2m6)或者相对于SEQ ID NO:245具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.g)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:246的CDR-H3(X2m7)或者相对于SEQ ID NO:246具有1、2、3、4或5个氨基酸改变的CDR-H3;(ii.h)重链可变结构域,其包含:SEQ ID NO:2的CDR-H1或者相对于SEQ ID NO:2具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:18的CDR-H2或者相对于SEQ ID NO:18具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:247的CDR-H3(X2m8)或者相对于SEQ ID NO:247具有1、2、3、4或5个氨基酸改变的CDR-H3。

[0120] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(xvii.a)重链可变结构域,其包含:SEQ ID NO:135的CDR-H1或者相对于SEQ ID NO:135具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:137的CDR-H2或者相对于SEQ ID NO:137具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:248的CDR-H3(X17m1)或者相对于SEQ ID NO:248具有1、2、3、4或5个氨基酸改变的CDR-H3;(xvii.b)重链可变结构域,其包含:SEQ ID NO:135的CDR-H1或者相对于SEQ ID NO:135具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:137的CDR-H2或者相对于SEQ ID NO:137具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:249的CDR-H3(X17m2)或者相对于SEQ ID NO:249具有1、2、3、4或5个氨基酸改变的CDR-H3;(xvii.c)重链可变结构域,其包含:SEQ ID NO:135的CDR-H1或者相对于SEQ ID NO:135具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:137的CDR-H2或者相对于SEQ ID NO:137具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:250的CDR-H3(X17m3)或者相对于SEQ ID NO:250具有1、2、3、4或5个氨基酸改变的CDR-H3;(xvii.d)重链可变结构域,其包含:SEQ ID NO:135的CDR-H1或者相对于SEQ ID NO:135具

有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:137的CDR-H2或者相对于SEQ ID NO:137具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:251的CDR-H3(X17m6)或者相对于SEQ ID NO:251具有1、2、3、4或5个氨基酸改变的CDR-H3。

[0121] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含:(xix)重链可变结构域,其包含:SEQ ID NO:147的CDR-H1或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:150的CDR-H2或者相对于SEQ ID NO:150具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:156的CDR-H3或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3;(xx)重链可变结构域,其包含:SEQ ID NO:148的CDR-H1或者相对于SEQ ID NO:148具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:151的CDR-H2或者相对于SEQ ID NO:151具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:157的CDR-H3或者相对于SEQ ID NO:157具有1、2、3、4或5个氨基酸改变的CDR-H3;(xxi)重链可变结构域,其包含:SEQ ID NO:149的CDR-H1或者相对于SEQ ID NO:149具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:152的CDR-H2或者相对于SEQ ID NO:152具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:158的CDR-H3或者相对于SEQ ID NO:158具有1、2、3、4或5个氨基酸改变的CDR-H3。

[0122] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含(xxii)重链可变结构域,其包含:SEQ ID NO:147的CDR-H1或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:153的CDR-H2或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:156的CDR-H3(3B2g1m1/3B2g2m1)或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3;(xxiii)重链可变结构域,其包含:SEQ ID NO:147的CDR-H1或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:154的CDR-H2或者相对于SEQ ID NO:154具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:156的CDR-H3(3B2g1m2/3B2g2m2)或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3;(xxi)重链可变结构域,其包含:SEQ ID NO:147的CDR-H1或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1,SEQ ID NO:155的CDR-H2或者相对于SEQ ID NO:155具有1、2、3、4或5个氨基酸改变的CDR-H2,和SEQ ID NO:156的CDR-H3(3B2g1m4/3B2g2m4)或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3。重链CDR的序列在表1中提供。

[0123] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(i)重链可变结构域,其包含SEQ ID NO:1的CDR-H1、SEQ ID NO:17的CDR-H2和SEQ ID NO:33的CDR-H3;(ii)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:34的CDR-H3;(iii)重链可变结构域,其包含SEQ ID NO:3的CDR-H1、SEQ ID NO:19的CDR-H2和SEQ ID NO:35的CDR-H3;(iv)重链可变结构域,其包含SEQ ID NO:4的CDR-H1、SEQ ID NO:20的CDR-H2和SEQ ID NO:36的CDR-H3;(v)重链可变结构域,其包含SEQ ID NO:5的CDR-H1、SEQ ID NO:21的CDR-H2和SEQ ID NO:37的CDR-H3;(vi)重链可变结构域,其包含SEQ ID NO:6的CDR-H1、SEQ ID NO:22的CDR-H2和SEQ ID NO:38的CDR-H3;(vii)重链可变结构域,其包含SEQ ID NO:7的CDR-H1、SEQ ID NO:23的CDR-H2和SEQ ID NO:39的CDR-H3;(viii)重链可变结构域,其包含SEQ ID NO:8的CDR-H1、SEQ ID NO:24的CDR-H2和SEQ ID NO:40的CDR-H3;(ix)重链可变结构域,其包含SEQ ID NO:9的CDR-H1、SEQ

ID NO:25的CDR-H2和SEQ ID NO:41的CDR-H3;(x)重链可变结构域,其包含SEQ ID NO:10的CDR-H1、SEQ ID NO:26的CDR-H2和SEQ ID NO:42的CDR-H3;(xi)重链可变结构域,其包含SEQ ID NO:11的CDR-H1、SEQ ID NO:27的CDR-H2和SEQ ID NO:43的CDR-H3;(xii)重链可变结构域,其包含SEQ ID NO:12的CDR-H1、SEQ ID NO:28的CDR-H2和SEQ ID NO:44的CDR-H3;(xiii)重链可变结构域,其包含SEQ ID NO:13的CDR-H1、SEQ ID NO:29的CDR-H2和SEQ ID NO:45的CDR-H3;(xiv)重链可变结构域,其包含SEQ ID NO:14的CDR-H1、SEQ ID NO:30的CDR-H2和SEQ ID NO:46的CDR-H3;(xv)重链可变结构域,其包含SEQ ID NO:15的CDR-H1、SEQ ID NO:31的CDR-H2和SEQ ID NO:47的CDR-H3;(xvi)重链可变结构域,其包含SEQ ID NO:16的CDR-H1、SEQ ID NO:32的CDR-H2和SEQ ID NO:48的CDR-H3;(xvii)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:139的CDR-H3;和(xviii)重链可变结构域,其包含SEQ ID NO:136的CDR-H1、SEQ ID NO:138的CDR-H2和SEQ ID NO:140的CDR-H3。重链CDR序列的序列在下表1中提供。

[0124] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(ii.a)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:240的CDR-H3(X2m1);(ii.b)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:241的CDR-H3(X2m2);(ii.c)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:242的CDR-H3(X2m3);(ii.d)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:243的CDR-H3(X2m4);(ii.e)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:244的CDR-H3(X2m5);(ii.f)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:245的CDR-H3(X2m6);(ii.g)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:246的CDR-H3(X2m7);(ii.h)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:247的CDR-H3(X2m8)。

[0125] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含:(xvii.a)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:248的CDR-H3(X17m1);(xvii.b)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:249的CDR-H3(X17m2);(xvii.c)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:250的CDR-H3(X17m3);(xvii.d)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:251的CDR-H3(X17m6)。

[0126] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含:(xix)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3;(xx)重链可变结构域,其包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3;(xxi)重链可变结构域,其包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3;

[0127] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含:(xxii)重链可变结构域,其包含SEQ ID

NO:147的CDR-H1、SEQ ID NO:153的CDR-H2和SEQ ID NO:156的CDR-H3(3B2g1m1/3B2g2m1)；(xxiii)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2和SEQ ID NO:156的CDR-H3(3B2g1m2/3B2g2m2)；(xxiv)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:155的CDR-H2和SEQ ID NO:156的CDR-H3(3B2g1m4/3B2g2m4)。重链CDR序列的序列在下表1中提供。

[0128] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2或者相对于SEQ ID NO:153具有至少0、1、2、3、4或5个改变的CDR-H2氨基酸序列,以及SEQ ID NO:156的CDR-H3(3B2g2m1)。在一个实施方案中,CDR-H2氨基酸序列相对于SEQ ID NO:153具有至少0、1、2、3、4或5个改变。根据该实施方案,CDR-H2氨基酸序列相对于SEQ ID NO:153具有至少0、1、2、3、4或5个改变,其中所述改变存在于残基1、2、6、7、8、9、10、11、12、13、14、15、16、17或其任意组合处。

[0129] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含：

[0130] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0131] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,以及

[0132] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)。

[0133] 在一个实施方案中,抗体的CDR-H2在第3位包含脯氨酸(P)、在第4位包含色氨酸(W)以及在第5位包含丝氨酸(S)或天冬酰胺(N)。

[0134] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域,其中重链可变结构域包含：

[0135] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0136] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,以及

[0137] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)。

[0138] 下表1中提供了重链CDR序列的序列。

[0139]

mAb/Fab 名称	HCDR1		HCDR2		HCDR3	
	序列	SEQ ID NO:	序列	SEQ ID NO:	序列	SEQ ID NO:
X1	SSSIH	1	SISSSSGSTSYADSVKG	17	KYWSQYYWAHYYGGLDY	33
X2	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGMDY	34
X2m1	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGFDY	240
X2m2	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGLDY	241
X2m3	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGFDY	242

[0140]

mAb/Fab 名称	HCDR1		HCDR2		HCDR3	
	序列	SEQ ID NO:	序列	SEQ ID NO:	序列	SEQ ID NO:
X2m4	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGLDY	243
X2m5	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYFGMDY	244
X2m6	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYSGMDY	245
X2m7	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGFDY	246
X2m8	SSSIH	2	SISSSYGSTSYADSVKG	18	SEGDRYVSGYMGLDY	247
X3	SSSIH	3	SISSSSGYTYYADSVKG	19	SWYEMWMSGYFGFDY	35
X4	SSSIH	4	SISSSSGSTYYADSVKG	20	GEHDYYVFGYLGMDY	36
X5	SSSIH	5	SISSSSGSTSYADSVKG	21	SYTMFYYGGWYGSGYFGMDY	37
X6	SSSIH	6	SISSYSGYTYYADSVKG	22	TYGSYYVSSYTGMDY	38
X7	SSSIH	7	SISSSYSSTYYADSVKG	23	LAGLYHYPGYLGLDY	39
X8	SSSIH	8	SISSSSGSTSYADSVKG	24	SWSYHPWYYHVGWYTGLDY	40
X9	SSSIH	9	SIYSSSGSTYYADSVKG	25	SGGEFYITSYYGMDY	41
X10	SSSIH	10	SISSSYSSTSYADSVKG	26	KYYRWRHNKYQGFDY	42
X11	SSSIH	11	SISSYSGSTYYADSVKG	27	SWGSYYVSGFVGFDY	43
X12	SSSIH	12	YISPSSGYTSYADSVKG	28	QYWVPQWWITQYFGMDY	44
X13	SSSIH	13	SISSSSGSTSYADSVKG	29	SSEHWYTIGYYGIDY	45
X14	SSSIH	14	SISSSSGYTYYADSVKG	30	GSHHWFLWIYSGLDY	46
X15	SSSIH	15	SISSSYGSTSYADSVKG	31	SEGDRYVSGYMGMDY	47
X16	SSSIH	16	SIYSSYGYTSYADSVKG	32	NWGYYMYWGWYYALDY	48
X17	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVFGYLGMDY	139
X17m1	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVSGYLGMDY	248
X17m2	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVYGYLGMDY	249
X17m3	YSSIH	135	SIYSSSGSTYYADSVKG	137	GDHGYYVSGYLGFDY	250
X17m6	YSSIH	135	SIYSSSGSTYYADSVKG	137	GEHGYYVSGYLGFDY	251
X18	SSSIH	136	SISSSSGYTSYADSVKG	138	KYSKRAYPDYYWRGLDY	140
14D10	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
7G4	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3C4	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3B2	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
3G3	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
31G2	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
31B7	DYGMS	147	AIPWNGGSTYYKESVKG	150	RSGRIAFGALDA	156
17H10	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157

[0141]

mAb/Fab 名称	HCDR1		HCDR2		HCDR3	
	序列	SEQ ID NO:	序列	SEQ ID NO:	序列	SEQ ID NO:
23B6	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
30E1	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
30A11	ARYYSWS	148	VIAYDGSTYYSPSLKS	151	GSSRVAAAFDS	157
16F11	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
4C11	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7A12	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7G12	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
7B8	LYYMN	149	VIDTHSIAYYADSVKG	152	GRTALVR	158
3B2g1m1	DYGMS	147	AIPWSGGSTYYKESVKG	153	RSGRIAFGALDA	156
3B2g1m2	DYGMS	147	AIPGSGGSTYYKESVKG	154	RSGRIAFGALDA	156
3B2g1m4	DYGMS	147	AIPWQGGSTYYKESVKG	155	RSGRIAFGALDA	156
3B2g2m1	DYGMS	147	AIPWSGGSTYYKESVKG	153	RSGRIAFGALDA	156
3B2g2m2	DYGMS	147	AIPGSGGSTYYKESVKG	154	RSGRIAFGALDA	156
3B2g2m4	DYGMS	147	AIPWQGGSTYYKESVKG	155	RSGRIAFGALDA	156

[0142] 在一些实施方案中,本文中公开的基于MuSK抗体的分子还包含轻链可变结构域。轻链可变结构域包含:

[0143] (i)互补决定区1(CDR-L1),其具有SEQ ID NO:49至64、141、142、159至169中任一个的氨基酸序列,或者SEQ ID NO:49至64、141、142或159至169中任一个的经修饰氨基酸序列,所述经修饰序列与SEQ ID NO:49至64、141、142或159至169中的任一个具有至少80%序列同一性;

[0144] (ii)互补决定区2(CDR-L2),其具有SEQ ID NO:65至80、143、144、170至179中任一个的氨基酸序列,或者SEQ ID NO:65至80、143、144或170至179中任一个的经修饰氨基酸序列,所述经修饰序列与SEQ ID NO:65至80、143、144或170至179中任一个具有至少80%序列同一性;和

[0145] (iii)互补决定区3(CDR-L3),其具有SEQ ID NO:81至96、145、146、180至195中任一个的氨基酸序列,或者SEQ ID NO:81至96、145、146或180至195中任一个的经修饰氨基酸序列,所述经修饰序列与SEQ ID NO:81至96、145、146或180至195中的任一个具有至少80%序列同一性。

[0146] 在一些实施方案中,本文中公开的基于MuSK抗体的分子还包含轻链可变结构域。轻链可变结构域包含:

[0147] (iv)互补决定区1(CDR-L1),其具有SEQ ID NO:49至64、141、142、159至169中任一个的氨基酸序列,或者SEQ ID NO:49至64、141、142或159至169中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:49至64、141、142或159至169中的任一个具有1、2、3、4或5个氨基酸改变;

[0148] (v)互补决定区2(CDR-L2),其具有SEQ ID NO:65至80、143、144、170至179中任一

个的氨基酸序列,或者SEQ ID NO:65至80、143、144或170至179中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:65至80、143、144或170至179中任一个具有1、2、3、4或5个氨基酸改变;和

[0149] (vi)互补决定区3(CDR-L3),其具有SEQ ID NO:81至96、145、146、180至195中任一个的氨基酸序列,或者SEQ ID NO:81至96、145、146或180至195中任一个的经修饰氨基酸序列,所述经修饰序列相对于SEQ ID NO:81至96、145、146或180至195中的任一个具有1、2、3、4或5个氨基酸改变。

[0150] 在一个实施方案中,本文中公开的基于MuSK抗体的分子的轻链可变结构域包含(i)轻链可变结构域,其包含:SEQ ID NO:49的CDR-L1或者相对于SEQ ID NO:49具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:65的CDR-L2或者相对于SEQ ID NO:65具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:81的CDR-L3或者相对于SEQ ID NO:81具有1、2、3、4或5个氨基酸改变的CDR-L3;(ii)轻链可变结构域,其包含:SEQ ID NO:50的CDR-L1或者相对于SEQ ID NO:50具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:66的CDR-L2或者相对于SEQ ID NO:66具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:82的CDR-L3或者相对于SEQ ID NO:82具有1、2、3、4或5个氨基酸改变的CDR-L3;(iii)轻链可变结构域,其包含:SEQ ID NO:51的CDR-L1或者相对于SEQ ID NO:51具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:67的CDR-L2或者相对于SEQ ID NO:67具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:83的CDR-L3或者相对于SEQ ID NO:83具有1、2、3、4或5个氨基酸改变的CDR-L3;(iv)轻链可变结构域,其包含:SEQ ID NO:52的CDR-L1或者相对于SEQ ID NO:52具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:68的CDR-L2或者相对于SEQ ID NO:68具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:84的CDR-L3或者相对于SEQ ID NO:84具有1、2、3、4或5个氨基酸改变的CDR-L3;(v)轻链可变结构域,其包含:SEQ ID NO:53的CDR-L1或者相对于SEQ ID NO:53具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:69的CDR-L2或者相对于SEQ ID NO:69具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:85的CDR-L3或者相对于SEQ ID NO:85具有1、2、3、4或5个氨基酸改变的CDR-L3;(vi)轻链可变结构域,其包含:SEQ ID NO:54的CDR-L1或者相对于SEQ ID NO:54具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:70的CDR-L2或者相对于SEQ ID NO:70具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:86的CDR-L3或者相对于SEQ ID NO:86具有1、2、3、4或5个氨基酸改变的CDR-L3;(vii)轻链可变结构域,其包含:SEQ ID NO:55的CDR-L1或者相对于SEQ ID NO:55具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:71的CDR-L2或者相对于SEQ ID NO:71具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:87的CDR-L3或者相对于SEQ ID NO:87具有1、2、3、4或5个氨基酸改变的CDR-L3;(viii)轻链可变结构域,其包含:SEQ ID NO:56的CDR-L1或者相对于SEQ ID NO:56具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:72的CDR-L2或者相对于SEQ ID NO:72具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:88的CDR-L3或者相对于SEQ ID NO:88具有1、2、3、4或5个氨基酸改变的CDR-L3;(ix)轻链可变结构域,其包含:SEQ ID NO:57的CDR-L1或者相对于SEQ ID NO:57具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:73的CDR-L2或者相对于SEQ ID NO:73具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:89的CDR-L3或者相对于SEQ ID NO:89具有1、2、3、4或5个氨基酸改变的CDR-L3;(x)轻链可变结构域,其包含:SEQ ID NO:58的CDR-L1

或者相对于SEQ ID NO:58具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:74的CDR-L2或者相对于SEQ ID NO:74具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:90的CDR-L3或者相对于SEQ ID NO:90具有1、2、3、4或5个氨基酸改变的CDR-L3;(xi)轻链可变结构域,其包含:SEQ ID NO:59的CDR-L1或者相对于SEQ ID NO:59具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:75的CDR-L2或者相对于SEQ ID NO:75具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:91的CDR-L3或者相对于SEQ ID NO:91具有1、2、3、4或5个氨基酸改变的CDR-L3;(xii)轻链可变结构域,其包含:SEQ ID NO:60的CDR-L1或者相对于SEQ ID NO:60具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:76的CDR-L2或者相对于SEQ ID NO:76具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:92的CDR-L3或者相对于SEQ ID NO:92具有1、2、3、4或5个氨基酸改变的CDR-L3;(xiii)轻链可变结构域,其包含:SEQ ID NO:61的CDR-L1或者相对于SEQ ID NO:61具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:77的CDR-L2或者相对于SEQ ID NO:77具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:93的CDR-L3或者相对于SEQ ID NO:93具有1、2、3、4或5个氨基酸改变的CDR-L3;(xiv)轻链可变结构域,其包含:SEQ ID NO:62的CDR-L1或者相对于SEQ ID NO:62具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:78的CDR-L2或者相对于SEQ ID NO:78具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:94的CDR-L3或者相对于SEQ ID NO:94具有1、2、3、4或5个氨基酸改变的CDR-L3;(xv)轻链可变结构域,其包含:SEQ ID NO:63的CDR-L1或者相对于SEQ ID NO:63具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:79的CDR-L2或者相对于SEQ ID NO:79具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:95的CDR-L3或者相对于SEQ ID NO:95具有1、2、3、4或5个氨基酸改变的CDR-L3;(xvi)轻链可变结构域,其包含:SEQ ID NO:64的CDR-L1或者相对于SEQ ID NO:64具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:80的CDR-L2或者相对于SEQ ID NO:80具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:96的CDR-L3或者相对于SEQ ID NO:96具有1、2、3、4或5个氨基酸改变的CDR-L3;(xvii)轻链可变结构域,其包含:SEQ ID NO:141的CDR-L1或者相对于SEQ ID NO:141具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:143的CDR-L2或者相对于SEQ ID NO:143具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:145的CDR-L3或者相对于SEQ ID NO:145具有1、2、3、4或5个氨基酸改变的CDR-L3;(xviii)轻链可变结构域,其包含:SEQ ID NO:142的CDR-L1或者相对于SEQ ID NO:142具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:144的CDR-L2或者相对于SEQ ID NO:144具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:146的CDR-L3或者相对于SEQ ID NO:146具有1、2、3、4或5个氨基酸改变的CDR-L3。轻链CDR的序列在下表2中提供。

[0151] 在一个实施方案中,本文中公开的基于MuSK抗体的分子的轻链可变结构域包含(xix)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:170的CDR-L2或者相对于SEQ ID NO:170具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:180的CDR-L3或者相对于SEQ ID NO:180具有1、2、3、4或5个氨基酸改变的CDR-L3;(xx)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:171的CDR-L2或者相对于SEQ ID NO:171具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:181的CDR-L3或者相对于SEQ ID NO:181具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxi)

轻链可变结构域,其包含:SEQ ID NO:160的CDR-L1或者相对于SEQ ID NO:160具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:172的CDR-L2或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:182的CDR-L3或者相对于SEQ ID NO:182具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxii)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:172的CDR-L2或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:183的CDR-L3或者相对于SEQ ID NO:183具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxiii)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:171的CDR-L2或者相对于SEQ ID NO:171具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:184的CDR-L3或者相对于SEQ ID NO:184具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxiv)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:173的CDR-L2或者相对于SEQ ID NO:173具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:185的CDR-L3或者相对于SEQ ID NO:185具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxv)轻链可变结构域,其包含:SEQ ID NO:159的CDR-L1或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:173的CDR-L2或者相对于SEQ ID NO:173具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:186的CDR-L3或者相对于SEQ ID NO:186具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxvi)轻链可变结构域,其包含:SEQ ID NO:161的CDR-L1或者相对于SEQ ID NO:161具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:174的CDR-L2或者相对于SEQ ID NO:174具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:187的CDR-L3或者相对于SEQ ID NO:187具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxvii)轻链可变结构域,其包含:SEQ ID NO:162的CDR-L1或者相对于SEQ ID NO:162具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:174的CDR-L2或者相对于SEQ ID NO:174具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:188的CDR-L3或者相对于SEQ ID NO:188具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxviii)轻链可变结构域,其包含:SEQ ID NO:163的CDR-L1或者相对于SEQ ID NO:163具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:174的CDR-L2或者相对于SEQ ID NO:174具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:188的CDR-L3或者相对于SEQ ID NO:188具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxix)轻链可变结构域,其包含:SEQ ID NO:164的CDR-L1或者相对于SEQ ID NO:164具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:174的CDR-L2或者相对于SEQ ID NO:174具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:189的CDR-L3或者相对于SEQ ID NO:189具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxx)轻链可变结构域,其包含:SEQ ID NO:165的CDR-L1或者相对于SEQ ID NO:165具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:175的CDR-L2或者相对于SEQ ID NO:175具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:190的CDR-L3或者相对于SEQ ID NO:190具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxxi)轻链可变结构域,其包含:SEQ ID NO:166的CDR-L1或者相对于SEQ ID NO:166具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:176的CDR-L2或者相对于SEQ ID NO:176具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:191的CDR-L3或者相对于SEQ ID NO:191具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxxi)轻链可变结构域,其包含:SEQ ID

NO:167的CDR-L1或者相对于SEQ ID NO:167具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:177的CDR-L2或者相对于SEQ ID NO:177具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:192的CDR-L3或者相对于SEQ ID NO:192具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxxii)轻链可变结构域,其包含:SEQ ID NO:168的CDR-L1或者相对于SEQ ID NO:168具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:178的CDR-L2或者相对于SEQ ID NO:178具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:193的CDR-L3或者相对于SEQ ID NO:193具有1、2、3、4或5个氨基酸改变的CDR-L3;(xxxiii)轻链可变结构域,其包含:SEQ ID NO:169的CDR-L1或者相对于SEQ ID NO:169具有1、2、3、4或5个氨基酸改变的CDR-L1,SEQ ID NO:179的CDR-L2或者相对于SEQ ID NO:179具有1、2、3、4或5个氨基酸改变的CDR-L2,和SEQ ID NO:194的CDR-L3或者相对于SEQ ID NO:194具有1、2、3、4或5个氨基酸改变的CDR-L3。

[0152] 在一个实施方案中,本文中公开的基于MuSK抗体的分子的轻链可变结构域包含:(i)轻链可变结构域,其包含SEQ ID NO:49的CDR-L1、SEQ ID NO:65的CDR-L2和SEQ ID NO:81的CDR-L3;(ii)轻链可变结构域,其包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3;(iii)轻链可变结构域,其包含SEQ ID NO:51的CDR-L1、SEQ ID NO:67的CDR-L2和SEQ ID NO:83的CDR-L3;(iv)轻链可变结构域,其包含SEQ ID NO:52的CDR-L1、SEQ ID NO:68的CDR-L2和SEQ ID NO:84的CDR-L3;(v)轻链可变结构域,其包含SEQ ID NO:53的CDR-L1、SEQ ID NO:69的CDR-L2和SEQ ID NO:85的CDR-L3;(vi)轻链可变结构域,其包含SEQ ID NO:54的CDR-L1、SEQ ID NO:70的CDR-L2和SEQ ID NO:86的CDR-L3;(vii)轻链可变结构域,其包含SEQ ID NO:55的CDR-L1、SEQ ID NO:71的CDR-L2和SEQ ID NO:87的CDR-L3;(viii)轻链可变结构域,其包含SEQ ID NO:56的CDR-L1、SEQ ID NO:72的CDR-L2和SEQ ID NO:88的CDR-L3;(ix)轻链可变结构域,其包含SEQ ID NO:57的CDR-L1、SEQ ID NO:73的CDR-L2和SEQ ID NO:89的CDR-L3;(x)轻链可变结构域,其包含SEQ ID NO:58的CDR-L1、SEQ ID NO:74的CDR-L2和SEQ ID NO:90的CDR-L3;(xi)轻链可变结构域,其包含SEQ ID NO:59的CDR-L1、SEQ ID NO:75的CDR-L2和SEQ ID NO:91的CDR-L3;(xii)轻链可变结构域,其包含SEQ ID NO:60的CDR-L1、SEQ ID NO:76的CDR-L2和SEQ ID NO:92的CDR-L3;(xiii)轻链可变结构域,其包含SEQ ID NO:61的CDR-L1、SEQ ID NO:77的CDR-L2和SEQ ID NO:93的CDR-L3;(xiv)轻链可变结构域,其包含SEQ ID NO:62的CDR-L1、SEQ ID NO:78的CDR-L2和SEQ ID NO:94的CDR-L3;(xv)轻链可变结构域,其包含SEQ ID NO:63的CDR-L1、SEQ ID NO:79的CDR-L2和SEQ ID NO:95的CDR-L3;(xvi)轻链可变结构域,其包含SEQ ID NO:64的CDR-L1、SEQ ID NO:80的CDR-L2和SEQ ID NO:96的CDR-L3;(xvii)轻链可变结构域,其包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3;(xviii)轻链可变结构域,其包含SEQ ID NO:142的CDR-L1、SEQ ID NO:144的CDR-L2和SEQ ID NO:146的CDR-L3。轻链CDR的序列在下表2中提供。

