



(51) International Patent Classification:

C07K 16/28 (2006.01) A61P 13/12 (2006.01)

A61K 39/395 (2006.01) A61P 19/02 (2006.01)

A61P 35/00 (2006.01) C12N 15/62 (2006.01)

A61P 19/08 (2006.01) C12N 15/63 (2006.01)

(21) International Application Number:

PCT/CN2021/131343

(22) International Filing Date:

18 November 2021 (18.11.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

202011307482.7 20 November 2020 (20.11.2020) CN

(71) Applicant: SUNSHINE LAKE PHARMA CO., LTD.

[CN/CN]; Northern Industrial Area, Songshan Lake, Dongguan, Guangdong 523000 (CN).

(72) Inventors: REN, Zhiheng; Dongyangguang Hi-tech Park,

Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). DONG, Junji; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

HE, Zhuandi; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). WANG, Kezhu; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

LU, Jielian; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). LIN, Shushan; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

LIU, Liang; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). LI, Xiang; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

ZHANG, Kuo; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). JIANG, Yan; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

LI, Xiaoping; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN). CHEN, Xiaofeng; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

LI, Wenjia; Dongyangguang Hi-tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

tech Park, Zhen An Road No. 368, Shang Sha, Chang An Town, Dongguan, Guangdong 523871 (CN).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: HUMANIZED ANTI-TRKA ANTIBODIES AND USES THEREOF

(57) Abstract: Provided is a humanized antibody or antigen-binding fragment thereof capable of specifically recognizing TrkA and uses thereof. The antibody comprises a heavy chain variable region with an amino acid sequence shown in any one of SEQ ID NO: 2-8, and a light chain variable region with an amino acid sequence shown in any one of SEQ ID NO: 10-13. The above-mentioned antibody according to the embodiments can specifically target and bind to the TrkA receptor and block the binding of NGF and TrkA.

HUMANIZED ANTI-TRKA ANTIBODIES AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This application claims the priority and benefits of Chinese Patent Application No. 202011307482.7, filed with the State Intellectual Property Office of China on November 20, 2020, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of biotechnology. Specifically, the present invention relates to humanized anti-TrkA antibodies and uses thereof. More specifically, the present invention relates to a humanized antibody or antigen-binding fragment thereof, nucleic acid molecule, expression vector, recombinant cell, pharmaceutical composition, pharmaceutical use and a kit for detecting TrkA that can specifically recognize TrkA.

BACKGROUND ART

[0003] At present, non-opioid analgesics are mainly used clinically for mild to moderate pain, such as non-steroidal anti-inflammatory drugs (NSAIDs); opioid analgesics are mainly used for moderate to severe pain. However, NSAIDs have a “ceiling effect”, and opioids only can effectively relieve less than 30% of non-tumor chronic pain, and 20% of patients with cancer pain have opioid resistance. In addition, NSAIDs have hidden dangers of gastrointestinal and cardiovascular safety, especially during long-term medication. For opioid analgesics, years of drug improvement experiments have failed to effectively reduce its addiction and many other side effects, and patients expect new safer and more effective drugs.

[0004] Nerve growth factor (NGF) is involved in the pathophysiological process of pain. It mainly activates the NGF/TrkA signaling pathway by binding to high-affinity tyrosine-nase (TrkA) receptors, which affects the release of inflammatory mediators, the opening of ion channels and the promotion of the growth of nerve fibers, thereby participating in the occurrence, conduction and sensitization process of pain. Studies have shown that blocking NGF-TrkA signaling pathway can effectively reduce pain and hyperalgesia, and NGF-TrkA signaling pathway is an effective target for the development of new analgesics. However, NGF may have a variety of undesirable agonist properties. TrkA monoclonal antibodies selectively target and bind to TrkA receptors,

which can not only block the activation of the TrkA signaling pathway by NGF, effectively inhibit the transmission of pain signals, but also avoid unpredictable side effects such as bone and joint necrosis caused by excessive neutralization of NGF by using anti-NGF antibodies. Therefore, TrkA-targeted analgesic drugs targeting NGF-TrkA may represent a better treatment option.

[0005] The therapeutic and diagnostic applications of animal-derived monoclonal antibodies in humans have basic contraindications, especially for treatment regimens that require repeated administration. Specifically, murine monoclonal antibodies have a relatively short half-life, and lack some basic functional properties of immunoglobulins when used in humans, such as complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. In addition, non-human monoclonal antibodies contain immunogenic amino acid sequences if injected into patients. Although so-called chimeric antibodies (variable murine regions linked to human constant regions) have produced some positive results, there are still immunogenicity problems.

SUMMARY

[0006] This application is based on the inventors' discovery of the following issues and facts:

[0007] The NGF-TrkA signaling pathway is an effective target for the development of new analgesics. If TrkA monoclonal antibodies selectively target and bind to TrkA receptors, it can not only block the activation of the TrkA signaling pathway by NGF, effectively inhibit the transmission of pain signals, but also avoid unpredictable side effects such as bone and joint necrosis caused by excessive neutralization of NGF by using anti-NGF antibodies. However, because the TrkA molecule is a receptor membrane protein, it is more difficult to screen for blocking anti-TrkA monoclonal antibodies. In addition, designing blocking TrkA receptor antibodies has safety risks due to antibody-mediated immune responses. Therefore, it is difficult to design and develop monoclonal antibodies against TrkA.

[0008] The inventors of the present application not only successfully screened a new type of anti-TrkA monoclonal antibody with long-acting analgesic effect, but more importantly, the inventors humanized the murine anti-TrkA monoclonal antibody that was screened out into a humanized monoclonal antibody. Specifically, the FR regions and constant regions of the murine anti-TrkA monoclonal antibodies screened by hybridoma technology were replaced with human ones, and the CDRs of the variable regions of the murine anti-TrkA monoclonal antibodies were retained, and a series of humanized monoclonal antibodies against TrkA were obtained. The

inventors found that the humanized antibody candidates obtained in this application had basically the same in vivo and in vitro activities as the human-mouse chimeric anti-TrkA monoclonal antibody 23E12, and not only could specifically target and bind to TrkA receptors, block the binding of NGF and TrkA, effectively inhibit pain, but also had lower immunogenicity and better pharmacokinetic parameters than the human-mouse chimeric anti-TrkA monoclonal antibody.

[0009] Among them, the human-mouse chimeric anti-TrkA monoclonal antibody 23E12 had the heavy chain variable region VH0 with the amino acid sequence shown in SEQ ID NO:1 and the light chain variable region VL0 with the amino acid sequence shown in SEQ ID NO:9.

[0010] In the first aspect of the present invention, the present invention provides a humanized antibody or antigen-binding fragment thereof capable of specifically recognizing TrkA. According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:42 or SEQ ID NO:43, and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:46 or SEQ ID NO:47, and the VL-CDR3 shown in SEQ ID NO:48.

GYAFTNYWLG (SEQ ID NO: 41).

DFYPRTGNTF (SEQ ID NO: 42).

GFYPRTGNTF (SEQ ID NO: 43).

ARAGTGFDY (SEQ ID NO: 44).

ENVGGYVS (SEQ ID NO: 45).

GASSRHT (SEQ ID NO: 46).

GASSRAT (SEQ ID NO: 47).

NYIYPFT (SEQ ID NO: 48).

[0011] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:42 and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:46 and the VL-CDR3 shown in SEQ ID NO:48.

[0012] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in

SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:43 and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:46 and the VL-CDR3 shown in SEQ ID NO:48.

[0013] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:42 and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:47 and the VL-CDR3 shown in SEQ ID NO:48.

[0014] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:43, and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:47 and the VL-CDR3 shown in SEQ ID NO:48.

[0015] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8, and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13. In the present application, the variable region includes murine CDRs and human framework regions.

[0016] In this application, SEQ ID NO: 2-8 are called VH1-VH7 in turn. SEQ ID NO: 10-13 are called VL1-VL4 in turn.

VH0:QVQLQQSGAELVRPGTSVKISCKASGYAFTNYWLGWMKQRPGHGLEWIGDF
YPRGTGNTFYNNFNGKGVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQG
TTLTVSS(SEQ ID NO:1).

VH1:EVQLLESQGGGLVQPGGSLKLSCKASGYAFTNYWLGWMKQRPGHGLEWIGDF
YPRGTGNTFYNNFNGKGVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQG
TTLTVSS(SEQ ID NO:2).

VH2:EVQLLESQGGGLVQPGGSLKLSCKASGYAFTNYWLGWMKQRPGHGLEWIGGF
YPRGTGNTFYNNFNGKGVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQG
TTLTVSS(SEQ ID NO:3).

VH3:EVQLLES^{GGGLVQPGGSLKLSCKAS}GYAFTNYWLGWMKQRP^{GHGLEWIGDF}
YPRTGNTFYNENFKGQVTMSVDKSITTAYLQWNSLKASDTAMYYCCARAGTGFDYWGQ
 GTTLTVSS(SEQ ID NO:4).

VH4:QVQLVQSGVEVKKPGASVKV^{SCKAS}GYAFTNYWLGWMKQRP^{GHGLEWIGD}
FYPRTGNTFYNENFKGQVTMSVDKSITTAYLQWNSLKASDTAMYYCCARAGTGFDYWG
 QGTTTLTVSS(SEQ ID NO:5).

VH5:QVQLVQSGVEVKKPGASVKV^{SCKAS}GYAFTNYWLGWMKQRP^{GHGLEWIGD}
FYPRTGNTFYNENFKGKVTITADKSITTAYMQLSSLKASDTAVYYCCARAGTGFDYWGQ
 TTLTVSS(SEQ ID NO:6).

VH6:QVQLVQSGVEVKKPGASVKV^{SCKAS}GYAFTNYWLGWVKQRP^{GHGLEWIGD}
FYPRTGNTFYNENFKGKVTITADKSITTAYMQLSSLKASDTAVYYCCARAGTGFDYWGQ
 TTLTVSS(SEQ ID NO:7).

VH7:QVQLVQSGVEVKKPGASVKV^{SCKAS}GYAFTNYWLGWMKQRP^{GHGLEWIGD}
FYPRTGNTFYNENFKGKVTLTADKSSNTAYMQLSSLTSEDSAVYLCCARAGTGFDYWGQ
 TTLTVSS(SEQ ID NO:8).

VL0:SIVMTQSPKSMSSVGERVTL^{SCKASE}ENVGGYVSWYQQKPDQSPKLLIYGAS
SRHTGVPDRFTGSGSETDFTLTISSVQAEDLAAYHCGQNYIYPFTFGGGTKLEIK(SEQ ID
 NO:9).

VL1:EIVMTQSPATLSLSVGERVTL^{SCKASE}ENVGGYVSWYQQKPDQSPKLLIYGASS
RHTGVPDRFTGSGSETDFTLTISSVQAEDLAAYHCGQNYIYPFTFGGGTKLEIK(SEQ ID
 NO:10).

VL2:EIVMTQSPATLSLSVGERVTL^{SCKASE}ENVGGYVSWYQQKPDQSPKLLIYGASS
RATGVPDRFTGSGSETDFTLTISSVQAEDLAAYHCGQNYIYPFTFGGGTKLEIK(SEQ ID
 NO:11).

VL3:EIVMTQSPATLSLSVGERVTL^{SCKASE}ENVGGYVSWYQQKPDQSPKLLIYGASS
RHTGVPARFSGSGSGTDFTLTISSLEPEDFAVYHCGQNYIYPFTFGGGTKLEIK(SEQ ID
 NO:12).

VL4:EIVLTQSPATLSLSPGERATL^{SCKASE}ENVGGYVSWYQQKPDQSPKLLIYGASSR
HTGVPDRFTGSGSETDFTLTISSVQAEDLAAYHCGQNYIYPFTFGGGTKLEIK(SEQ ID
 NO:13).

[0017] Among them, the underlined parts are the CDR sequence of the heavy chain variable region and the CDR sequence of the light chain variable region, respectively.

[0018] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region

selected from:

- (a) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 2 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 10;
- (b) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 4 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 10; or
- (c) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 4 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 11.

[0019] According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof specifically recognizes the extracellular region of TrkA.

[0020] According to an embodiment of the present invention, the antibody comprises at least one of a heavy chain framework region sequence and a light chain framework region sequence, and both the heavy chain framework region sequence and the light chain framework region sequence are derived from human IgG antibodies or their mutants. Furthermore, the immunogenicity of the antibody can be effectively reduced.

[0021] According to an embodiment of the present invention, the light chain constant region of the antibody is derived from a human Kappa light chain constant region; the heavy chain constant region is derived from a human IgG4 heavy chain constant region.

[0022] According to an embodiment of the present invention, the Fc region of the antibody has S10P, F16A, L17A, R191K mutations and 229 K deletion mutations compared with human IgG4 wild-type Fc. Wherein, the location of the above-mentioned amino acid position is based on the amino acid sequence shown in SEQ ID NO: 16 of the human IgG4 wild-type Fc sequence. For example, S10P means that the 10th S of the amino acid sequence shown in SEQ ID NO: 16 is mutated to P, and so on. The inventors found that after the Fc region of the antibody had the above mutations and deletions, the safety and stability of the antibody could be significantly improved, and the half-life of the antibody in the body was also significantly prolonged.

ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK(SEQ ID
NO:16).

[0023] According to an embodiment of the present invention, the full-length sequence of the

constant region of the antibody is as shown in SEQ ID NO: 14 or 15.

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:14).

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:15).

[0024] Wherein, the full-length sequence of the constant region of the antibody shown in SEQ ID NO: 14 is an IgG4 light chain constant region. The full-length sequence of the constant region of the antibody shown in SEQ ID NO: 15 includes the IgG4 heavy chain constant region and the Fc region, wherein the IgG4 heavy chain constant region sequence is ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRV, the sequence of the Fc region is ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG.

[0025] According to an embodiment of the present invention, the antibody comprises a heavy chain with the amino acid sequence shown in any one of SEQ ID NO: 17-23 and a light chain with the amino acid sequence shown in any one of SEQ ID NO: 24-27. In this application, SEQ ID NO: 17-23 are called H1-H7 in turn. SEQ ID NO: 24-27 are called L1-L4 in turn. In addition, the human-mouse chimeric anti-TrkA monoclonal antibody 23E12 has a heavy chain H0 with the amino acid sequence shown in SEQ ID NO: 28 and a light chain L0 with the amino acid sequence shown in SEQ ID NO: 29.

H1:EVQLLESQGGGLVQPQGGSLKLCKASGYAFTNYWLGWMKQRPQGHGLEWIGDFYPRGTNTFYNENFKGKVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQ

FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:17).

