

Antibody drugs, especially monoclonal antibodies, have achieved good efficacy in the treatment of various diseases. Preparing safe and effective anti-NGF monoclonal antibodies can provide a new class of analgesic drugs in the treatment of chronic pain and cancer pain different from opiates, non-steroids, *etc.* that are addictive or cause side effects in digestive tract. Currently, Tanezumab, developed by Pfizer Pharmaceuticals Co., Ltd., has shown good analgesic effects in preclinical and clinical studies. However, there is still a lack of other anti-NGF antibodies with higher activity.

SUMMARY

The object of the present disclosure is to overcome the defects of low activity and species differences of antibodies against nerve growth factor in the prior art, and to provide a monoclonal antibody against nerve growth factor with high activity and eliminating species difference, and an encoding gene and use thereof, as well as use thereof in preparing detection kits and various drugs.

In order to achieve the above object of the disclosure, the present disclosure provides a monoclonal antibody that can specifically bind to human nerve growth factor, comprising heavy chains and light chains, the heavy chain comprises a heavy chain constant region and a heavy chain variable region, and the light chain comprises a light chain constant region and a light chain variable region.

In one aspect, the present disclosure refers to a monoclonal antibody against nerve growth factor (NGF), comprising a heavy chains comprising a heavy chain constant region and a heavy chain variable region, and a light chains comprising a light chain constant region and a light chain variable region, or an antigen binding fragment thereof, wherein the heavy chain variable region comprises three complementarity determining regions HCDR1, HCDR2 and HCDR3, and the light chain variable region comprises three complementarity determining regions LCDR1, LCDR2 and LCDR3;

the amino acid sequence of the complementarity determining region HCDR1 is set forth in SEQ ID NO: 5;

the amino acid sequence of the complementarity determining region HCDR2 is set forth in SEQ ID NO: 6;

the amino acid sequence of the complementarity determining region HCDR3 is set forth in SEQ ID NO: 7;

the amino acid sequence of the complementarity determining region LCDR1 is set forth in SEQ ID NO: 8;

the amino acid sequence of the complementarity determining region LCDR2 is set forth in SEQ ID NO: 9; and

the amino acid sequence of the complementarity determining region LCDR3 is set forth in SEQ ID NO: 10.

The variable regions of the light chain and heavy chain determine the binding of the antigen; the variable region of each chain contains three hypervariable regions, called complementarity determining regions (CDRs) (the CDRs of the heavy chain (H) include HCDR1, HCDR2, HCDR3, and the CDRs of the light chain (L) include LCDR1, LCDR2, LCDR3; which were named by Kabat et al., see *Sequences of Proteins of Immunological Interest*, Fifth Edition (1991), Volumes 1-3, NIH Publication 91-3242, Bethesda Md).

In the present disclosure, the heavy chain variable region comprises three complementarity determining regions HCDR1, HCDR2 and HCDR3, and the light

region is set forth in SEQ ID NO: 2; the amino acid sequence of the light chain variable region is set forth in SEQ ID NO: 4.

As a further preferred technical scheme of the monoclonal antibody against nerve growth factor of the present disclosure, the nucleotide sequence encoding the heavy chain variable region is set forth in SEQ ID NO: 1; the nucleotide sequence encoding the light chain variable region is set forth in SEQ ID NO: 3.

In one example of the monoclonal antibody against nerve growth factor of the present disclosure, the monoclonal antibody against nerve growth factor is selected from a Fab, a Fab', an F(ab')₂, an Fd, an Fv, a dAb, a complementarity determining region fragment, a single chain antibody (*e.g.*, scFv), a humanized antibody, a chimeric antibody, and a diabody.

In one example of the monoclonal antibody against nerve growth factor of the present disclosure, the monoclonal antibody against nerve growth factor binds to NGF protein with an EC₅₀ of less than 100 nM (*e.g.*, less than about 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, or less). Wherein, EC₅₀ can be measured by ELISA sandwich method.

In one example of the monoclonal antibody against nerve growth factor of the present disclosure, the monoclonal antibody against nerve growth factor comprises a non-CDR region, and the non-CDR region is from a species other than murine, for example, from a human antibody.

In order to achieve the above object of the disclosure, the present disclosure also provides a gene encoding the above monoclonal antibody against nerve growth factor.

In another aspect, the present disclosure refers to a nucleotide sequence encoding the monoclonal antibody [or antigen binding fragment thereof](#) against nerve growth factor as disclosed herein.

In order to achieve the above object of the disclosure, the present disclosure also provides a vector comprising the nucleotide sequence of the heavy chain variable region and/or the nucleotide sequence of the light chain variable region.

In another aspect, the present disclosure refers to a vector, comprising the nucleotide sequence as disclosed herein.

In order to achieve the above object of the disclosure, the present disclosure also provides a host cell comprising the nucleotide sequence of the heavy chain variable region and/or the nucleotide sequence of the light chain variable region;

or

a vector comprising the nucleotide sequence of the heavy chain variable region and/or the nucleotide sequence of the light chain variable region.