[0153] 在一个实施方案中,本文中公开的基于MuSK抗体的分子的轻链可变结构域包含(xix)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:170的CDR-L2和SEQ ID NO:180的CDR-L3;(xx)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:181的CDR-L3;(xxi)轻链可变结构域,其包含SEQ ID NO:160的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:182的CDR-L3;(xxii)轻链可变结构域,其包含

SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3;(xxiii)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:184的CDR-L3;(xxiv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:185的CDR-L3;(xxv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:186的CDR-L3;(xxvi)轻链可变结构域,其包含SEQ ID NO:161的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:187的CDR-L3;(xxvii)轻链可变结构域,其包含SEQ ID NO:162的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3;(xxviii)轻链可变结构域,其包含SEQ ID NO:163的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3;(xxix)轻链可变结构域,其包含SEQ ID NO:164的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:189的CDR-L3;(xxx)轻链可变结构域,其包含SEQ ID NO:165的CDR-L1、SEQ ID NO:175的CDR-L2和SEQ ID NO:190的CDR-L3;(xxxi)轻链可变结构域,其包含SEQ ID NO:166的CDR-L1、SEQ ID NO:176的CDR-L2和SEQ ID NO:191的CDR-L3;(xxxi)轻链可变结构域,其包含SEQ ID NO:167的CDR-L1、SEQ ID NO:177的CDR-L2和SEQ ID NO:192的CDR-L3;(xxxii)轻链可变结构域,其包含SEQ ID NO:168的CDR-L1、SEQ ID NO:178的CDR-L2和SEQ ID NO:193的CDR-L3;(xxxiii)轻链可变结构域,其包含SEQ ID NO:169的CDR-L1、SEQ ID NO:179的CDR-L2和SEQ ID NO:194的CDR-L3。

[0154] 在一个实施方案中,本文中公开的基于MuSK抗体的分子的轻链可变结构域包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3或者相对于SEQ ID NO:195的氨基酸序列具有1、2、3、4或5个氨基酸改变的CDR-L3,其中所述改变存在于残基1、2、6、7、8、9、10、11、12、13、14、15、16、17或其任意组合处。

[0155] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含轻链可变结构域,其中轻链可变结构域含有:

[0156] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0157] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,以及

[0158] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0159] 在一个实施方案中,CDR-L1、CDR-L2、CDR-L3氨基酸序列相对于SEQ ID NO:159、172或195(分别)具有至少0、1、2、3、4或5个氨基酸改变。

[0160] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含轻链可变结构域,其中轻链可变结构域含有:

[0161] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0162] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,以及

[0163] 包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0164] 下表2中提供了轻链CDR序列的序列。

[0165]

mAb/Fab 名称	LCDR1		LCDR2		LCDR3	
	序列	SEQ ID NO:	序列	SEQ ID NO:	序列	SEQ ID NO:
X1	RASQSVSSAVA	49	SASSLYS	65	QQSSSSLIT	81
X2	RASQSVSSAVA	50	SASSLYS	66	QQSGVWLIT	82
X3	RASQSVSSAVA	51	SASSLYS	67	QQSSSSLIT	83
X4	RASQSVSSAVA	52	SASSLYS	68	QQSYKPGALIT	84
X5	RASQSVSSAVA	53	SASSLYS	69	QQSSSSLIT	85
X6	RASQSVSSAVA	54	SASSLYS	70	QQSSSSLIT	86
X7	RASQSVSSAVA	55	SASSLYS	71	QQSSRSSLLT	87
X8	RASQSVSSAVA	56	SASSLYS	72	QQSSSSLIT	88
X9	RASQSVSSAVA	57	SASSLYS	73	QQSSSSLIT	89
X10	RASQSVSSAVA	58	SASSLYS	74	QQSLWYPVT	90
X11	RASQSVSSAVA	59	SASSLYS	75	QQNSYYLIT	91
X12	RASQSVSSAVA	60	SASSLYS	76	QQSSSSLIT	92
X13	RASQSVSSAVA	61	SASSLYS	77	QQSYGSFSLIT	93
X14	RASQSVSSAVA	62	SASSLYS	78	QQGSYHLIT	94
X15	RASQSVSSAVA	63	SASSLYS	79	QQSGVWLIT	95
X16	RASQSVSSAVA	64	SASSLYS	80	QQWSSAQALIT	96
X17	RASQSVSSAVA	141	SASSLYS	143	QQSYKPGALIT	145
X18	RASQSVSSAVA	142	SASSLYS	144	QQSYWWPIT	146

[0166]

mAb/Fab 名称	LCDR1		LCDR2		LCDR3	
	序列	SEQ ID NO:	序列	SEQ ID NO:	序列	SEQ ID NO:
14D10	GLSSGSVTSSNYPD	159	TTNSRHS	170	ALYMGGGSNVYV	180
7G4	GLSSGSVTSSNYPD	159	STNSRHS	171	ALYMGRGSNKDYV	181
3C4	GLSSGSVTASNYPD	160	STDSRHS	172	ALYMYSDSKLYV	182
3B2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3G3	GLSSGSVTSSNYPD	159	STNSRHS	171	ALYMGSDIRNYV	184
31G2	GLSSGSVTSSNYPD	159	STNSRLS	173	ALYMGSGSRNYV	185
31B7	GLSSGSVTSSNYPD	159	STNSRLS	173	ALYMGSESRNYV	186
17H10	GGNRIGGKSVQ	161	ADSRRPS	174	HVWGSTASAD	187
23B6	GGDNIGSKNAQ	162	ADSRRPS	174	HVWDSSTNAW	188
30E1	GGDNIGSKNTQ	163	ADSRRPS	174	HVWDSSTNAW	188
30A11	GGDNIASKNVQ	164	ADSRRPS	174	QVWDSSTNVAV	189
16F11	KSSQSVVFGSNQKSYLN	165	YASTQES	175	QQAYSAPT	190
4C11	RSSQSVLYSSNQKNYLN	166	WASARES	176	QQSYKPPYG	191
7A12	ESSQSVLYNQKNYLN	167	WASTRQS	177	QQAYNAPLT	192
7G12	KSSQRVQLGSNQKSYLN	168	YASTQQS	178	QQGYSAPFT	193
7B8	KSSQSVLYNQKNYLA	169	WASTRES	179	QQGYSVPYT	194
3B2g1m1	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g1m2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g1m4	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYMYSGSKNYV	183
3B2g2m1	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195
3B2g2m2	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195
3B2g2m4	GLSSGSVTSSNYPD	159	STDSRHS	172	GLYSYSGSKNYV	195

[0167] 对本文中公开的基于MuSK抗体的分子的重链CDR序列和/或轻链CDR序列的合适的氨基酸修饰包括,例如导致变体CDR序列具有与上述的本文中公开的CDR序列的结合特征相似或增强的结合特征的保守替换或功能等同的氨基酸残基替换。本发明涵盖表1和表2的CDR,其包含维持或增强抗体的MuSK结合的1、2、3、4、5个或更多个氨基酸改变(取决于CDR的长度)。对表1的重链CDR序列和/或表1和表2的轻链CDR序列合适的氨基酸修饰包括,例如导致变体CDR序列具有与表1和表2中CDR序列的结合特征相似或增强的结合特征的保守替换或功能等同的氨基酸残基替换。保守替换是在其侧链相关的氨基酸家族中发生的那些。遗传编码的氨基酸可分为四个家族:(1)酸性(天冬氨酸、谷氨酸);(2)碱性(赖氨酸、精氨酸、组氨酸);(3)非极性(丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、甲硫氨酸、色氨酸);和(4)不带电荷的极性(甘氨酸、天冬酰胺、谷氨酰胺、半胱氨酸、丝氨酸、苏氨酸、酪氨酸)。苯丙氨酸、色氨酸和酪氨酸有时共同归类为芳香族氨基酸。或者,氨基酸库可分为(1)酸性(天冬氨酸、谷氨酸);(2)碱性(赖氨酸、精氨酸组氨酸),(3)脂肪族(甘氨酸、丙氨酸、缬氨酸、亮氨酸、异亮氨酸、丝氨酸、苏氨酸),其中丝氨酸和苏氨酸任选地单独分为脂肪族-羟基;(4)芳香族(苯丙氨酸、酪氨酸、色氨酸);(5)酰胺(天冬酰胺、谷氨酰胺);和(6)含硫(半

胱氨酸和甲硫氨酸)(Stryer(ed.),Biochemistry,2nd ed,WH Freeman and Co.,1981,其在此通过引用整体并入)。还可以对表1的重链CDR序列和表2的轻链CDR序列进行非保守替换。非保守替换涉及用来自不同类别的氨基酸的一个或更多个氨基酸残基替换CDR的一个或更多个氨基酸残基以改善或增强CDR的结合特性。表1的重链CDR和/或表2的轻链CDR的氨基酸序列还可以包含维持或增强MuSK结合的一个或更多个内部中性氨基酸插入或缺失。

[0168] 在一个实施方案中,基于MuSK抗体的分子含有:

[0169] (i)包含SEQ ID NO:1的CDR-H1、SEQ ID NO:17的CDR-H2和SEQ ID NO:33的CDR-H3的重链可变结构域,以及包含SEQ ID NO:49的CDR-L1、SEQ ID NO:65的CDR-L2和SEQ ID NO:81的CDR-L3的轻链可变结构域;

[0170] (ii)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:34的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域;

[0171] (iii)包含SEQ ID NO:3的CDR-H1、SEQ ID NO:19的CDR-H2和SEQ ID NO:35的CDR-H3的重链可变结构域,以及包含SEQ ID NO:51的CDR-L1、SEQ ID NO:67的CDR-L2和SEQ ID NO:83的CDR-L3的轻链可变结构域;

[0172] (iv)包含SEQ ID NO:4的CDR-H1、SEQ ID NO:20的CDR-H2和SEQ ID NO:36的CDR-H3的重链可变结构域,以及包含SEQ ID NO:52的CDR-L1、SEQ ID NO:68的CDR-L2和SEQ ID NO:84的CDR-L3的轻链可变结构域;

[0173] (v)包含SEQ ID NO:5的CDR-H1、SEQ ID NO:21的CDR-H2和SEQ ID NO:37的CDR-H3的重链可变结构域,以及包含SEQ ID NO:53的CDR-L1、SEQ ID NO:69的CDR-L2和SEQ ID NO:85的CDR-L3的轻链可变结构域;

[0174] (vi)包含SEQ ID NO:6的CDR-H1、SEQ ID NO:22的CDR-H2和SEQ ID NO:38的CDR-H3的重链可变结构域,以及包含SEQ ID NO:54的CDR-L1、SEQ ID NO:70的CDR-L2和SEQ ID NO:86的CDR-L3的轻链可变结构域;

[0175] (vii)包含SEQ ID NO:7的CDR-H1、SEQ ID NO:23的CDR-H2和SEQ ID NO:39的CDR-H3的重链可变结构域,以及包含SEQ ID NO:55的CDR-L1、SEQ ID NO:71的CDR-L2和SEQ ID NO:87的CDR-L3的轻链可变结构域;

[0176] (viii)包含SEQ ID NO:8的CDR-H1、SEQ ID NO:24的CDR-H2和SEQ ID NO:40的CDR-H3的重链可变结构域,以及包含SEQ ID NO:56的CDR-L1、SEQ ID NO:72的CDR-L2和SEQ ID NO:88的CDR-L3的轻链可变结构域;

[0177] (ix)包含SEQ ID NO:9的CDR-H1、SEQ ID NO:25的CDR-H2和SEQ ID NO:41的CDR-H3的重链可变结构域,以及包含SEQ ID NO:57的CDR-L1、SEQ ID NO:73的CDR-L2和SEQ ID NO:89的CDR-L3的轻链可变结构域;

[0178] (x)包含SEQ ID NO:10的CDR-H1、SEQ ID NO:26的CDR-H2和SEQ ID NO:42的CDR-H3的重链可变结构域,以及包含SEQ ID NO:58的CDR-L1、SEQ ID NO:74的CDR-L2和SEQ ID NO:90的CDR-L3的轻链可变结构域;

[0179] (xi)包含SEQ ID NO:11的CDR-H1、SEQ ID NO:27的CDR-H2和SEQ ID NO:43的CDR-H3的重链可变结构域,以及包含SEQ ID NO:59的CDR-L1、SEQ ID NO:75的CDR-L2和SEQ ID NO:91的CDR-L3的轻链可变结构域;

[0180] (xii)包含SEQ ID NO:12的CDR-H1、SEQ ID NO:28的CDR-H2和SEQ ID NO:44的CDR-H3的重链可变结构域,以及包含SEQ ID NO:60的CDR-L1、SEQ ID NO:76的CDR-L2和SEQ ID NO:92的CDR-L3的轻链可变结构域;

[0181] (xiii)包含SEQ ID NO:13的CDR-H1、SEQ ID NO:29的CDR-H2和SEQ ID NO:45的CDR-H3的重链可变结构域,以及包含SEQ ID NO:61的CDR-L1、SEQ ID NO:77的CDR-L2和SEQ ID NO:93的CDR-L3的轻链可变结构域;

[0182] (xiv)包含SEQ ID NO:14的CDR-H1、SEQ ID NO:30的CDR-H2和SEQ ID NO:46的CDR-H3的重链可变结构域,以及包含SEQ ID NO:62的CDR-L1、SEQ ID NO:78的CDR-L2和SEQ ID NO:94的CDR-L3的轻链可变结构域;

[0183] (xv)包含SEQ ID NO:15的CDR-H1、SEQ ID NO:31的CDR-H2和SEQ ID NO:47的CDR-H3的重链可变结构域,以及包含SEQ ID NO:63的CDR-L1、SEQ ID NO:79的CDR-L2和SEQ ID NO:95的CDR-L3的轻链可变结构域;

[0184] (xvi)包含SEQ ID NO:16的CDR-H1、SEQ ID NO:32的CDR-H2和SEQ ID NO:48的CDR-H3的重链可变结构域,以及包含SEQ ID NO:64的CDR-L1、SEQ ID NO:80的CDR-L2和SEQ ID NO:96的CDR-L3的轻链可变结构域;

[0185] (xvii)包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:139的CDR-H3的重链可变结构域,以及包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3的轻链可变结构域;以及

[0186] (xviii)包含SEQ ID NO:136的CDR-H1、SEQ ID NO:138的CDR-H2和SEQ ID NO:140的CDR-H3的重链可变结构域,以及包含SEQ ID NO:142的CDR-L1、SEQ ID NO:144的CDR-L2和SEQ ID NO:146的CDR-L3的轻链可变结构域。

[0187] 在一个实施方案中,基于MuSK抗体的分子含有:

[0188] (ii.a)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:240的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m1);

[0189] (ii.b)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:241的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m2);

[0190] (ii.c)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:242的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m3);

[0191] (ii.d)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:243的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m4);

[0192] (ii.e)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:244的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m5);

[0193] (ii.f)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:245的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ

ID NO:82的CDR-L3的轻链可变结构域(X2m6)；

[0194] (ii.g)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:246的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m7)；

[0195] (ii.f)包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:247的CDR-H3的重链可变结构域,以及包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3的轻链可变结构域(X2m8)。

[0196] 在一个实施方案中,基于MuSK抗体的分子含有：

[0197] (xvii.a)包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:248的CDR-H3的重链可变结构域,以及包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3的轻链可变结构域(X17m1)；

[0198] (xvii.b)包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:249的CDR-H3的重链可变结构域,以及包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3的轻链可变结构域(X17m2)；

[0199] (xvii.c)包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:250的CDR-H3的重链可变结构域,以及包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3的轻链可变结构域(X17m3)；

[0200] (xvii.d)包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:251的CDR-H3的重链可变结构域,以及包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3的轻链可变结构域(X17m6)。

[0201] 在一个实施方案中,基于MuSK抗体的分子含有：

[0202] (i)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:170的CDR-L2和SEQ ID NO:180的CDR-L3的轻链可变结构域(14D10)；

[0203] (ii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:181的CDR-L3的轻链可变结构域(7G4)；

[0204] (iii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:160的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:182的CDR-L3的轻链可变结构域(3C4)；

[0205] (iv)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2)；

[0206] (v)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:184的CDR-L3的轻链可变结构域(3G3)；

[0207] (vi)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:185的CDR-L3的轻链可变结构域(31G2)；

[0208] (vii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:186的CDR-L3的轻链可变结构域(31B7);

[0209] (viii)包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3的重链可变结构域,以及包含SEQ ID NO:161的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:187的CDR-L3的轻链可变结构域(17H10);

[0210] (ix)包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3的重链可变结构域,以及包含SEQ ID NO:162的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3的轻链可变结构域(23B6);

[0211] (x)包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3的重链可变结构域,以及包含SEQ ID NO:163的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3的轻链可变结构域(30E1);

[0212] (xi)包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3的重链可变结构域,以及包含SEQ ID NO:164的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:189的CDR-L3的轻链可变结构域(30A11);

[0213] (xii)包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3的重链可变结构域,以及包含SEQ ID NO:165的CDR-L1、SEQ ID NO:175的CDR-L2和SEQ ID NO:190的CDR-L3的轻链可变结构域(16F11);

[0214] (xiii)包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3的重链可变结构域,以及包含SEQ ID NO:166的CDR-L1、SEQ ID NO:176的CDR-L2和SEQ ID NO:191的CDR-L3的轻链可变结构域(4C11);

[0215] (xiv)包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3的重链可变结构域,以及包含SEQ ID NO:167的CDR-L1、SEQ ID NO:177的CDR-L2和SEQ ID NO:192的CDR-L3的轻链可变结构域(7A12);

[0216] (xv)包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3的重链可变结构域,以及包含SEQ ID NO:168的CDR-L1、SEQ ID NO:178的CDR-L2和SEQ ID NO:193的CDR-L3的轻链可变结构域(7G12);

[0217] (xvi)包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3的重链可变结构域,以及包含SEQ ID NO:169的CDR-L1、SEQ ID NO:179的CDR-L2和SEQ ID NO:194的CDR-L3的轻链可变结构域(7B8);

[0218] (xvii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2g1m1);

[0219] (xviii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2g1m2);

[0220] (xvix)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:155的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2g1m4);

[0221] (xx)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3的轻链可变结构域(3B2g2m1);

[0222] (xxi)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3的轻链可变结构域(3B2g2m2);以及

[0223] (xxii)包含SEQ ID NO:147的CDR-H1、SEQ ID NO:155的CDR-H2和SEQ ID NO:156的CDR-H3的重链可变结构域,以及包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3的轻链可变结构域(3B2g2m4)。

[0224] 在一个优选的实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域和轻链可变结构域,其中重链可变结构域含有:

[0225] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0226] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0227] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0228] 其中轻链可变结构域含有:

[0229] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0230] -包含SEQ ID NO:172或者相对于SEQ ID NO:172的1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0231] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0232] 在一个实施方案中,抗体的CDR-H2在第3位包含脯氨酸(P)、在第4位包含色氨酸(W)以及在第5位包含丝氨酸(S)或天冬酰胺(N)。

[0233] 在一个更优选的实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域和轻链可变结构域,其中重链可变结构域含有:

[0234] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0235] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0236] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0237] 其中轻链可变结构域含有:

[0238] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0239] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0240] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0241] 本文中所述的基于MuSK抗体的分子可包含可变轻(VL)链、可变重(VH)链或VL和VH链的组合。在一些实施方案中,基于MuSK抗体的分子的VH链包含下表3中提供的VH氨基酸序列中的任一者,或包含与表3中列出的VH氨基酸序列中的任一者具有至少60%、至少61%、

至少62%、至少63%、至少64%、至少65%、至少66%、至少67%、至少68%、至少69%、至少70%、至少71%、至少72%、至少73%、至少74%、至少75%、至少76%、至少77%、至少78%、至少79%、至少80%、至少81%、至少82%、至少83%、至少84%、至少85%、至少86%、至少87%、至少88%、至少89%、至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%同一性或相似性的氨基酸序列。在一些实施方案中,基于MuSK抗体的分子的VL链包含下表3中提供的VL氨基酸序列中的任一者,或包含与表3中列出的VL氨基酸序列中的任一者具有至少60%同一性或相似性的氨基酸序列。在一些实施方案中,同一性或相似性为至少61%、至少62%、至少63%、至少64%、至少65%、至少66%、至少67%、至少68%、至少69%、至少70%、至少71%、至少72%、至少73%、至少74%、至少75%、至少76%、至少77%、至少78%、至少79%、至少80%、至少81%、至少82%、至少83%、至少84%、至少85%、至少86%、至少87%、至少88%、至少89%、至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%。

[0242]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
X1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKY WSQYYWAHYYGGLDYWGQGTLVTVSS	97
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	98
X2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGMDYWGQGTLVTVSS	99
X2m1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGFDYWGQGTLVTVSS	252
X2m2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGLDYWGQGTLVTVSS	253
X2m3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGFDYWGQGTLVTVSS	254
X2m4	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGLDYWGQGTLVTVSS	255
X2m5	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYFGMDYWGQGTLVTVSS	256
X2m6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYSGMDYWGQGTLVTVSS	257
X2m7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGFDYWGQGTLVTVSS	258
X2m8	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGLDYWGQGTLVTVSS	259

[0243]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
X2	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSGVWLITFGQGT KVEIK	100
X3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSSSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSWY EMWMSGYFGFDYWGQGTLVTVSS	101
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	102
X4	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGE HDYYVFGYLGMDYWGQGTLVTVSS	103
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYKPGALITFGQ GTKVEIK	104
X5	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFYSSSIHWVRQAPGKGLEWVA SISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSY TMFYYGGWYGSGYFGMDYWGQGTLVTVSS	105
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	106
X6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSYSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTY GSYYVSSYTGMDYWGQGTLVTVSS	107
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	108
X7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTLYSSSIHWVRQAPGKGLEWVA SISSSYSSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARLA GLYHYPGYLGLDYWGQGTLVTVSS	109
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSRSSLLTFGQG TKVEIK	110
X8	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSW SYHPWYYHVGWYTGLDYWGQGTLVTVSS	111
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	112

[0244]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
X9	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SIYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSG GEFYITSYYGMDYWGQGTLVTVSS	113
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	114
X10	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSYSSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKY YRWRHNKYQGFDYWGQGTLVTVSS	115
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSLWYPVTFGQG TKVEIK	116
X11	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSWG SYYVSGFVGFDYWGQGTLVTVSS	117
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQNSYYLITFGQGT KVEIK	118
X12	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAY ISPSSGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARQYW VPQWWITQYFGMDYWGQGTLVTVSS	119
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSSSLITFGQGT KVEIK	120
X13	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSSSGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSSE HWYTIGYYGIDYWGQGTLVTVSS	121
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYGSFSLITFGQ GTKVEIK	122
X14	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SISSSSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGS HHWFLWIYSGLDYWGQGTLVTVSS	123
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQGSYHLITFGQGT KVEIK	124
X15	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVA SISSSYGSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSE GDRYVSGYMGMDYWGQGTLVTVSS	125

[0245]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSGVWLITFGQGT KVEIK	126
X16	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSSIHWVRQAPGKGLEWVA SIYSSYGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARNW GYYMYWGWYYALDYWGQGTLVTVSS	127
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQWSSAQALITFGQ GTKVEIK	128
X17	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVAS IYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDH GYYVFGYLGMDYWGQGTLVTVSS	131
X17m1	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVAS IYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDH GYYVSGYLGMDYWGQGTLVTVSS	260
X17m2	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVAS IYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDH GYYVYGYLGMDYWGQGTLVTVSS	261
X17m3	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVAS IYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGDH GYYVSGYLGFDYWGQGTLVTVSS	262
X17m6	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISYSSIHWVRQAPGKGLEWVAS IYSSSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGEH GYYVSGYLGFDYWGQGTLVTVSS	263
X17	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYKPGALITFGQ GTKVEIK	132
X18	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISSSSIHWVRQAPGKGLEWVAS ISSSSGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARKYS KRAYPDYYWRGLDYWGQGTLVTVSS	133
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSA SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSYWWPITFGQGT KVEIK	134
14D10	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	196
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YTTNSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMGGGSNV YVFGGGTKLTVL	197

[0246]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
7G4	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	198
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMGRGSNK DYVFGGGTKLTVL	199
3C4	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	200
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTASNYPDWYQQTPGQAPRGLI YSTDSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALYMYSDSKLY VFGGGTKLTVL	201
3B2	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	202
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRGLI YSTDSRHSGVPSRFSGSISGNKAALTITGAQSEDEADYYCGLYMYSGSKN YVFGGGTKLTVL	203
3G3	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	204
	VL	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRHSGVPSRFSGSTSGNKAALTITGAQPEDEADYYCALYMGSDIRN YVFGGGTKLTVL	205
31G2	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	206
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRLSGVPSRFSGSFSGNKAALTITGAQPEDEADYYCALYMGSGSRN YVFGGGTKLTVL	207
31B7	VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV SAIPWNGGSTYYKESVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	208
	VL	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRALI YSTNSRLSGVPSRFSGSFSGNKAALTITGAQPEDEADYYCALYMGSESRN YVFGGGTKLTVL	209
17H10	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	210