H2:EVQLLESGGGLVQPGGSLKLCKASGYAFTNYWLGWMKQRPBGHGLEWIGGFY
PRTGNTFYNNFKGKVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQGT
LTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:18).

H3:EVQLLESGGGLVQPGGSLKLCKASGYAFTNYWLGWMKQRPBGHGLEWIGDFY
PRTGNTFYNNFKGQVTMSVDKSITTAYLQWNSLKASDTAMYYCARAGTGFDYWGQG
TTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
AVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:19).

H4:QVQLVQSGVEVKKPGASVKVSCKASGYAFTNYWLGWMKQRPBGHGLEWIGDF
YPRTGNTFYNNFKGQVTMSVDKSITTAYLQWNSLKASDTAMYYCARAGTGFDYWGQ
GTTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:20).

H5:QVQLVQSGVEVKKPGASVKVSCKASGYAFTNYWLGWMKQRPBGHGLEWIGDF
YPRTGNTFYNNFKGKVTITADKSITTAYMQLSSLKASDTAVYYCARAGTGFDYWGQGT
TLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAA
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS

QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:21).

H6:QVQLVQSGVEVKKPGASVKVSCKASGYAFTNYWLGWVKQRPGHGLEWIGDF
YPRTGNTFYNNENFKGKVTITADKSITTAYMQLSSLKASDTAVYYCARAGTGFDYWGGQT
TLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAA
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:22).

H7:QVQLVQSGVEVKKPGASVKVSCKASGYAFTNYWLGWMKQRPGHGLEWIGDF
YPRTGNTFYNNENFKGKVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGGQ
TTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG(SEQ ID NO:23).

L1:EIVMTQSPATLSLISVGERVTLISCKASENVGGYVSWYQQKPDQSPKLLIYGASSR
HTGVPDRFTGSGSETDFTLTISVQAEDLAAYHCGQNYIYPFTFGGGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:24).

L2:EIVMTQSPATLSLISVGERVTLISCKASENVGGYVSWYQQKPDQSPKLLIYGASSR
ATGVPDRFTGSGSETDFTLTISVQAEDLAAYHCGQNYIYPFTFGGGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:25).

L3:EIVMTQSPATLSLISVGERVTLISCKASENVGGYVSWYQQKPDQSPKLLIYGASSR
HTGVPARFSGSGSGTDFTLTISLEPEDFAVYHCGQNYIYPFTFGGGTKLEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:26).

L4:EIVLTQSPATLSLSPGERATLSCKASENVGGYVSWYQQKPDQSPKLLIYGASSRH

TGVPDRFTGSGSETDFTLTISVQAEDLAAYHCGQNYIYPFTFGGGTKLEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:27).

H0:QVQLQQSGAELVRPGTSVKISCKASGYAFTNYWLGWMKQRPBGHGLEWIGDFY
PRTGNTFYNNFKGKVTLTADKSSNTAYMQLSSLTSEDSAVYLCARAGTGFDYWGQGT
LTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVESKYGPPCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQEGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO:28).

L0:SIVMTQSPKSMMSVGERVTLSCKASENVGGYVSWYQQKPDQSPKLLIYGASS
RHTGVPDRFTGSGSETDFTLTISVQAEDLAAYHCGQNYIYPFTFGGGTKLEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:29).

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:49).

[0026] The full-length sequence of the constant region of the antibody shown in SEQ ID NO: 49 includes the IgG1 heavy chain constant region and the Fc region, wherein the IgG1 heavy chain constant region sequence is ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV and the Fc region sequence is EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

[0027] According to an embodiment of the present invention, the antibody comprises a heavy chain and a light chain selected from the group selected from:

- (a) the heavy chain with the amino acid sequence shown in SEQ ID NO: 17 and the light chain with the amino acid sequence shown in SEQ ID NO: 24;
- (b) the heavy chain with the amino acid sequence shown in SEQ ID NO: 19 and the light chain with the amino acid sequence shown in SEQ ID NO: 24; or
- (c) the heavy chain with the amino acid sequence shown in SEQ ID NO: 19 and the light chain with the amino acid sequence shown in SEQ ID NO: 25.

[0028] In this application, the humanized monoclonal antibody composed of the above-mentioned H1 and L1 with the IGHG4 heavy chain isotype and the Kappa isotype light chain is called H1L1-IgG4, the antibody composed of the above H3 and L1 with the IGHG4 heavy chain isotype and the Kappa isotype light chain is called H3L1-IgG4, and the antibody composed of the above H3 and L2 with the IGHG4 heavy chain isotype and the Kappa isotype light chain is called H3L2-IgG4, the humanized monoclonal antibody composed of the above-mentioned H1 and L1 with the IGHG1 heavy chain isotype and the Kappa isotype light chain is called H1L1-IgG1, and so on.

[0029] According to an embodiment of the present invention, the antibody is a single-chain antibody, a multimeric antibody, or a CDR-grafted antibody.

[0030] According to an embodiment of the present invention, the single-chain antibody comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8 and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13, wherein the C-terminus of the heavy chain variable region is connected to the N-terminus of the light chain variable region through a connecting peptide linker, or the C-terminus of the light chain variable region is connected to the N-terminus of the heavy chain variable region through a connecting peptide linker. It should be noted that the “connecting peptide linker” of the single-chain antibody described in this application is a connecting peptide used to connect the heavy chain variable region and the light chain variable region of the antibody. It can be a commonly used connecting peptide linker for the preparation of single-chain antibodies, or it can be a connecting peptide linker modified by scientific researchers. In some embodiments, the connecting peptide may be a G-rich polypeptide, for example, it may be selected from (G)₃-S (*i.e.*, “GGGS”), (G)₄-S (*i.e.*, “GGGGS”) and (G)₅-S (*i.e.*, “GGGGGS”), such as GGGGSGGGGSGGGGS.

[0031] According to an embodiment of the present invention, the antigen-binding fragment

comprises at least one of Fab, Fab', F(ab)₂, F(ab')₂, Fv, scFv-Fc fusion protein, scFv-Fv fusion protein, and minimum recognition unit.

[0032] In the second aspect of the present invention, the present invention provides a nucleic acid molecule. According to an embodiment of the present invention, the nucleic acid molecule encodes the aforementioned antibody or antigen-binding fragment thereof. The antibody or antigen-binding fragment encoded by the nucleic acid molecule according to the embodiment of the present invention can specifically target and bind to TrkA and block the binding of NGF and TrkA.

[0033] According to an embodiment of the present invention, the aforementioned nucleic acid molecule may further include at least one of the following additional technical features:

[0034] According to an embodiment of the present invention, the nucleic acid molecule is DNA.

[0035] According to an embodiment of the present invention, the nucleic acid molecule comprises the nucleotide sequence shown in any one of SEQ ID NO: 30-36 or comprises the nucleotide sequence shown in any one of SEQ ID NO: 37-40.

GAGGTGCAGCTGCTGGAGTCTGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCT
GAAGCTGTCCTGCAAGGCCAGCGGCTACGCTTTCACCAACTATTGGCTGGGATGGAT
GAAGCAGAGGCCAGGACACGGACTGGAGTGGATCGGCGACTTTTACCCTCGGACCG
GCAACACATTCTATAACGAGAACTTCAAGGGCAAGGTGACCCTGACAGCCGATAAGT
CCAGCAATACCGCTTACATGCAGCTGTCTTCCCTGACATCCGAGGACTCCGCCGTGTA
CCTGTGCGCTAGGGCTGGAACCGGATTTCGATTATTGGGGCCAGGGCACCACTGAC
AGTGAGCTCTGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTGTAGCCG
GTCCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGA
ACCCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGACACCTTCCC
CGCTGTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCT
CCAGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACC
AAGGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTGTCCCCCTTGCCCCGCC
CCCGAGGCCGCTGGCGGACCCTCCGTGTTCTTCTTCCCCCCTTGAACCAAGGACACT
TTAATGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCCAAGAA
GACCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAA
GACCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCAC

CGTGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACA
AGGGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGG
GAACCTCAAGTTTATACACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTT
TCTTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGT
CCAATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCGATG
GTTCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAA
CGTGTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCT
TTATCTCTGTCTTTAGGC(SEQ ID NO:30).

GAGGTGCAGCTGCTGGAGAGCGGGCGGCGGCCTGGTGCAGCCCGGCGGCAGCCT
GAAGCTGAGCTGCAAGGCCAGCGGCTACGCCTTCACCAACTACTGGCTGGGCTGGAT
GAAGCAGAGGCCCCGGCCACGGCCTGGAGTGGATCGGCGGCTTCTACCCAGGACCG
GCAACACCTTCTACAACGAGAACTTCAAGGGCAAGGTGACCCTGACCGCCGACAAG
AGCAGCAACACCGCCTACATGCAGCTGAGCAGCCTGACCAGCGAGGACAGCGCCGT
GTACCTGTGCGCCAGGGCCGGCACCGGCTTCGACTACTGGGGCCAGGGCACCACT
GACCGTGAGCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTGTAG
CCGGTCCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCC
CGAACCCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTT
CCCCGCTGTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTT
CCTCCAGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACA
CCAAGGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTGTCCCCCTTGCCCCG
CCCCGAGGCCGCTGGCGGACCCTCCGTGTTCTCTTCCCCCCCCAAACCCAAGGACA
CTTTAATGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCAAG
AAGACCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCC
AAGACCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTC
ACCGTGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAA
CAAGGGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCC
GGGAACCTCAAGTTTATACACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAA
GTTTCTTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGG
AGTCCAATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCG
ATGGTTCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGG
CAACGTGTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAA

GTCTTTATCTCTGTCTTTAGGC(SEQ ID NO:31).

GAGGTGCAGCTGCTGGAGTCCGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCT
GAAGCTGTCCTGCAAGGCCAGCGGCTACGCTTTCACCAACTATTGGCTGGGATGGAT
GAAGCAGAGGCCAGGACACGGACTGGAGTGGATCGGCGACTTTTACCCTCGGACCG
GCAATACATTCTATAACGAGAACTTCAAGGGCCAGGTGACAATGTCTGTGGATAAGTC
CATCACCACAGCCTACCTGCAGTGGAAACAGCCTGAAGGCCTCTGACACCGCTATGTA
CTATTGTGCCAGGGCTGGCACAGGCTTCGATTATTGGGGCCAGGGCACCACACTGAC
CGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCTCTGGCTCCTTGTAGCCG
GTCCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGA
ACCCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTTCCC
CGCTGTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCT
CCAGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACC
AAGGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTGTCCCCCTTGCCCCGCC
CCCGAGGCCGCTGGCGGACCCTCCGTGTTCTTCCCCCCCCAAACCAAGGACACT
TTAATGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCCAAGAA
GACCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAA
GACCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCAC
CGTGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACA
AGGGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGG
GAACCTCAAGTTTATACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTT
TCTTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGT
CCAATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCGATG
GTTCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAA
CGTGTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCT
TTATCTCTGTCTTTAGGC(SEQ ID NO:32).

CAAGTGCAACTGGTTCAATCTGGAGTGGAAAGTTAAGAAGCCTGGTGCCAGCGTT
AAAGTGAGTTGCAAAGCCAGCGGATATGCCTTTACCAACTATTGGCTGGGCTGGATG
AAACAGAGGCCTGGCCATGGTCTGGAATGGATCGGAGACTTTTATCCACGCACCGGC
AACACATTCTATAACGAGAACTTCAAAGGTCAGGTGACCATGTCCGTGGATAAGAGC
ATCACTACCGCTTACCTCCAGTGGAAACAGTCTGAAGGCTTCTGACACCGCCATGTACT
ACTGCGCTAGGGCAGGCACCGGGTTCGACTACTGGGGTCAAGGGACCACCTCACC

GTGAGTAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTG TAGCCGG
TCCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAA
CCCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTTCCCC
GCTGTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCTC
CAGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACCA
AGGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTG TCCCCCTTGCCCCGCCC
CCGAGGCCGCTGGCGGACCCTCCGTGTTCTCTTCCCCCCCCAAACCCAAGGACACTT
TAATGATCTCCCGGACCCCCGAAGTGACTTG TGTGGTGGTGGACGTGTCCCAAGAAG
ACCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAAG
ACCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCACC
GTGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAA
GGGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGGG
AACCTCAAGTTTATACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTTT
CTTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGTC
CAATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCGTGCTGGACTCCGATGG
TTCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAAC
GTGTTCTCTTG TAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCTT
TATCTCTGTCTTTAGGC(SEQ ID NO:33).

CAAGTGCAGCTGGTTCAAAGTGGTGTGAAGTTAAGAAGCCTGGAGCTAGTGTG
AAGGTGTCCTGTAAGGCCTCCGGCTATGCCTTTACAACTACTGGCTCGGGTGGATGA
AGCAGCGCCCAGGACACGGTCTGGAATGGATTGGCGACTTTTACCCACGGACAGGA
AATACATTCTATAATGAAACTTCAAAGGCCAAAGTGACCATCACAGCCGATAAGTCCA
TTACCACTGCATACATGCAGCTCAGTAGTCTCAAAGCTAGTGATACAGCAGTGTATTA
CTGCGCCAGGGCCGGCACCGGGTTCGACTACTGGGGGCAGGGAACCAACCTCACCG
TGAGCTCTGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTG TAGCCGGT
CCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAAC
CCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTTCCCCG
CTGTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCTCC
AGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACCAA
GGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTG TCCCCCTTGCCCCGCCCC
CGAGGCCGCTGGCGGACCCTCCGTGTTCTCTTCCCCCCCCAAACCCAAGGACACTTT

AATGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCCAAGAAGA
CCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAAGA
CCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCACCG
TGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAG
GGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGGGA
ACCTCAAGTTTATACACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTTTC
TTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGTCC
AATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCGATGGT
TCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAACG
TGTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCTTT
ATCTCTGTCTTTAGGC(SEQ ID NO:34).