In another aspect, the present disclosure refers to a host cell, comprising the nucleotide sequence as disclosed herein, or the vector as disclosed herein.

In order to achieve the above object of the disclosure, the present disclosure also provides a method for preparing the monoclonal antibody against nerve growth factor, which includes culturing the host cell of the present disclosure under suitable conditions, and isolating the monoclonal antibody against nerve growth factor from the cell culture.

In another aspect, the present disclosure refers to a monoclonal antibody conjugate, comprising the monoclonal antibody or antigen binding fragment thereof against nerve growth factor as disclosed herein and a conjugated portion conjugated thereto, wherein the conjugated portion is a detectable label. Preferably, the conjugated portion is a radioisotope, a luminescent substance, a colored substance or an enzyme.

In another aspect, the present disclosure refers to a kit, comprising the monoclonal antibody or antigen binding fragment thereof against nerve growth factor as disclosed herein and/or the monoclonal antibody conjugate as disclosed herein.

As a preferred technical scheme of the kit of the present disclosure, the kit further comprises a secondary antibody which specifically recognizes the monoclonal antibody against nerve growth factor; in addition, the secondary antibody further comprises a detectable label, such as a radioisotope, a luminescent substance, a colored substance, or an enzyme.

The present disclosure also provides use of the monoclonal antibody against nerve growth factor and/or the monoclonal antibody conjugate in the kit that can detect the presence and/or level of nerve growth factor. The kit is used to detect the presence or level of NGF in a sample.

In another aspect, the present disclosure refers to use of the monoclonal antibody or antigen binding fragment thereof against nerve growth factor as disclosed herein and/or the monoclonal antibody conjugate as disclosed herein in preparing a kit as disclosed herein for detecting the presence and/or level of nerve growth factor.

The present disclosure also provides a drug comprising the monoclonal antibody against nerve growth factor and/or the monoclonal antibody conjugate; optionally, it further comprises pharmaceutically acceptable carriers and/or excipients.

In another aspect, the present disclosure refers to a drug, comprising the monoclonal antibody or antigen binding fragment thereof against nerve growth factor as disclosed herein and/or the monoclonal antibody conjugate as disclosed herein as an active ingredient, and a pharmaceutically acceptable carrier or excipient.

The above drug specifically binds to nerve growth factor and can be used to inhibit nerve growth factor-mediated biological effects, such as the proliferation of TF-1 cells; and/or

to treat or prevent neuropathic pain, chronic pain, and inflammatory pain.

In another aspect, the present disclosure refers to use of the ~~monoclonal antibody against nerve growth factor~~ drug as disclosed herein ~~and/or the monoclonal antibody conjugate as disclosed herein~~ in the manufacture of a medicament for:

~~specifically binding to nerve growth factor;~~

~~inhibiting the proliferation of nerve growth factor-dependent TF-1 cells;~~

~~and/or~~

treating or preventing at least one of neuropathic pain, chronic pain, and inflammatory pain and cancer pain, preferably wherein the drug specifically binds to nerve growth factor to inhibit nerve growth factor-dependent proliferation of TF-1 cells.

In another aspect, the present disclosure refers to the drug as disclosed herein for use in therapy.

The present disclosure, through *in vivo* experiments, has found that the monoclonal antibody against nerve growth factor of the present disclosure can improve the change of walking behavior of the affected limb and the condition of weight loss of the animal in the knee arthritis pain model of Lenti-IL-1 β -NIH/3T3 mice.

In the present disclosure, unless otherwise defined, the scientific and technical terms used herein have the meanings generally understood by those skilled in the art. In addition, the laboratory operations of cell culture, molecular genetics, nucleic acid chemistry and immunology used in the present disclosure are the routine operations widely used in the corresponding fields. Meanwhile, in order to better understand the present disclosure, the definitions and explanations of the relevant terms are provided below.

As used in the present disclosure, the term "antibody" refers to an immunoglobulin molecule that generally consists of two pairs of polypeptide chains (each pair with one "light" (L) chain and one "heavy" (H) chain). Antibody light chains are classified as κ and λ light chains. Heavy chains are classified as μ , δ , γ , α , or ϵ . And isotypes of antibodies are defined as IgM, IgD, IgG, IgA, and IgE, respectively. In light chains and heavy chains, the variable region and constant region are linked by a "J" region of about 12 or more amino acids, and the heavy chain also comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region consists of three domains (C_{H1} , C_{H2} and C_{H3}). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). The light chain constant region consists of one domain C_L . The constant region of the antibody can mediate the binding of immunoglobulins to host tissues or factors, including the