[0247]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
	VL	SYELTQSPSVSVALRQTAKITCGGNRIGGKSVQWYQQKPGQAPMLVIYAD SRRPSGIPERFTGSNSGNTATLTITGAQAEDEADYYCHVWGSTASADFGG GTHLTVL	211
23B6	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	212
	VL	SYELTQSPSVSVALRQTAKITCGGDNIGSKNAQWYQQKPGQAPVMVLYAD SRRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCHVWDSSTNAWFGG GTHLTVL	213
30E1	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	214
	VL	SYELTQSPSVSVALRRTAKITCGGDNIGSKNTQWYQQKPGQAPVLVIYADS RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCHVWDSSTNAWFGGG THLTVL	215
30A11	VH	QVQVQESGPGLVKPSQTLSLTCTVSGGSITARYYSWSWIRQPPGKGLEW MGVIAYDGSTYYSPSLKSRTSISRDTSKNQFSLHLSSVTPDDTAVYYCARG SSRVAAAFDSWGQGTQVTVSS	216
	VL	SYELTQSPSVTVALRQTAKITCGGDNIASKNVQWYQQKPGQAPSLVIWAD SRRPSGIPVRFSGSNFGNTATLTISGAQAEDEADYYCQVWDSSTNVAVFG GGTHLTVL	217
16F11	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	218
	VL	DIVMTQSPSSVTASVGEKVTINCKSSQSVVFGSNQKSYLNWYQQRPGQSP RLLIYYASTQESGIPDRFSGSGSTTDFTLTISSVQPEDAAVYYCQQAYSAPT FGSGTRLEIK	219
4C11	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	220
	VL	DIVMTQSPSSVTASAGERVTINCRSSQSVLYSSNQKNYLNWYQQRLGQSP RLLIYWASARESGVPDRFSGSGSTTNFTLTISSFQPEDAAVYYCQQSYKPP YGFGSGTRLEIK	221
7A12	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	222
	VL	EIVLTQSPSSVTASIGEKVTINCESSQSVLYNQKNYLNWYQQRPGQSPRLLI YWASTRQSGVPDRFSGSGSGSTTDFTLTISSFQPEDVAVYYCQQAYNAPL TFGPGTKVELK	223

[0248]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
7G12	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	224
	VL	EIVLTQSPNSVTASVGEKVTINCKSSQRVQLGSNQKSYLNWYQQRPGQSP RLLIYYASTQQSGIPDRFSGSGSATDFTLTINSVQPEDAAVYYCQQGYSAP FTFGQGTKVELK	225
7B8	VH	EVQLVESGGGLVQPGGSLSLSCVASGFTFSLYYMNWVRQAPGKGLEWLS VIDTHSIAYYADSVKGRFTISRDNVKNTLYLQLNNLKPEDTALYYCVLGRTAL VRWGQGTQVTVSS	226
	VL	EIVLTQSPSSVTASAGEKVTINCKSSQSVLYNQKNYLAWYQQRPGQSPRLL IYWASTRESGVPDRFSGSGSTTDFTLTISSFQPEDVAVYYCQQGYSVPYTF GSGTRLEIK	227
3B2g1m1	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	228
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	229
3B2g1m2	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPGSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	230
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	231
3B2g1m4	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWQGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	232
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYMYSGSKN YVFGGGTKLTVL	233
3B2g2m1	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	234
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	235
3B2g2m2	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPGSGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRS GRIAFGALDAWGQGTLVTVSS	236

[0249]

mAb/Fab 名称	结构域	序列	SEQ ID NO:
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	237
3B2g2m4	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYGMSWVRQAPGKGLEWVS AIPWQGGSTYYKESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKR SGRIAFGALDAWGQGTLVTVSS	238
	VL	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVTSSNYPDWYQQTPGQAPRTLI YSTDSRHSGVPDRFSGSILGNKAALTITGAQADDESDYYCGLYSYSGSKNY VFGGGTKLTVL	239

[0250] 在一个实施方案中,本文中公开的基于MuSK抗体的分子含有:包含与SEQ ID NO:97具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:98具有至少80%同一性的氨基酸序列的轻链可变结构域;(ii)包含与SEQ ID NO:99和252至259中任一个具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:100具有至少80%同一性的氨基酸序列的轻链可变结构域;(iii)包含与SEQ ID NO:101具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:102具有至少80%同一性的氨基酸序列的轻链可变结构域;(iv)包含与SEQ ID NO:103具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:104具有至少80%同一性的氨基酸序列的轻链可变结构域;(v)包含与SEQ ID NO:105具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:106具有至少80%同一性的氨基酸序列的轻链可变结构域;(vi)包含与SEQ ID NO:107具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:108具有至少80%同一性的氨基酸序列的轻链可变结构域;或(vii)包含与SEQ ID NO:109具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:110具有至少80%同一性的氨基酸序列的轻链可变结构域;(viii)包含与SEQ ID NO:111具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:112具有至少80%同一性的氨基酸序列的轻链可变结构域;(ix)包含与SEQ ID NO:113具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:114具有至少80%同一性的氨基酸序列的轻链可变结构域;(x)包含与SEQ ID NO:115具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:116具有至少80%同一性的氨基酸序列的轻链可变结构域;(xi)包含与SEQ ID NO:117具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:118具有至少80%同一性的氨基酸序列的轻链可变结构域;(xii)包含与SEQ ID NO:119具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:120具有至少80%同一性的氨基酸序列的轻链可变结构域;(xiii)包含与SEQ ID NO:121具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:122具有至少80%同一性的氨基酸序列的轻链可变结构域;(xiv)包含与SEQ ID NO:123具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:124具有至少80%同一性的氨基酸序列的轻链可变结构域;(xv)包含与SEQ ID NO:125具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:126具有至少80%同一性的氨基酸序列的轻链可变结构域;(xvi)包含与SEQ ID NO:127具有至少80%同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:128具有至少80%同一性的氨基酸序列的轻链可变结构域;(xvii)包含与SEQ ID NO:

131和260至263中的任一个具有至少80％同一性的氨基酸序列的重链可变结构域,和包含与SEQ ID NO:132具有至少80％同一性的氨基酸序列的轻链可变结构域;以及(xviii)包含与SEQ ID NO:133具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:134具有至少80％同一性的氨基酸序列的轻链可变结构域。

[0251] 在一些实施方案中,本文中公开的基于MuSK抗体的分子含有:(i)包含与SEQ ID NO:196具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:197具有至少80％同一性的氨基酸序列的轻链可变结构域;(ii)包含与SEQ ID NO:198具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:199具有至少80％同一性的氨基酸序列的轻链可变结构域;(iii)包含与SEQ ID NO:200具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:201具有至少80％同一性的氨基酸序列的轻链可变结构域;(iv)包含与SEQ ID NO:202具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:203具有至少80％同一性的氨基酸序列的轻链可变结构域;(v)包含与SEQ ID NO:204具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:205具有至少80％同一性的氨基酸序列的轻链可变结构域;(vi)包含与SEQ ID NO:206具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:207具有至少80％同一性的氨基酸序列的轻链可变结构域;(vii)包含与SEQ ID NO:208具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:209具有至少80％同一性的氨基酸序列的轻链可变结构域;(viii)包含与SEQ ID NO:210具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:211具有至少80％同一性的氨基酸序列的轻链可变结构域;(vix)包含与SEQ ID NO:212具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:213具有至少80％同一性的氨基酸序列的轻链可变结构域;(x)包含与SEQ ID NO:214具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:215具有至少80％同一性的氨基酸序列的轻链可变结构域;(xi)包含与SEQ ID NO:216具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:217具有至少80％同一性的氨基酸序列的轻链可变结构域;(xii)包含与SEQ ID NO:218具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:219具有至少80％同一性的氨基酸序列的轻链可变结构域;(xiii)包含与SEQ ID NO:220具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:221具有至少80％同一性的氨基酸序列的轻链可变结构域;(xiv)包含与SEQ ID NO:222具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:223具有至少80％同一性的氨基酸序列的轻链可变结构域;(xv)包含与SEQ ID NO:224具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:225具有至少80％同一性的氨基酸序列的轻链可变结构域;(xvi)包含与SEQ ID NO:226具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:227具有至少80％同一性的氨基酸序列的轻链可变结构域(xvii)包含与SEQ ID NO:228具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:229具有至少80％同一性的氨基酸序列的轻链可变结构域;(xviii)包含与SEQ ID NO:230具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:231具有至少80％同一性的氨基酸序列的轻链可变结构域;(xix)包含与SEQ ID NO:232具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:233具有至少80％同一性的氨基酸序列的轻链可变结构域;(xx)包

含与SEQ ID NO:234具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:235具有至少80％同一性的氨基酸序列的轻链可变结构域;(xxi)包含与SEQ ID NO:236具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:237具有至少80％同一性的氨基酸序列的轻链可变结构域;(xxii)包含与SEQ ID NO:238具有至少80％同一性的氨基酸序列的重链可变结构域和包含与SEQ ID NO:239具有至少80％同一性的氨基酸序列的轻链可变结构域。

[0252] 在一个优选的实施方案中,本文中所公开的基于MuSK抗体的分子(或抗MuSK抗体或其抗原结合片段)包含含有与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列的重链可变结构域(VH)以及含有与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列的轻链可变结构域(VL)。

[0253] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0254] 在一个优选的实施方案中,本文中所公开的基于MuSK抗体的分子包含含有氨基酸序列SEQ ID NO:234的重链可变结构域和含有氨基酸序列SEQ ID NO:235的轻链可变结构域。

[0255] 在一个优选的实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0256] 其中重链可变结构域含有:

[0257] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0258] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,以及

[0259] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0260] 其中轻链可变结构域含有:

[0261] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0262] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,以及

[0263] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0264] 在一个更优选的实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0265] 其中重链可变结构域含有:

[0266] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0267] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0268] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0269] 其中轻链可变结构域含有:

[0270] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0271] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0272] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0273] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区与SEQ ID NO:266或SEQ ID NO:267包含至少80%序列同一性,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且其中重链可变结构域含有:

[0274] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0275] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0276] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0277] 其中轻链可变结构域含有:

[0278] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0279] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0280] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0281] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0282] 在一个实施方案中,抗体的CDR-H2在第3位包含脯氨酸(P)、在第4位包含色氨酸(W)以及在第5位包含丝氨酸(S)或天冬酰胺(N)。

[0283] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0284] 其中重链可变结构域含有:

[0285] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0286] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的

CDR-H2氨基酸序列,和

[0287] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0288] 其中轻链可变结构域含有:

[0289] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0290] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0291] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0292] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0293] 在一个实施方案中,抗体的CDR-H2在第3位包含脯氨酸(P)、在第4位包含色氨酸(W)以及在第5位包含丝氨酸(S)或天冬酰胺(N)。

[0294] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267,其中重链可变结构域包含SEQ ID NO:234并且轻链可变结构域包含SEQ ID NO:235,并且

[0295] 其中重链可变结构域含有:

[0296] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0297] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0298] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0299] 其中轻链可变结构域含有:

[0300] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0301] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0302] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0303] 在一个实施方案中,抗体的CDR-H2在第3位包含脯氨酸(P)、在第4位包含色氨酸(W)以及在第5位包含丝氨酸(S)或天冬酰胺(N)。

[0304] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267,其中重链可变结构域包含SEQ ID NO:234并且轻链可变结构域包含SEQ ID NO:235,并且

[0305] 其中重链可变结构域含有:

[0306] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0307] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0308] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0309] 其中轻链可变结构域包含:

[0310] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0311] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0312] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0313] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0314] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,

[0315] 其中野生型人IgG恒定Fc区与SEQ ID NO:266或SEQ ID NO:267包含至少80%序列同一性,其中a),其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A,并且其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0316] 其中重链可变结构域含有:

[0317] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0318] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0319] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0320] 其中轻链可变结构域含有:

[0321] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0322] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0323] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0324] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0325] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,

[0326] 其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267,其中根据EU编号系统编号的L234A和/或L235A替换引入到所述Fc区,并且

[0327] 其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0328] 其中重链可变结构域含有:

[0329] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0330] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0331] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0332] 其中轻链可变结构域含有:

[0333] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0334] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0335] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0336] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0337] 在一个实施方案中,与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267,其中根据EU编号系统编号的L234A和/或L235A被引入到所述Fc区,并且其中重链可变结构域包含SEQ ID NO:234并且轻链可变结构域包含SEQ ID NO:235,以及

[0338] 其中重链可变结构域含有:

[0339] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0340] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0341] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0342] 其中轻链可变结构域含有:

[0343] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0344] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0345] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0346] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0347] 在一个实施方案中,抗MuSK抗体或其抗原结合片段,包含:

[0348] a)全长重链,其包含与SEQ ID NO:268具有至少80%同一性或相似性的氨基酸序列,和

[0349] b)全长轻链,其包含与SEQ ID NO:269具有至少80%同一性或相似性的氨基酸序列,并且

[0350] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0351] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0352] 在一个实施方案中,抗MuSK抗体或其抗原结合片段,包含:

[0353] a)全长重链,其包含SEQ ID NO:268和

[0354] b)全长轻链,其包含SEQ ID NO:269,并且

[0355] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0356] 在一个实施方案中,抗MuSK抗体或其抗原结合片段,包含:

[0357] a)全长重链,其包含与SEQ ID NO:270具有至少80%同一性或相似性的氨基酸序列和

[0358] b)全长轻链,其包含与SEQ ID NO:271具有至少80%同一性或相似性的氨基酸序列,并且

[0359] c)其中以下突变(均根据EU编号系统编号)中的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0360] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、

87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0361] 在一个实施方案中,抗MuSK抗体或其抗原结合片段,包含:

[0362] a)全长重链,其包含SEQ ID NO:270和

[0363] b)全长轻链,其包含SEQ ID NO:271,并且

[0364] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0365] 多核苷酸

[0366] 本发明的另一方面涉及编码本文中所述的基于MuSK抗体的分子的分离的多核苷酸。在一个实施方案中,编码本发明的MuSK抗体的多核苷酸包含编码上述CDR中的任意一种、任意两种、任意三种、任意四种、任意五种或任意六种的核苷酸序列,包括SEQ ID NO:1至48、135至140、147至158、240至251的重链CDR和SEQ ID NO:49至96、141至146和159至195的轻链CDR。

[0367] 因此,本发明提供了用于在人对象中治疗神经肌肉疾病的多核苷酸,该多核苷酸包含编码抗MuSK抗体或抗原结合片段或其VH、VL或CDR结构域的核苷酸序列。

[0368] 在一个实施方案中,多核苷酸包含编码VH结构域的核苷酸序列,其中所述VH结构域包含:(i)重链可变结构域,其包含SEQ ID NO:1的CDR-H1、SEQ ID NO:17的CDR-H2和SEQ ID NO:33的CDR-H3;(ii)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:34的CDR-H3;(iii)重链可变结构域,其包含SEQ ID NO:3的CDR-H1、SEQ ID NO:19的CDR-H2和SEQ ID NO:35的CDR-H3;(iv)重链可变结构域,其包含SEQ ID NO:4的CDR-H1、SEQ ID NO:20的CDR-H2和SEQ ID NO:36的CDR-H3;(v)重链可变结构域,其包含SEQ ID NO:5的CDR-H1、SEQ ID NO:21的CDR-H2和SEQ ID NO:37的CDR-H3;(vi)重链可变结构域,其包含SEQ ID NO:6的CDR-H1、SEQ ID NO:22的CDR-H2和SEQ ID NO:38的CDR-H3;(vii)重链可变结构域,其包含SEQ ID NO:7的CDR-H1、SEQ ID NO:23的CDR-H2和SEQ ID NO:39的CDR-H3;(viii)重链可变结构域,其包含SEQ ID NO:8的CDR-H1、SEQ ID NO:24的CDR-H2和SEQ ID NO:40的CDR-H3;(ix)重链可变结构域,其包含SEQ ID NO:9的CDR-H1、SEQ ID NO:25的CDR-H2和SEQ ID NO:41的CDR-H3;(x)重链可变结构域,其包含SEQ ID NO:10的CDR-H1、SEQ ID NO:26的CDR-H2和SEQ ID NO:42的CDR-H3;(xi)重链可变结构域,其包含SEQ ID NO:11的CDR-H1、SEQ ID NO:27的CDR-H2和SEQ ID NO:43的CDR-H3;(xii)重链可变结构域,其包含SEQ ID NO:12的CDR-H1、SEQ ID NO:28的CDR-H2和SEQ ID NO:44的CDR-H3;(xiii)重链可变结构域,其包含SEQ ID NO:13的CDR-H1、SEQ ID NO:29的CDR-H2和SEQ ID NO:45的CDR-H3;(xiv)重链可变结构域,其包含SEQ ID NO:14的CDR-H1、SEQ ID NO:30的CDR-H2和SEQ ID NO:46的CDR-H3;(xv)重链可变结构域,其包含SEQ ID NO:15的CDR-H1、SEQ ID NO:31的CDR-H2和SEQ ID NO:47的CDR-H3;(xvi)重链可变结构域,其包含SEQ ID NO:16的CDR-H1、SEQ ID NO:32的CDR-H2和SEQ ID NO:48的CDR-H3;(xvii)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:139的CDR-H3;和(xviii)重链可变结构域,其包含SEQ ID NO:136的CDR-H1、SEQ ID NO:138的CDR-H2和SEQ ID NO:140的CDR-H3。

[0369] 在一些实施方案中,多核苷酸包含编码VH结构域的核苷酸序列,其中所述VH结构域包含:(ii.a)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:240的CDR-H3(X2m1);(ii.b)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、

SEQ ID NO:18的CDR-H2和SEQ ID NO:241的CDR-H3(X2m2);(ii.c)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:242的CDR-H3(X2m3);(ii.d)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:243的CDR-H3(X2m4);(ii.e)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:244的CDR-H3(X2m5);(ii.f)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:245的CDR-H3(X2m6);(ii.g)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:246的CDR-H3(X2m7);(ii.h)重链可变结构域,其包含SEQ ID NO:2的CDR-H1、SEQ ID NO:18的CDR-H2和SEQ ID NO:247的CDR-H3(X2m8)。

[0370] 在一些实施方案中,多核苷酸包含编码VH结构域的核苷酸序列,其中所述VH结构域包含:(xvii.a)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:248的CDR-H3(X17m1);(xvii.b)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:249的CDR-H3(X17m2);(xvii.c)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:250的CDR-H3(X17m3);(xvii.d)重链可变结构域,其包含SEQ ID NO:135的CDR-H1、SEQ ID NO:137的CDR-H2和SEQ ID NO:251的CDR-H3(X17m6)。

[0371] 在一个实施方案中,多核苷酸包含编码VH结构域的核苷酸序列,其中所述VH结构域包含:(xix)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2和SEQ ID NO:156的CDR-H3;(xx)重链可变结构域,其包含SEQ ID NO:148的CDR-H1、SEQ ID NO:151的CDR-H2和SEQ ID NO:157的CDR-H3;(xxi)重链可变结构域,其包含SEQ ID NO:149的CDR-H1、SEQ ID NO:152的CDR-H2和SEQ ID NO:158的CDR-H3。

[0372] 在一个实施方案中,多核苷酸包含编码VH结构域的核苷酸序列,其中所述VH结构域包含:(xxii)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2和SEQ ID NO:156的CDR-H3;(xxiii)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2和SEQ ID NO:156的CDR-H3;(xxiv)重链可变结构域,其包含SEQ ID NO:147的CDR-H1、SEQ ID NO:155的CDR-H2和SEQ ID NO:156的CDR-H3。

[0373] 在一个实施方案中,多核苷酸包含编码VL结构域的核苷酸序列,其中所述VL结构域包含:(i)轻链可变结构域,其包含SEQ ID NO:49的CDR-L1、SEQ ID NO:65的CDR-L2和SEQ ID NO:81的CDR-L3;(ii)轻链可变结构域,其包含SEQ ID NO:50的CDR-L1、SEQ ID NO:66的CDR-L2和SEQ ID NO:82的CDR-L3;(iii)轻链可变结构域,其包含SEQ ID NO:51的CDR-L1、SEQ ID NO:67的CDR-L2和SEQ ID NO:83的CDR-L3;(iv)轻链可变结构域,其包含SEQ ID NO:52的CDR-L1、SEQ ID NO:68的CDR-L2和SEQ ID NO:84的CDR-L3;(v)轻链可变结构域,其包含SEQ ID NO:53的CDR-L1、SEQ ID NO:69的CDR-L2和SEQ ID NO:85的CDR-L3;(vi)轻链可变结构域,其包含SEQ ID NO:54的CDR-L1、SEQ ID NO:70的CDR-L2和SEQ ID NO:86的CDR-L3;(vii)轻链可变结构域,其包含SEQ ID NO:55的CDR-L1、SEQ ID NO:71的CDR-L2和SEQ ID NO:87的CDR-L3;(viii)轻链可变结构域,其包含SEQ ID NO:56的CDR-L1、SEQ ID NO:72的CDR-L2和SEQ ID NO:88的CDR-L3;(ix)轻链可变结构域,其包含SEQ ID NO:57的CDR-L1、SEQ ID NO:73的CDR-L2和SEQ ID NO:89的CDR-L3;(x)轻链可变结构域,其包含SEQ ID NO:58的CDR-L1、SEQ ID NO:74的CDR-L2和SEQ ID NO:90的CDR-L3;(xi)轻链可变结构

域,其包含SEQ ID NO:59的CDR-L1、SEQ ID NO:75的CDR-L2和SEQ ID NO:91的CDR-L3;(xii)轻链可变结构域,其包含SEQ ID NO:60的CDR-L1、SEQ ID NO:76的CDR-L2和SEQ ID NO:92的CDR-L3;(xiii)轻链可变结构域,其包含SEQ ID NO:61的CDR-L1、SEQ ID NO:77的CDR-L2和SEQ ID NO:93的CDR-L3;(xiv)轻链可变结构域,其包含SEQ ID NO:62的CDR-L1、SEQ ID NO:78的CDR-L2和SEQ ID NO:94的CDR-L3;(xv)轻链可变结构域,其包含SEQ ID NO:63的CDR-L1、SEQ ID NO:79的CDR-L2和SEQ ID NO:95的CDR-L3;(xvi)轻链可变结构域,其包含SEQ ID NO:64的CDR-L1、SEQ ID NO:80的CDR-L2和SEQ ID NO:96的CDR-L3;(xvii)轻链可变结构域,其包含SEQ ID NO:141的CDR-L1、SEQ ID NO:143的CDR-L2和SEQ ID NO:145的CDR-L3;和(xviii)轻链可变结构域,其包含SEQ ID NO:142的CDR-L1、SEQ ID NO:144的CDR-L2和SEQ ID NO:146的CDR-L3。

[0374] 在一个实施方案中,多核苷酸包含编码VL结构域的核苷酸序列,其中所述VL结构域包含:(xix)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:170的CDR-L2和SEQ ID NO:180的CDR-L3;(xx)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:181的CDR-L3;(xxi)轻链可变结构域,其包含SEQ ID NO:160的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:182的CDR-L3;(xxii)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3;(xxiii)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:171的CDR-L2和SEQ ID NO:184的CDR-L3;(xxiv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:185的CDR-L3;(xxv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:173的CDR-L2和SEQ ID NO:186的CDR-L3;(xxvi)轻链可变结构域,其包含SEQ ID NO:161的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:187的CDR-L3;(xxvii)轻链可变结构域,其包含SEQ ID NO:162的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3;(xxviii)轻链可变结构域,其包含SEQ ID NO:163的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:188的CDR-L3;(xxix)轻链可变结构域,其包含SEQ ID NO:164的CDR-L1、SEQ ID NO:174的CDR-L2和SEQ ID NO:189的CDR-L3;(xxx)轻链可变结构域,其包含SEQ ID NO:165的CDR-L1、SEQ ID NO:175的CDR-L2和SEQ ID NO:190的CDR-L3;(xxxi)轻链可变结构域,其包含SEQ ID NO:166的CDR-L1、SEQ ID NO:176的CDR-L2和SEQ ID NO:191的CDR-L3;(xxxi)轻链可变结构域,其包含SEQ ID NO:167的CDR-L1、SEQ ID NO:177的CDR-L2和SEQ ID NO:192的CDR-L3;(xxxii)轻链可变结构域,其包含SEQ ID NO:168的CDR-L1、SEQ ID NO:178的CDR-L2和SEQ ID NO:193的CDR-L3;(xxxiii)轻链可变结构域,其包含SEQ ID NO:169的CDR-L1、SEQ ID NO:179的CDR-L2和SEQ ID NO:194的CDR-L3。

[0375] 在一个实施方案中,多核苷酸包含编码VL结构域的核苷酸序列,其中所述VL结构域包含:(xxxiv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2、和SEQ ID NO:183的CDR-L3;(xxxv)轻链可变结构域,其包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3。

[0376] 在一个实施方案中,编码基于MuSK抗体的分子的分离的多核苷酸编码如下表3中提供的VH和/或VL结构域序列中的任一者。本文中所述的核酸分子包括分离的多核苷酸、表达载体的部分或线性DNA序列的部分,包括用于体外转录/翻译的线性DNA序列、以及与本文中所述的抗体或其结合片段的原核、真核或丝状噬菌体表达、分泌和/或展示相容的载体。

[0377] 在一个优选的实施方案中,多核苷酸包含这样的核苷酸序列,其编码包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列的VH。在另一个更优选的实施方案中,多核苷酸包含这样的核苷酸序列,其编码包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列的VL。

[0378] 在一个优选的实施方案中,多核苷酸包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的核苷酸序列,所述分子包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0379] 其中重链可变结构域含有:

[0380] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0381] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0382] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0383] 其中轻链可变结构域含有:

[0384] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0385] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0386] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0387] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0388] 在一个更优选的实施方案中,多核苷酸包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的核苷酸序列,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且其中重链可变结构域含有:

[0389] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0390] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0391] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0392] 其中轻链可变结构域含有:

[0393] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0394] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0395] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0396] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、

87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0397] 在一个更优选的实施方案中,多核苷酸包含SEQ ID NO:264具有至少80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的核苷酸序列或由其组成。在一个甚至更优选的实施方案中,多核苷酸包含SEQ ID NO:264或由SEQ ID NO:264组成。

[0398] 在另一个更优选的实施方案中,多核苷酸包含与SEQ ID NO:265具有至少80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的核苷酸序列或由其组成。在一个甚至更优选的实施方案中,多核苷酸包含SEQ ID NO:265或由SEQ ID NO:265组成。

[0399] 在一个更优选的实施方案中,多核苷酸包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的核苷酸序列,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域由与SEQ ID NO:264具有至少80％同一性的核苷酸序列编码并且轻链可变结构域由与SEQ ID NO:265具有至少80％同一性的核苷酸序列编码。在一个实施方案中,同一性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0400] 在一个实施方案中,多核苷酸包含:

[0401] a)与编码全长重链的SEQ ID NO:276具有至少80％同一性的核苷酸序列,以及

[0402] b)与编码全长轻链的SEQ ID NO:278具有至少80％同一性的核苷酸序列。

[0403] 在一个实施方案中,同一性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0404] 在一个实施方案中,多核苷酸包含:

[0405] a)与编码重链可变结构域的SEQ ID NO:277具有至少80％同一性的核苷酸序列,以及

[0406] b)与编码轻链可变结构域的SEQ ID NO:279具有至少80％同一性的核苷酸序列。

[0407] 在一个实施方案中,同一性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0408] 在一个实施方案中,多核苷酸包含:

[0409] a)与编码全长重链的SEQ ID NO:276具有至少80％同一性的核苷酸序列,其中所述核苷酸序列包含与编码重链可变结构域的SEQ ID NO:277具有至少80％同一性的核苷酸序列,以及