CAAGTCCAACTGGTTCAATCTGGCGTGGAAGTCAAGAAGCCCGGAGCCTCCGTG
AAGGTGAGCTGCAAGGCAAGCGGCTATGCATTCACTAACTACTGGCTCGGATGGGTG
AAACAACGGCCAGGACATGGCCTGGAATGGATCGGCGACTTCTACCCTAGGACTGGC
AACACTTTCTATAACGAGAACTTTAAGGGCAAGGTCACCATTACAGCTGATAAGAGTA
TCACTACCGCCTACATGCAGCTGTCTTCCCTGAAAGCTAGTGATACAGCCGTTTATTAC
TGTGCTCGGGCTGGCACAGGATTCGATTATTGGGGACAGGGTACCACACTCACAGTG
TCCTCTGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTG TAGCCGGTCC
ACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAACCC
GTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTTCCCCGCT
GTGCTCCAGTCCTCCGGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCTCCAG
CCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACCAAGG
TGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTG TCCCCCTTGCCCCGCCCCCG
AGGCCGCTGGCGGACCCTCCGTGTTCTCTTCCCCCCCCAAACCCAAGGACACTTTAA
TGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCCAAGAAGACC
CCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAAGACC
AAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCACCGTG
CTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGG
ACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGGGAAC
CTCAAGTTTATACACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTTTCTT
TAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGTCCA

ATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCGATGGTT
CTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAACGT
GTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCTTTA
TCTCTGTCTTTAGGC(SEQ ID NO:35).

CAAGTTCAGCTGGTGCAATCCGGTGTCTGAGGTGAAGAAACCAGGCGCAAGCGT
GAAAGTCTCCTGCAAGGCTTCTGGCTATGCCTTCACTAACTACTGGCTCGGCTGGATG
AAGCAGAGGCCCGGACATGGGCTGGAGTGGATCGGAGACTTCTATCCCAGAACTGG
AAACACCTTTTACAACGAGAATTTCAAGGGCAAGGTCACCCTGACTGCCGACAAATC
CTCTAACACAGCTTACATGCAGCTGAGCAGTCTGACATCCGAAGACTCTGCAGTTTAC
CTGTGTGCTCGGGCAGGCACAGGCTTCGATTATTGGGGGCAAGGGACCACTCTGACT
GTGTCTTCCGCCAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCTCCTTGTAGCCGG
TCCACCTCCGAGTCCACAGCTGCTCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAA
CCCGTTACCGTGAGCTGGAATAGCGGCGCTTTAACCTCCGGAGTGCACACCTTCCCC
GCTGTGCTCCAGTCCTCCGTTTATACTCTTTATCCTCCGTGGTGACCGTGCCTTCCTC
CAGCCTCGGCACCAAGACCTACACTTGTAACGTGGACCACAAGCCCAGCAACACCA
AGGTGGACAAGAGGGTGGAGTCCAAGTACGGACCTCCTTGTCCCCCTTGCCCCGCCC
CCGAGGCCGCTGGCGGACCCTCCGTGTTCTTCCCCCCCCAAACCCAAGGACACTT
TAATGATCTCCCGGACCCCCGAAGTGACTTGTGTGGTGGTGGACGTGTCCCAAGAAG
ACCCCGAGGTGCAGTTTAACTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAAG
ACCAAGCCTAGGGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTCACC
GTGCTGCATCAAGATTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAA
GGGACTGCCCAGCTCCATCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCCGGG
AACCTCAAGTTTATACACTGCCCCCAGCCAAGAAGAGATGACCAAGAACCAAGTTT
CTTTAACTTGTTTAGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGGAGTGGGAGTC
CAATGGCCAGCCCGAAAACAATTATAAGACCACCCCCCCCCGTGCTGGACTCCGATGG
TTCTTTTTTTTTTATACTCCAAGCTGACAGTGGACAAGTCTCGTTGGCAAGAAGGCAAC
GTGTTCTCTTGTAGCGTGATGCACGAGGCTTTACACAACCACTACACCCAGAAGTCTT
TATCTCTGTCTTTAGGC(SEQ ID NO:36).

GAGATCGTGATGACCCAGTCCCCAGCCACACTGAGCCTGTCTGTGGGAGAGAGG
GTGACCCTGTCTTGCAAGGCTTCCGAGAACGTGGGCGGCTACGTGAGCTGGTATCAG
CAGAAGCCCGACCAGTCTCCTAAGCTGCTGATCTACGGAGCCTCCAGCAGGCACACA

GGAGTGCCAGACCGGTTACCGGATCCGGAAGCGAGACAGACTTCACCCTGACAAT
CTCTTCCGTGCAGGCTGAGGATCTGGCCGCTTATCATTGTGGCCAGAATTACATCTATC
CCTTCACCTTTGGCGGCGGCACAAAGCTGGAGATCAAGCGGACCGTGGCTGCCCCCT
CCGTGTTTCATCTTCCCCCTTCCGACGAGCAGCTGAAGTCCGGCACCGCTAGCGTGG
TGTGTTTACTGAACAACTTCTACCCTCGTGAGGCCAAGGTGCAGTGGAAGGTGGACA
ACGCTTTACAGTCCGGCAACTCCCAAGAATCCGTGACCGAGCAAGATTCCAAGGACT
CCACCTACTCTTTATCCTCCACTTTAACTTTATCCAAGGCCGACTACGAGAAGCACAA
GGTGTACGCTTGTGAGGTGACCCATCAAGGTTTATCCTCCCCCGTGACCAAGTCCTTC
AATCGTGGCGAGTGC(SEQ ID NO:37).

GAGATCGTGATGACCCAGTCCCCAGCCACACTGAGCCTGTCTGTGGGAGAGAGG
GTGACCCTGTCTTGCAAGGCTTCCGAGAACGTGGGCGGCTACGTGAGCTGGTATCAG
CAGAAGCCCGACCAGTCTCCTAAGCTGCTGATCTACGGAGCCTCCAGCAGGGCTACA
GGAGTGCCAGACCGGTTACCGGATCCGGAAGCGAGACAGACTTCACCCTGACAAT
CTCTTCCGTGCAGGCCGAGGATCTGGCCGCTTATCACTGTGGCCAGAATTACATCTAT
CCCTTCACCTTTGGCGGCGGCACAAAGCTGGAGATCAAGCGGACCGTGGCTGCCCCC
TCCGTGTTTCATCTTCCCCCTTCCGACGAGCAGCTGAAGTCCGGCACCGCTAGCGTG
GTGTGTTTACTGAACAACTTCTACCCTCGTGAGGCCAAGGTGCAGTGGAAGGTGGAC
AACGCTTTACAGTCCGGCAACTCCCAAGAATCCGTGACCGAGCAAGATTCCAAGGAC
TCCACCTACTCTTTATCCTCCACTTTAACTTTATCCAAGGCCGACTACGAGAAGCACA
AGGTGTACGCTTGTGAGGTGACCCATCAAGGTTTATCCTCCCCCGTGACCAAGTCCTT
CAATCGTGGCGAGTGC(SEQ ID NO:38).

GAGATCGTGATGACCCAGAGCCCTGCCACACTGAGCCTGTCTGTGGGCGAGAGG
GTGACCCTGTCCTGCAAGGCCTCCGAGAACGTGGGCGGCTACGTGTCTTGGTATCAG
CAGAAGCCCGACCAGTCCCCTAAGCTGCTGATCTACGGAGCCTCCAGCAGGCACACC
GGAGTGCCAGCTCGGTTCTCCGGAAGCGGATCTGGCACAGACTTTACCCTGACAATC
TCTTCCCTGGAGCCAGAGGATTCGCCGTGTATCATTGTGGCCAGAATTACATCTATCC
CTTCACCTTTGGCGGCGGCACAAAGCTGGAGATCAAGCGGACCGTGGCTGCCCCCTC
CGTGTTTCATCTTCCCCCTTCCGACGAGCAGCTGAAGTCCGGCACCGCTAGCGTGGT
GTGTTTACTGAACAACTTCTACCCTCGTGAGGCCAAGGTGCAGTGGAAGGTGGACAA
CGCTTTACAGTCCGGCAACTCCCAAGAATCCGTGACCGAGCAAGATTCCAAGGACTC
CACCTACTCTTTATCCTCCACTTTAACTTTATCCAAGGCCGACTACGAGAAGCACAAG

GTGTACGCTTGTGAGGTGACCCATCAAGGTTTATCCTCCCCCGTGACCAAGTCCTTCA
ATCGTGGCGAGTGC(SEQ ID NO:39).

GAAATTGTGCTGACTCAGTCTCCTGCTACTCTGTCCCTGTCTCCTGGTGAACGGG
CCACTCTGAGCTGCAAGGCCAGTGAAAATGTGGGTGGCTATGTTAGCTGGTATCAGC
AAAAGCCCGACCAGTCTCCCAAAGTCTGCTGATCTACGGCGCTTCCAGTCGGCACACAG
GCGTGCCAGATCGCTTTACTGGGAGCGGCTCTGAGACTGACTTCACACTGACCATTA
GCAGTGTCCAGGCCGAAGATCTCGCAGCCTATCATTGCGGCCAGAACTACATCTATCC
ATTCACCTTCGGTGGAGGAACCAAAGTGGAAATCAAGCGGACCGTGGCTGCCCCCTC
CGTGTTTCATCTTCCCCCTTCCGACGAGCAGCTGAAGTCCGGCACCGCTAGCGTGGT
GTGTTTACTGAACAACCTTCTACCCTCGTGAGGCCAAGGTGCAGTGGAAGGTGGACAA
CGCTTTACAGTCCGGCAACTCCCAAGAATCCGTGACCGAGCAAGATTCCAAGGACTC
CACCTACTCTTTATCCTCCACTTTAACTTTATCCAAGGCCGACTACGAGAAGCACAAG
GTGTACGCTTGTGAGGTGACCCATCAAGGTTTATCCTCCCCCGTGACCAAGTCCTTCA
ATCGTGGCGAGTGC(SEQ ID NO:40).

[0036] The nucleotide sequences shown in SEQ ID NO: 30-36 respectively encode heavy chains H1-H7, and the nucleotide sequences shown in SEQ ID NO: 37-40 respectively encode light chains L1-L4. The underlined parts encode heavy chain variable regions VH1~VH7, and light chain variable regions VL1~VL4, respectively.

[0037] In the third aspect of the present invention, the present invention provides an expression vector. According to an embodiment of the present invention, the expression vector carries the aforementioned nucleic acid molecule. After the expression vector according to the embodiment of the present invention is introduced into a suitable recipient cell, it can effectively realize the expression of the humanized antibody or its antigen-binding fragment that specifically recognizes TrkA under the mediation of the regulatory system, thereby realizing the large-scale in vitro acquisition of the humanized antibody or antigen-binding fragment.

[0038] According to an embodiment of the present invention, the aforementioned expression vector may further include at least one of the following additional technical features:

[0039] According to an embodiment of the present invention, the expression vector is a eukaryotic expression vector. Furthermore, the aforementioned humanized antibody or its antigen-binding fragment that specifically recognizes TrkA can be expressed in eukaryotic cells, such as CHO cells.

[0040] In the fourth aspect of the present invention, the present invention provides a recombinant cell. According to an embodiment of the present invention, the recombinant cell carries the aforementioned nucleic acid molecule, or expresses the aforementioned humanized antibody or antigen-binding fragment thereof. The recombinant cells according to the embodiments of the present invention can be used for in vitro expression and large-scale acquisition of the aforementioned humanized antibody or antigen-binding fragment thereof that specifically recognizes TrkA.

[0041] According to an embodiment of the present invention, the aforementioned recombinant cell may further include at least one of the following additional technical features:

[0042] According to an embodiment of the present invention, the recombinant cell is obtained by introducing the aforementioned expression vector into a host cell.

[0043] According to an embodiment of the present invention, wherein the expression vector is introduced into the host cell by electrotransduction.

[0044] According to an embodiment of the present invention, the recombinant cell is a eukaryotic cell.

[0045] According to an embodiment of the present invention, the recombinant cell is a mammalian cell.

[0046] In the fifth aspect of the present invention, the present invention provides a pharmaceutical composition. According to an embodiment of the present invention, the pharmaceutical composition comprises the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector or the aforementioned recombinant cell. The humanized antibody or expressed humanized antibody contained in the pharmaceutical composition according to the embodiment of the present invention has the same in vivo and in vitro activities as the human-mouse chimeric anti-TrkA monoclonal antibody 23E12. It not only can specifically target and bind to TrkA receptor, block the binding of NGF and TrkA, effectively inhibit pain, basically without the characteristics of antibody-dependent cell-mediated cytotoxicity (ADCC), but also has lower immunogenicity and better pharmacokinetic parameters than the human-mouse chimeric anti-TrkA monoclonal antibody 23E12.

[0047] In the sixth aspect of the present invention, the present invention provides use of the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector, the aforementioned recombinant cell, or the aforementioned pharmaceutical

composition in the manufacture of a medicament for the treatment or prevention of pain, cancer, inflammation or inflammatory diseases, neurodegenerative diseases, Sjogren's syndrome, endometriosis, diabetic peripheral neuropathy, prostatitis, pelvic pain syndrome, and diseases related to the unbalanced regulation of bone remodeling, and diseases caused by abnormal signal conduction of connective tissue growth factor.

[0048] According to an embodiment of the present invention, the aforementioned use may further include at least one of the following additional technical features:

[0049] According to an embodiment of the present invention, the medicament is used to treat or prevent neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

[0050] In the sixth aspect of the present invention, the present invention provides a method of treating or preventing a disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA in a subject comprising administering to the subject a therapeutically effective amount of the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector, the aforementioned recombinant cell, or the aforementioned pharmaceutical composition.

[0051] According to an embodiment of the present invention, the disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA includes neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis,

scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

[0052] In the sixth aspect of the present invention, the present invention provides the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector, the aforementioned recombinant cell, or the aforementioned pharmaceutical composition for use in treating or preventing a disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA in a subject.

[0053] According to an embodiment of the present invention, the present invention provides the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector, the aforementioned recombinant cell, or the aforementioned pharmaceutical composition for use in treating or preventing a disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA in a subject, wherein the disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA includes neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

[0054] In the seventh aspect of the present invention, the present invention provides a kit for detecting TrkA. According to an embodiment of the present invention, the kit includes any one of the aforementioned antibodies. The aforementioned TrkA antibody can specifically target and bind to TrkA. The kit according to the embodiment of the present invention can realize the specific detection of TrkA. For example, when the antibody is bound with a fluorescent group, a fluorescent detection device can be used to realize the localization or real-time detection of TrkA.

[0055] In the eighth aspect of the present invention, the present invention provides use of the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector or the aforementioned recombinant cell in the preparation of a kit for detecting TrkA or diagnosing a TrkA-related disease.

[0056] In the eighth aspect of the present invention, the present invention provides a method of detecting TrkA or diagnosing a TrkA-related disease in a subject using a kit comprising the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector or the aforementioned recombinant cell.