CLAIMS

1. _____ A monoclonal antibody against nerve growth factor, ~~comprising heavy chains (NGF)~~ comprising a heavy chain ~~comprising a heavy chain~~ constant region and a heavy chain variable region; and ~~a light chain~~ comprising a light chain constant region and a light chain variable region, ~~or an antigen binding fragment thereof~~, wherein the heavy chain variable region comprises three complementarity determining regions HCDR1, HCDR2 and HCDR3, and the light chain variable region comprises three complementarity determining regions LCDR1, LCDR2 and LCDR3;
the amino acid sequence of the complementarity determining region HCDR1 is set forth in SEQ ID NO: 5;
the amino acid sequence of the complementarity determining region HCDR2 is set forth in SEQ ID NO: 6;
the amino acid sequence of the complementarity determining region HCDR3 is set forth in SEQ ID NO: 7;
the amino acid sequence of the complementarity determining region LCDR1 is set forth in SEQ ID NO: 8;
the amino acid sequence of the complementarity determining region LCDR2 is set forth in SEQ ID NO: 9; and
the amino acid sequence of the complementarity determining region LCDR3 is set forth in SEQ ID NO: 10.
2. _____ The monoclonal antibody ~~against nerve growth factor~~ ~~or antigen binding fragment thereof~~ according to claim 1, wherein the amino acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 2; the amino acid sequence of the light chain variable region is set forth in SEQ ID NO: 4.

3. _____ The monoclonal antibody ~~against nerve growth factor~~ or antigen binding fragment thereof according to claim 1 or 2, wherein the monoclonal antibody ~~against nerve growth factor~~ is selected from a Fab, a Fab', an F(ab')₂, an Fd, an Fv, a dAb, a complementarity determining region fragment, a single chain antibody, a humanized antibody, a chimeric antibody, and a diabody.
4. _____ The monoclonal antibody ~~against nerve growth factor~~ or antigen binding fragment thereof according to ~~claim 1~~ any one of claims 1-3, wherein the monoclonal antibody ~~against nerve growth factor~~ binds to NGF ~~protein~~ with an EC₅₀ of less than 100 nM.
5. _____ A nucleotide sequence encoding the monoclonal antibody ~~against nerve growth factor~~ or antigen binding fragment thereof according to any one of claims 1-~~or 2~~-4.
6. _____ The nucleotide sequence according to claim 5, wherein the nucleotide sequence encoding the heavy chain variable region is set forth in SEQ ID NO: 1; and the nucleotide sequence encoding the light chain variable region is set forth in SEQ ID NO: 3.
7. _____ A vector_; comprising the nucleotide sequence according to ~~claims~~ claim 5 or 6.
8. _____ A host cell_; comprising the nucleotide sequence according to ~~claims~~ claim 5, or 6, or the vector of claim 7.
9. _____ A monoclonal antibody conjugate_; comprising the monoclonal antibody ~~against nerve growth factor~~ or antigen binding fragment thereof according

to any one of claims 1 ~~or 2-4~~, and a conjugated portion conjugated thereto, wherein the conjugated portion is a detectable label.

10. The monoclonal antibody conjugate of claim 9, wherein the conjugated portion is a radioisotope, a luminescent substance, a colored substance or an enzyme.

~~10~~11. A kit, comprising the monoclonal antibody or antigen binding fragment thereof against nerve growth factor according to any one of claims 1 ~~4 or 2~~ and/or the monoclonal antibody conjugate according to claim 9 or 10.

~~11~~12. The kit according to claim ~~10~~11, further comprising a secondary antibody specifically recognizing the monoclonal antibody or antigen binding fragment thereof against nerve growth factor according to any one of claims 1 ~~or 2-4~~.

~~12~~13. Use of the monoclonal antibody or antigen binding fragment thereof against nerve growth factor according to any one of claims 1 ~~4 or 2~~ and/or the monoclonal antibody conjugate according to claim 9 or 10 or the kit according to claim 11 or 12 in preparing a kit for detecting the presence and/or level of nerve growth factor.

~~13. Use of the monoclonal antibody against nerve growth factor according to claims 1 or 2 and/or the monoclonal antibody conjugate according to claim 9 in the manufacture of a medicament for:~~
~~specifically binding to nerve growth factor;~~
~~inhibiting the proliferation of nerve growth factor dependent TF-1 cells;~~
~~and/or~~
~~treating or preventing at least one of neuropathic pain, chronic pain, and inflammatory pain.~~

14. ___ A drug, comprising the monoclonal antibody ~~against nerve growth factor or~~
~~antigen binding fragment thereof~~ according to any one of claims 1 ~~or 2-4~~ and/or
the monoclonal antibody conjugate according to claim 9 or 10 as an active
ingredient, and a pharmaceutically acceptable carrier or excipient.

15. ~~The~~ Use of the drug according to claim 14, in the manufacture of a
medicament for treating or preventing at least one of neuropathic pain, chronic
pain, inflammatory pain, and cancer pain, preferably wherein the drug specifically
binds to nerve growth factor to inhibit nerve growth factor-dependent proliferation
of TF-1 cells;

~~and/or~~

~~to treat or prevent at least one of neuropathic pain, chronic pain, and inflammatory~~
~~pain.~~

16. The drug according to claim 14 for use in therapy.