[0410] b)与编码全长轻链的SEQ ID NO:278具有至少80％同一性的核苷酸序列,其中所述核苷酸序列包含与编码轻链可变结构域的SEQ ID NO:279具有至少80％同一性的核苷酸序列。

[0411] 在一个实施方案中,同一性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0412] 在一个实施方案中,多核苷酸包含:

[0413] a)核苷酸序列SEQ ID NO:276,和

[0414] b)核苷酸序列SEQ ID NO:278。

[0415] 本发明的多核苷酸可以通过化学合成例如在自动化多核苷酸合成仪上的固相多

核苷酸合成来产生并且组装成完整的单链或双链分子。或者,多核苷酸可以通过其他技术例如PCR,随后是常规克隆来产生。用于产生或获得给定序列的多核苷酸的技术是本领域公知的。

[0416] 多核苷酸可以包含至少一种非编码序列,例如启动子或增强子序列、内含子、多腺苷酸化信号、促进RepA结合的顺式序列等。多核苷酸序列还可以包含另外的序列,其编码例如接头序列、标志物或标签序列(例如组氨酸标签或HA标签)以促进纯化或检测蛋白质、信号序列、融合蛋白配偶体(例如RepA、Fc部分)或噬菌体外壳蛋白(例如pIX或pIII)。

[0417] 载体

[0418] 在另一个方面中,提供了用于在人对象中治疗神经肌肉病症的载体(优选表达载体),其包含编码本文中所述的基于MuSK抗体的分子(或抗MuSK抗体或其抗原结合片段)的多核苷酸。

[0419] 这样的载体包括但不限于质粒载体、病毒载体,包括但不限于牛痘载体、慢病毒载体、腺病毒载体、腺相关病毒载体、用于杆状病毒表达的载体、基于转座子的载体或这样的任何其他载体,所述其他载体适用于将本文中所述的多核苷酸通过任何方式引入到给定生物体或遗传背景以促进编码的抗体多肽的表达。在一个实施方案中,编码重链可变结构域的多核苷酸,单独或与编码如本文中所述的轻链可变结构域的多核苷酸一起,与启动子、翻译起始区段(例如,核糖体结合序列和起始密码子)、3'非翻译区、多聚腺苷酸化信号、终止密码子和转录终止的序列组合以形成一个或更多个表达载体构建体。

[0420] 在一个实施方案中,载体是腺病毒相关病毒(adenoviral-associated viral,AAV)载体。许多适合于将编码本文中所述抗体的多核苷酸递送至中枢神经系统的治疗性AAV载体是本领域已知的。参见例如,Deverman et al.,"Gene Therapy for Neurological Disorders:Progress and Prospects,"Nature Rev.17:641-659(2018),Nature Rev.17:641-659(2018),其在此通过引用整体并入。合适的AAV载体包括以天然形式或针对增强的趋向性而改造的血清型AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、或AAV11。特别适合于本文中所述MuSK抗体的治疗性表达的已知对CNS具有趋向性的AAV载体包括以天然形式或针对增强的趋向性而改造的AAV1、AAV2、AAV4、AAV5、AAV8和AAV9。在一个实施方案中,AAV载体是AAV2载体。在另一个实施方案中,AAV载体是AAV5载体(Vitale et al.,"Anti-tau Conformational scFv MC1 Antibody Efficiently Reduces Pathological Tau Species in Adult JNPL3 Mice,"Acta Neuropathol.Commun.6:82(2018),其在此通过引用整体并入)。在另一个实施方案中,AAV载体是AAV9载体(Haiyan et al.,"Targeting Root Cause by Systemic scAAV9-hIDS Gene Delivery:Functional Correction and Reversal of Severe MPSII in Mice,"Mol.Ther.Methods Clin.Dev.10:327-340(2018),其在此通过引用整体并入)。在另一个实施方案中,AAV载体是AAVrh10载体(Liu et al.,"Vectored Intracerebral Immunizations with the Anti-Tau Monoclonal Antibody PHF1 Markedly Reduces Tau Pathology in Mutant Transgenic Mice,"J.Neurosci.36(49):12425-35(2016),其在此通过引用整体并入)。

[0421] 在另一个实施方案中,AAV载体是杂合载体,其包含一种血清型(例如AAV2)的基因组和另一种血清型(例如AAV1或AAV3至9)的衣壳蛋白以控制趋向性。参见例如,Broekman et al.,"Adeno-associated Virus Vectors Serotyped with AAV8 Capsid are More

Efficient than AAV-1or-2Serotypes for Widespread Gene Delivery to the Neonatal Mouse Brain,"Neuroscience 138:501-510(2006),其在此通过引用整体并入。在一个实施方案中,AAV载体是AAV2/8杂合载体(Ising et al.,"AAV-mediated Expression of Anti-Tau ScFv Decreases Tau Accumulation in a Mouse Model of Tauopathy,"J.Exp.Med.214(5):1227(2017),其在此通过引用整体并入)。在另一个实施方案中,AAV载体是AAV2/9杂合载体(Simon et al.,"A Rapid Gene Delivery-Based Mouse Model for Early-Stage Alzheimer Disease-Type Tauopathy,"J.Neuropath.Exp.Neurol.72(11):1062-71(2013),其在此通过引用整体并入)。

[0422] 在另一个实施方案中,AAV载体是由于其以下而经改造或经选择的AAV载体:在实质内施用之后增强的CNS转导,例如AAV-DJ(Grimm et al.,J.Viol.82:5887-5911(2008),其在此通过引用整体并入);对神经干细胞和祖细胞的转导提高,例如SCH9和AAV4.18(Murlidharan et al.,J.Virol.89:3976-3987(2015)和Ojala et al.,Mol.Ther.26:304-319(2018),其在此通过引用整体并入);增强的逆行转导,例如rAAV2-retro(Muller et al.,Nat.Biotechnol.21:1040-1046(2003),其在此通过引用整体并入);选择性转导至脑内皮细胞中,例如AAV-BRI(Korbelin et al.,EMBO Mol.Med.8:609-625(2016),其在此通过引用整体并入);或者在IV施用之后增强的成体CNS的转导,例如AAV-PHP.B和AAVPHP.eB(Deverman et al.,Nat.Biotechnol.34:204-209(2016)和Chan et al.,Nat.Neurosci.20:1172-1179(2017),其在此通过引用整体并入。

[0423] 根据该实施方案,编码基于MuSK抗体的分子的表达载体构建体包含编码重链多肽、其功能片段、其变体、或其组合的多核苷酸。表达构建体可替代地包含编码轻链多肽、其功能片段、其变体、或其组合的核酸序列。在一个实施方案中,表达载体构建体包含编码重链多肽、其功能片段或其变体和轻链多肽、其功能片段或其变体的核酸序列。

[0424] 在一个实施方案中,表达构建体还包含适合于驱动基于MuSK抗体的分子的表达的启动子序列。合适的启动子序列包括但不限于延伸因子1-α启动子(EF1a)启动子、磷酸甘油酸激酶-1启动子(PGK)启动子、巨细胞病毒即早基因启动子(CMV)、嵌合肝特异性启动子(liver-specific promoter,LSP)、巨细胞病毒增强子/鸡β-肌动蛋白启动子(CAG)、四环素响应性启动子(TRE)、甲状腺素转运蛋白启动子(TTR)、猿病毒40启动子(SV40)和CK6启动子。本领域已知的适合于驱动哺乳动物细胞中基因表达的其他启动子也适合于并入到本文中公开的表达构建体中。

[0425] 在一个实施方案中,表达构建体(或表达载体)还编码接头序列。接头序列可以编码在空间上分离和/或连接表达构建体的一个或更多个组件(所编码抗体的重链和轻链组件)的氨基酸序列。

[0426] 在一个优选的实施方案中,表达载体包含:编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合并且含有重链可变结构域和轻链可变结构域的基于抗体的分子的多核苷酸,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且其中重链可变结构域含有:

[0427] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0428] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0429] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0430] 其中轻链可变结构域含有:

[0431] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0432] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0433] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0434] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0435] 在一个更优选的实施方案中,表达载体包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合并且含有重链可变结构域和轻链可变结构域的基于抗体的分子的核苷酸,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且其中重链可变结构域含有:

[0436] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0437] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0438] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0439] 其中轻链可变结构域含有:

[0440] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0441] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0442] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0443] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0444] 在一个实施方案中,表达载体包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的多核苷酸并且包含:

[0445] a)与编码全长重链的SEQ ID NO:276具有至少80%同一性的核苷酸序列,以及

[0446] b)与编码全长轻链的SEQ ID NO:278具有至少80%同一性的核苷酸序列。

[0447] 在一个实施方案中,同一性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0448] 在一个实施方案中,表达载体包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的多核苷酸并且包含:

[0449] a)与编码重链可变结构域的SEQ ID NO:277具有至少80%同一性的核苷酸序列,以及

[0450] b)与编码轻链可变结构域的SEQ ID NO:279具有至少80%同一性的核苷酸序列。

[0451] 在一个实施方案中,同一性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0452] 在一个更优选的实施方案中,表达载体包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的多核苷酸并且包含:

[0453] c)与编码全长重链的SEQ ID NO:276具有至少80%同一性的核苷酸序列,其中所述核苷酸序列包含与编码重链可变结构域的SEQ ID NO:277具有至少80%同一性的核苷酸序列,以及

[0454] d)与编码全长轻链的SEQ ID NO:278具有至少80%同一性的核苷酸序列,其中所述核苷酸序列包含与编码轻链可变结构域的SEQ ID NO:279具有至少80%同一性的核苷酸序列。

[0455] 在一个实施方案中,同一性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0456] 在一个更优选的实施方案中,表达载体包含编码与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子的多核苷酸并且包含:

[0457] a)核苷酸序列SEQ ID NO:276,和

[0458] b)核苷酸序列SEQ ID NO:278。

[0459] 宿主细胞

[0460] 本发明的另一个方面是用于在人对象中治疗神经肌肉疾病的宿主细胞或无细胞表达系统,其中细胞含有编码MuSK抗体(或其抗原结合片段)的表达载体并任选地产生本文中描述的所述MuSK抗体。

[0461] 本文中所述的基于MuSK抗体的分子可以任选地由细胞系、混合细胞系、永生化细胞或永生化细胞的克隆群体产生,如本领域公知的(参见例如,

[0462] Ausubel et al.,ed.,Current Protocols in Molecular Biology,John Wiley& Sons,Inc.,NY,N.Y.(1987-2001);Sambrook et al.,Molecular Cloning:A Laboratory Manual,2nd Edition,Cold Spring Harbor,N.Y.(1989);Harlow and Lane,Antibodies,a Laboratory Manual,Cold Spring Harbor,N.Y.(1989);Colligan et al.,eds.,Current Protocols in lmmunology,John Wiley&Sons,lnc.,NY(1994-2001);Colligan et al., Current Protocols in Protein Science,John Wiley&Sons,NY,N.Y.,(1997-2001),

[0463] 其在此通过引用整体并入)。

[0464] 在一些实施方案中,选择用于表达的宿主细胞可以是哺乳动物来源的。合适的哺乳动物宿主细胞包括但不限于COS-1细胞、COS-7细胞、HEK293细胞、BHK21细胞、CHO细胞、BSC-1细胞、HeG2细胞、SP2/0细胞、HeLa细胞、哺乳动物骨髓瘤细胞、哺乳动物淋巴瘤细胞或其任何衍生的、永生化的或转化的细胞。其他合适的宿主细胞包括但不限于酵母细胞、昆虫细胞和植物细胞。或者,宿主细胞可以选白不能使多肽糖基化的物种或生物体,例如原核细胞或生物体,例如BL21、BL21(DE3)、BL21-GOLD(DE3)、XL1-Blue、JM109、HMS174、HMS174(DE3),以及任何天然的或经改造的大肠杆菌属(E.coli spp)、克雷伯菌属(Klebsiella spp)或假单胞菌属(Pseudomonas spp)菌株。

[0465] 本文中所述的基于MuSK抗体的分子可以通过多种技术中的任一种使用上文所述的分离的多核苷酸、载体和宿主细胞来制备。一般而言,抗体可以通过细胞培养技术产生,

包括通过常规技术产生单克隆抗体,或者通过将抗体基因、重链和/或轻链转染到合适的细菌或哺乳动物细胞宿主中,以允许产生抗体,其中抗体可以是重组的。在一个实施方案中,本文中所述的基于MuSK抗体的分子是单克隆抗体或其功能结合片段。标准分子生物学技术用于制备重组表达载体、转染宿主细胞、选择转化体、培养宿主细胞并从培养基中回收抗体。可以使用通常用于将外源DNA引入原核或真核宿主细胞的多种技术进行对宿主细胞的转染,例如通过电穿孔、磷酸钙沉淀、DEAE-葡聚糖转染等。尽管可以在原核或真核宿主细胞中表达本文中所述的抗体,但有时优选在真核细胞,特别是在哺乳动物细胞中表达抗体,因为这样的真核细胞(并且特别是哺乳动物细胞)比原核细胞更有可能组装和分泌适当折叠且具有免疫活性的抗体。

[0466] 如上所述,用于表达本发明的重组抗体的示例性哺乳动物宿主细胞包括中国仓鼠卵巢(CHO细胞)(包括dhfr-CHO细胞,描述于Urlaub and Chasin,Proc.Natl.Acad.Sci.USA,77:4216-4220(1980),其在此通过引用整体并入)。另一些合适的哺乳动物宿主细胞包括但不限于NSO骨髓瘤细胞、COS细胞和SP2细胞。当将编码抗体基因的重组表达载体引入到哺乳动物宿主细胞中时,通过将宿主细胞培养足以使抗体在宿主细胞中表达或者更优选使抗体分泌到其中培养宿主细胞的培养基中的一段时间来产生抗体。

[0467] 宿主细胞也可用于产生功能性抗体片段,例如Fab片段或scFv分子。可以理解,上述操作的变化也在本发明的范围内。例如,可期望用编码本文中所述抗体的轻链和/或重链的功能片段的DNA来转染宿主细胞。重组DNA技术也可用于去除编码轻链和重链中的任一者或两者的DNA中的对于与目的抗原结合不是必需的一些或全部。从这样的截短的DNA分子表达的分子也涵盖在本文中所述的抗体中。

[0468] 抗体和抗体结合片段通过已知方法从重组细胞培养物中回收和纯化,所述方法包括但不限于蛋白A纯化、硫酸铵或乙醇沉淀、酸提取、阴离子或阳离子交换色谱、磷酸纤维素色谱、疏水相互作用色谱、亲和色谱、羟基磷灰石色谱和凝集素色谱。高效液相色谱("HPLC")也可用于纯化。

[0469] 在一个优选的实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0470] 其中重链可变结构域含有:

[0471] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0472] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0473] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0474] 其中轻链可变结构域含有:

[0475] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0476] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的

CDR-L2氨基酸序列,和

[0477] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0478] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0479] 在一个更优选的实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0480] 其中重链可变结构域含有:

[0481] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0482] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0483] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0484] 其中轻链可变结构域含有:

[0485] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0486] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,以及

[0487] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0488] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0489] 在一个实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含:

[0490] a)与编码全长重链的SEQ ID NO:276具有至少80%同一性的核苷酸序列,以及

[0491] b)与编码全长轻链的SEQ ID NO:278具有至少80%同一性的核苷酸序列。

[0492] 在一个实施方案中,同一性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0493] 在一个实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含:

[0494] a)与编码重链可变结构域的SEQ ID NO:277具有至少80%同一性的核苷酸序列,以及

[0495] b)与编码轻链可变结构域的SEQ ID NO:279具有至少80%同一性的核苷酸序列。

[0496] 在一个实施方案中,同一性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0497] 在一个实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含:

[0498] a)与编码全长重链的SEQ ID NO:276具有至少80%同一性的核苷酸序列,其中所述核苷酸序列包含与编码重链可变结构域的SEQ ID NO:277具有至少80%同一性的核苷酸序列,以及

[0499] b)与编码全长轻链的SEQ ID NO:278具有至少80%同一性的核苷酸序列,其中所

述核苷酸序列包含与编码轻链可变结构域的SEQ ID NO:279具有至少80％同一性的核苷酸序列。

[0500] 在一个实施方案中,同一性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0501] 在一个实施方案中,宿主细胞表达与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的基于抗体的分子,其包含:

[0502] a)核苷酸序列SEQ ID NO:276,和

[0503] b)核苷酸序列SEQ ID NO:278。

[0504] 包含基于MuSK抗体的分子的组合物

[0505] 可将本发明的基于MuSK抗体的分子或编码基于MuSK抗体的分子的多核苷酸有利地作为组合物施用。

[0506] 因此,在另一个方面中,提供了用于在人对象中治疗神经肌肉病症的组合物,所述组合物包含本文中所定义的抗体或抗原结合片段、多核苷酸、表达载体或宿主细胞或无细胞表达系统。

[0507] 在一个实施方案中,所述组合物是药物组合物。在一个实施方案中,所述药物组合物包含至少一种可药用载体或赋形剂。

[0508] 在一个实施方案中,这样的组合物是包含活性治疗剂(即,MuSK抗体)和多种其他可药用组分中的一种或更多种的药物组合物。参见Remington:The Science and Practice ofPharmacy(21st Edition)(2005)(Troy,D.B.et al.(Eds.)Lippincott Williams& Wilkins(Publs.),Baltimore MD),其在此通过引用整体并入。优选的形式取决于预期的施用方式和治疗应用。取决于所期望的制剂,组合物还可以包含可药用无毒载体、赋形剂、稀释剂、填充剂、盐、缓冲剂、洗涤剂(例如,非离子型洗涤剂,例如吐温-20或吐温-80)、稳定剂(例如,糖或不含蛋白质的氨基酸)、防腐剂、组织固定剂、增溶剂和/或适合于包含在药物组合物中的其他物质,并且它们是通常用于配制用于动物或人施用的药物组合物的载剂。选择稀释剂以不影响组合的生物活性。这样的稀释剂的一些实例是蒸馏水、生理磷酸缓冲盐水、林格溶液(Ringer's solution)、右旋糖溶液和Hank溶液。另外,药物组合物或制剂还可以包含其他载体,或无毒、非治疗性、非免疫原性稳定剂等。可用于本发明药物组合物的合适的水性和非水性载体的实例包括水、盐水、磷酸缓冲盐水、乙醇、右旋糖、多元醇(例如甘油、丙二醇、聚乙二醇等)及其合适的混合物、植物油(例如橄榄油、玉米油、花生油、棉籽油和芝麻油)、羧甲基纤维素胶体溶液、黄蓍胶和可注射的有机酯(例如油酸乙酯),和/或多种缓冲液。其他载体在制药领域是公知的。

[0509] 可药用载体包括无菌水溶液或分散体以及用于临时制备无菌可注射溶液或分散体的无菌粉末。这样的介质和试剂用于药物活性物质的用途在本领域中是已知的。除非任何常规介质或试剂与活性化合物不相容,否则考虑其在本发明的药物组合物中的用途。

[0510] 组合物还可以包含大的、缓慢代谢的大分子,例如蛋白质、多糖(如壳聚糖)、聚乳酸、聚乙醇酸和共聚物(例如,胶乳官能化琼脂糖、琼脂糖、纤维素等)、聚合物氨基酸、氨基酸共聚物和脂质聚集体(例如油滴或脂质体)。基于对本发明的基于活性抗体的分子的所期望生物学特性没有显著负面影响(例如,对抗原结合小于显著影响(例如,10％或更少的相对抑制、5％或更少的相对抑制等))来确定药物组合物的载体和其他组分的适用性。

[0511] 本发明的药物组合物还可以包含可药用抗氧化剂,例如(1)水溶性抗氧化剂,例如抗坏血酸、盐酸半胱氨酸、硫酸氢钠、焦亚硫酸钠、亚硫酸钠等;(2)油溶性抗氧化剂,例如抗坏血酸棕榈酸酯、丁基羟基茴香醚(BHA)、丁基羟基甲苯(BHT)、卵磷脂、没食子酸丙酯、α-生育酚等;以及(3)金属螯合剂,例如柠檬酸、乙二胺四乙酸(EDTA)、山梨糖醇、酒石酸、磷酸等。

[0512] 本发明的药物组合物还可以在组合物中包含等张剂,例如糖、多元醇,例如甘露糖醇、山梨糖醇、甘油或氯化钠。

[0513] 本发明的药物组合物还可以包含适合于所选施用途径的一种或更多种辅料,例如防腐剂、润湿剂、乳化剂、分散剂、防腐剂或缓冲剂,它们可以提高药物组合物的保质期或有效性。本发明的抗体可以与将保护抗体免于快速释放的载体一起制备,所述载体例如控制释放制剂,包括植入物、经皮贴剂和微囊化递送系统。这样的载体可以包括明胶,单硬脂酸甘油酯,二硬脂酸甘油酯,生物可降解的、生物相容的聚合物,例如乙烯醋酸乙烯酯、聚酸酐、聚乙醇酸、胶原蛋白、聚原酸酯和聚乳酸,其单独地或与蜡一起,或本领域公知的其他物质。用于制备这样的制剂的方法通常是本领域技术人员已知的。参见例如,SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS,J.R.Robinson,ed.,Marcel Dekker,Inc.,New York,1978。

[0514] 在一个实施方案中,本发明的抗体可配制成确保在体内适当分布。用于肠胃外施用的可药用载体包括无菌水溶液或分散体以及用于临时制备无菌可注射溶液或分散体的无菌粉末。这样的介质和试剂用于药物活性物质的用途在本领域中是已知的。

[0515] 注射用药物组合物通常必须是无菌的并且在制备和储存条件下是稳定的。组合物可以配制成溶液剂、微乳剂、脂质体或适合实现高药物浓度的其他有序结构。载体可以是水性或非水性溶剂或分散介质,其含有例如水、乙醇、多元醇(例如甘油、丙二醇、聚乙二醇等)及其合适的混合物、植物油(例如橄榄油)和可注射的有机酯,例如油酸乙酯。恰当的流动性可以例如通过使用包衣(例如卵磷脂)来维持,在分散体的情况下通过维持所需的颗粒尺寸来维持,以及通过使用表面活性剂来维持。在许多情况下,将优选在所述组合物中包含等张剂,例如糖、多元醇(例如甘油、甘露糖醇、山梨糖醇)或氯化钠。通过在组合物中包含延迟吸收的药剂,例如单硬脂酸盐和明胶,可实现可注射组合物的延长吸收。无菌可注射溶液可通过将所需量的活性化合物与例如上文列举的成分之一或组合(根据需要)一起并入合适的溶剂中,随后灭菌微滤来制备。通常,分散体通过将活性化合物并入到含有基本分散介质和所需其他成分的无菌载剂中来制备。在用于制备无菌可注射溶液的无菌粉末的情况下,制备方法的实例是真空干燥和冷冻干燥(冻干),其产生活性成分加上来自其先前无菌过滤溶液的任何另外的所期望成分的粉末。

[0516] 对于肠胃外施用,本发明的药剂通常与药用载体一起配制成物质在生理上可接受的稀释剂中的可注射剂量的溶液剂或混悬剂,所述药用载体可以是无菌液体,例如水、油、盐水、甘油、或乙醇。另外,组合物中可以存在辅助物质,例如润湿剂或乳化剂、表面活性剂、pH缓冲物质等。药物组合物的另一些组分是石油、动物、植物或合成来源的组分。花生油、大豆油和矿物油都是可用物质的实例。一般而言,二醇类(例如丙二醇或聚乙二醇)是优选的液体载体,特别是对于可注射溶液。本发明的药剂可以以储库注射剂或植入制剂的形式施用,其可以以允许活性成分持续释放的这样的方式配制。

[0517] 通常,组合物制备为可注射剂,作为液体溶液剂或混悬剂;也可以制备适合于在注射之前溶解在或悬浮在液体载剂中的固体形式。制剂也可以乳化或包封在脂质体或微粒中,例如聚丙交酯、聚乙交酯或共聚物,以增强辅料效果(Langer,et al.,Science 249:1527(1990);Hanes,et al.,Advanced Drug Delivery Reviews 28:97-119(1997),其在此通过引用整体并入)。适合于另一些施用方式的另外制剂包括经口、鼻内和肺部制剂、栓剂和经皮应用。

[0518] 在一个实施方案中,组合物包含含有以下的抗MuSK抗体(或抗原结合片段):包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2、和SEQ ID NO:156的CDR-H3的重链可变结构域,和包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3的轻链可变结构域(3B2g2m1)。

[0519] 在一个实施方案中,组合物包含含有以下的抗MuSK抗体(或抗原结合片段):包含SEQ ID NO:147的CDR-H1、SEQ ID NO:153的CDR-H2、和SEQ ID NO:156的CDR-H3的重链可变结构域,和包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2g1m1)。

[0520] 在一个实施方案中,抗MuSK抗体(或抗原结合片段)含有:包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2、和SEQ ID NO:156的CDR-H3的重链可变结构域,和包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2g1m2)。

[0521] 在一个实施方案中,抗MuSK抗体(或抗原结合片段)含有:包含SEQ ID NO:147的CDR-H1、SEQ ID NO:154的CDR-H2、和SEQ ID NO:156的CDR-H3的重链可变结构域,和包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:195的CDR-L3的轻链可变结构域(3B2g2m2)。

[0522] 在一个实施方案中,抗MuSK抗体(或抗原结合片段)含有:包含SEQ ID NO:147的CDR-H1、SEQ ID NO:150的CDR-H2、和SEQ ID NO:156的CDR-H3的重链可变结构域,和包含SEQ ID NO:159的CDR-L1、SEQ ID NO:172的CDR-L2和SEQ ID NO:183的CDR-L3的轻链可变结构域(3B2)。

[0523] 在一个优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0524] 其中重链可变结构域含有:

[0525] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0526] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0527] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0528] 其中轻链可变结构域含有:

[0529] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的

CDR-L1氨基酸序列,

[0530] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0531] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0532] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0533] 在一个更优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0534] 其中重链可变结构域含有:

[0535] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0536] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0537] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0538] 其中轻链可变结构域含有:

[0539] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0540] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0541] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0542] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0543] 在一个优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0544] 其中重链可变结构域含有:

[0545] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0546] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0547] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0548] 其中轻链可变结构域含有:

[0549] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0550] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的

CDR-L2氨基酸序列,和

[0551] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0552] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0553] 在一个更优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0554] 其中重链可变结构域含有:

[0555] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0556] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0557] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0558] 其中轻链可变结构域含有:

[0559] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0560] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0561] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0562] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0563] 在一个优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0564] 其中重链可变结构域含有:

[0565] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0566] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0567] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0568] 其中轻链可变结构域含有:

[0569] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0570] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0571] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0572] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0573] 在一个更优选的实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0574] 其中重链可变结构域含有:

[0575] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0576] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0577] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0578] 其中轻链可变结构域含有:

[0579] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0580] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0581] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0582] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0583] 在一个实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其含有:

[0584] a)包含与SEQ ID NO:268具有至少80%同一性或相似性的氨基酸序列的全长重链和

[0585] b)包含与SEQ ID NO:269具有至少80%同一性或相似性的氨基酸序列的全长轻链,并且

[0586] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变

是L234A或L235A,更优选地突变是L234A和L235A。

[0587] 在一个实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含:

[0588] a)全长重链,其包含SEQ ID NO:268,和

[0589] b)全长轻链,其包含SEQ ID NO:269,并且

[0590] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0591] 在一个实施方案中,与在其人IgG恒定Fc区中不具有任何氨基酸替换的抗体与效应物配体的结合相比,与相同配体的结合降低至少10%、20%、30%、40%、50%、60%、70%、80%、90%,或不再可检测。

[0592] 在一个实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含:

[0593] a)全长重链,其包含与SEQ ID NO:270具有至少80%同一性或相似性的氨基酸序列,和

[0594] b)全长轻链,其包含与SEQ ID NO:271具有至少80%同一性或相似性的氨基酸序列,并且

[0595] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0596] 在一个实施方案中,组合物包含与人肌肉特异性酪氨酸蛋白激酶(MuSK)结合的抗体或抗原结合片段,其包含:

[0597] a)全长重链,其包含SEQ ID NO:270,和

[0598] b)全长轻链,其包含SEQ ID NO:271,并且

[0599] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0600] 在一个实施方案中,与在其人IgG恒定Fc区中不具有任何氨基酸替换的抗体与效应物配体的结合相比,与相同配体的结合降低至少10%、20%、30%、40%、50%、60%、70%、80%、90%,或不再可检测。

[0601] 本发明的基于MuSK抗体的分子可以通过肠胃外、表面、经口或鼻内方式施用以用于治疗性治疗。肌内注射(例如,注射到臂或腿肌肉中)和静脉内输注是本发明分子的优选施用方法。在一些方法中,这样的分子作为缓慢释放组合物或装置施用,例如Medipad™装置(Elan Pharm.Technologies,Dublin,Ireland)。在一些方法中,本文中公开的抗体直接注射到特定组织中,例如颅内注射。

[0602] 在一个实施方案中,本发明的药物组合物是肠胃外施用的。本文中使用的短语“肠

胃外施用”及其变形表示除肠内和表面施用以外的施用方式,通常通过注射,并且包括经表皮、静脉内、肌内、动脉内、鞘内、囊内、颅内、眶内、心内、皮内、腹膜内、腱内、经气管、皮下、表皮下、关节内、囊下、蛛网膜下、脊柱内、颅内、胸内、硬膜外和胸骨内注射、皮下和输注。在一个实施方案中,药物组合物通过静脉内或皮下注射或输注施用。

[0603] 在一个实施方案中,根据本发明应用的抗MuSK抗体或其抗原结合片段(或者多核苷酸、表达载体、宿主细胞或者组合物)与抗胆碱能化合物组合施用。抗胆碱能化合物是能够抑制神经递质乙酰胆碱在突触或神经效应器接头(例如神经肌肉接头)处的作用的化合物。优选地,抗胆碱能化合物是能够抑制毒蕈碱乙酰胆碱受体活性的化合物。

[0604] 抗胆碱能化合物也可配制在作为抗MuSK抗体或抗原结合片段的组合物中。本文中所公开的抗MuSK抗体或抗原结合片段的组合物类型也可用于包含抗胆碱能化合物的组合物。这两种化合物可存在于单个组合物中。或者,它们可以配制在单独的组合物中。

[0605] 导致激活、诱导促进NMJ(神经肌肉接头)稳定性和/或修复的机制的化合物的使用对于任何神经肌肉疾病的治疗都是有吸引力的,尤其是在这样的NMJ受到影响的情况下。在一个优选的实施方案中,使用两种不同的化合物,每种化合物都激活、诱导促进NMJ稳定性和/或修复的机制,甚至是更有吸引力的,因为已证明这样的组合治疗是协同的。因此,所述组合对于神经肌肉疾病的治疗是高度有益的,特别是对于具有受影响NMJ的神经肌肉疾病或病症,例如ALS。

[0606] 在一个实施方案中,根据前述权利要求中任一项所述应用的抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞或者组合物,其中神经肌肉病症的特征在于受损的神经肌肉传递和/或NMJ去神经。

[0607] 受损的神经肌肉传递的特征在于以下中的至少一种:

[0608] a.毒蕈碱过度兴奋,

[0609] b.运动神经元死亡,

[0610] c.NMJ去神经以及

[0611] d.突触传递受损。

[0612] 在一个实施方案中,受损的神经肌肉传递或受损的突触传递的特征可在于MuSK信号传导不足、MuSK二聚化不足、MuSK磷酸化不足、MuSK信号传导不足和/或乙酰胆碱受体聚集不足。

[0613] 在一个实施方案中,受损的神经肌肉传递或受损的突触传递的特征可在于运动表现较差、握力降低、NMJ处肌肉的收缩特性较差、对肌肉疲劳的抗性较差、肌肉重量降低。

[0614] 在一个实施方案中,通过电生理学评估;药效学评估;在血清、血浆和/或脑脊液(cerebrospinal fluid,CSF)中的神经丝(例如神经丝轻链(neurofilament light chain,NFL))的水平;或NMJ活检来分析、评估或诊断神经肌肉病症。

[0615] 神经肌肉病症可选自以下:肌萎缩侧索硬化(amyotrophic lateral sclerosis,ALS)、脊髓性肌萎缩(spinal muscular atrophy,SMA)、重症肌无力(myasthenia gravis,MG)、先天性肌无力、兰伯特-伊顿肌无力综合征(Lambert-Eaton myasthenic syndrome,LEMS)、莱姆病、脊髓灰质炎、脊髓灰质炎后(post-poliomyelitis)、重金属中毒、肯尼迪综合征(Kennedy syndrome)、成年发作的泰-萨克斯病(adult-onset Tay-Sachs disease)、遗传性痉挛性截瘫、多灶性神经病、颈椎病、髓外肿瘤伴压迫性神经根病和脊髓病、包涵体

肌炎、进行性延髓麻痹、进行性肌萎缩、运动神经元综合征和甲状腺毒性肌病。优选的神经肌肉病症是ALS。

[0616] 在一个实施方案中,本文中所定义的抗MuSK抗体或其抗原结合片段可施用于无症状ALS对象。这意味着这样的抗体或抗原结合片段可在所述对象中的ALS发作之前施用。这同样适用于其他神经肌肉病症。

[0617] 在本上下文中,无症状ALS对象可能是已被诊断为易发生神经肌肉病症或疾病(例如ALS)的对象。鉴定患有神经肌肉病症的个体(或对象)可意指使用诊断方法进行鉴定。这样的对象可能是在疾病发作时或疾病发作之后被诊断的有症状的对象,或者易发生神经肌肉病症或疾病(即,在疾病发作之前诊断的无症状对象,其与疾病发作前同义)。

[0618] 神经肌肉病症可由遗传缺陷引起。遗传缺陷全部或部分是由基因组DNA序列相对于没有患有所述遗传缺陷的相应个体或对象的基因组DNA序列的变化引起的。遗传缺陷可以是由一个基因中的突变(单基因障碍)、多个基因中的突变(多因素遗传病症)、基因突变和环境因素的组合或染色体损伤(整个染色体的数目或结构、携带基因的结构中的变化)引起的。基因突变的类型包括碱基替换、缺失和插入。

[0619] 在一个实施方案中,人对象被鉴定为患有(或为易于发生)由遗传缺陷引起的神经肌肉疾病。在一个实施方案中,神经肌肉疾病是ALS,并且遗传缺陷在SOD1基因中。易发生ALS的个体或人对象包括那些具有一种或更多种发生ALS风险因素(包括年龄增长、具有个人或家族病史,或者一种或更多种SOD-1相关疾病的遗传倾向)的个体或人对象。ALS的一个潜在遗传原因或倾向是人SOD1基因中的突变。因此,可以通过使用本领域已知的测定(例如基因测序)对对象的SOD1基因进行基因测试来鉴定患有ALS、易患ALS或易于发生ALS的对象。本领域中已知的人SOD1中至少180个突变与ALS有关。在一个实施方案中,SOD1突变是选白以下的一种或更多种突变:

[0620] A4V,H46R,G93S,A4T,G141X,D133A,V148G,N139K,G85R,G93A,V14G,C6S,I113T,D49K,G37R,

[0621] A89V,E100G,D90A,T137A,E100K,G41A,G41D,G41S,G13R,G72S,L8V,F20C,Q22L,H48R,T54R,

[0622] S591,V87A,T88δTAD,A89T,V97M,S105δSL,VI 18L,D124G,LI 14F,D90A,G12R G147R和G37R

[0623] 在一个实施方案中,SOD1基因中的突变是G37R。

[0624] 因此,当所述对象具有选白以下的一个SOD1突变时,可(在疾病发作之前)鉴定无症状个体或对象:

[0625] A4V,H46R,G93S,A4T,G141X,D133A,V148G,N139K,G85R,G93A,V14G,C6S,I 113T,D49K,G37R,A89V,E100G,D90A,T137A,E100K,G41A,G41D,G41S,G13R,G72S,L8V,F20C,Q22L,H48R,T54R,S591,V87A,T88δTAD,A89T,V97M,S105δSL,VI 18L,D124G,LI 14F,D90A,G12R G147R和G37R。

[0626] 对象对ALS疾病易感性的分析(即易发生ALS的无症状对象)可通过分析对象的ALS的家族病史来进行。家族病史分析可包括记录ALS的三代系谱、家庭成员的病历和尸检报告的回顾、以及SOD1突变的常染色体显性模式的鉴定。

[0627] 对ALS无症状(但易于发生这样的疾病)的个体或对象的鉴定也可通过ALS标志物

进行分析。例如,ALS特异性标记物可以是循环微RNA、环状RNA(circRNA)或信使RNA(mRNA)、TDP-42聚集体、8-氧代-脱氧鸟苷(8-oxodG)、15-F2t-异前列烷(IsoP)、血浆TNF-a、IL-10、TRAIL、血浆IL-1b、CSF TRAIL、促炎性T辅助(Th)17细胞、Th1细胞、抗炎性Th2、调节性T细胞(Treg)、促炎性IL-1b、IL-6、IFN-g、抗炎性IL-10、胆固醇、LDL胆固醇、载脂蛋白B、HDL胆固醇、载脂蛋白AI、血浆肌酐(PCr)、血浆铁蛋白、转铁蛋白、铁调素、壳三糖苷酶-1(CHIT1)、类几丁质酶-3样蛋白2(CHI3L2/YKL39)、总τ(tTu)、磷酸化τ(pTau)、淀粉样蛋白b(Ab)、新型INHAT阻遏物(novel INHAT repressor,NIR)、泛素C端水解酶L1(UCHL1)、微管相关蛋白2、加帽肌动蛋白、类凝溶胶蛋白(CAPG)或糖蛋白非转移性黑素瘤蛋白B(glycoprotein nonmetastatic melanoma protein B,GPNMB)。当这样的标志物测量中的至少一个与相同年龄但未患ALS的正常人对象偏离至少10%、20%、30%、40%、50%、60%、70%、80%、90%时,人对象可被认为易患ALS疾病。对象对ALS疾病易感性的分析也可以通过成像进行分析。例如,这样的成像分析可以是骨骼肌的MRI评估、成像衍生的功能性肌肉评分、或者与MRI组合或不组合的舌超声预测的延髓进展。

[0628] 在一个实施方案中,抗胆碱能化合物与抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞、无细胞表达系统或者组合物分别、顺序或同时施用。

[0629] 在一个实施方案中,抗胆碱能化合物在疾病发作时或在疾病发作之后1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21天内或1、2、3、4、5、6、7周内;或1、2、3、4、5、6、7、8、9、10、11或12个月内施用。在一个实施方案中,抗胆碱能化合物在疾病发作时或在疾病发作之后一周内施用。出乎意料地,当在疾病发作时或在疾病发作之后尽可能快地施用抗胆碱能化合物时,获得了有吸引力的结果。本领域技术人员应当理解,抗胆碱能化合物优选地不用于减少、减轻与神经肌肉病症(例如ALS)相关的症状。在一个实施方案中,抗胆碱能化合物不用于减少、减轻尿急。在一个实施方案中,抗胆碱能化合物不用于减少或减轻神经肌肉疾病(例如ALS)中的尿急。

[0630] 在一个实施方案中,抗胆碱能化合物在疾病发作时施用,或在在疾病发作之后1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20或21天内;1、2、3、4、5、6或7周内;或1、2、3、4、5、6、7、8、9、10、11或12个月内,但在疾病诊断之前施用。在一些优选的实施方案中,施用在诊断与在诊断之前1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20或21天;1、2、3、4、5、6或7周内;或者1、2、3、4、5、6、7、8、9、10、11或12个月内之间。

[0631] 在一个实施方案中,在疾病发作之前或在疾病发作时施用抗MuSK抗体或其抗原结合片段、多核苷酸、表达载体、宿主细胞或者组合物。疾病发作之前可意指疾病发作之前1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21天,或疾病发作之前1、2,3、4、5、6,7、8周,或疾病发作之前1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20个月。在本上下文中,发作之前的人对象可意指没有针对所述神经肌肉病症(例如ALS)的症状的人对象。

[0632] 因此,在本发明的另一个方面中,提供了用于在人对象中治疗ALS的抗MuSK抗体或其抗原结合片段(多核苷酸、表达载体、宿主细胞、无细胞表达系统或组合物),其中所述抗体或抗原结合片段在疾病发作之前施用,优选地在疾病发作之前1、2、3、4、5或6个月内施用。在本上下文中,在发作之前的人对象可意指没有针对ALS的症状的人对象。在一个实施方案中,对象被诊断为易于发生神经肌肉病症或疾病,例如ALS。

[0633] 在一个实施方案中,抗体或抗原结合片段结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列。

[0634] 在一个实施方案中,抗体或其抗原结合片段包含野生型人IgG恒定Fc区,其与SEQ ID NO:266或SEQ ID NO:267包含至少80%序列同一性。

[0635] 在一个实施方案中,抗体或抗原结合片段为激动剂MuSK抗体并且/或者效应物功能已被降低或消除。

[0636] 在一个实施方案中,效应物功能的降低或消除是通过将以下突变(均根据EU编号系统编号)的一个或更多个引入本文中所述的基于抗体的分子的人IgG恒定Fc区SEQ ID NO:266或SEQ ID NO:267中获得的:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换或P396L替换。

[0637] 在一个优选的实施方案中,L234A或L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。在一个更优选的实施方案中,L234A和L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。该实施方案产生具有由SEQ ID NO:268或SEQ ID NO:270表示的重链的基于抗体的分子。

[0638] 在一个实施方案中,抗体或抗原结合片段包含:

[0639] a)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列以及

[0640] b)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

[0641] 在一个实施方案中,抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

[0642] 其中所述VH含有:

[0643] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0644] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0645] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0646] 其中所述VL含有:

[0647] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0648] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0649] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0650] 在一个实施方案中,抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

[0651] -其中所述VH包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,并且所述VL包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,以及

[0652] -其中所述VH含有:

[0653] ○包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0654] ○包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0655] ○包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且-其中所述VL含有:

[0656] ○包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0657] ○包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0658] ○包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0659] 在一个实施方案中,抗体或抗原结合片段包含:

[0660] a)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,和

[0661] b)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

[0662] 在一个实施方案中,抗体或抗原结合片段包含:

[0663] a)全长重链,其包含SEQ ID NO:268,和

[0664] b)全长轻链,其包含SEQ ID NO:269,并且

[0665] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0666] 出乎意料地,当在疾病发作之前施用抗MuSK抗体(宿主细胞的表达载体或多核苷酸或抗原结合片段)时,获得了有吸引力的结果。在一个实施方案中,人对象因此没有针对所述神经肌肉病症(例如ALS)的症状。在这样的实施方案中,基于他/她的家族病史、遗传背景,或者基于如在他/她的血清或脑脊液(CSF)中确定的神经丝(例如神经丝轻链(NFL))的提高的水平,或者基于ALS相关基因突变的阳性基因测试,或者基于ALS的生物标志物水平的变化,或者其组合,人对象已被诊断为易于发生神经肌肉病症(例如ALS)。然而,他/她还没有发生任何明显的症状;他/她是无症状的。在一个优选的实施方案中,在被诊断出患有遗传缺陷或与ALS相关遗传突变但尚未发生任何可见症状(即无症状对象)之后,尽可能早地向人对象施用。在一个更优选的实施方案中,在被诊断出患有遗传缺陷或与ALS相关遗传突变但尚未发生任何可见症状之后(并且所述人对象具有ALS家族病史),尽可能早地向人对象施用。在本上下文中,“立即”可意指在1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、

17、18、19、20、21、22、23、24小时内,或者在1、2、3、4、5、6或7天内,或者1、2,3或4周内。在本上下文中,这样的治疗在ALS诊断没有证实的情况下最终显示出有限的或没有毒性和/或副作用,或者任何有害影响。

[0667] 在本发明的另一个方面中,提供了包含本文中所述的抗MuSK抗体或其抗原结合片段和抗胆碱能化合物的组合。所述组合优选地用于在人对象中治疗神经肌肉疾病。

[0668] 所述神经肌肉病症的特征在于神经肌肉传递受损和/或NMJ(神经肌肉接头)处的去神经。神经肌肉病症的特征在于以下的至少一种:

[0669] a.毒蕈碱过度兴奋,

[0670] b.运动神经元死亡,

[0671] c.神经肌肉接头(NMJ)去神经以及

[0672] d.突触传递受损。

[0673] 在一个实施方案中,神经肌肉病症选自以下:肌萎缩侧索硬化(ALS)、脊髓性肌萎缩(SMA)、重症肌无力(MG)、先天性肌无力、兰伯特-伊顿肌无力综合征(LEMS)、莱姆病、脊髓灰质炎、脊髓灰质炎后、重金属中毒、肯尼迪综合征、成年发作的泰-萨克斯病、遗传性痉挛性截瘫、多灶性神经病、颈椎病、髓外肿瘤伴压迫性神经根病和脊髓病、包涵体肌炎、进行性延髓麻痹、进行性肌萎缩、运动神经元综合征和甲状腺毒性肌病。在优选的实施方案中,神经肌肉疾病是ALS。

[0674] 在本上下文中,组合不需要物理上一起存在于一种组合物中的本文中所述的抗MuSK抗体或其抗原结合片段和抗胆碱能化合物。

[0675] 在一个实施方案中,抗胆碱能化合物单独、顺序或同时施用。在一个实施方案中,抗体或抗原结合片段在疾病发作之前施用,或者在疾病发作之前1、2、3、4、5或6个月内施用。在一个实施方案中,所述抗体或抗原结合片段在疾病发作之前施用,或者优选地在疾病发作之前1、2、3、4、5或6个月内施用,并且/或者其中抗胆碱能化合物在疾病发作时或者在疾病发作之后1、2、3、4、5、6或7周内施用。在本上下文中,在发作之前的人对象可意指没有针对所述神经肌肉病症的症状的人对象。在一个实施方案中,用抗体治疗的对象已首先被诊断为易于发生神经肌肉病症或疾病。

[0676] 在一个实施方案中,抗MuSK抗体或其抗原结合片段结合SEQ ID NO:129的MuSK卷曲(Fz)样结构域序列。

[0677] 在一个实施方案中,抗体或其抗原结合片段包含野生型人IgG恒定Fc区,其与SEQ ID NO:266或SEQ ID NO:267包含至少80%序列同一性。

[0678] 在一个实施方案中,抗体或抗原结合片段为激动剂MuSK抗体并且/或者效应物功能已被降低或消除。

[0679] 在一个实施方案中,效应物功能的降低或消除是通过将以下突变(均根据EU编号系统编号)的一个或更多个引入本文中所述的基于抗体的分子的人IgG恒定Fc区SEQ ID NO:266或SEQ ID NO:267中获得的:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替

换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换或P396L替换。

[0680] 在一个优选的实施方案中,L234A或L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。在一个更优选的实施方案中,L234A和L235A替换被引入到本文中所述的基于抗体的分子的人IgG恒定Fc区中。该实施方案产生具有由SEQ ID NO:268或SEQ ID NO:270表示的重链的基于抗体的分子。

[0681] 在一个实施方案中,抗体或抗原结合片段包含:

[0682] a)重链可变结构域(VH),其包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,和

[0683] b)轻链可变结构域(VL),其包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列。

[0684] 在一个实施方案中,抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

[0685] 其中所述VH含有:

[0686] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0687] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0688] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0689] 其中所述VL包含:

[0690] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0691] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0692] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0693] 在一个实施方案中,抗体或抗原结合片段包含重链可变结构域(VH)和轻链可变结构域(VL):

[0694] -其中所述VH包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列,并且所述VL包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,以及

[0695] -其中所述VH含有:

[0696] ○包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0697] ○包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0698] ○包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且-其中所述VL包含:

[0699] ○包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0700] ○包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0701] ○包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0702] 在一个实施方案中,抗体或抗原结合片段包含:

[0703] a)全长重链,其包含SEQ ID NO:268,和

[0704] b)全长轻链,其包含SEQ ID NO:269,并且

[0705] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0706] 在一个实施方案中,抗体或抗原结合片段包含:

[0707] a)全长重链,其包含SEQ ID NO:270,和

[0708] b)全长轻链,其包含SEQ ID NO:271,并且

[0709] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0710] 在一个实施方案中,抗MuSK抗体或其抗原片段(多核苷酸、表达载体、宿主细胞、无细胞表达系统或组合物)在疾病发作之前(例如疾病发作之前1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20或21天或者疾病发作之前1、2、3、4、5、6或7周)施用,并且抗胆碱能化合物在疾病发作时(例如在疾病发作之后1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20或21天内或者1、2、3、4、5、6或7周内)施用。在本上下文中,在发作之前的人对象可意指没有针对所述神经肌肉病症(例如ALS)的症状的人对象。

[0711] 在一个优选的实施方案中,疾病发作包括至少一种选自以下的症状:肌肉颤搐、肌肉痉挛、痉挛、肌无力、口齿不清和/或鼻音、咀嚼困难或吞咽困难(difficulty chewing or swallowing)、吞咽困难(dysphagia)、构音障碍和呼吸困难。在一个更优选的实施方案中,疾病是ALS并且疾病发作包括至少一种选自以下的症状:肌肉颤搐、肌肉痉挛、痉挛、肌无力、口齿不清和/或鼻音、咀嚼困难或吞咽困难、吞咽困难、构音障碍和呼吸困难。

[0712] 疾病发作可由医师或兽医进行评估。在一个实施方案中,重量减轻的开始被认为是疾病发作。

[0713] 在一个优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0714] 其中重链可变结构域含有:

[0715] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0716] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0717] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0718] 其中轻链可变结构域含有:

[0719] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0720] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0721] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0722] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0723] 在一个更优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0724] 其中重链可变结构域含有:

[0725] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0726] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0727] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0728] 其中轻链可变结构域含有:

[0729] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0730] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0731] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0732] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0733] 在一个优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0734] 其中重链可变结构域含有:

[0735] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0736] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0737] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0738] 其中轻链可变结构域含有:

[0739] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0740] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的

CDR-L2氨基酸序列,和

[0741] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0742] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0743] 在一个更优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0744] 其中重链可变结构域含有:

[0745] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0746] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0747] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0748] 其中轻链可变结构域含有:

[0749] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0750] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,以及

[0751] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0752] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0753] 在一个优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0754] 其中重链可变结构域含有:

[0755] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0756] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0757] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0758] 其中轻链可变结构域含有:

[0759] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0760] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0761] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0762] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0763] 在一个更优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0764] 其中重链可变结构域含有:

[0765] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0766] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0767] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0768] 其中轻链可变结构域含有:

[0769] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0770] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0771] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0772] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0773] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0774] a)全长重链,其包含与SEQ ID NO:268具有至少80％同一性或相似性的氨基酸序列,和

[0775] b)全长轻链,其包含与SEQ ID NO:269具有至少80％同一性或相似性的氨基酸序列,并且

[0776] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0777] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0778] a)全长重链,其包含SEQ ID NO:268,和

[0779] b)全长轻链,其包含SEQ ID NO:269,并且

[0780] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0781] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0782] a)全长重链,其包含与SEQ ID NO:270具有至少80%同一性或相似性的氨基酸序列,和

[0783] b)全长轻链,其包含与SEQ ID NO:271具有至少80%同一性或相似性的氨基酸序列,并且

[0784] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0785] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0786] a)全长重链,其包含SEQ ID NO:270,和

[0787] b)全长轻链,其包含SEQ ID NO:271,并且

[0788] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0789] 在一些实施方案中,抗胆碱能化合物是毒蕈碱受体拮抗剂。毒蕈碱受体也称为毒蕈碱乙酰胆碱受体或mAchR,是在某些神经元和其他细胞的细胞膜中形成G蛋白受体复合物的乙酰胆碱受体。毒蕈碱受体在介导神经递质乙酰胆碱的作用中发挥数种作用。例如,毒蕈碱受体包含在神经肌肉接头的体细胞神经元的突触前膜中,在那里它们参与乙酰胆碱释放的调节。

[0790] 毒蕈碱受体的五种亚型M1至M5是普遍认可的。这种分类源于它们对某些激动剂和拮抗剂的不同选择性。M1、M3和M5受体与细胞膜中的Gq蛋白偶联,而M2和M4受体与细胞膜中的Gi/o蛋白偶联。不受该理论的约束,基因CHRM1-5分别编码M1至M5受体。

[0791] 毒蕈碱受体的基础活性或组成型活性定义为在不存在乙酰胆碱、毒蕈碱受体激动剂和毒蕈碱受体拮抗剂的情况下受体的物理、生物和/或化学活性。

[0792] 毒蕈碱受体的激动剂(agonist of a muscarinic receptor),也称为毒蕈碱受体激动剂(muscarinic receptor agonist),定义为当其接触受体时提高受体的物理、生物和/或化学活性的化合物。提高的活性意指与将受体与乙酰胆碱接触所引起的活性相似的活性。

[0793] 毒蕈碱受体的拮抗剂(antagonist of a muscarinic receptor),也称为毒蕈碱受体拮抗剂(muscarinic receptor antagonist),定义为毒蕈碱受体中性拮抗剂或毒蕈碱负性拮抗剂。

[0794] 毒蕈碱受体中性拮抗剂是这样的化合物,所述化合物与毒蕈碱受体中性激动剂或与毒蕈碱受体负性拮抗剂竞争与受体结合,从而阻断激动剂或负性拮抗剂的作用(即提高

或降低活性),而中性拮抗剂单独结合时不显著改变受体的基础活性。

[0795] 在一些实施方案中,抗胆碱能化合物是毒蕈碱受体中性拮抗剂。

[0796] 毒蕈碱受体负性拮抗剂是这样的化合物,当所述化合物接触受体时,即使在不存在毒蕈碱受体激动剂的情况下,也会降低受体的物理、生物和/或化学活性。降低的活性意指与将受体与乙酰胆碱接触所引起的活性相反的活性。