[0057] In the eighth aspect of the present invention, the present invention provides the aforementioned antibody, the aforementioned nucleic acid molecule, the aforementioned expression vector or the aforementioned recombinant cell for use in the preparation of a kit for detecting TrkA or diagnosing a TrkA-related disease.

DESCRIPTION OF THE DRAWINGS

[0058] Figure 1 is a diagram showing the results of the purity of monomer of humanized antibodies evaluated by the SEC-HPLC purity detection method according to an embodiment of the present invention;

[0059] Figure 2 is a diagram showing the experimental results of the binding ability of humanized antibodies and Human-TrkA detected by flow cytometry according to an embodiment of the present invention;

[0060] Figure 3 is a diagram showing the experimental results of the binding ability of humanized antibodies and Mouse-TrkA detected by flow cytometry according to an embodiment of the present invention;

[0061] Figure 4 is a diagram showing the results of the inhibitory effect of humanized antibodies on the binding of Human-NGF and Human-TrkA detected by flow cytometry according to an embodiment of the present invention;

[0062] Figure 5 is a diagram showing the results of the inhibitory effect of humanized antibodies on the binding of Mouse-NGF and Mouse-TrkA detected by flow cytometry according to an embodiment of the present invention;

[0063] Figure 6A-6D are diagrams showing the results of the specificity of the binding of humanized antibodies to the target Human-TrkA detected by flow cytometry according to an

embodiment of the present invention;

[0064] Figure 7 is a diagram showing the results of ADA of humanized antibodies in mice evaluated by the ELISA method according to an embodiment of the present invention;

[0065] Figure 8 is a diagram showing the results of pharmacokinetics of humanized antibodies in mice evaluated by the ELISA method according to an embodiment of the present invention;

[0066] Figure 9 is a diagram showing the results of ADCC activity of humanized antibodies detected by the luciferase reporter gene system according to an embodiment of the present invention;

[0067] Figure 10 is a diagram showing the results of in vivo analgesic activity of humanized antibodies evaluated by a complete Freund's adjuvant-induced inflammatory pain model according to an embodiment of the present invention;

[0068] Figure 11 is a diagram showing the results of the CDC activity of humanized antibodies detected by NIH-3T3-TrkA cell model according to an embodiment of the present invention;

[0069] Figure 12 is a diagram showing the results of the in vitro activity of humanized antibodies evaluated by the NIH-3T3-TrkA cell model according to an embodiment of the present invention.

EXAMPLES

[0070] The embodiments of the present invention are described in detail below. Examples of the embodiments are shown in the accompanying drawings, wherein the same or similar reference numerals represent the same or similar elements or elements having the same or similar functions throughout. The embodiments described below with reference to the drawings are exemplary and are intended to explain the present invention, but should not be construed as limiting the present invention.

[0071] In the course of describing the present invention, the terms used herein are explained. These explanations are only for the convenience of understanding the scheme, and should not be regarded as limiting the protection scheme of the present invention.

Antibody

[0072] As used herein, the term "antibody" is an immunoglobulin molecule capable of binding to a specific antigen. It consists of two light chains with a lighter molecular weight and

two heavy chains with a heavier molecular weight. The heavy (H) and light (L) chains are linked by disulfide bonds to form a tetrapeptide chain molecule. Among them, the amino acid sequence of the amino terminal (N-terminal) of the peptide chain changes greatly, which is called the variable region (V region). The carboxyl terminal (C-terminal) is relatively stable with little change, which is called the constant region (C region). The constant region of the antibody can mediate the binding of the immunoglobulin to host tissues or factors. The host tissues or factors include various cells of the immune system (for example, effector cells) and the first component of the classical complement system (C1q). The V regions of the L and H chains are referred to as VL and VH, respectively.

[0073] In the variable region, the amino acid composition and arrangement order of certain regions have a higher degree of variation, which is called the hypervariable region (Hypervariable region, HVR). Hypervariable region is where antigen and antibody binds, so it is also called complementarity-determining region (CDR). They are interspersed in more conserved regions of the called framework regions (FR). Each VH and VL can be composed of three CDRs and four FR regions, which can be arranged in the following order from the amino terminus to the carboxy terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

[0074] The present invention utilizes the extracellular segment of TrkA to obtain anti-TrkA Fab (antigen-binding fragment) antibody fragments with high specificity and high affinity through immunization. The antibody fragment can specifically bind to the TrkA antigen, which can target the treatment of diseases such as pain or tumors.

[0075] In some embodiments, the present invention provides a humanized antibody or antigen-binding fragment, wherein the humanized antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8, and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13. The inventors can obtain the CDR region of the heavy chain variable region sequence and the CDR region of the light chain variable region sequence through the antibody sequence alignment database (NCBI, IMGT). In other embodiments, the heavy chain variable region sequence of the antibody or antigen-binding fragment comprises conservative amino acid substitutions compared to the amino acid sequence shown in SEQ ID NO: 2-8. In some embodiments, the light chain variable region sequence of the antibody or antigen-binding fragment comprises conservative amino acid substitutions compared to the amino acid sequence shown in

SEQ ID NO: 10-13. “Antigen-binding fragment” refers to an antibody fragment that retains the ability to specifically bind to an antigen (ROR2). Examples of antigen-binding fragments include, but are not limited to, at least one of Fv fragments, disulfide bond-stabilized Fv fragments (dsFv), Fab fragments, (Fab)₂ fragments, scFv-Fc fusion proteins, scFv-Fv fusion proteins, Fv-Fc fusion proteins, multispecific antibodies formed from antigen-binding fragments, single domain antibodies, domain antibodies, bivalent domain antibodies, or minimal recognition units. “Conservative amino acid substitution” refers to the substitution of an amino acid with a residue that is biologically, chemically or structurally similar to another amino acid. Of course, these conservative amino acid substitutions will not change the biological function of the antibody or antigen-binding fragment. In some specific ways, these conservative amino acid substitutions can occur on amino acids other than the CDR regions in the heavy chain variable region and the light chain variable region. Biological similarity means that the substitution does not destroy the TrkA antibody or biological activity with the TrkA antigen. Structural similarity refers to that amino acids have side chains of similar length, such as alanine, glycine, or serine, or side chains of similar size. Chemical similarity means that amino acids have the same charge or are both hydrophilic or hydrophobic. For example, the hydrophobic residues isoleucine, valine, leucine or methionine are substituted with each other. Alternatively, polar amino acids can be substituted for each other, such as lysine is substituted with arginine, aspartic acid is substituted with glutamic acid, asparagine is substituted with glutamine, threonine is substituted with serine, etc.

[0076] The term “murine antibody” usually refers to that the B cells derived from immunized mice are fused with myeloma cells, and then the mouse hybrid fusion cells that can proliferate immortally and secrete antibodies are screened, and then the antibody is screened, prepared and purified.

[0077] The term “chimeric antibody” refers to an antibody obtained by combining non-human genetic material with human genetic material. “Chimeric antibody” or “chimeric anti-TrkA antibody” herein includes antibodies in which the variable region sequence is derived from one species and the constant region sequence is derived from another species. For example, the variable region sequence is derived from a mouse antibody and the constant region sequence is derived from a human antibody.

[0078] The term “humanized antibody” refers to an antibody that is derived from a non-human species but whose protein sequence has been modified to increase its similarity to human

naturally-occurring antibodies. Specifically, a humanized antibody refers to a molecule having an antigen binding site that is essentially derived from an immunoglobulin of a non-human species, wherein the remaining immunoglobulin structure of the molecule is based on the structure and/or sequence of a human immunoglobulin. The antigen binding site may comprise a complete variable domain fused to a constant domain or only a complementarity determining region (CDR) grafted to an appropriate framework region in the variable domain. The antigen binding site can be wild-type or modified by one or more amino acid substitutions, for example, modifications are made to be more similar to human immunoglobulins. Some forms of humanized antibodies retain all CDR sequences (*e.g.*, a humanized mouse antibody that contains all six CDRs from a mouse antibody). Other forms have one or more CDRs that have changed relative to the original antibody.

[0079] In some preferred embodiments, the present invention provides a humanized anti-TrkA antibody. The antibody has a heavy chain with the amino acid sequence shown in any one of SEQ ID NO: 17-23 and a light chain with the amino acid sequence shown in any one of SEQ ID NO: 24-27.

[0080] In some preferred embodiments, the present invention provides a humanized anti-TrkA single chain antibody. The single-chain antibody comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8 and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13, wherein the C-terminus of the heavy chain variable region is connected to the N-terminus of the light chain variable region through a connecting peptide linker, or the C-terminus of the light chain variable region is connected to the N-terminus of the heavy chain variable region through a connecting peptide linker.

Nucleic acid molecule, expression vector, recombinant cell

[0081] In the process of preparing or obtaining these antibodies, nucleic acid molecules expressing these antibodies can be connected to different vectors and then expressed in different cells to obtain corresponding antibodies.

[0082] To this end, the present invention also provides an isolated nucleic acid molecule, which encodes the antibody or antigen-binding fragment described above.

[0083] In some embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence shown in any one of SEQ ID NO: 30-36 or has the nucleotide sequence shown in any one of SEQ ID NO: 37-40.

[0084] In some embodiments, the isolated nucleic acid molecule comprises at least more than 90% homology with the nucleotide sequence shown in SEQ ID NO: 30-36, preferably has more than 95% homology, and more preferably comprises more than 98% and 99% homology. In at least some embodiments, the isolated polynucleotide comprises at least more than 90% homology with the nucleotide sequence shown in SEQ ID NO: 37-40, preferably comprises more than 95% homology, and more preferably comprises more than 98% and 99% homology. These sequences that comprise homology with the nucleotide sequences shown in SEQ ID NO: 30~36 or SEQ ID NO: 37~40 can express amino acid sequences similar to SEQ ID NO: 17~23 or SEQ ID NO: 24~27, so that they can specifically bind to the TrkA antigen to achieve the targeting function of the antibody.

[0085] In some preferred embodiments, the isolated nucleic acid molecule comprises the heavy chain nucleotide sequence shown in SEQ ID NO: 30~36 and the light chain nucleotide sequence shown in SEQ ID NO: 37~40. These nucleotide sequences are optimized for species and are more easily expressed in mammalian cells.

[0086] The present invention also provides an expression vector, which contains the aforementioned isolated nucleic acid molecule. When the aforementioned isolated polynucleotide is ligated to a vector, the polynucleotide can be directly or indirectly connected to control elements on the vector, as long as these control elements can control the translation and expression of the polynucleotide. Of course, these control elements can come directly from the vector itself, or they can be exogenous, that is, not from the vector itself. Of course, the polynucleotide may be operably linked to the control element. "Operably linked" herein refers to the connection of a exogenous gene to a vector, so that control elements in the vector, such as transcription control sequences and translation control sequences, can exert its expected function of regulating the transcription and translation of exogenous genes. Of course, the polynucleotides used to encode the heavy and light chains of the antibodies can be inserted into different vectors independently, and they are usually inserted into the same vector. Commonly used vectors can be, for example, plasmids, phages, and the like. For example, plasmid-X plasmid.

[0087] The invention also provides a recombinant cell, which contains the expression vector. The expression vector can be introduced into mammalian cells, constructed to obtain recombinant cells, and then these recombinant cells can be used to express the humanized antibodies or antigen-binding fragments provided by the present invention. By culturing the recombinant cells,

corresponding antibodies can be obtained. These usable mammalian cells may be, for example, CHO cells and the like.

Pharmaceutical composition, kit and pharmaceutical uses and uses in the preparation of kits

[0088] The invention also provides a pharmaceutical composition, which comprises the antibody or antigen-binding fragment described above and a pharmaceutically acceptable carrier.

[0089] The anti-TrkA humanized antibodies provided herein can be incorporated into a pharmaceutical composition suitable for administration to a subject. Generally, these pharmaceutical compositions include the anti-TrkA humanized antibodies provided herein as well as a pharmaceutically acceptable carrier. A “pharmaceutically acceptable carrier” may include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and delayed absorption agents, and the like that are physiologically compatible. Specific examples may be one or more of water, saline, phosphate buffered saline, glucose, glycerol, ethanol, and the like, and combinations thereof. In many cases, pharmaceutical compositions include isotonic agents, such as sugars, polyalcohols (such as mannitol, sorbitol), or sodium chloride. Of course, pharmaceutically acceptable carriers may also include minor amounts of auxiliary substances, such as wetting or emulsifying agents, preservatives or buffering agents, to extend the shelf life or efficacy of the antibody.

[0090] For example, the antibodies of the invention can be incorporated into pharmaceutical compositions suitable for parenteral administration (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). These pharmaceutical compositions can be prepared in various forms. Examples are liquid, semi-solid, and solid dosage forms, including, but not limited to, liquid solutions (*e.g.*, injection solutions and infusion solutions), dispersing or suspending agents, tablets, pills, powders, liposomes, and suppositories. Typical pharmaceutical compositions are in the form of injection solutions or infusion solutions. The antibodies can be administered by intravenous infusion or injection or intramuscular or subcutaneous injection.

[0091] Of course, the anti-TrkA humanized antibodies herein can also be made into kits or part of other diagnostic reagents as needed. According to the embodiment of the present invention, the present invention also provides a kit comprising the above-mentioned TrkA antibody. The kit provided by the present invention can be used, for example, the kit can be used for immunoblotting, immunoprecipitation, etc., which involves detection using the specific binding properties of TrkA antigen and antibodies. These kits may include any one or more of the following: antagonists, anti-

TrkA humanized antibodies or drug reference materials; protein purification columns; immunoglobulin affinity purification buffers; cell assay diluents; instructions or literature, etc. Anti-TrkA humanized antibodies can be used for different types of diagnostic tests, such as the detection of various diseases or the presence of drugs, toxins or other proteins in vitro or in vivo. For example, it can be used to test related diseases by testing the serum or blood of the subject. Such related diseases may include TrkA-related diseases such as pain, cancer, inflammation or inflammatory diseases, neurodegenerative diseases, Sjogren's syndrome, endometriosis, diabetic peripheral neuropathy, prostatitis, pelvic pain syndrome, and diseases related to the unbalanced regulation of bone remodeling, and diseases caused by abnormal signal conduction of connective tissue growth factor, and the like. Of course, the antibodies provided herein can also be used for radioimmunodetection and radioimmunotherapy of the above diseases.

[0092] Specifically, the aforementioned pain, inflammation or inflammatory disease, neurodegenerative diseases, Sjogren's syndrome, endometriosis, diabetic peripheral neuropathy, prostatitis, pelvic pain syndrome, diseases related to the regulation of bone remodeling imbalance and diseases caused by abnormal signaling of connective tissue growth factor include neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis.