[0797] 在一些实施方案中,抗胆碱能化合物是毒蕈碱受体负性拮抗剂。

[0798] 如果拮抗剂(阻断激动剂、阻断负性拮抗剂(negative antagonist)或降低活性)的作用仅在接触一种或更多种亚型的毒蕈碱受体时显著,而在接触另一种亚型的毒蕈碱受体后作用显著较小或没有作用,则毒蕈碱受体拮抗剂被定义为对一种或更多种毒蕈碱受体亚型M1、M2、M3、M4和/或M5具有选择性。因此,毒蕈碱受体拮抗剂被称为是对毒蕈碱受体M3具有选择性,应当理解,当拮抗剂与亚型M1、M2、M4或M5的毒蕈碱受体接触时,获得显著更小的作用或没有作用。在本上下文中,显著更小可以是至多1/10、1/20、1/30、1/40、1/50、1/60、1/70、1/80、1/90、1/100、1/200、1/300、1/400、1/500、1/600、1/700、1/800、1/900、1/1000、1/10000、1/100000或1/1000000小,或至少小

[0799] 10%,20%,30%,40%,50%,60%,70%,80%,90%,100%,120%,140%,160%,180%,200%,220%,240%,260%,280%,300%,320%,340%,360%,380%,400%,420%,440%,460%,480%,500%,600%,700%,800%,900%,1000%,1500%,2000%,2500%,3000%,3500%,4000%,4500%,5000%,5500%,6000%,6500%,7000%,7500%,8000%,8500%,9000%,9500%,10000%,20000%,30000%,40000%,50000%,60000%,70000%,80000%,90000%,100000%,1000000%,10000000%或100000000%。

[0800] 毒蕈碱受体(优选为M1、M3和M5亚型)的活性可使用动态Ca2+成像进行测量。这些受体调节IP3的水平,其然后控制Ca2+从内部储存的释放[7]。

[0801] 在一些实施方案中,抗胆碱能化合物是毒蕈碱受体拮抗剂,其:

[0802] -对毒蕈碱受体M1具有选择性,或

[0803] -对毒蕈碱受体M3具有选择性,或

[0804] -对毒蕈碱受体M5具有选择性,或

[0805] -对毒蕈碱受体M1和毒蕈碱受体M3具有选择性,或

[0806] -对毒蕈碱受体M1和毒蕈碱受体M5具有选择性,或

[0807] -对毒蕈碱受体M3和毒蕈碱受体M5具有选择性,或

[0808] -对毒蕈碱受体M1、毒蕈碱受体M3和毒蕈碱受体M5具有选择性。

[0809] 在一些实施方案中,抗胆碱能化合物是毒蕈碱受体拮抗剂,其:

[0810] -对毒蕈碱受体M3具有选择性,或

[0811] -对毒蕈碱受体M1和毒蕈碱受体M3具有选择性,或

[0812] -对毒蕈碱受体M3和毒蕈碱受体M5具有选择性,或

[0813] -对毒蕈碱受体M1、毒蕈碱受体M3和毒蕈碱受体M5具有选择性。

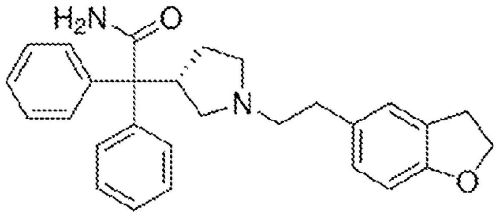
[0814] 在一些实施方案中,抗胆碱能化合物为达非那新(darifenacin)、异丙托溴铵(ipratropium bromide)、噻托溴铵(tiotropium bromide)、曲司氯铵(trospium)、格隆溴铵(glycopyrronium)、阿地溴铵(aclidinium)、芜美溴铵(umeclidinium)、索非那新(solifenacin)、双环胺(dicylomine)、弗斯特罗定(fesoterodine)、黄酮哌酯

(fiavoxate)、甘罗溴铵(glycopyrrolate)、普鲁本辛(propantheline)、1R,2R,4S,5S,7S)-7-[({4-氟-2-(噻吩-2-基)苯基}氨基甲酰基)氧基]-9,9-二甲基-3-氧杂-9-氮杂三环[3.3.1.02,4]壬烷-9-甲酸锎([38]中的BS46)、N-(2-[3-([3R]-1-(环己基甲基)-3-哌啶基]甲基氨基)-3-氧代丙基]氨基-2-氧代乙基)-3,3,3-三苯基-丙酰胺([39]中的J-115311),在三苯基丙酸部分和哌啶基甲胺部分之间具有一个或两个氨基酸残基的3,3,3-三苯基丙酰胺衍生物([40]),OrM3([41])或(3R)-3-[[[(3-氟苯基)[(3,4,5-三氟苯基)甲基]氨基]羰基]氧基]-1-[2-氧代-2-(2-噻吩基)乙基]-1-氮阳离子二环[2.2.2]辛烷溴化物([41]中的CHF 5407)。不受该理论的约束,这些化合物可被认为是毒蕈碱受体拮抗剂。

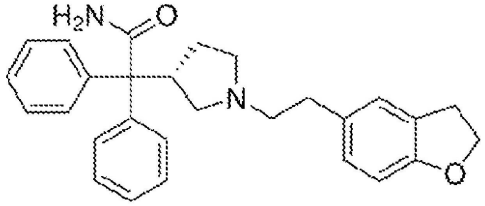
[0815] 在一些实施方案中,抗胆碱能化合物为达非那新、异丙托溴铵、噻托溴铵、曲司氯铵、格隆溴铵、阿地溴铵、芜美溴铵、索非那新、双环胺、弗斯特罗定、黄酮哌酯、甘罗溴铵或普鲁本辛。不受该理论的约束,达非那新、异丙托溴铵和噻托溴铵可被认为是毒蕈碱受体拮抗剂。

[0816] 在一些实施方案中,抗胆碱能化合物为达非那新、异丙托溴铵、噻托溴铵或曲司氯铵。不受该理论的约束,达非那新、异丙托溴铵和噻托溴铵可被认为是毒蕈碱受体拮抗剂。

[0817] 在一个优选的实施方案中,抗胆碱能化合物是达非那新。达非那新可由以下结构表示:

[0818] 

[0819] 优选地,达非那新是氢溴酸达非那星。氢溴酸达非那新可由以下结构表示:

[0820] 

[0821] 在一些实施方案中,上述实施方案中所公开的任何抗胆碱能化合物可作为其可药用盐存在。特别地,抗胆碱能化合物是达非那新或其可药用盐。

[0822] 可药用盐的实例包括但不限于碱金属(例如钠、钾或锂)盐或碱土金属(例如钙)盐;然而,当施用于所治疗的对象时通常非毒性且有效的任何盐是可接受的。其他盐可包括但不限于:(1)酸加成盐,其可通过母体化合物的游离碱与无机酸(例如盐酸、氢溴酸、硝酸、磷酸、硫酸和高氯酸等)或有机酸(例如乙酸、草酸、(D)或(L)苹果酸、马来酸、氨基甲酸酯磺酸、乙磺酸、对甲苯磺酸、水杨酸、酒石酸、柠檬酸、琥珀酸或丙二酸等)反应获得;或者(2)当母体化合物中存在的酸性质子被金属离子(例如碱金属离子、碱土金属离子或铝离子)替代,或与有机碱(例如乙醇胺、二乙醇胺、三乙醇胺、三甲胺、N-甲基葡糖胺等)配位时形成的盐。可药用盐是本领域技术人员公知的,并且可考虑与本文中所述的实施方案结合的任何这样的可药用盐。

[0823] 可接受的盐可使用本领域已知的标准操作获得,包括(但不限于)使足够酸性的化合物与提供生理学上可接受的阴离子的合适的碱反应。合适的酸加成盐由形成非毒性盐的

酸形成。说明性但非限制性的实例包括乙酸盐、天冬氨酸盐、苯甲酸盐、苯磺酸盐、碳酸氢盐/碳酸盐、硫酸氢盐/硫酸盐、硼酸盐、樟脑磺酸盐、柠檬酸盐、乙二磺酸盐、乙磺酸盐、甲酸盐、富马酸盐、葡庚糖酸盐、葡糖酸盐、葡糖醛酸盐、六氟磷酸盐、海苯酸盐(hibenzate)、盐酸盐/氯化物、氢溴酸盐/溴化物、氢碘酸盐/碘化物、乳酸盐、苹果酸盐、马来酸盐、丙二酸盐、甲磺酸盐、甲基硫酸盐、萘酸盐、2-萘磺酸盐、烟酸盐、硝酸盐、乳清酸盐、草酸盐、棕榈酸盐、扑酸盐、磷酸盐/磷酸氢盐/磷酸二氢盐、蔗糖盐、硬脂酸盐、琥珀酸盐、酒石酸盐、甲苯磺酸盐和三氟乙酸盐。本文中所述化合物的合适碱盐由形成无毒盐的碱形成,说明性但非限制性的实例包括精氨酸、苄星(benzathine)、钙、胆碱、二乙胺、二乙醇胺、甘氨酸、赖氨酸、镁、葡甲胺、乙醇胺、钾、钠、氨丁三醇和锌盐。也可以形成酸和碱的半盐,例如半硫酸盐和半钙盐。

[0824] 上述实施方案中所公开的抗胆碱能化合物可作为组合物施用,优选地作为治疗组合物施用。在一些实施方案中,组合物被配制为一天一次的用于经口应用的延长释放片剂,所述延长释放片剂包含达非那新,优选地氢溴酸达非那新。优选地,组合物包含以下赋形剂的一种或更多种:无水磷酸氢钙、羟丙甲纤维素、硬脂酸镁、二氧化钛、氧化铁黄、氧化铁红、PEG 400和/或滑石。在一个实施方案中,组合物被称为ENABLEX™。ENABLEX™被配制为7.5mg或15mg达非那新(氢溴酸达非那新)。

[0825] 在一个优选的实施方案中,神经肌肉病症是ALS,抗MuSK抗体或抗原结合片段包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0826] 其中重链可变结构域含有:

[0827] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0828] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0829] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0830] 其中轻链可变结构域含有:

[0831] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0832] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0833] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)

[0834] 并且使用了抗胆碱能化合物。

[0835] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0836] 在一个更优选的实施方案中,神经肌肉病症是ALS,抗MuSK抗体或抗原结合片段包含重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至

少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0837] 其中重链可变结构域含有:

[0838] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0839] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0840] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0841] 其中轻链可变结构域含有:

[0842] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0843] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0844] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)

[0845] 并且使用了抗胆碱能化合物。

[0846] 在一个实施方案中,同一性或相似性为至少81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0847] 在一个优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0848] 其中重链可变结构域含有:

[0849] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0850] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0851] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0852] 其中轻链可变结构域含有:

[0853] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0854] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0855] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0856] 在一个更优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80％同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80％同一性或相似性的氨基酸序列,并且

[0857] 其中重链可变结构域含有:

[0858] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0859] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0860] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0861] 其中轻链可变结构域含有:

[0862] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0863] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,和

[0864] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0865] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0866] 在一个优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0867] 其中重链可变结构域含有:

[0868] -包含SEQ ID NO:147或者相对于SEQ ID NO:147具有1、2、3、4或5个氨基酸改变的CDR-H1氨基酸序列,

[0869] -包含SEQ ID NO:153或者相对于SEQ ID NO:153具有1、2、3、4或5个氨基酸改变的CDR-H2氨基酸序列,和

[0870] -包含SEQ ID NO:156或者相对于SEQ ID NO:156具有1、2、3、4或5个氨基酸改变的CDR-H3氨基酸序列(3B2g2m1)并且

[0871] 其中轻链可变结构域含有:

[0872] -包含SEQ ID NO:159或者相对于SEQ ID NO:159具有1、2、3、4或5个氨基酸改变的CDR-L1氨基酸序列,

[0873] -包含SEQ ID NO:172或者相对于SEQ ID NO:172具有1、2、3、4或5个氨基酸改变的CDR-L2氨基酸序列,和

[0874] -包含SEQ ID NO:195或者相对于SEQ ID NO:195具有1、2、3、4或5个氨基酸改变的CDR-L3氨基酸序列(3B2g2m1)。

[0875] 在一个更优选的实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含野生型人IgG恒定Fc区(其中根据EU编号系统编号的L234A和/或L235A替换被引入到所述Fc区中)、重链可变结构域和轻链可变结构域,其中野生型人IgG恒定Fc区包含SEQ ID NO:266或SEQ ID NO:267、重链可变结构域和轻链可变结构域,其中重链可变结构域包含与SEQ ID NO:234具有至少80%同一性或相似性的氨基酸序列并且轻链可变结构域包含与SEQ ID NO:235具有至少80%同一性或相似性的氨基酸序列,并且

[0876] 其中重链可变结构域含有:

[0877] -包含SEQ ID NO:147或由SEQ ID NO:147组成的CDR-H1氨基酸序列,

[0878] -包含SEQ ID NO:153或由SEQ ID NO:153组成的CDR-H2氨基酸序列,和

[0879] -包含SEQ ID NO:156或由SEQ ID NO:156组成的CDR-H3氨基酸序列(3B2g2m1)并且

[0880] 其中轻链可变结构域含有:

[0881] -包含SEQ ID NO:159或由SEQ ID NO:159组成的CDR-L1氨基酸序列,

[0882] -包含SEQ ID NO:172或由SEQ ID NO:172组成的CDR-L2氨基酸序列,以及

[0883] -包含SEQ ID NO:195或由SEQ ID NO:195组成的CDR-L3氨基酸序列(3B2g2m1)。

[0884] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0885] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0886] a)全长重链,其包含与SEQ ID NO:268具有至少80%同一性或相似性的氨基酸序列,和

[0887] b)全长轻链,其包含与SEQ ID NO:269具有至少80%同一性或相似性的氨基酸序列,并且

[0888] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0889] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0890] a)全长重链,其包含SEQ ID NO:268,和

[0891] b)全长轻链,其包含SEQ ID NO:269,并且

[0892] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0893] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0894] a)全长重链,其包含与SEQ ID NO:270具有至少80%同一性或相似性的氨基酸序列,和

[0895] b)全长轻链,其包含与SEQ ID NO:271具有至少80%同一性或相似性的氨基酸序列,并且

[0896] c)其中以下突变(均根据EU编号系统编号)的一个或更多个已被引入全长重链:N297A替换;N297Q替换;L234A替换;L234D替换;L234E替换;L234G替换;L234H替换;L234F替换;L234K替换;L234Q替换;L234R替换;L234S替换;L234T替换;L235A替换;L235D替换;L235E替换;L235F替换;L235G替换;L235V替换;L235H替换;L235I替换;L235K替换;L235R替换;L235S替换;L235T替换;L235Q替换;L237A替换;S239D替换;E233P替换;L234V替换;C236缺失;G236E替换;G236R替换;G236K替换;G237A替换;P238A替换;F243L替换;D265A替换;S267E替换;H268A替换;R292P替换;Y300L替换;K322A替换;K322Q替换;A327Q替换;L328F替

换;L328R替换;P329A替换;P329G替换;A330L替换;A330S替换;P331S替换;I332E替换;P396L替换;或者在本申请的第四实施方案中前面描述的突变的组合的每一者,优选地突变是L234A或L235A,更优选地突变是L234A和L235A。

[0897] 在一个实施方案中,同一性或相似性为至少81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%。

[0898] 在一个实施方案中,神经肌肉病症是ALS并且抗MuSK抗体或抗原结合片段包含:

[0899] a)全长重链,其包含SEQ ID NO:270,和

[0900] b)全长轻链,其包含SEQ ID NO:271,并且

[0901] c)其中所述全长重链包含根据EU编号系统编号的L234A和L235A突变。

[0902] 在一个人对象或患者已被诊断为患有神经肌肉病症(例如上文公开的那些之一)的实施方案中,任选地与抗胆碱能化合物组合的本发明的MuSK抗体以足以治愈、治疗或至少部分抑制疾病症状(例如通过生物化学、组织学和/或行为评估得出)(包括其并发症和疾病发展中的中间病理表型)的量施用于这样的患者。在一些实施方案中,施用本发明的治疗性分子减轻或消除神经肌肉病症。

[0903] 用于治疗上述病症的本发明所提供的治疗性分子(即抗MuSK抗体或其抗原结合片段和抗胆碱能化合物)的有效剂量可根据许多不同因素而变化,包括施用方式、靶部位、患者的生理状态、所施用的其他药物。通常滴定治疗剂量以优化其安全性和效力。在给予剂量的任何给定日期,本文中所述的基于MuSK抗体的分子的剂量可以为约0.0001mg/kg至约100mg/kg患者体重,并且更通常为约0.01mg/kg至约20mg/kg患者体重。例如,剂量可以是1mg/kg体重或10mg/kg体重或在1mg/kg至10mg/kg体重范围内。因此示例性剂量包括:约0.1mg/kg至约10mg/kg体重、约0.1mg/kg至约5mg/kg体重、约0.1mg/kg至约2mg/kg体重、约0.1mg/kg至约1mg/kg体重,例如约0.15mg/kg体重、约0.2mg/kg体重、约0.5mg/kg体重、约1mg/kg体重、约1.5mg/kg体重、约2mg/kg体重、约5mg/kg体重、或约10mg/kg体重。

[0904] 具有本领域普通技术的医师或兽医可以容易地确定和开出所需药物组合物的有效量。例如,医师或兽医可以以低于为达到所期望治疗作用所需水平的水平开始药物组合物中的基于抗体的分子的剂量,并逐渐提高剂量直至达到所期望效果。一般而言,本发明组合物的合适日剂量将是有效产生治疗作用的最低剂量的化合物的量。这样的有效剂量将通常取决于上述因素。施用可以例如是静脉内的、肌内的、腹膜内的或皮下的,并且例如靠近靶部位施用。如果期望的话,药物组合物的有效日剂量可以作为两个、三个、四个、五个、六个或更多个亚剂量在全天以适当的间隔分开施用来施用,任选地以单位剂型施用。虽然本发明的基于抗体的分子可以单独施用,但优选将基于抗体的分子作为药物组合物施用,如上所述。

[0905] 出于治疗目的,本发明的基于MuSK抗体的分子(以及任选的抗胆碱能化合物)通常在多个时刻施用。单次剂量(例如,推注或输注)之间的间隔可以是每周、每月或每年。在一些实施方案中,本发明的基于MuSK抗体的分子(以及任选的抗胆碱能化合物)在四个月的过程中向人对象施用至少1次、至少2次、至少3次、至少4次、至少5次、至少6次、至少7次、至少8次、至少9次、至少10次。

[0906] 在某些实施方案中,向人对象施用药物组合物的一个或更多个负荷剂量,随后施用一个或更多个维持剂量。在一些情况下,施用三个负荷剂量,其中负荷剂量间隔两周,例

如在第1天、第15天和第29天。在一些情况下,在第三次负荷剂量之后4周开始,每4周施用维持剂量(例如,持续1个月、2个月、三个月、四个月、五个月、六个月、七个月、八个月、九个月、十个月)。

[0907] 在某些实施方案中,向人对象施用三个负荷剂量的药物组合物,然后施用至少一个(例如,1、2、3、4、5、6、7、8、9、10、11、12)维持剂量。在一些情况下,三个负荷剂量间隔两周施用。在一些情况下,三个负荷剂量间隔14天施用。在一些情况下,从第三个负荷剂量之后4周开始,每4周施用一个/更多个维持剂量。在一些情况下,在第三个负荷剂量之后1个月开始,每个月施用一个/更多个维持剂量。在一些情况下,在第三个负荷剂量之后28天开始,每28天施用一个/更多个维持剂量。

[0908] 在一些方法中,调整剂量以达到1ng/ml至1000μg/mL的血浆浓度,优选1至1000μg/mL的血浆浓度,更优选25至300μg/mL的血浆浓度。或者,本发明的治疗分子可以作为缓慢释放制剂施用,在这种情况下需要较低频率的施用。剂量和频率取决于抗体在患者中的半衰期而变化。一般而言,人抗体显示出最长的半衰期,其次是人源化抗体、嵌合抗体和非人抗体。scFv分子通常具有短的血清半衰期。

[0909] 在另一个实施方案中,包含编码如本文中所述的基于MuSK抗体的分子(以及任选地与抗胆碱能化合物组合)的重组核酸序列的药物组合物施用于对象以促进基于抗体的分子的体内表达和形成以治疗由减少的信号传导和/或MuSK的磷酸化介导的病症。适用于本发明的该实施方案的表达载体构建体在上文进行了描述。

[0910] 多核苷酸组合物可使得在向对象施用组合物的至少约1小时、2小时、3小时、4小时、5小时、6小时、7小时、8小时、9小时、10小时、11小时、12小时、13小时、14小时、15小时、20小时、25小时、30小时、35小时、40小时、45小时、50小时、或60小时内在对象中产生基于MuSK抗体的分子。所述组合物可以使得在向对象施用组合物的至少约1天、2天、3天、4天、5天、6天、7天、8天、9天或10天之内在对象中产生基于抗体的分子。所述组合物可使得在向对象施用组合物的约1小时至约6天、约1小时至约5天、约1小时至约4天、约1小时至约3天、约1小时至约2天、约1小时至约1天、约1小时至约72小时、约1小时至约60小时、约1小时至约48小时、约1小时至约36小时、约1小时至约24小时、约1小时至约12小时或约1小时至约6小时内在对象中产生基于抗体的分子。

[0911] 所述组合物在施用于有此需要的对象时可使得在对象中持续产生基于抗体的分子。组合物可以使得在对象中持续至少约1天、2天、3天、4天、5天、6天、7天、8天、9天、10天、11天、12天、13天、14天、15天、16天、17天、18天、19天、20天、21天、22天、23天、24天、25天、26天、27天、28天、29天、30天、31天、32天、33天、34天、35天、36天、37天、38天、39天、40天、41天、42天、43天、44天、45天、46天、47天、48天、49天、50天、51天、52天、53天、54天、55天、56天、57天、58天、59天、或60天产生基于抗体的分子。

[0912] 本文中使用的术语“治疗”及其变形意指改善、减缓或逆转疾病或病症的进展或严重程度,或改善、减缓或逆转这样的疾病或病症的一种或更多种症状或副作用。出于本发明的目的,“治疗”或其变形还意指用于获得有益或期望的临床结果的方法,其中“有益或期望的临床结果”包括但不限于部分或全部地,可检出或不可检出地,缓解症状、减轻病症或疾病程度、稳定(即,不恶化)疾病或病症状态、延迟或减慢疾病或病症状态的进展、改善或减轻疾病或病症状态、以及缓解疾病或病症。

[0913] 因此,在一个实施方案中,根据本发明的抗MuSK抗体或抗原结合片段或者根据本发明的组合物用于在人对象中治疗神经肌肉病症,其中所述治疗导致所述病症的稳定。稳定可持续至少1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月或12个月或者至少1年、2年或3年。本文中进一步表征的每一种治疗性效果都可以被视为病症的稳定。

[0914] 在一个实施方案中,抗MuSK抗体或抗原结合片段(或多核苷酸、表达载体、宿主细胞、组合物)的使用对本文中所限定的经治疗的人对象表现出治疗性效果。

[0915] 这样的治疗作用可以是以下公开的作用中的至少一种。

[0916] 通过与MuSK的表位结合,本发明的抗MuSK抗体或抗原结合片段能够引发激动性MuSK活性。在本申请的上下文中,"引发激动性MuSK活性"可用"激活MuSK"替代。激动性MuSK活性或MuSK的激活可在分子和/或细胞水平和/或在更生物复杂的系统中(如NMJ、突触、活生物体)触发。在本申请的上下文中,激动性MuSK活性可通过触发MuSK诱导的信号或通过在NMJ处的肌细胞中诱导MuSK激活来替代。MuSK诱导的信号(或MuSK激活或MuSK活性)可以是以下的至少一种:MuSK二聚化的诱导、MuSK酪氨酸磷酸化的诱导、在NMJ处聚集的AChR(或在肌管AChR斑块(patch)中体外聚集)的诱导的诱导或提高、完全受神经支配的NMJ的数目或百分比的提高、完全去神经的NMJ的数目或百分比的提高的降低、完全受神经支配的NMJ的数目或百分比的维持(疾病稳定/疾病进展稳定)、突触传递的可靠性的改善、运动表现的改善、运动神经元死亡的预防/稳定或甚至减少/降低、经治疗对象的寿命延长。

[0917] 本发明的通过抗MuSK抗体的MuSK诱导的信号可以是MuSK二聚化的诱导,其可通过Western印迹来评估。在本发明的上下文中,在使用本发明的抗体的实验中,通过与没有任何抗体或有阴性对照或有阴性对照抗体的相同实验设置相比,当MuSK二聚化的诱导提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%或更多时,可评估MuSK的激动活性。或者,在本发明的上下文中,当在使用本发明抗体的实验中,通过与没有阳性对照抗体的相同实验设置相比,MuSK二聚化的诱导相同或大约相同(20%以下、10%以下或相同或10%以上或20%以上)时,可评估MuSK抗体的激动活性。这样的MuSK二聚化可在没有突触蛋白聚糖(agrin)的情况下进行评估。MuSK二聚化评估中的阳性对照是突触蛋白聚糖。

[0918] 本发明的通过抗MuSK抗体的MuSK诱导的信号可以是MuSK酪氨酸磷酸化和这样的磷酸化的诱导,其可使用对酪氨酸磷酸化具有特异性的抗体通过Western印迹来评估。在本发明的上下文中,在使用本发明的抗体的实验中,通过与没有任何抗体的相同实验设置相比,当MuSK酪氨酸磷酸化的诱导提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%、100%、120%、150%、180%、200%或更多时,可评估MuSK的激动活性。或者,在本发明的上下文中,在使用本发明抗体的实验中,通过与没有阳性对照抗体的相同实验设置相比,当MuSK酪氨酸磷酸化的诱导相同或大约相同(低20%、低10%或相同或高10%或高20%)时,可评估MuSK抗体的激动活性。这样的MuSK酪氨酸磷酸化可在没有突触蛋白聚糖的情况下进行评估。MuSK酪氨酸磷酸化评估中的阳性对照是突触蛋白聚糖。

[0919] 通过本发明的抗MuSK抗体的MuSK诱导的信号可以是在NMJ处乙酰胆碱受体(AChR)聚集的诱导,并且这样的聚集可以通过使用与AChR特异性结合的抗体对AChR进行染色并使用本领域技术人员已知的技术在荧光显微镜中可视化这样的染色来评估。或者,可在肌管AChR斑块中体外评估聚集。用于可视化AChR聚集的优选抗体是对AChR具有特异性的抗体。

更优选的抗体是AlexaFluor488缀合的α-银环蛇毒素(B13422,ThermoFisher)。通常将待分析的区域固定在多聚甲醛中,并在室温下与本发明的相关抗体或者与阳性或阴性对照一起孵育,并且随后用PBS洗涤每个区域并在落射荧光显微镜下观察。在本发明的上下文中,在使用本发明抗体的实验中,通过与没有任何抗体的相同实验设置相比,当NMJ处AChR聚集的诱导相同或大约相同(即低20%、低10%或相同或高10%或高20%)或者提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可评估MuSK抗体的激动活性。这样的AchR聚集可在没有突触蛋白聚糖的情况下进行评估。AchR聚集评估中的阳性对照是突触蛋白聚糖。

[0920] 在一个优选的实施方案中,本发明的抗MuSK抗体表现出在NMJ处聚集乙酰胆碱受体的诱导或提高的诱导,并且与没有MuSK激动剂抗体所获得的染色相比,这样的聚集可通过可视化小鼠膈肌NMJ处的AchR的染色或增加的染色来评估。在一个实施方案中,在NMJ处的AchR聚集的这种诱导或提高导致维持突触神经支配和/或突触前和突触后排列(alignment)的更正常/生理的NMJ形态。

[0921] 在NMJ处的肌细胞中的通过本发明的抗MuSK抗体的MuSK诱导的信号可以是完全受神经支配的NMJ的数目或百分比的提高、完全去神经的NMJ的数目或百分比的提高的降低、完全受神经支配的NMJ的数目或百分比的维持(疾病稳定/疾病进展稳定)、突触传递的可靠性的改善、运动神经元死亡的预防/稳定或甚至减少/降低。这些特征中的每一个都可以使用本领域技术人员已知的技术来评估,例如使用本文中先前限定的a-银环蛇毒素抗体对AchR进行染色,通过荧光共聚焦显微镜进行突触前标记和量化神经支配,EMG单纤维EMG,单个突触的电生理学,骨髓特异性区域中运动神经元细胞体的染色。所有这些测定都已在Cantor S et al2018(Elife,2018;7:e34375)中进行了描述。

[0922] 抗MuSK抗体或抗原结合片段可改善经治疗对象的运动表现和/或握力。在使用本发明的抗MuSK抗体的实验中,与没有任何抗体的相同实验设置相比,当这样的运动表现或握力可提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可认为经治疗对象的运动表现和握力已得到改善。可使用本领域技术人员已知的测定来评估经治疗对象的运动表现(或握力)。实验部分公开了一些示例性的方法。

[0923] 抗MuSK抗体或抗原结合片段可改善经治疗对象NMJ处肌肉的收缩特性。在使用本发明的抗MuSK抗体的实验中,与没有任何抗体的相同实验设置相比,当这样的肌肉的收缩特性可提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可认为经治疗对象肌肉的收缩特性已得到改善。可使用本领域技术人员已知的测定来评估经治疗对象的(NMJ处)肌肉的收缩特性。实验部分公开了一些示例性的方法。在这种情况下,对象可以是动物。

[0924] 抗MuSK抗体或抗原结合片段可改善经治疗对象NMJ处肌肉的对疲劳的抗性。在使用本发明的抗MuSK抗体的实验中,与没有任何抗体的相同实验设置相比,当这样的肌肉的疲劳特性可改善至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可认为经治疗对象肌肉的对疲劳的抗性已得到改善。可使用本领域技术人员已知的测定来评估经治疗对象(NMJ处)肌肉的疲劳特性。实验部分公开了一些示例性的方法。在这种情况下,对象可以是动物。

[0925] 抗MuSK抗体或抗原结合片段可诱导经治疗对象NMJ处肌肉重量的提高。在使用本

发明的抗MuSK抗体的实验中,与没有任何抗体的相同实验设置相比,当这样的重量可提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可认为经治疗对象肌肉重量已得到改善。实验部分公开了一些示例性的方法。在这种情况下,对象可以是动物。

[0926] 通过本发明的抗MuSK抗体的MuSK诱导的信号或作用的特征可在于经治疗对象的生活质量的改善或生活质量恶化出现的延迟。生活质量可以通过对象的体重来量化。生活质量的改善或生活质量恶化出现的延迟可以是至少1天、1周、2周、3周、4周、1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月、1年或更长。这是与患有相同病症并且未用本发明抗体治疗的对象的预期生活质量(或预期生活质量恶化的出现)进行比较来评估的。在这种情况下,对象可以是动物。

[0927] 通过本发明的抗MuSK抗体的MuSK诱导的信号或作用的特征可在于经治疗对象的寿命。延长可以是至少1天、1周、2周、3周、4周、1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月、1年或更长。这是与患有相同病症并且未用本发明抗体治疗的对象的预期寿命进行比较来评估的。在这种情况下,对象可以是动物。

[0928] 本文中所述的抗MuSK抗体的特性可根据本文中所述的测定进行测量。MuSK激动剂抗体的激活活性可相对于对照(例如可不结合MuSK的阴性对照抗体(例如同种型对照))来测量。不与MuSK结合的优选对照抗体是靶向RSV的莫维珠单抗(综述,MAbs,1(5),439-442,Sept-Octo 2009,DOI:10.4161/mabs.1.5.9496)。优选的阳性对照激动剂MuSK抗体是来自Genentech的mAb#13。用于证明激活MuSK活性的另一个优选的阳性对照分子是突触蛋白聚糖(来自R&D系统的大鼠突触蛋白聚糖,550-AG)。

[0929] 在另一个实施方案中,与本文中先前限定的抗胆碱能化合物组合的抗MuSK抗体或其抗原结合片段(或多核苷酸、表达载体、宿主细胞、组合物)在本文中限定的经治疗的人对象中表现出治疗作用。在一个优选的实施方案中,与使用抗MuSK抗体或抗原结合片段(或多核苷酸、表达载体、宿主细胞、组合物)作为独立治疗相比,当使用这两种化合物时,会引发另外的并且更优选的协同治疗作用。

[0930] 另外的治疗作用可能是突触周围施万细胞(perisynaptic Schwann cell,PSC)毒蕈碱活性的降低(“抑制”),NMJ修复。这样的另外的治疗作用可以是PSC毒蕈碱活性的特异性降低(“抑制”)。这样的另外的治疗作用可以是在神经肌肉病症的情况下PSC的过度兴奋的降低(“抑制”)。本发明的化合物或组合特异性作用于毒蕈碱受体。本发明的化合物或组合似乎对PSC上表达的纯化受体没有任何影响。

[0931] NMJ修复可以是神经芽生(nerve sprouting)的诱导或提高和/或NMJ神经支配状态的提高。可以使用技术人员已知的技术来评估这些作用中的每一个。此外,抑制PSC的毒蕈碱活性可有助于维持NMJ神经支配。

[0932] 在本发明的上下文中,在使用抗胆碱能化合物的实验中,与没有所述化合物的相同实验设置相比,当在NMJ处的神经芽生(或NMJ的神经支配状态)的诱导提高至少10%、20%、30%、40%、50%、60%、70%、80%、90%或100%时,可以评估神经芽生(或NMJ的神经支配状况)的诱导或提高。可使用神经肌肉制剂的免疫组织化学来评估神经芽生或神经支配状态。实验部分公开了如何获得这样的神经肌肉制剂。

[0933] 在本发明的上下文中,在使用抗胆碱能化合物的实验中,与没有所述化合物的相同实验设置相比,当PSC的毒蕈碱活性(或者毒蕈碱过度兴奋(hyperexcitability)或过度

兴奋(overexcitability))降低了至少10％、20％、30％、40％、50％、60％、70％、80％、90％或100％时,可评估PSC的毒蕈碱活性的降低(或者毒蕈碱过度兴奋(hyperexcitability)或过度兴奋(overexcitability)的降低)。

[0934] 因此,在一个实施方案中,抗MuSK抗体或抗原结合片段(或多核苷酸、表达载体、宿主细胞、组合物)的使用,优选地与本文中先前限定的抗胆碱能化合物组合,表现出以下一种或更多种治疗作用:

[0935] -所述对象中完全受神经支配的NMJ的数目或百分比的提高,所述对象中完全受神经支配NMJ的数目或百分比的维持,所述对象中完全去神经的NMJ的数目或百分比的降低,突触传递的可靠性的改善,所述对象中运动神经元死亡的预防、稳定或降低;以及/或者

[0936] -所述对象的运动表现和/或握力的改善;以及/或者

[0937] -所述对象的NMJ处的肌肉的收缩特性的改善;以及/或者

[0938] -所述对象的NMJ处的肌肉的对疲劳的抗性的改善;以及/或者

[0939] -诱导所述对象的NMJ处的肌肉重量增加;以及/或者

[0940] -所述对象生活质量的改善或生活质量恶化出现的延迟;以及/或者

[0941] -所述对象中的突触周围施万细胞(PSC)的毒蕈碱活性的降低(或者毒蕈碱过度兴奋(hyperexcitability)或过度兴奋(overexcitability)的降低)或所述对象中的NMJ修复。

[0942] 如实验部分中所表明的,当使用两种化合物时,获得协同治疗作用。这些协同作用包括以下参数/症状的改善/提高:运动功能和握力、NMJ处肌肉的收缩特性、对肌肉的疲劳的抗性、肌肉重量、对一般生活状况(例如体重)的影响。

[0943] 基于抗体的分子的“有效量”是指在必要的剂量和时间段内足以实现预期的生物学作用或期望的治疗结果(包括但不限于临床结果)的量。当应用于本发明的基于抗体的分子时,短语“治疗有效量”旨在表示足以改善、减轻、稳定、逆转、减缓或延迟病症或疾病状态的进展或病症或疾病的症状的进展的抗体的量。在一个实施方案中,本发明的方法提供了与其他化合物组合的基于抗体的分子的施用。在这样的情况下,“有效量”是足以引起预期生物学作用的组合的量。

[0944] 在另一个方面中,提供了用于预防和/或治疗神经肌肉疾病和/或障碍和/或病症的方法,其包括向有此需要的对象施用抗MuSK抗体或其抗原结合片段(多核苷酸、表达载体、宿主细胞或组合物,均如本文中先前所限定),以及优选地抗胆碱能化合物。此方法的所有特征已在本文中先前进行了限定。

[0945] 在另一个方面中,提供了抗MuSK抗体或其抗原结合片段(多核苷酸、表达载体、宿主细胞或组合物,均如本文中先前所限定)以及优选地抗胆碱能化合物用于制备预防和/或治疗神经肌肉疾病和/或障碍和/或病症的药物的用途。此用途的所有特征已在本文中先前进行了限定。

[0946] 本说明书中引用的所有文件在此均通过引用整体并入。除非另有定义,否则用于公开本发明的所有术语(包括技术术语和科学术语)具有如本发明所属领域普通技术人员通常所理解的含义。通过进一步的指导,包括术语定义以更好地理解本发明的教导。除非另外指明,否则本文中所述的每个实施方案可以与本文中所述的任何其他实施方案组合在一起。

[0947] 通过以下实施例进一步描述本发明,所述实施例不应被解释为对本发明范围的限制。

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[0986] 实施例

[0987] 实施例1:方法

[0988] 动物

[0989] 从杰克逊实验室(Jackson Laboratory)获得过表达人突变的$SOD1^{G37R}$转基因的小鼠,系29,并在蒙特利尔大学(Universitéde Montréal)动物设施在C57BL/6背景下饲养。这种小鼠模型是发作晚、进展缓慢的ALS模型,它重现了该疾病的人表型。该品系表型的表征先前已发表在数项ALS研究中(5、7、20、31、32)。所有实验都是根据加拿大动物保护委员会和蒙特利尔大学动物伦理委员会(Comitéde déontologie animale of Universitéde Montréal.)的指南进行的。

[0990] 临床前试验设计

[0991] 根据ALS/MND中的临床前动物研究指南(33)进行临床前试验设计。这项研究是以双盲方式进行的。将来自$SOD1^{G37R}$背景的15只雄性小鼠随机分配为三组。

[0992] 1.ARGX-119(3B2g2m1-hIgG1LALAdelk:具有降低的效应物功能的全长重链SEQ ID NO:268和具有降低的效应物功能的全长轻链SEQ ID:269):和达非那新

[0993] 2.ARGX-119(3B2g2m1-hIgG1LALAdelk)和载剂(达非那新对照)

[0994] 3.同种型对照mAb+载剂(达非那新对照)

[0995] ARGX-119处理在发作之前(出现症状之前或无症状)开始,并且达非那新处理在发作时(出现症状)开始,并持续到处死。使用一组神经学评分(1至5级,附录1)来确定症状的发作以及疾病进展期间症状的进展和严重程度。通过以下来评估疾病的发作:重量减轻的开始(34)和震颤的出现,相当于神经评分为1,而本研究的终点在晚期有症状阶段,相当于神经评分为3至5。

[0996] 在P400以20mg/kg的初始剂量开始腹膜内注射ARGX-119MuSK抗体(3B2g2m1-hlgG1LALAdelk)或安慰剂(莫维珠单抗-hlgG1LALAdelk:具有降低的效应物功能的全长重链SEQ ID NO:272和具有降低的效应物功能的全长轻链SEQ ID:273),并随后每周以10mg/kg的剂量进行腹膜内注射,直至处死。在疾病发作时(约P425)开始经口给予达非那新(10mg/kg,在DMSO中稀释,5天/周)。达非那新的安慰剂是单独的DMSO。小鼠接受这两种处理约4个月,直到约520天的年龄,这是它们通常达到严重疾病终点的中位年龄。

[0997] 莫维珠单抗-hlgG1LALAdelk:SEQ ID NO:272来源于SEQ ID NO:274并且SEQ ID:273来源于SEQ ID:275。

[0998] 处理、行为监测、实验和结果分析以盲法进行。每周进行标准ALS行为测量,以测量不同研究组的疾病进展。这包括转棒仪测试、握力测量、重量测量和尾悬吊测试,以评估后肢伸展反射。在处死时,将趾长伸肌(Extensor Digitorum Longus,EDL)和比目鱼肌(Soleus,SOL)及其神经支配解剖并放置在生理室中。获得了两组测量值。首先,使用力传感器确定肌肉的功能特性(强度和疲劳)。其次,固定肌肉并确定肌肉质量。

[0999] 转棒仪加速方案

[1000] 使用转棒仪(TSE转棒仪,TSE系统Gmbh,Germany)评估运动协调、力量和平衡。将动物以4rpm的起始速度放置在转轮上,在300秒内提高到40rpm。在加速方案期间,小鼠每组(block)有两次尝试,并且每个环节(session)有两组(组之间有休息时间)来保持在转棒仪上,并对两个最长的掉落的等待时间取平均值。

[1001] 握力

[1002] 为了测量小鼠的肢的整体力量,使用了握力计(图1C)。小鼠每个环节有九次尝试(3组,每组3次尝试;每组之间间隔1分钟的休息期),并且对每组的最佳三个值进行平均。

[1003] 神经-肌肉标本(preparation)

[1004] 将EDL和SOL肌肉及其支配神经的标本在如下的含氧Ree's溶液(以mM计)中解剖:110NaCl、5KCl、1$MgCl_2$、25$NaHCO_3$、2$CaCl_2$、11葡萄糖、0.3谷氨酸、0.4谷氨酰胺、5BES(N,N-双(2-羟基乙基)-2-氨基乙磺酸钠盐)、0.036氯化胆碱和4.34×10^{-7}辅羧酶。在解剖之后,用含氧Ree's溶液(95% O_2,5% CO_2)不断灌注神经肌肉标本。

[1005] 神经肌肉特性的测量

[1006] 使用手术缝合线将EDL和SOL神经-肌肉样品垂直附接到固定的力传感器(型号

402A-500mN,Aurora Scientific Inc.)上。将样品在肌腱水平上附接到一端的触感器上,并在另一端附接到适应性钩上(图2A)。然后将铂参比电极与肌肉并置,定位在靠近肌腱的肌肉末端附近。为了刺激肌肉,将第二个铂电极并置在肌肉的另一端。为了引发运动神经的肌肉收缩和神经肌肉活动,将胫神经(SOL)或腓深神经(EDL)吸入到由PE管制成的电极中,并用生理溶液填充。因此,该系统被设计用于从肌肉和/或神经刺激二者中引发肌肉收缩。通过在运动神经上施加的500mV、0.1ms脉冲的单个超大方波引发神经肌肉收缩基础力响应。通过15V,1ms的方脉冲刺激引发肌肉收缩基础力响应。通过逐渐拉伸肌肉直到获得最大收缩力输出来确定最佳肌肉长度。

[1007] 力-频率曲线:进行神经和肌肉刺激以生成标准力-频率图。以多个频率(5Hz,10Hz,20Hz,30Hz,40Hz,50Hz,60Hz,70Hz,80Hz,90Hz,100Hz,120Hz,140Hz,160Hz,180Hz,200Hz,250Hz,和300Hz,)进行500ms的交替神经和肌肉刺激,并监测产生的力。每次刺激之间有2分钟的休息期。在神经刺激时神经肌肉系统使用的肌肉能力比例表示为收缩能力比,并对每个频率进行如下计算:

[1008] $$\frac{力_{神经}}{力_{肌肉}} X\,100$$

[1009] 最大力:在50Hz和80Hz的频率下获得由交替的神经和肌肉刺激产生的最大力,持续2秒,每次间隔2(SOL)或5(EDL)分钟。

[1010] 肌肉疲劳:疲劳方案如图4A所示。疲劳方案因其内在特性的差异而适用于每种肌肉。对于EDL,使用在120Hz的频率下引发的持续时间为300ms的一回180次神经刺激来测试疲劳。每次刺激之间的休息期为700ms,总方案持续时间为3分钟。每10次刺激将神经刺激叠加在肌肉刺激上(18次同时的神经-肌肉刺激),以评价肌肉储备。针对SOL的疲劳方案由以下组成:在50Hz下进行一回300次神经刺激持续500ms,其中两次刺激之间的休息期为600ms,总持续时间为5分30。每10次刺激将神经刺激叠加在肌肉刺激上(30次同时的神经-肌肉刺激)。

[1011] 肌肉恢复:每个疲劳方案之后都有30分钟的恢复期,在所述恢复期期间,在疲劳方案之后5s、10s、15s、30s、45s、1分钟、1.5分钟、2分钟、2.5分钟、5分钟、10分钟、20分钟和30分钟测量神经肌肉收缩力和神经肌肉+肌肉收缩力(针对EDL为120Hz-300ms并且针对SOL为50Hz-500ms)。

[1012] 肌肉重量

[1013] 在每次实验之后,将肌肉固定(10分钟,PFA)并洗涤(3次洗涤,每次5分钟,PBS1X)。然后,切割两根肌腱并对肌肉进行称重,并将其储存在4℃下用于进一步处理。

[1014] 统计

[1015] 结果表示为平均值±SEM,其中动物数目确定为N(重复的数目),并且肌肉数目表示为n(观察的数目)。在比较三个或四个不同组的大多数情况下,使用单因素ANOVAKruskal-Wallis检验和多重t检验。使用重复单因素ANOVA和事后Bonferroni多重比较试验来比较来自不同组中的相同动物在多个频率或随时间变化获得的值。研究中使用的置信水平为95%(α=0.05)。所有分析均使用GraphPad 8软件(Prism)进行。

[1016] 实施例2:结果

[1017] 与达非那新组合的ARGX-119抗体改善运动功能和握力

[1018] 测试动物的运动功能和总体力量,以研究用ARGX-119抗体和达非那新的组合治疗是否能改善肌肉功能。

[1019] 首先,使用转棒仪上的标准加速方案测量运动表现、平衡和协调(图1A),已知随着疾病的进展,这会揭示ALS运动缺陷(34)。图1B示出了如转棒仪掉落的等待时间较短所揭示的单独ARGX-119、单独达非那新或安慰剂处理组的小鼠运动表现的逐渐下降,显示了ALS运动表型的预期进展。然而,与ARGX-119+DMSO($p<0.001$)、PBS+达非那新($p<0.001$)和安慰剂处理的小鼠($p<0.001$)相比,用ARGX-119抗体和达非那新组合处理的小鼠的运动表现不太明显,导致运动表现显著改善(图1B;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,PBS+达非那新N=5;安慰剂N=5、单因素ANOVA、Kruskal-Wallis's检验和多重t检验)。当接近终点阶段时,这一点尤其明显,其中与安慰剂组相比,对经组合处理的小鼠观察到在P475龄至P525龄时获得的评分显著更高。事实上,在这个晚期症状阶段,大多数安慰剂小鼠不再能够在转轮上奔跑,而达非那新处理组中超过一半的小鼠仍然能够奔跑。有趣的是,从P510龄到P525龄,ARGX-119+DMSO处理的小鼠在转棒仪有改善的运动行为的趋势(分别为$p=0.07$、$p=0.06$和$p=0.053$)。这些结果表明,与其他单一处理和安慰剂组相比,组合处理具有有益影响。

[1020] 其次,测量握力,以评估组合处理是否改善了动物的总体力量(图1C)。所有组的小鼠都以相似的握力开始试验。然而,经组合处理的小鼠表现好于安慰剂组的小鼠,如P460时产生的更大握力所示,直到临床前试验结束(图1D;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,PBS+达非那新N=5,安慰剂N=5,单因素ANOVA,$p<0.05$,Tukey's检验和多重t检验)。有趣的是,在P507时,与安慰剂组相比,PBS+达非那新处理的小鼠握力显著增加。

[1021] 第三,测试ARGX-119抗体与达非那新组合处理是否对小鼠的一般状况产生影响。为此,监测了动物体重的变化,其是与疾病进展和存活直接相关的指标,其中动物在症状发作之后表现出重要的体重逐渐减轻。然而,两组之间没有观察到差异(ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,PBS+达非那新N=5,安慰剂N=5,$p>0.05$,单因素ANOVA,Tukey's检验和多重t检验)。

[1022] 实施例2.1:组合处理改善神经肌肉收缩肌力和NMJ效力

[1023] 改善的收缩肌肉特性是肌肉和NMJ功能也应通过组合处理而改善的有力指标。研究了两种具有不同特性和对疾病的抗性的肌肉。EDL被用作易受疾病影响的快速颤搐易疲劳肌肉,并且SOL被用作对疾病更具有抗性的慢速颤搐疲劳抗性肌肉。肌力传感器用于测量肌肉在刺激运动神经和/或直接肌肉刺激时产生的力(参见图2A)。使用该系统,以多个频率刺激运动神经通过NMJ效力引发肌肉收缩,仅反映与受神经支配的NMJ相关的收缩纤维的强度。相反,肌肉刺激使所有肌肉纤维去极化,并反映所有肌肉的最大颤搐力,与神经支配状态无关。这种方法尤其适用于对表现NMJ和肌肉缺陷的疾病(如ALS)进行表征(35,36)。

[1024] 快速-易疲劳EDL肌肉

[1025] 首先,执行标准的刺激方案,以生成力频率曲线(5Hz至300Hz),以表征ARGX-119和达非那新慢性处理之后的NMJ效力。在方案期间,由运动神经刺激和NMJ激活引发的收缩产生的肌力显著高于安慰剂组($p<0.001$)或ARGX-119+DMSO组($p<0.05$)的EDL(图2B;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5;重复单因素ANOVA,Bonferroni事后检验)。事实上,组合处理产生的颤搐力为66.3±15.7mN,安慰剂组为47.2±12.4mN并且ARGX-

119+DMSO组为53.6±14.9mN。与安慰剂处理的小鼠相比,ARGX-119抗体组没有显著差异。

[1026] 对于直接肌肉刺激,在更高的频率下观察到组合处理小鼠和其他组之间的显著差异(图2C)。与ARGX-119和DMSO组($p<0.05$)以及安慰剂组($p<0.001$)相比,组合处理组显示出显著的高峰值力(mN)。这表明EDL的快速颤搐特性的保持(37,38)(图2C,ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,重复单因素ANOVA,Bonferroni事后检验和多重t检验)。有趣的是,如图2E所示,与安慰剂组相比,在经组合处理的动物中也观察到收缩力的提高,并且EDL肌肉重量的保持更好。

[1027] 接下来,确定了在神经刺激时神经肌肉系统使用的肌肉能力的比例。这表示为收缩能力比。在WT小鼠中,这一比例为100%,表明肌肉的神经元控制募集了其100%的收缩能力。因此,如果处理改善了NMJ神经支配,导致产生的力提高,则认为与其他组相比,经组合处理动物的EDL肌肉中的该比例应该更高。如图2D所示,与经组合处理小鼠的52.3±1.8%($p<0.001$)和安慰剂组的53.3±3.6%($p<0.0001$)相比,ARGX-119+DMSO小鼠的EDL比例显著更高,为61.1±1.0%(ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,单因素ANOVA,Kruskal-Wallis's检验)。

[1028] 慢速颤搐SOL肌肉

[1029] 然后,执行相同的方案,但针对SOL肌肉(图3)。在神经刺激方案期间,经组合处理的组表现出与经ARGX-119+DMSO处理的组($p<0.0001$)和安慰剂组($p<0.0001$)相比显著更高的颤搐力(图3A;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,重复单因素ANOVA和多重t检验)。然而,与安慰剂相比,经ARGX-119+DMSO处理的组产生了较小的收缩力($p<0.05$)。组合处理产生的颤搐力为114.5±3.5mN,安慰剂组的为83.1±4.5mN并且ARGX-119+DMSO组的为64.6±5.1mN。在直接肌肉刺激的情况下,在神经刺激方案期间,经组合处理的组再次表现出与经ARGX-119+DMSO处理的组(98.3±2.1mN;$p<0.01$)和安慰剂组(119.5±1.6mN;$p<0.001$)相比显著更高的颤搐力(140.6±2.1mM)(图3B;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,重复单因素ANOVA)。经ARGX-119+DMSO处理的组与安慰剂组之间也有显著差异($p<0.05$)。

[1030] 与经ARGX-119处理的组的收缩能力比68.4±7.8%($p<0.0001$)和对照小鼠的收缩能力比56.1±相比,经组合处理的小鼠的收缩能力比(图3C)显著更高,为82.0±2.8%,($p<0.0001$;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,重复单因素ANOVA)。有趣的是,在经处理的动物的SOL肌肉重量保持较好的情况下,观察到收缩力和收缩能力比的提高。事实上,与安慰剂组相比,经ARGX-119+达非那新处理的小鼠具有更好地保持的肌肉重量($p<0.05$;ARGX-119+DMSO N=5,ARGX-119+达非那新N=4,安慰剂N=5,单因素ANOVA)。有趣的是,ARGX-119+DMSO处理组和安慰剂组之间也存在显著差异($p<0.001$),其中来自ARGX-119的SOL产生了比安慰剂组的SOL更好的收缩能力比。

[1031] 总体而言,这些结果表明,组合处理改善了两种肌肉的肌肉和神经肌肉收缩力以及肌肉重量,但仅对于ARGX-119+DMSO组,EDL和SOL的收缩能力比提高。