[0093] These cancers or tumors can be any unregulated cell growth. Specifically, it may be non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, prostate cancer, neuroblastoma, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer, etc.

[0094] When using the anti-TrkA humanized antibody provided by the present invention to treat the above-mentioned diseases, the anti-TrkA humanized antibody provided by the present invention may be provided to a subject. To this end, the present invention provides a method for treating the above-mentioned diseases, comprising administering an antibody or an antigen-binding fragment thereof provided by the present invention to a subject in need.

Example 1 Humanized design of the variable region of the murine anti-TrKA monoclonal antibody 23E12

[0095] The immunogenicity analysis of the B cell epitope was carried out against the variable region of the murine anti-TrKA monoclonal antibody 23E12 by using the B cell epitope analysis software AbEpiMax, and the sequence of the antibody FR region with a strong B cell epitope was found.

[0096] Then, the sequence of the human antibody FR library that had high 3D structural homology with the original sequence and had a weaker B cell epitope was used to replace the sequence of the antibody FR region with a strong B cell epitope. The sequences of heavy and light chain variable region of the murine anti-TrKA monoclonal antibody 23E12 and modified heavy and light chain variable region of 23E12 were shown in Table 1.

Table 1

	Heavy chain variable region	Sequence	Light chain variable region	Sequence
Variable region of the murine anti-TrKA monoclonal antibody 23E12	VH0	SEQ ID NO:1	VL0	SEQ ID NO:9
Variable region of the humanized anti-TrKA monoclonal antibody 23E12 modified	VH1	SEQ ID NO:2	VL1	SEQ ID NO:10
	VH2	SEQ ID NO:3	VL2	SEQ ID NO:11
	VH3	SEQ ID NO:4	VL3	SEQ ID NO:12
	VH4	SEQ ID NO:5	VL4	SEQ ID NO:13
	VH5	SEQ ID NO:6		
	VH6	SEQ ID NO:7		
	VH7	SEQ ID NO:8		

Example 2 Construction of vector

[0097] A series of humanized antibody expression vectors (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) were constructed by molecular cloning, and the humanized antibody was expressed recombinantly in the CHO expression system. Nucleotide sequences encoding a series of humanized monoclonal antibody light and heavy chains (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) were obtained by entrusting GenScript Biotechnology Co., Ltd. through chemical synthesis. After the sequences were double digested, they were inserted between the same restriction sites of the

eukaryotic expression vector to construct a series of humanized monoclonal antibody expression vectors (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4). Then a series of verified and correct expression vectors were extracted by Invitrogen plasmid extraction kit, linearized with restriction enzymes, purified and recovered, and then stored at -20°C.

Example 3 Transfection of vector encoding a series of humanized antibodies and expression in cells

[0098] After the CHO host cells were resuscitated and cultured with CD CHO medium, the cells were collected for transfection when the density of the cells was about 8×10^5 cell/mL. The transfected cells were about 1×10^7 cell, the vector was about 40 μ g, and the transfection method is by electroporation (Bio-Rad, Gene pulser Xcell). After the electric shock, the cells were cultured in 20mL CD CHO medium. On the second day of culture, the cells were collected by centrifugation and resuspended in 20 mL CD CHO medium added with MSX to a final concentration of 50 μ M. When the density of the cells was about 0.6×10^6 cell/mL, the obtained mixed clones were passaged with CD CHO medium, and the density of the cells was about 0.2×10^6 cell/mL. When the survival rate of the cells was about 90%, the cell culture fluid was collected.

Example 4 Collection of cell fermentation broth and purification of humanized antibody

[0099] A series of humanized monoclonal antibodies were tested at the translation level. The collected cell culture fluid was purified by a Protein A chromatography column, and the absorption peak was collected for mass spectrometry. Mass spectrometry detected that a series of chimeric antibodies had a molecular weight of about 150KD, which was consistent with the theoretical molecular weight and was in the form of a dimer. At the same time, the collected samples were detected by 10% SDS-PAGE electrophoresis after reduction and non-reduction, respectively. The reduced SDS-PAGE electrophoresis pattern showed two bands, about 25KD and 50KD, respectively. The non-reduced SDS-PAGE electrophoresis pattern showed a single band, around 150KD. The band size of the electrophoresis pattern was consistent with the theory. After purification, the sample was dialyzed overnight at 4°C with a 0.01M PBS buffer of pH 7.0.

Example 5 Evaluation of the purity of monomer of humanized antibodies by the SEC-HPLC purity detection method

[00100] The humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) samples and the chimeric antibody (H0L0-IgG4) samples were centrifuged. About 80 μ g of the supernatant was taken and injected into HPLC for detection. The monomer peak area percentage of the humanized

antibody was detected by SEC-HPLC. The higher the peak area percentage, the higher the purity of the monomer. The results were shown in Figure 1. The results in the Figure 1 showed that the monomer peak area percentages of the humanized antibodies H1L1-IgG4, H3L1-IgG4, and H3L2-IgG4 were 99.847%, 99.738%, and 99.836%, respectively, and the monomer peak area percentage of the chimeric antibody H0L0-IgG4 was 99.621%. It showed that the humanized antibodies H1L1-IgG4, H3L1-IgG4, H3L2-IgG4 and the chimeric antibody H0L0-IgG4 had high monomer purity.

Example 6 Evaluation of the binding ability of humanized antibodies and Human-TrKA by flow cytometry

[00101] Lentiviral technology was used to construct HEK293T-HumanTrkA cell model. The humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) samples and the chimeric antibody (H0L0-IgG4) samples were diluted with PBS buffer to 11 concentration gradients (20 μ g/mL, 10 μ g/mL, 5 μ g/mL, 2.5 μ g/mL, 1.25 μ g/mL, 0.625 μ g/mL, 0.313 μ g/mL, 0.156 μ g/mL, 0.078 μ g/mL, 0.039 μ g/mL, 0.019 μ g/mL). Flow cytometry was used to detect the binding of each concentration gradient humanized antibody to the Human-TrKA receptor on the surface of HEK293T-HumanTrKA cells, and the binding ability of each humanized antibody to Human-TrKA was evaluated at the cellular level. The results were shown in Figure 2. In the Figure 2, the EC50 (half binding concentration) value reflected the binding ability of the antibody to Human-TrKA; the smaller the EC50 value, the stronger the binding ability of the antibody to Human-TrKA, and the higher the affinity of the antibody. It was generally believed that the EC50 value of high-affinity antibodies was lower than 1.5 μ g/mL. The results in the Figure 2 showed that the EC50 values of the humanized antibodies H1L1-IgG4, H3L1-IgG4, and H3L2-IgG4 were 0.1307 μ g/mL, 0.1268 μ g/mL, 0.1683 μ g/mL, respectively, and the EC50 value of chimeric antibody H0L0-IgG4 was 0.08669 μ g/mL. It showed that the humanized antibodies H1L1-IgG4, H3L1-IgG4, H3L2-IgG4 and the chimeric antibody H0L0-IgG4 had strong binding abilities to Human-TrKA; compared with the chimeric antibody H0L0-IgG4, the affinity of humanized antibodies H1L1-IgG4, H3L1-IgG4, H3L2-IgG4 binding to Human-TrKA remained basically unchanged.

Example 7 Evaluation of the binding ability of humanized antibodies and Mouse-TrKA by flow cytometry

[00102] Lentiviral technology was used to construct HEK293T-MouseTrkA cell model. The humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) samples and the chimeric

antibody (H0L0-IgG4) samples were diluted with PBS buffer to 11 concentration gradients (20 μ g/mL, 10 μ g/mL, 5 μ g/mL, 2.5 μ g/mL, 1.25 μ g/mL, 0.625 μ g/mL, 0.313 μ g/mL, 0.156 μ g/mL, 0.078 μ g/mL, 0.039 μ g/mL, 0.019 μ g/mL). Flow cytometry was used to detect the binding of each concentration gradient humanized antibody to the Human-TrKA receptor on the surface of HEK293T-MouseTrKA cells, and the binding ability of each humanized antibody to Mouse-TrKA was evaluated at the cellular level. The results were shown in Figure 3. In the Figure 3, the EC₅₀ (half binding concentration) value reflected the binding ability of the antibody to Mouse-TrKA; the smaller the EC₅₀ value, the stronger the binding ability of the antibody to Mouse-TrKA, and the higher the affinity of the antibody. It was generally believed that the EC₅₀ value of high-affinity antibodies was lower than 1.5 μ g/mL. The results in the Figure 3 showed that the EC₅₀ values of the humanized antibodies H1L1-IgG4, H3L1-IgG4, and H3L2-IgG4 were 0.1341 μ g/mL, 0.1110 μ g/mL, 0.1254 μ g/mL, respectively, and the EC₅₀ value of chimeric antibody H0L0-IgG4 was 0.1048 μ g/mL. It showed that the humanized antibodies H1L1-IgG4, H3L1-IgG4, H3L2-IgG4 and the chimeric antibody H0L0-IgG4 had strong binding abilities to Mouse-TrKA; compared with the chimeric antibody H0L0-IgG4, the affinity of humanized antibodies H1L1-IgG4, H3L1-IgG4, H3L2-IgG4 binding to Mouse-TrKA remained basically unchanged.

Example 8 The inhibitory effect of humanized antibodies on the binding of Human-NGF and Human-TrKA detected by flow cytometry

[00103] Human-NGF was biotinylated, and Human-NGF could bind to the extracellular region of Human-TrkA protein on HEK293T-HumanTrkA cells, and anti-TrkA monoclonal antibodies could also bind to the extracellular region of Human-TrkA protein on HEK293T-HumanTrkA cells. Competitive experiments were designed to detect the binding of Human-NGF to the extracellular region of Human-TrkA protein on HEK293T-HumanTrkA cells under the action of different concentrations (20 μ g/mL, 10 μ g/mL, 5 μ g/mL, 2.5 μ g/mL, 1.25 μ g/mL, 0.625 μ g/mL, 0.313 μ g/mL, 0.156 μ g/mL, 0.078 μ g/mL, 0.039 μ g/mL, 0.019 μ g/mL) of humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibodies (H0L0-IgG4) by flow cytometry, and to study the inhibitory effect of each humanized antibody on the binding of Human-NGF and Human-TrkA. The experimental results were shown in Figure 4. In the Figure 4, the parent% value reflected the Human-NGF signal that bound to the extracellular region of the Human-TrkA protein on HEK293T-HumanTrkA cells. The lower the reading, the weaker the Human-NGF signal that bound to the extracellular region of the Human-TrkA protein on

HEK293T-HumanTrkA cells, and the greater the effect of the antibody in inhibiting the binding of Human-NGF and Human-TrkA; as shown in the Figure 4, as the concentrations of each humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) increased, the parent% value gradually decreased until it approached zero, that is, the Human-NGF signal that bound to the extracellular region of the Human-TrkA protein gradually decreased until there was no Human-NGF to bind to the extracellular region of Human-TrkA protein, and the binding of Human-NGF to Human-TrkA was completely inhibited. The IC₅₀ of the humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) were 0.7963μg/mL, 0.7405μg/mL, 0.6653μg/mL, respectively, and the IC₅₀ of the chimeric antibody (H0L0-IgG4) was 0.8810μg/mL; it could be seen that each humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) could dose-dependently inhibit the binding of Human-NGF and Human-TrkA at the cellular level within a concentration range; compared with the chimeric antibody (H0L0-IgG4), the inhibitory effect of humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) on the binding of Human-NGF and Human-TrkA remained basically unchanged.

Example 9 The inhibitory effect of humanized antibodies on the binding of Mouse-NGF and Mouse-TrkA detected by flow cytometry

[00104] Mouse-NGF was biotinylated, and Mouse-NGF could bind to the extracellular region of Mouse-TrkA protein on HEK293T-MouseTrkA cells, and anti-TrkA monoclonal antibodies could also bind to the extracellular region of Mouse-TrkA protein on HEK293T-MouseTrkA cells. Competitive experiments were designed to detect the binding of Mouse-NGF to the extracellular region of Mouse-TrkA protein on HEK293T-MouseTrkA cells under the action of different concentrations (20μg/mL, 10μg/mL, 5μg/mL, 2.5μg/mL, 1.25μg/mL, 0.625μg/mL, 0.313μg/mL, 0.156μg/mL, 0.078μg/mL, 0.039μg/mL, 0.019μg/mL) of humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibodies (H0L0-IgG4) by flow cytometry, and to study the inhibitory effect of each humanized antibody on the binding of Mouse-NGF and Mouse-TrkA. The experimental results were shown in Figure 5. In the Figure 5, the parent% value reflected the Mouse-NGF signal that bound to the extracellular region of the Mouse-TrkA protein on HEK293T-MouseTrkA cells. The lower the reading, the weaker the Mouse-NGF signal that bound to the extracellular region of the Mouse-TrkA protein on HEK293T-MouseTrkA cells, and the greater the effect of the antibody in inhibiting the binding of Mouse-NGF and Mouse-TrkA; as shown in the Figure 5, as the concentrations of each humanized antibody (H1L1-IgG4, H3L1-

IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) increased, the parent% value gradually decreased until it approached zero, that is, the Mouse-NGF signal that bound to the extracellular region of the Mouse-TrkA protein gradually decreased until there was no Mouse-NGF to bind to the extracellular region of Mouse-TrkA protein, and the binding of Mouse-NGF to Mouse-TrkA was completely inhibited. The IC₅₀ of the humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) were 0.3848μg/mL, 0.2826μg/mL, 0.2524μg/mL, respectively, and the IC₅₀ of the chimeric antibody (H0L0-IgG4) was 0.3959 μg/mL; it could be seen that each humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) could dose-dependently inhibit the binding of Mouse-NGF and Mouse-TrkA at the cellular level within a concentration range; compared with the chimeric antibody (H0L0-IgG4), the inhibitory effect of humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) on the binding of Mouse-NGF and Mouse-TrkA remained basically unchanged .

Example 10 Evaluation of the specificity of humanized antibody binding to the target Human-TrkA by ELISA method

[00105] The TrkA receptor family belonged to receptor tyrosine kinases (RTKs), including TrkA, TrkB, and TrkC, which had high homology. TrkA was a receptor tyrosine kinase of nerve growth factor (NGF) that selectively bound to NGF and was a functional receptor for NGF. In addition to the high-affinity receptor TrkA, NGF could also bind to its low-affinity receptor p75. In the test, the binding of different concentrations (20μg/mL, 10μg/mL, 5μg/mL, 2.5μg/mL, 1.25μg/mL, 0.625μg/mL, 0.313μg/mL, 0.156μg/mL, 0.078μg/mL, 0.039μg/mL, 0.019μg/mL) of humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) to TrkA, TrkB, TrkC, and P75 was detected by the ELISA method respectively, and the specificity of the binding of the tested antibody to the target Human-TrkA was evaluated. The results were shown in Figure 6. In the Figure 6, at a certain concentration of the antibody, the OD₄₅₀ value reflected the strength of the binding between the antibody and the protein. The larger the reading, the stronger the binding between the antibody and the protein. The experimental results showed that humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) had good binding to TrkA receptors (The concentration of the tested antibody increased from 0μg/mL to 20μg/mL, and the OD₄₅₀ value gradually increased until it approaches stable, close to about 3), and basically did not bind to TrkB, TrkC, P75 (the concentration of each tested antibody increased from 0μg/mL to 20μg/mL, the OD₄₅₀ value was

basically unchanged, close to 0). It could be seen that the specificity of the tested antibody binding to the target Human-TrKA was very good.

Example 11 Evaluation of ADA of humanized antibodies in mice evaluated by the ELISA method

[00106] In the test, 5 mice were immunized with humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) respectively, and tail vein blood was collected on the 14th day after administration. Each humanized antibody (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) and chimeric antibody (H0L0-IgG4) were diluted to 1 µg/mL coated microplate with PBS, 100 µL of which was added to each well and reacted at 4℃ overnight; the plate was washed 3 times with PBS solution, and blocked with 5% milk-PBS for 1hr at room temperature; then the plate was washed once with PBS solution; 5% Milk-PBS buffer was used to gradiently dilute mouse tail vein blood (1:500, 1:1000, 1:5000, 1:10000, 1:50000), and the mouse tail vein blood was placed at room temperature for 1hr, and then the pre-reacted tail vein blood was added to the microplate with 100 µL per well. A negative control (NC) was set. The mixture was reacted at room temperature for 1hr, then the plate was washed 3 times with PBS solution and patted dry. 1:2000 diluted HRP-labeled goat anti-mouse IgG (Fc) secondary antibody was added, and reacted at room temperature for 1hr; the plate was washed 5 times with PBS solution and patted dry, then 100µL of substrate color developing solution TMB was added, and reacted for 20 min under dark conditions at room temperature; then 50µL of stop solution was added and the OD450 value was read on the microplate reader after mixing. The results were shown in Figure 7 below. In the Figure 7, the OD450 value reflected the strength of the generated ADA. The larger the reading, the stronger the generated ADA. The experimental results showed that compared with chimeric antibodies (H0L0-IgG4), humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) produced weaker ADA; it could be seen that humanized antibodies (H1L1-IgG4, H3L1-IgG4, H3L2-IgG4) had lower immunogenicity than chimeric antibodies (H0L0-IgG4).

Example 12 Evaluation of the pharmacokinetics of humanized antibodies in mice evaluated by the ELISA method

[00107] 12 Male ICR mice were randomly divided into 4 groups and 3 mice/group. The chimeric antibody H0L0-IgG4 and humanized antibody H1L1-IgG4 were injected intravenously or subcutaneously at 1mg/kg. Blood was collected at 1, 6, 24, 72, 168, 336, 504, and 672h after administration, and plasma was separated (EDTA-K2 anticoagulation). In the intravenous

administration group, blood was collected for an additional 0.25h. The indirect ECLA method was used to analyze the concentration of H0L0-IgG4 or H1L1-IgG4 in each sample. The pharmacokinetic parameters were calculated based on the plasma drug concentration. The main PK parameter results were shown in Table 2, and the drug-time curve was shown in Figure 8. The results showed that after intravenous injection of H0L0-IgG4 or H1L1-IgG4 in ICR mice, the average plasma half-life was 97h and 143h, respectively; after subcutaneous injection of H0L0-IgG4 or H1L1-IgG4, the average peak time was 40 h and 56 h, the average C_{max} was 6.61 μ g/mL and 9.49 μ g/mL, the average AUC_{last} was 2120 μ g·h/mL and 3020 μ g·h/mL, the average plasma half-life was 75 h and 122 h, and the absolute bioavailability was 89% and 101%, respectively. It could be seen that the humanized antibody H1L1-IgG4 had better pharmacokinetic parameters in mice compared with the chimeric antibody H0L0-IgG4.

Table 2

Group	Tested substance	batch number	Mode of administration	dose	t _{1/2}	T _{max}	C _{max}	AUC _{last}	V _{as}	CL	MRT	F
				mg/kg	h	h	μ g/mL	μ g·h/mL	mL/kg	mL/h/kg	h	%
1	H0L0	20200524	i.v.	1	97	0.25	28.6	2390	76	0.47	170	/
2	H0L0	20200524	s.c.	1	75	40	6.61	2120	/	/	213	89
3	H1L1	20200524	i.v.	1	143	0.25	31.7	3000	71	0.33	200	/
4	H1L1	20200524	s.c.	1	122	56	9.49	3020	/	/	221	101

Figure 13 ADCC activity of humanized antibodies detected by the luciferase reporter gene system

[00108] Antibody-dependent cell-mediated cytotoxicity (ADCC) means that when IgG antibody specifically binds to antigenic determinants on the surface of target cells through the Fab segment, its Fc segment can bind to effector cells such as Fc γ R killer cells (NK cells, monocytes-macrophages, neutrophils) to trigger the killing activity of effector cells, and directly kill target cells. In the experiment, the Jurkat-NFAT-Luc-CD16 luciferase reporter cell line stably transfected with CD16 receptor and NFAT (Nuclear Factor of Activated T-cells) reaction original was used. When the Fab segment of the test antibody bound to the antigen on the target cell HEK293T-HumanTrKA cell, the Fc segment of the antibody bound to (Fc γ RIIIA) on the surface of the effector cell Jurkat-NFAT-Luciferase-CD16 cell, causing activation of NFAT-related signaling pathways in Jurkat-NFAT-Luciferase-CD16 cells, which in turn led to an increase in expression level of luciferase. The ADCC activity of humanized antibodies was evaluated by detecting the expression level of luciferase of effector cells Jurkat-NFAT-Luciferase-CD16 under the action of different concentrations (100 μ g/mL, 20 μ g/mL, 4 μ g/mL, 0.8 μ g/mL, 0.16 μ g/mL, 0.032 μ g/mL, 0.0064 μ g/mL, 0.00128 μ g/mL, 0.000256 μ g/mL, 0.0000512 μ g/mL) of humanized antibodies

(H1L1-IgG4, H1L1-IgG1). The results were shown in the Figure 9 below. In the Figure 9, Mean Value reflected the expression level of luciferase. The larger the reading, the higher the expression level and the stronger the ADCC activity of the corresponding antibody. The experimental results showed that as the concentration of the antibody increased, the Mean Value of the negative control fusion protein Dulaglutide-IgG4 and the humanized antibody H1L1-IgG4 remained basically unchanged and close to zero, while the Mean Value of the humanized antibody H1L1-IgG1 gradually increased until reaching the plateau value, and half of the peak concentration EC50 was 0.02281 μ g/mL; it could be seen that the humanized antibody H1L1-IgG1 had strong ADCC activity, and H1L1-IgG4 basically had no ADCC activity.

Example 14 Evaluation of in vivo analgesic activity of humanized antibodies by a complete Freund's adjuvant-induced inflammation pain model

[00109] Complete Freund's adjuvant-induced inflammatory pain model is a pain model that produces a pain model of chronic inflammatory pain stimulation similar to osteoarthritis and responses by injecting complete Freund's adjuvant in the palms of mice. The pain is measured by the mechanical pain test. The greater the intensity of the mechanical stimulus, the more resistant the animal is to pain. In the experiment, 18-25g male C57BL/6 mice were selected, and 10 μ L of CFA was injected into the center of the sole of the right hind foot of the mouse. After 24 hours of modeling, the mechanical hyperalgesia method was used to test, and the animals with the withdrawal threshold less than 0.5 gram force were screened out. Based on their pain sensitivity, they were randomly divided into solvent control group, naproxen 100 mg/kg dose group, Tanezumab 2mg/kg dose group, MNAC13 2 mg/kg dose group, H1L1-IgG4 2 mg/kg dose group. There were a total of 5 groups, n=10 per group. Among them, Tanezumab was an anti-NGF monoclonal antibody, and MNAC13 was an anti-TrkA monoclonal antibody. The solvent control group, Tanezumab, MNAC13, and H1L1-IgG4 dose groups were administered by subcutaneous injection, and the mechanical hyperalgesia test was performed after 42hr and 96hr respectively. The naproxen dose group was administered intragastrically 2 hours before the test. The results were shown in Figure 10. The ordinate represented the intensity of mechanical stimulation. The greater the pressure threshold of the mouse paw withdrawal, the better the analgesic effect. The results showed that the positive control group naproxen was tested 2 hours after orally administration at a dose of 100 mg/kg and showed inhibition of mechanical hyperalgesia induced by C57BL/6 mouse CFA model; Tanezumab was tested 42 hours after subcutaneous administration

at a dose of 2 mg/kg and showed inhibition of mechanical hyperalgesia induced by C57BL/6 mouse CFA model, while Tanezumab which was tested 96 hours after administration did not show such inhibition; MNAC13 was tested 42 and 96 hours after subcutaneous administration at a dose of 2 mg/kg, and none of them showed inhibition of mechanical hyperalgesia induced by C57BL/6 mouse CFA model. H1L1-IgG4 was tested 96 hours after subcutaneous administration at a dose of 2 mg/kg and showed inhibition of mechanical hyperalgesia induced by the C57BL/6 mouse CFA model, while H1L1-IgG4 which was tested 42 hours after administration did not show such inhibition. Conclusion: H1L1-IgG4 significantly inhibited the mechanical hyperalgesia induced by the C57BL/6 mouse CFA model after 96 hours of subcutaneous administration, and had the activity of relieving inflammatory pain.

Example 15 CDC activity of humanized antibodies detected by NIH-3T3-TrkA cell model

[00110] Complement-dependent cytotoxicity (CDC) refers to the cytotoxic effect of complement, that is, through specific antibody binding with the corresponding antigen on the cell membrane surface to form a complex to activate the classical pathway of complement, the formed membrane attack complex exerts a lytic effect on target cells. In the experiment, the cell viability of the target cell NIH-3T3-TrkA was detected by the CCK8 method under the action of different concentrations (16.67 μ g/mL, 5.56 μ g/mL, 1.85 μ g/mL, 0.62 μ g/mL, 0.21 μ g/mL, 0.069 μ g/mL, 0.023 μ g/mL, 0.008 μ g/mL, 0.003 μ g/mL) of humanized antibody (H1L1-IgG4, H1L1-IgG1) and the negative control fusion protein Dulaglutide-IgG4, and the CDC activity of the humanized Anti-TrkA antibody was evaluated. The results were shown in Figure 11. The results in Figure 11 showed that with the increase of antibody concentration, the killing effect of humanized antibody H1L1-IgG1 on the target cells NIH-3T3-TrkA cells gradually increased, and the half-peak concentration IC₅₀ was 0.2219 μ g/mL; the humanized antibody H1L1-IgG4 and the negative control fusion protein Dulaglutide-IgG4 had basically no killing effect on the target cells NIH-3T3-TrkA cells; it could be seen that the humanized antibody H1L1-IgG1 had strong CDC activity, and H1L1-IgG4 basically had no CDC activity.

Example 16 Evaluation of in vitro activity of humanized antibodies by NIH-3T3-TrkA cell model

[00111] Under NGF stimulation, the level of TrkA protein tyrosine phosphorylation on the NIH-3T3-TrkA cell membrane is up-regulated, and the downstream signaling pathway of TrkA is

activated. Humanized Anti-TrkA antibody can bind to the TrkA protein on the surface of NIH-3T3-TrkA cell membrane, inhibit NGF stimulation, and down-regulate the level of TrkA protein tyrosine phosphorylation. In the experiment, the AlphaLISA method was used to detect the down-regulation of the level of TrkA protein tyrosine phosphorylation under the action of different concentrations (1000 μ g/mL, 333.33 μ g/mL, 111.11 μ g/mL, 37.04 μ g/mL, 12.35 μ g/mL, 4.12 μ g/mL, 1.37 μ g/mL, 0.45 μ g/mL, 0.15 μ g/mL, 0.05 μ g/mL, 0.017 μ g/mL, 0.005 μ g/mL) of humanized antibodies, and the in vitro activity of the tested antibody was evaluated. The test results of p-TrkA were shown in Figure 12. The experimental results showed that the humanized Anti-TrkA antibody H1L1-IgG4 could inhibit the NGF-TrkA signaling pathway and down-regulate the level of TrkA protein tyrosine phosphorylation in a dose-dependent manner. The IC₅₀ value was 0.02072 μ g/mL. It could be seen that the humanized Anti-TrkA antibody H1L1-IgG4 could inhibit the activation of the downstream signaling pathway of TrkA by NGF.

[00112] The solution of the present invention will be explained below in conjunction with examples. Those skilled in the art will understand that the following examples are only used to illustrate the present invention, and should not be regarded as limiting the scope of the present invention. If the specific technology or condition is not indicated in the examples, the technology or condition described in the literature in the art or the product description is performed. If the reagents or instruments used are not specified by the manufacturer, they are all conventional products that are commercially available.

1. A humanized antibody or antigen-binding fragment thereof capable of specifically recognizing TrkA, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the VH-CDR1 shown in SEQ ID NO:41, the VH-CDR2 shown in SEQ ID NO:42 or SEQ ID NO:43, and the VH-CDR3 shown in SEQ ID NO:44; and

a light chain variable region with the VL-CDR1 shown in SEQ ID NO:45, the VL-CDR2 shown in SEQ ID NO:46 or SEQ ID NO:47, and the VL-CDR3 shown in SEQ ID NO:48.

2. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8, and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13.

3. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region selected from:

(a) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 2 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 10;

(b) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 4 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 10; or

(c) the heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 4 and the light chain variable region with the amino acid sequence shown in SEQ ID NO: 11.

4. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody or antigen-binding fragment thereof specifically recognizes the extracellular region of TrkA.

5. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody comprises at least one of a heavy chain constant region and a light chain constant region, and both the heavy chain constant region and the light chain constant region are derived from human IgG antibodies or their mutants.

6. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the light chain constant region of the antibody is derived from a human Kappa light chain constant region; the heavy chain constant region is derived from a human IgG4 heavy chain constant region.