[1032] 实施例2.2:ARGX-119抗体与达非那新组合保持肌肉疲劳特性

[1033] 除了产生的力外,肌肉的特征还在于对疲劳的抗性。例如,与慢速颤搐肌肉(如SOL)相比,主要由快速易疲劳的运动单元构成的快速颤搐肌肉(如EDL)表现出更高的疲劳(37)。在ALS中,神经支配类型的改变(从快速到慢速颤搐)和肌肉本身特性的改变会改变疲

劳特性,使其更具抗性。由于ARGX-119+达非那新组合的处理显著保持了肌力,接下来研究了EDL和SOL肌肉对疲劳的抗性。

[1034] 使用疲劳刺激方案,随后是30分钟的恢复期(参见图4A)。来自经安慰剂处理的组的EDL肌肉在神经被直接刺激时表现出非典型的对疲劳的抗性。然而,如延迟恢复所揭示的,来自经ARGX-119+达非那新组合处理的小鼠的EDL肌肉表现出对这种类型的快速颤搐肌肉更典型的疲劳水平(图4B;ARGX-119+达非那新N=4,ARGX-119+DMSO N=5,安慰剂N=5;$p<0.05$,单因素ANOVA,Kruskal-Wallis's检验和多重t检验)。有趣的是,在恢复过程中,ARGX-119处理和安慰剂组之间存在显著差异($p<0.05$),其中经ARGX-119+DMSO处理的肌肉表现出更典型的疲劳。这表明,组合处理以及单一处理(如ARGX-119抗体)的使用改善了肌肉特性。

[1035] 然而,当所有肌纤维被募集时,没有发现神经+肌肉刺激的疲劳和恢复率的差异(图4C;ARGX-119+达非那新N=4,ARGX-119+DMSO N=5,安慰剂N=5;$p>0.05$;单因素ANOVA,Kruskal-Wallis's检验)。

[1036] SOL是慢性颤搐和抗性肌肉,与快速颤搐肌肉EDL相比,预计其对退行性疾病中观察到的去神经也更具抗性,并且恢复更快。对于SOL肌肉的神经刺激,与ARGX-119+达非那新处理组相比,如通过较慢的疲劳恢复所揭示的,安慰剂组表现出更明显的疲劳,这对于这种抗疲劳肌肉来说是非典型的。因此,组合处理恢复了SOL肌肉的抗疲劳特性。(图4D至E;ARGX-119+达非那新N=4,ARGX-119+DMSO N=5,安慰剂N=5;$p<0.01$;单因素ANOVA,Kruskal-Wallis's检验和多重t检验)。对于神经+肌肉刺激来说,ARGX-119+达非那新和ARGX-119+DMSO组之间存在显著差异($p<0.001$),并且ARGX-119+DMSO和安慰剂组之间存在显著差异($p<0.0001$)。

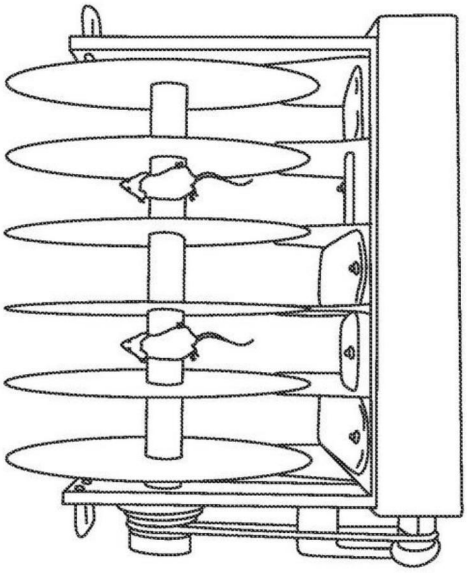

图1A

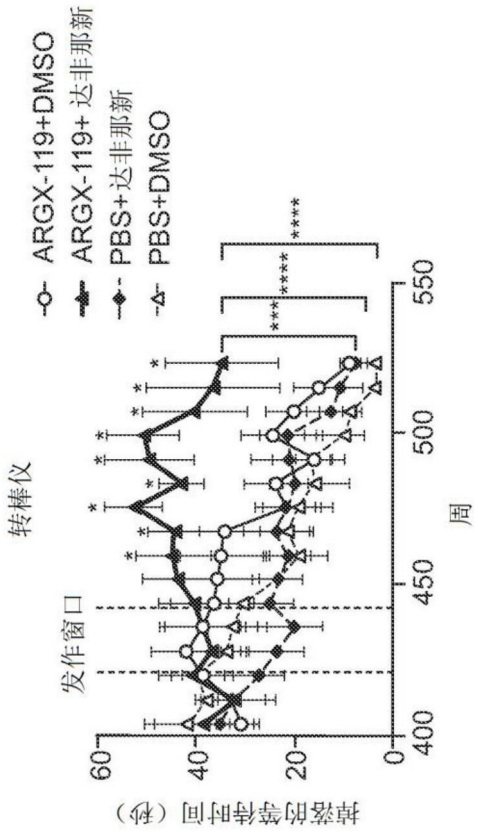
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+达非那新
PBS+DMSO
转棒仪
发作窗口
掉落的等待时间（秒）
周
0
20
40
60
400
450
500
550
*
**
***
****

图1B

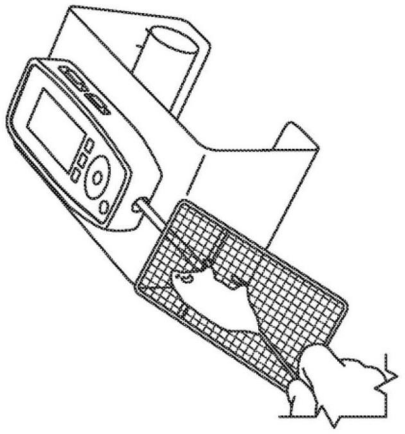

图1C

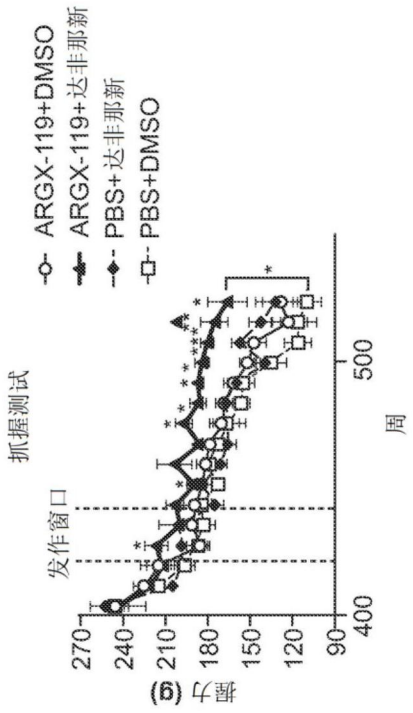
ARGX-119+DMSO
ARGX-119+达非那新
PBS+达非那新
PBS+DMSO
抓握测试
发作窗口
握力 (g)
270
240
210
180
150
120
90
400
500
周

图1D

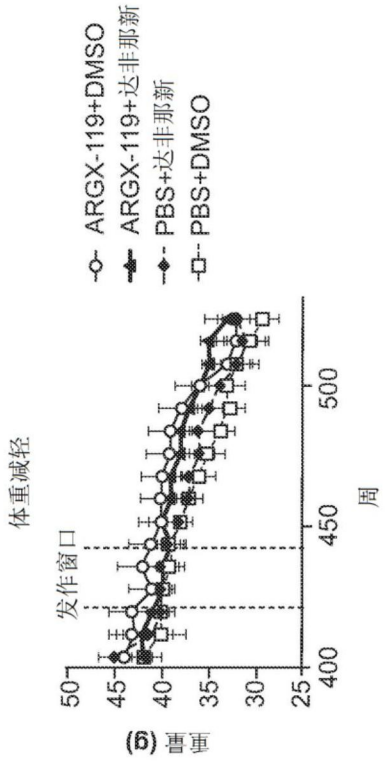
ARGX-119+DMSO
ARGX-119+达非那新
PBS+达非那新
PBS+DMSO
体重减轻
发作窗口
50
45
40
35
30
25
400
450
500
周
(g) 重量

图1E

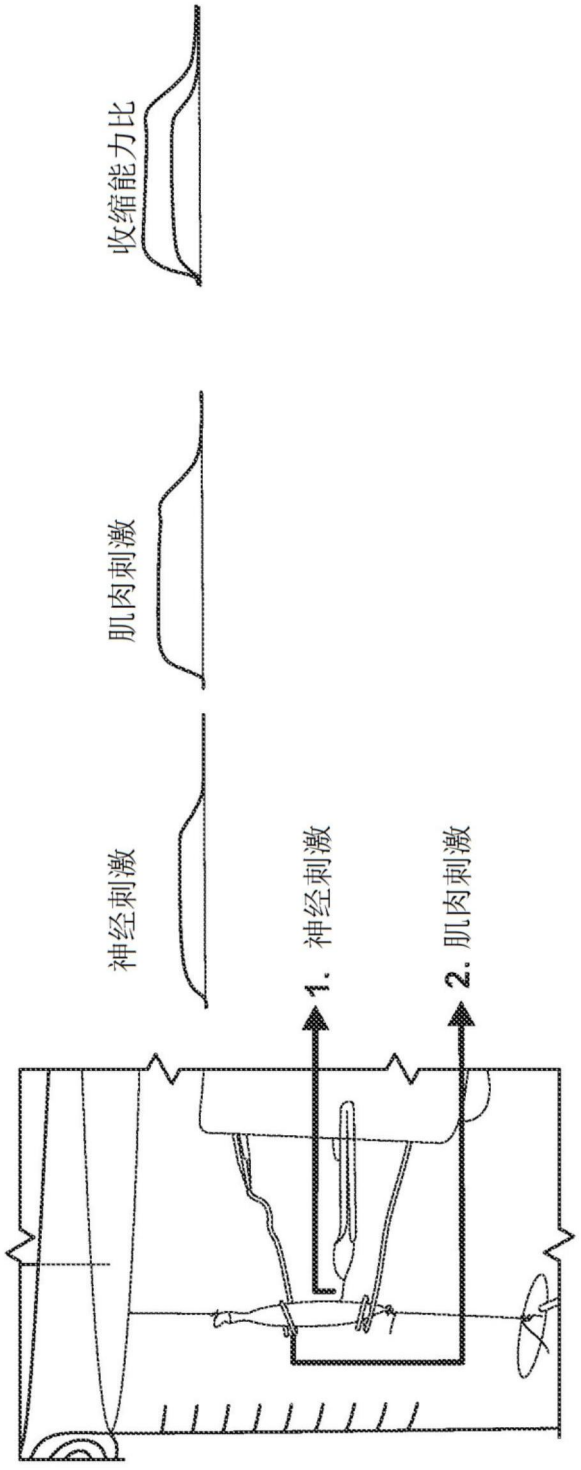

图2A

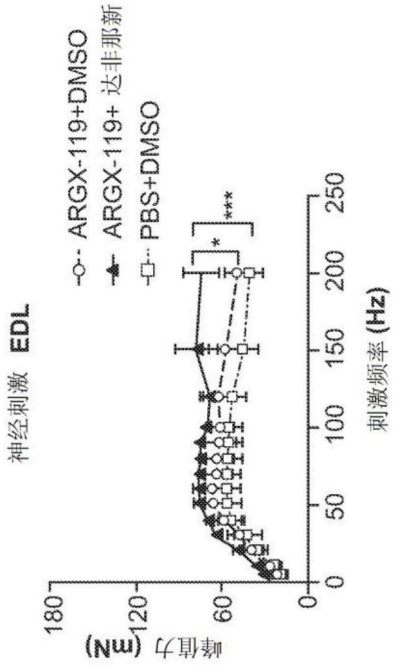
神经刺激 EDL
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
峰值力 (mN)
刺激频率 (Hz)
0
60
120
180
0
50
100
150
200
250
*
***

图2B

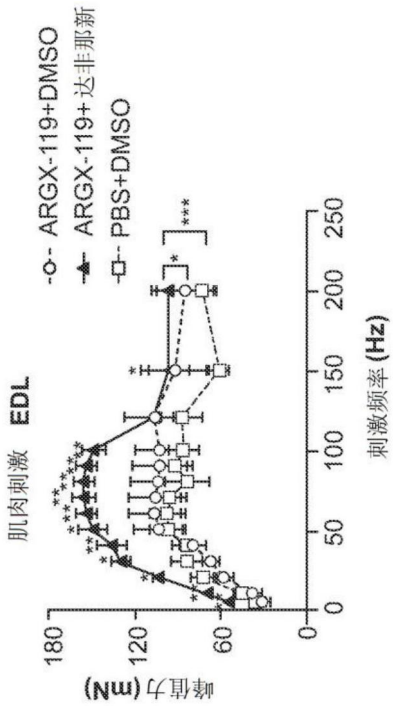
肌肉刺激 EDL
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
峰值力 (mN)
刺激频率 (Hz)
0
60
120
180
0
50
100
150
200
250
*
***

图2C

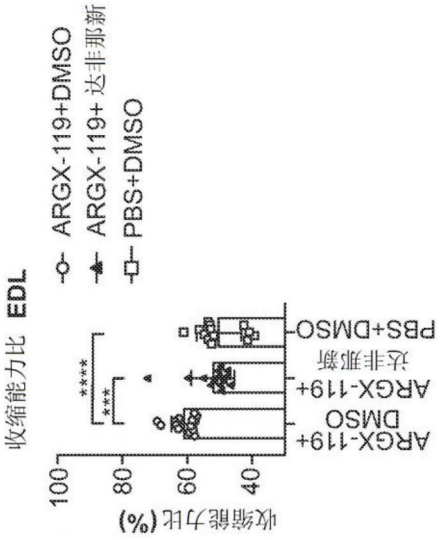
收缩能力比 EDL
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
收缩能力比(%)
100
80
60
40
***
****
ARGX-119+ DMSO
ARGX-119+ 达非那新
PBS+DMSO

图2D

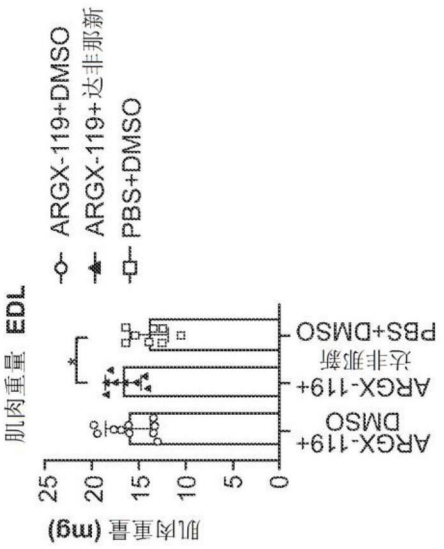
肌肉重量 EDL
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
肌肉重量 (mg)
25
20
15
10
5
0
*
ARGX-119+ DMSO
ARGX-119+ 达非那新
PBS+DMSO

图2E

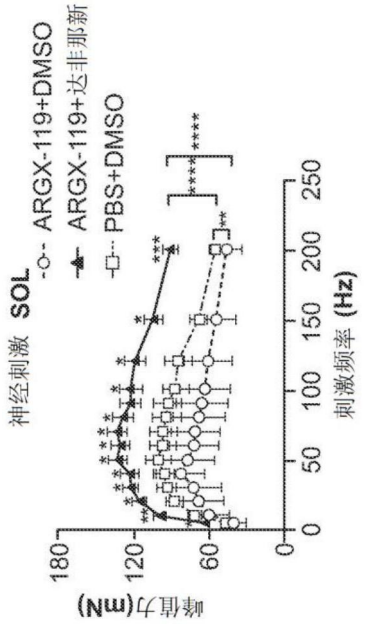
神经刺激 SOL
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
峰值力(mN)
刺激频率 (Hz)

图3A

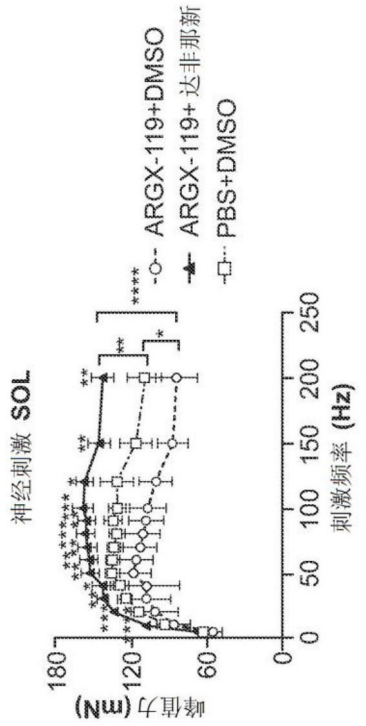
神经刺激 SOL
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
峰值力(mN)
刺激频率 (Hz)

图3B

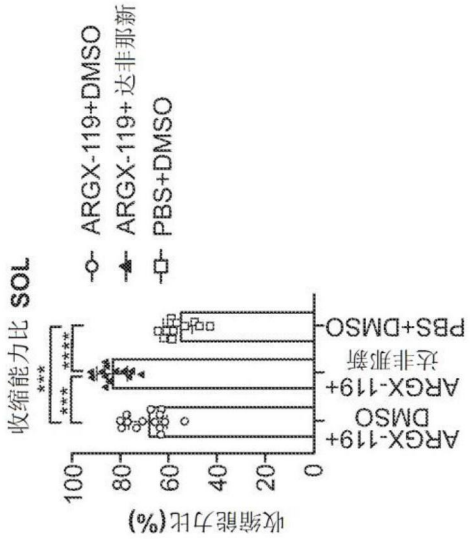
收缩能力比 SOL
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
收缩能力比(%)
100
80
60
40
20
0
***
****
ARGX-119+ DMSO
ARGX-119+ 达非那新
PBS+DMSO

图3C

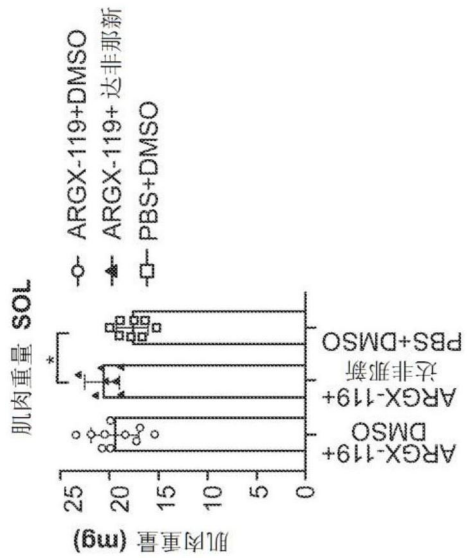
肌肉重量 SOL
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
肌肉重量 (mg)
25
20
15
10
5
0
*
ARGX-119+ DMSO
ARGX-119+ 达非那新
PBS+DMSO

图3D

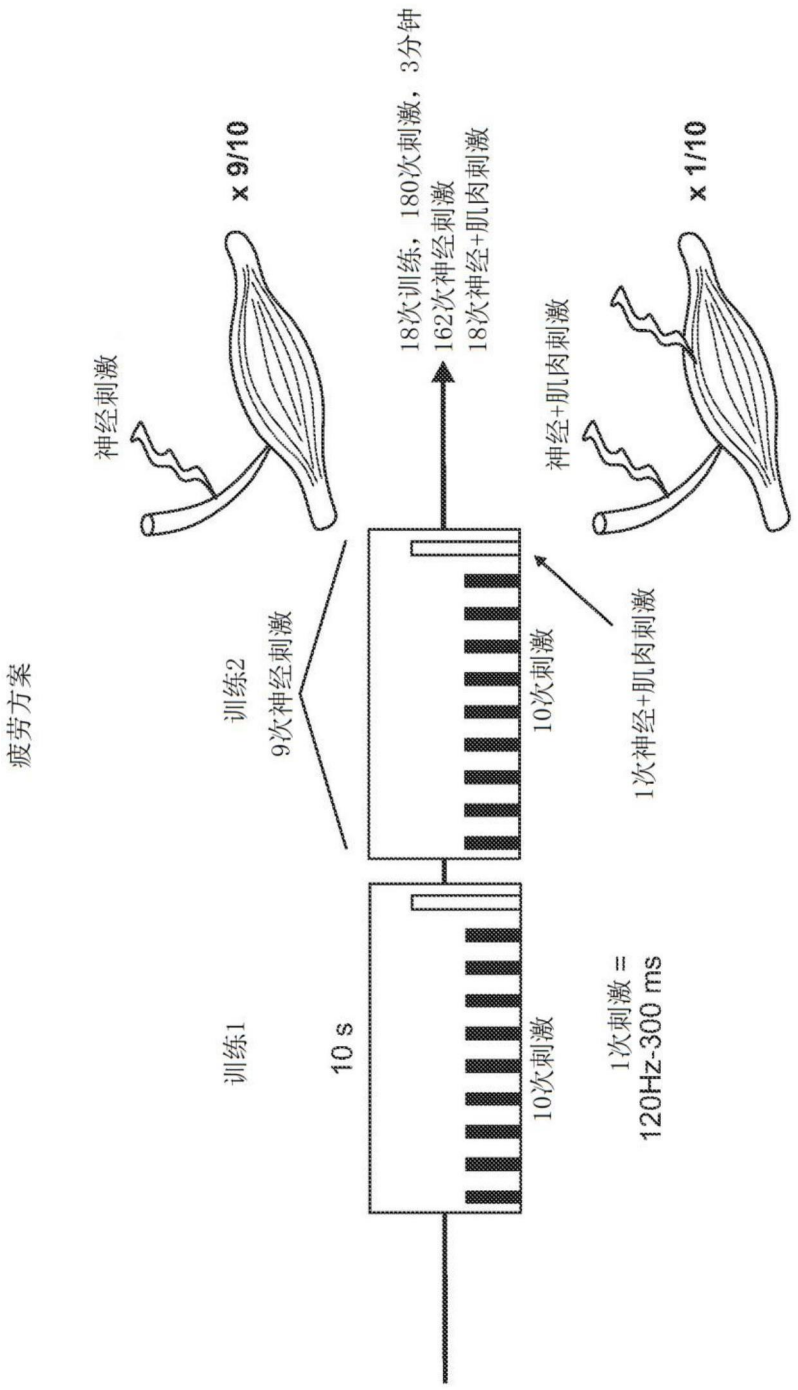
疲劳方案
训练1
10 s
10次刺激
训练2
9次神经刺激
神经刺激
x 9/10
10次刺激
18次训练，180次刺激，3分钟
162次神经刺激
18次神经+肌肉刺激
1次神经+肌肉刺激
神经+肌肉刺激
x 1/10
1次刺激 =
120Hz-300 ms

图4A

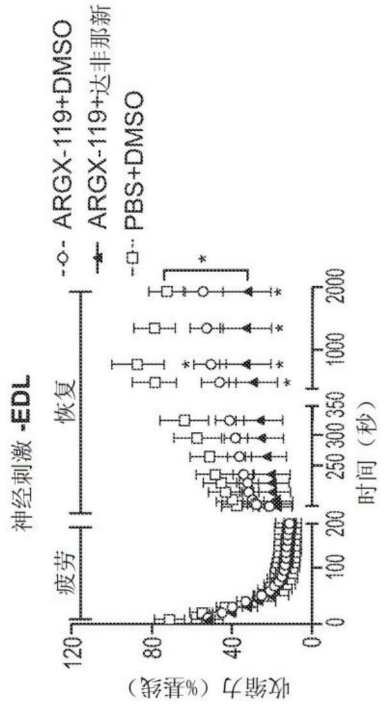
神经刺激 -EDL
疲劳
恢复
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
收缩力（%基线）
时间（秒）
120
80
40
0
0
100
200
250
300
350
1000
2000
*

图4B

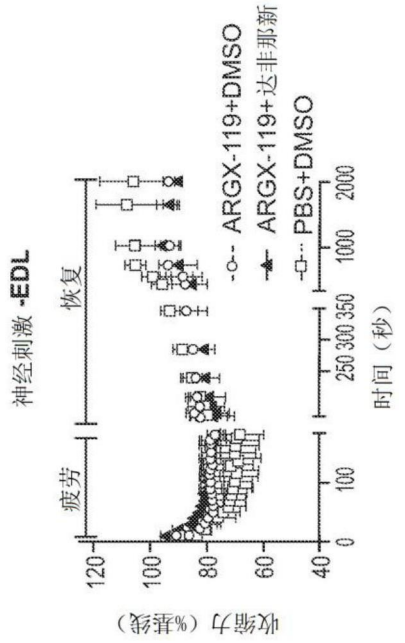
神经刺激 -EDL
疲劳
恢复
ARGX-119+DMSO
ARGX-119+达非那新
PBS+DMSO
收缩力（%基线）
时间（秒）
120
100
80
60
40
0
100
250
300
350
1000
2000

图4C

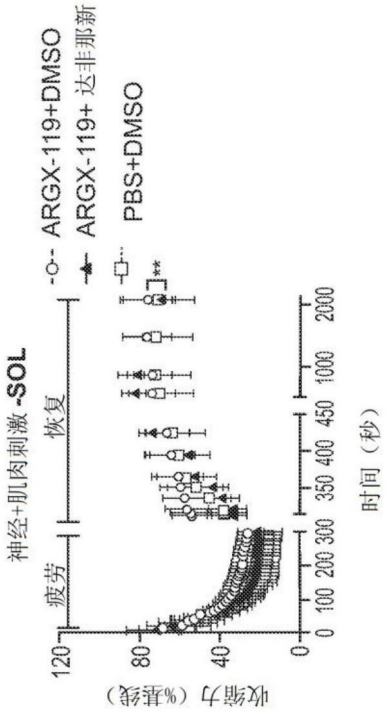
神经+肌肉刺激 -SOL
疲劳
恢复
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
**
收缩力（%基线）
时间（秒）
120
80
40
0
0 100 200 300 350 400 450 1000 2000

图4D

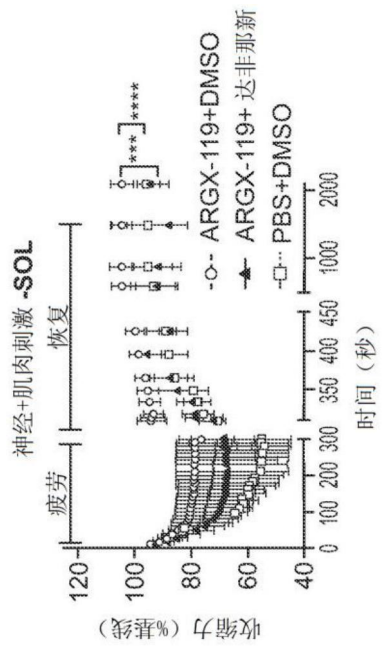
神经+肌肉刺激 -SOL
疲劳
恢复
ARGX-119+DMSO
ARGX-119+ 达非那新
PBS+DMSO
***
****
收缩力（%基线）
时间（秒）
120
100
80
60
40
0 100 200 300 350 400 450 1000 2000

图4E