7. The humanized antibody or antigen-binding fragment thereof according to claim 6, wherein the Fc region of the antibody has S10P, F16A, L17A, R191K mutations and 229 K deletion

mutations compared with the human IgG4 wild-type Fc, wherein the human IgG4 wild-type Fc has the amino acid sequence shown in SEQ ID NO:16.

8. The humanized antibody or antigen-binding fragment thereof according to claim 6, wherein the full-length sequence of the constant region of the antibody is as shown in SEQ ID NO: 14 or 15.

9. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody comprises a heavy chain with the amino acid sequence shown in any one of SEQ ID NO: 17 to 23 and a light chain with the amino acid sequence shown in any one of SEQ ID NO: 24 to 27.

10. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody is selected from the following heavy chain and light chain:

(a) the heavy chain with the amino acid sequence shown in SEQ ID NO: 17 and the light chain with the amino acid sequence shown in SEQ ID NO: 24;

(b) the heavy chain with the amino acid sequence shown in SEQ ID NO: 19 and the light chain with the amino acid sequence shown in SEQ ID NO: 24; or

(c) the heavy chain with the amino acid sequence shown in SEQ ID NO: 19 and the light chain with the amino acid sequence shown in SEQ ID NO: 25.

11. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody is a single-chain antibody, a multimeric antibody, or a CDR-grafted antibody.

12. The humanized antibody or antigen-binding fragment thereof according to claim 11, wherein the single-chain antibody comprises a heavy chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 2-8 and a light chain variable region with the amino acid sequence shown in any one of SEQ ID NO: 10-13, wherein the C-terminus of the heavy chain variable region is connected to the N-terminus of the light chain variable region through a connecting peptide linker, or the C-terminus of the light chain variable region is connected to the N-terminus of the heavy chain variable region through a connecting peptide linker.

13. The humanized antibody or antigen-binding fragment thereof according to claim 1, wherein the antigen-binding fragment comprises at least one of Fab, Fab', F(ab)₂, F(ab')₂, Fv, scFv-Fc fusion protein, scFv-Fv fusion protein, and minimum recognition unit.

14. A nucleic acid molecule encoding the humanized antibody or antigen-binding fragment thereof according to any one of claims 1 to 13.

15. The nucleic acid molecule of claim 14, wherein the nucleic acid molecule is DNA.
16. The nucleic acid molecule according to claim 15, wherein the nucleic acid molecule comprises the nucleotide sequence shown in any one of SEQ ID NO: 30-36 or comprises the nucleotide sequence shown in any one of SEQ ID NO: 37-40.
17. An expression vector carrying the nucleic acid molecule according to any one of claims 14 to 16.
18. The expression vector of claim 17, wherein the expression vector is a eukaryotic expression vector.
19. A recombinant cell carrying the nucleic acid molecule according to any one of claims 14 to 16, or expressing the humanized antibody or antigen-binding fragment thereof according to any one of claims 1 to 13.
20. The recombinant cell according to claim 19, wherein the recombinant cell is obtained by introducing the expression vector according to claim 17 or 18 into a host cell.
21. The recombinant cell of claim 20, wherein the expression vector is introduced into the host cell by electrotransduction.
22. The recombinant cell of claim 19, wherein the recombinant cell is a eukaryotic cell.
23. The recombinant cell of claim 19, wherein the recombinant cell is a mammalian cell.
24. A pharmaceutical composition comprising the humanized antibody or antigen-binding fragment thereof according to any one of claims 1 to 13, the nucleic acid molecule according to any one of claims 14 to 16, the expression vector according to any one of claims 17 to 18, or the recombinant cell according to any one of claims 19 to 23.
25. Use of the humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, the recombinant cell of any one of claims 19 to 23, or the pharmaceutical composition of claim 24 in the manufacture of a medicament for the treatment or prevention of pain, cancer, inflammation or inflammatory diseases, neurodegenerative diseases, Sjogren's syndrome, endometriosis, diabetic peripheral neuropathy, prostatitis, pelvic pain syndrome, diseases related to the unbalanced regulation of bone remodeling, and diseases caused by abnormal signal conduction of connective tissue growth factor.
26. The use of claim 25, wherein the medicament is used to treat or prevent neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory

lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

27. A method of treating or preventing a disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA in a subject comprising administering to the subject a therapeutically effective amount of the humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, the recombinant cell of any one of claims 19 to 23, or the pharmaceutical composition of claim 24.

28. The method of claim 27, wherein the disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA includes neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

29. The humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, the recombinant cell of any one of claims 19 to 23, or the pharmaceutical composition of claim 24 for use in treating or preventing a disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA in a subject.

30. The humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any

one of claims 14 to 16, the expression vector of any one of claims 17 to 18, the recombinant cell of any one of claims 19 to 23, or the pharmaceutical composition of claim 24 for use in treating or preventing a disease, wherein the disease caused by abnormal expression of NGF, abnormal expression of TrkA, or abnormal activity of TrkA includes neuropathic pain, inflammatory pain, cancer-related pain, fracture-related pain, surgery-related pain, inflammatory lung disease, interstitial cystitis, painful bladder syndrome, inflammatory bowel disease, inflammatory skin disease, Raynaud's syndrome, idiopathic pulmonary fibrosis, scar (hypertrophy, keloid type and other forms), sclerosis, endocardial myocardial fibrosis, atrial fibrosis, bone marrow fibrosis, progressive massive fibrosis (lung), renal-derived systemic fibrosis, scleroderma, systemic sclerosis, joint fibrosis, ocular fibrosis, non-small cell lung cancer, papillary thyroid cancer, glioblastoma multiforme, colorectal cancer, melanoma, bile duct cancer or sarcoma, acute myeloid leukemia, large cell neuroendocrine cancer, neuroblastoma, prostate cancer, pancreatic cancer, melanoma, head and neck squamous cell carcinoma or gastric cancer.

31. A kit for detecting TrkA comprising the humanized antibody according to any one of claims 1 to 13.

32. Use of the humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, or the recombinant cell of any one of claims 19 to 23 in the preparation of a kit for detecting TrkA or diagnosing a TrkA-related disease.

33. A method of detecting TrkA or diagnosing a TrkA-related disease in a subject using a kit comprising the humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, or the recombinant cell of any one of claims 19 to 23.

34. The humanized antibody of any one of claims 1 to 13, the nucleic acid molecule of any one of claims 14 to 16, the expression vector of any one of claims 17 to 18, or the recombinant cell of any one of claims 19 to 23 for use in the preparation of a kit for detecting TrkA or diagnosing a TrkA-related disease.

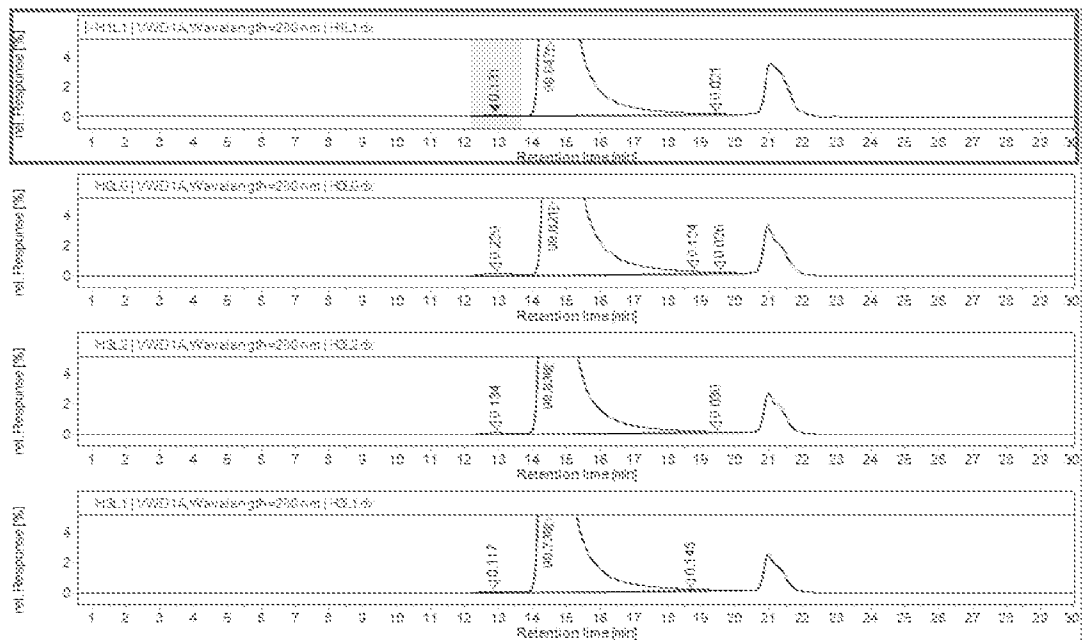


Figure 1

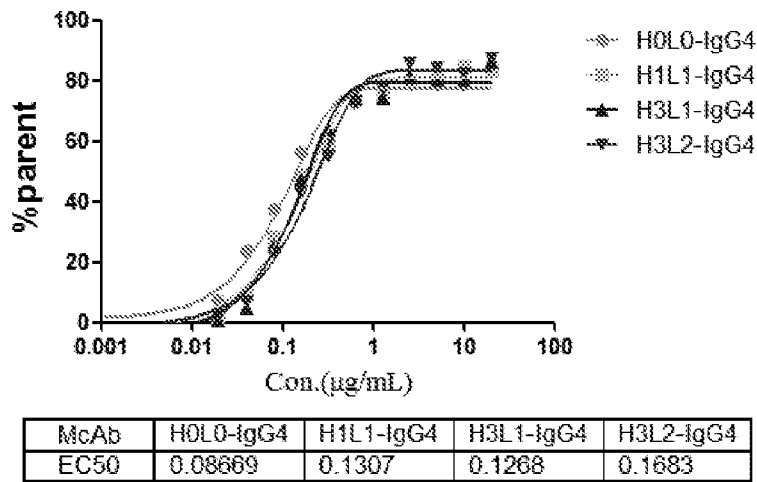


Figure 2

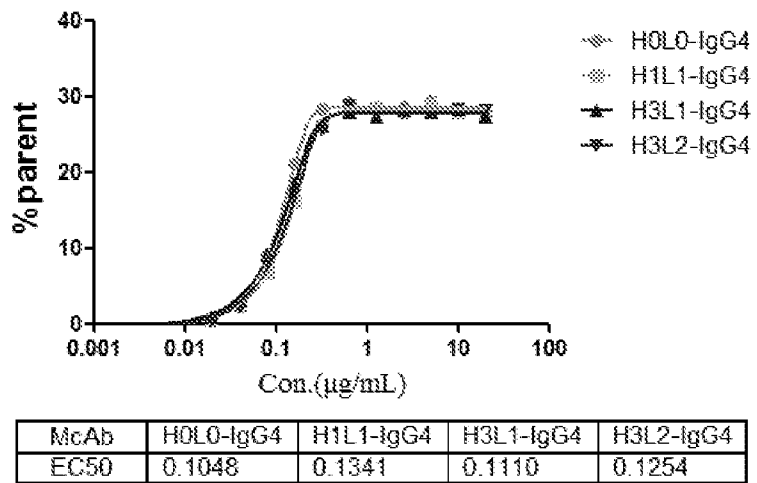


Figure 3

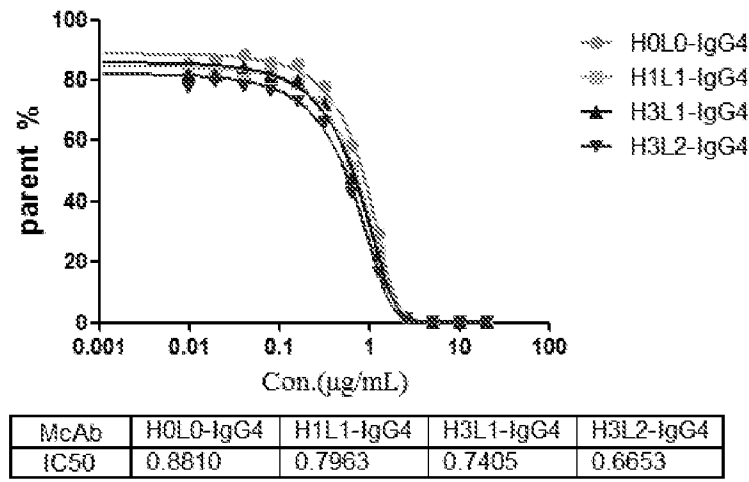


Figure 4

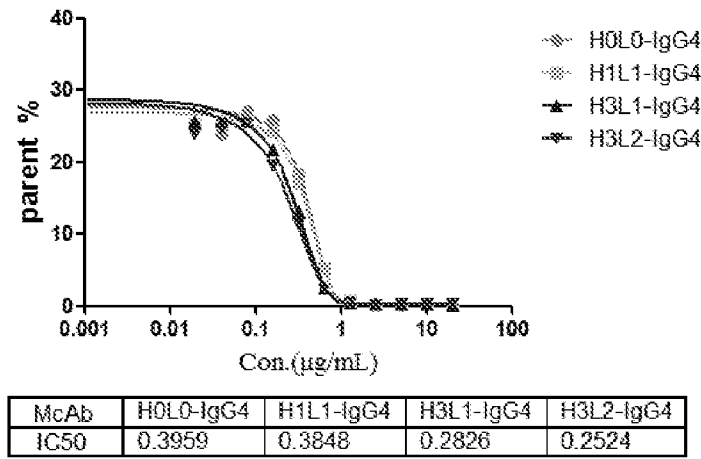


Figure 5

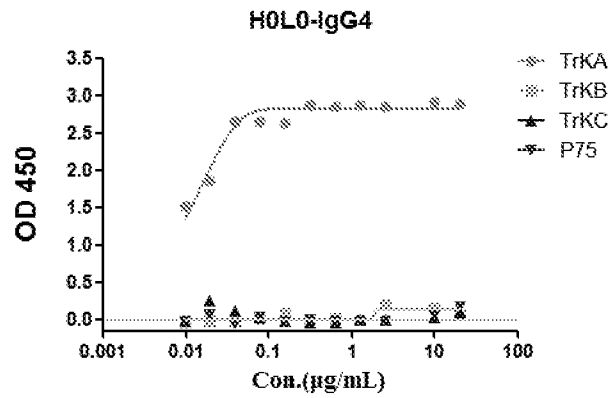


Figure 6A

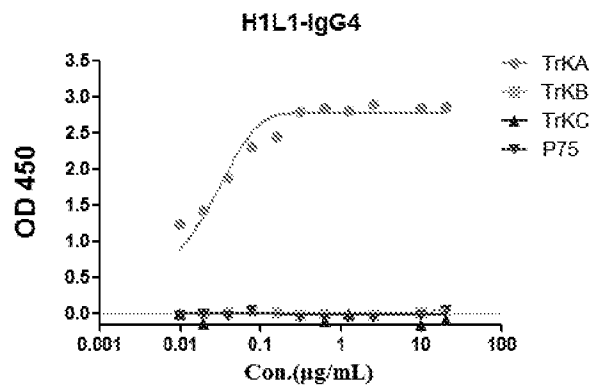


Figure 6B

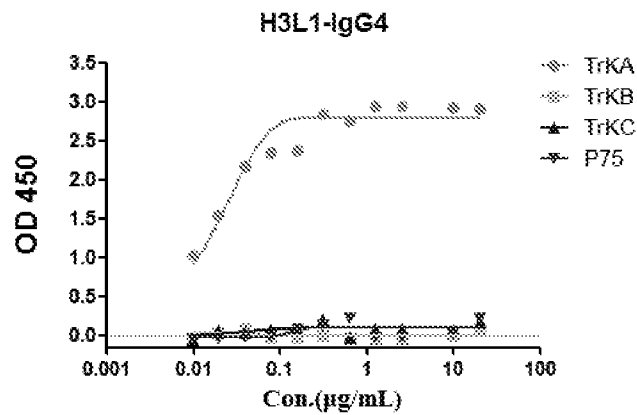


Figure 6C

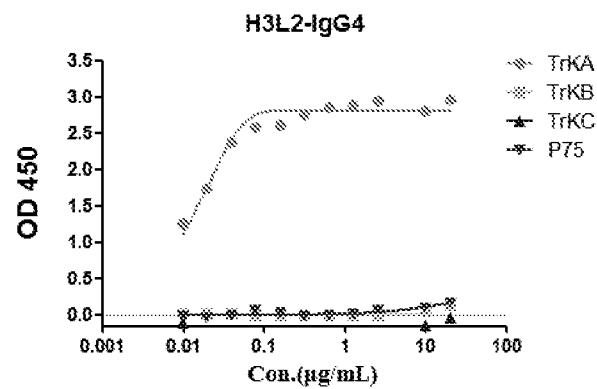


Figure 6D

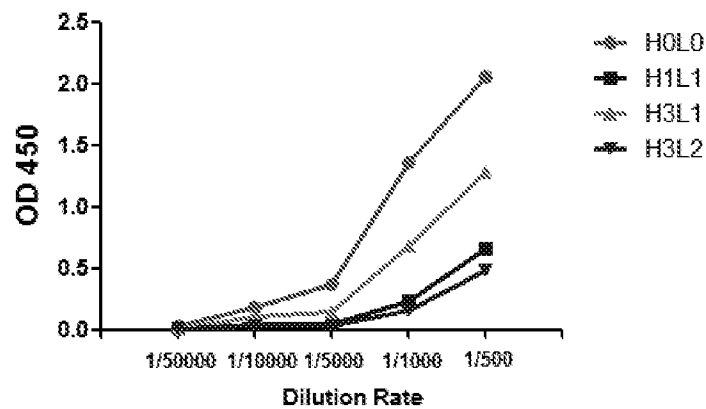


Figure 7

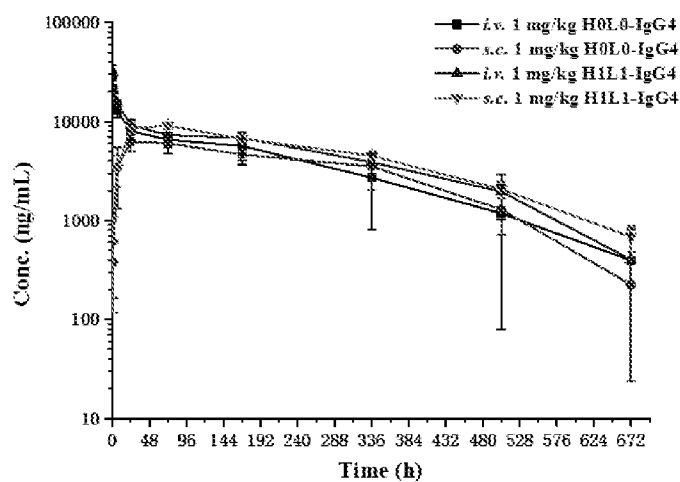


Figure 8

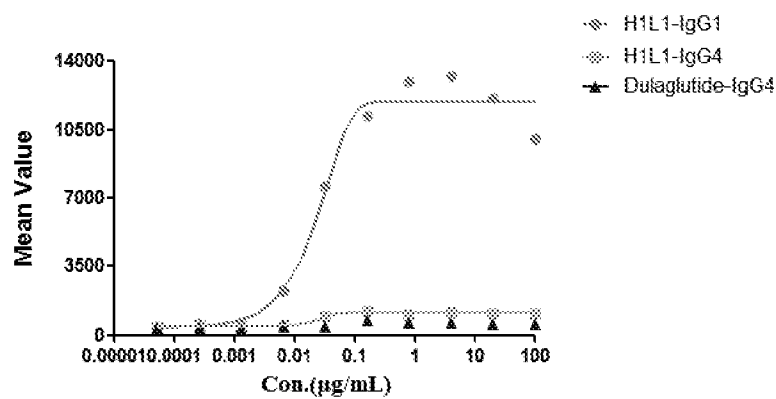


Figure 9

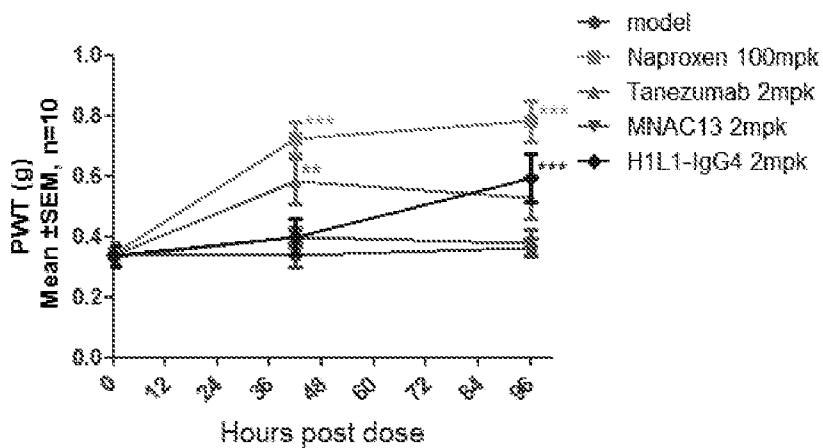


Figure 10

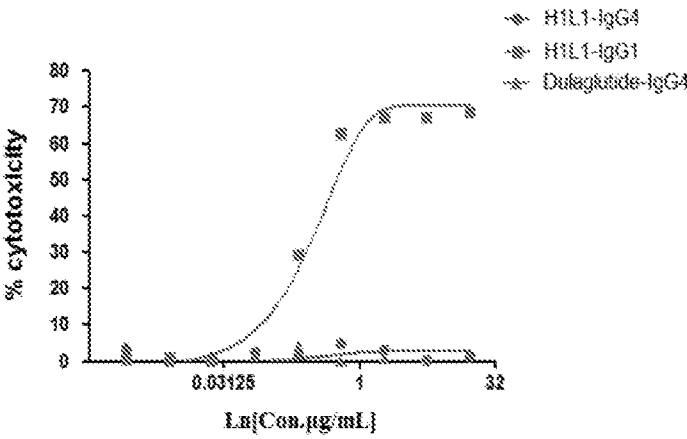


Figure 11

NIH3T3-TrkA stable cell line , p-TrkA AlphaLISA

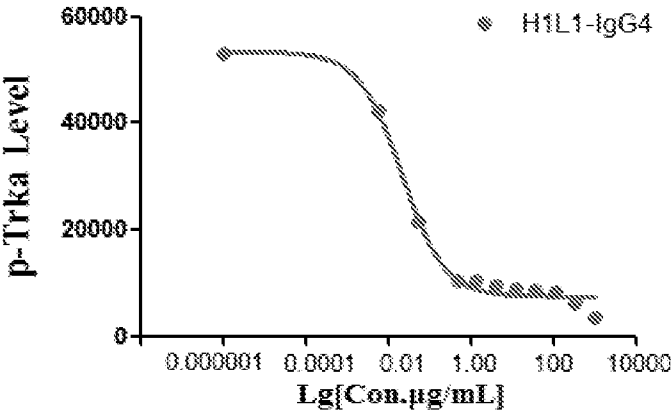


Figure 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/131343

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i; A61P 19/08(2006.01)i; A61P 13/12(2006.01)i;
A61P 19/02(2006.01)i; C12N 15/62(2006.01)i; C12N 15/63(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K, A61P; C12N

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

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

CNABS, VEN, CNKI, CNTXT, USTXT, EPTXT, PUBMED, ISI WEB, NCBI, EMBL-EBI, STNnext, SUNSHINE LAKE PHARMA, tyrosine-nase, TrkA, nerve growth factor, NGF, antibody+, humanized, heavy chain, light chain, variable region?, CDR, pain, cancer, inflammation, SEQ ID NO: 2-8, 10-27,30-48

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017362327 A1 (GLENMARK PHARMACEUTICALS S. A.) 21 December 2017 (2017-12-21) the whole document	1-34
A	US 2013344068 A1 (Leon F. Garcia-Martinez et al.) 26 December 2013 (2013-12-26) the whole document	1-34
A	US 2011145941 A1 (LAY LINE GENOMICS S.P.A.) 16 June 2011 (2011-06-16) the whole document	1-34
A	CN 104520330 A (GLENMARK PHARMACEUTICALS S. A.) 15 April 2015 (2015-04-15) the whole document	1-34
A	COVACEUSZACH, S. et al. "Neutralization of NGF-TrkA Receptor Interaction by the Novel Antagonistic Anti-TrkA Monoclonal Antibody MNAC13: A Structural Insight" <i>PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS</i> , Vol. 58, 29 December 2004 (2004-12-29), pages 717-727	1-34



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier application or patent but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
 "&" document member of the same patent family

Date of the actual completion of the international search

27 January 2022

Date of mailing of the international search report

15 February 2022

Name and mailing address of the ISA/CN

**National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088, China**

Facsimile No. (86-10)62019451

Authorized officer

WANG,Yao

Telephone No. 86-(10)-53961926

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/131343

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABDICHE, Y. N. et al. "Probing the binding mechanism and affinity of tanezumab, a recombinant humanized anti-NGF monoclonal antibody, using a repertoire of biosensors" <i>PROTEIN SCIENCE</i> , Vol. 17, No. 8, 02 January 2009 (2009-01-02), pages 1326-1335	1-34
A	UGOLINI, G. et al. "The function neutralizing anti-TrkA antibody MNAC13 reduces inflammatory and neuropathic pain" <i>PNAS</i> , Vol. 104, No. 8, 20 February 2007 (2007-02-20), pages 2985-2990	1-34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/131343

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 filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/131343**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.: **27-30**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 27-30 relate to a method of treating or preventing a disease, or the humanized antibody, the nucleic acid molecule, the expression vector, the recombinant cell, or the pharmaceutical composition for use in treating or preventing a disease. The subject matter of claims 27-30 relates to the treatment of human bodies, and therefore does not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). An international search has been carried out and based on the manufacturing of a medicament.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/131343

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)		Publication date (day/month/year)
US	2017362327	A1	21 December 2017	None		
US	2013344068	A1	26 December 2013	US	9539324 B2	10 January 2017
				US	2020062838 A1	27 February 2020
				US	2017137508 A1	18 May 2017
				US	10457727 B2	29 October 2019
US	2011145941	A1	16 June 2011	US	9751947 B2	05 September 2017
				MX	2010008571 A	02 December 2010
				AU	2009211340 A1	13 August 2009
				AU	2009211340 B2	12 September 2013
				IL	207390 D0	30 December 2010
				IL	207390 A	30 June 2015
				ZA	201006218 B	31 August 2011
				EP	2252633 A1	24 November 2010
				EP	2252633 B1	14 August 2013
				EP	2252633 B9	19 February 2014
				EA	201070888 A1	29 April 2011
				EA	021284 B1	29 May 2015
				JP	2011514314 A	06 May 2011
				JP	5719596 B2	20 May 2015
				ES	2435917 T3	26 December 2013
				ES	2435917 T9	02 April 2014
				CA	2713786 A1	13 August 2009
				BR	PI0905955 A2	04 August 2015
				BR	PI0905955 A8	08 May 2018
				PT	2252633 E	19 November 2013
				DK	2252633 T3	11 November 2013
				NZ	587701 A	28 March 2013
				PL	2252633 T3	28 February 2014
				WO	2009098238 A1	13 August 2009
				CN	101939337 A	05 January 2011
				CN	101939337 B	09 March 2016
				KR	20100114536 A	25 October 2010
				KR	101782857 B1	28 September 2017
				AP	201005379 A0	31 August 2010
				AP	2010005379 A0	31 August 2010
				AP	2929 A	30 June 2014
CN	104520330	A	15 April 2015	UA	113879 C2	27 March 2017
				MX	2014015043 A	20 February 2015
				MX	362394 B	15 January 2019
				PE	20150212 A1	27 February 2015
				IL	235994 A	29 January 2015
				IL	235994 D0	29 January 2015
				AU	2013273115 A2	22 January 2015
				AU	2013273115 A1	22 January 2015
				AU	2013273115 B2	01 March 2018
				HK	1209435 A1	01 April 2016
				CO	7240431 A2	17 April 2015
				US	2013344064 A1	26 December 2013
				CA	2875703 A1	12 December 2013
				AP	201408145 D0	31 December 2014

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/131343

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
	WO	2013183032 A2	12 December 2013
	WO	2013183032 A3	20 February 2014
	EP	2859019 A2	15 April 2015
	EP	2859019 B1	21 February 2018
	ES	2666126 T3	03 May 2018
	AU	2001273115 A1	13 June 2002
	KR	20150023679 A	05 March 2015
	CL	2014003339 A1	10 April 2015
	EA	201492163 A1	31 August 2015
	AP	2014008145 A0	31 December 2014
	AP	201408145 A0	31 December 2014
	SG	11201408037S A	29 January 2015
	US	2017218087 A1	03 August 2017
	PH	12014502722 A1	02 February 2015
	JP	2015520182 A	16 July 2015
	IN	2656MUN2014 A	21 August 2015