

CLAIMS

1. An antigen-binding molecule, comprising:
at least one first antigen-binding domain that specifically binds to RANKL, and
5 at least one second antigen-binding domain that specifically binds to NGF;
the first antigen-binding domain that specifically binds to RANKL comprises a heavy chain variable region and a light chain variable region, wherein:
- 10 i) the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 7; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; and
the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and
a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
- 15 ii) the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 7; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 15; and
the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and
a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
- 20 iii) the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 13; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3; and
the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and
25 a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6.
2. The antigen-binding molecule according to claim 1, wherein the first antigen-binding domain that specifically binds to RANKL comprises a heavy chain variable region and a light chain variable region, wherein:
- 30 the heavy chain variable region comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 37, 36 or 38; and
the light chain variable region comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 40;
preferably,
- 35 the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 37, 36 or 38; and/or
the light chain variable region comprises the amino acid sequence of SEQ ID NO: 40;

more preferably,

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 37;

and/or

the light chain variable region comprises the amino acid sequence of SEQ ID NO: 40.

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3. The antigen-binding molecule according to claim 1 or 2, wherein the second antigen-binding domain that specifically binds to NGF comprises a heavy chain variable region and a light chain variable region, wherein:

the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 58; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 59; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 60; and

the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 61; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 62; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 63.

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4. The antigen-binding molecule according to claim 3, wherein the second antigen-binding domain that specifically binds to NGF comprises a heavy chain variable region and a light chain variable region, wherein:

the heavy chain variable region comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 64; and/or

the light chain variable region comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 65;

preferably,

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 64;

25 and/or

the light chain variable region comprises the amino acid sequence of SEQ ID NO: 65.

5. The antigen-binding molecule according to any one of claims 1 to 4, comprising an Fc region, wherein the Fc region comprises two subunits capable of associating;

30 the Fc region is preferably an IgG₄ Fc region;

more preferably, the Fc region is a human IgG₄ Fc region, and the amino acid residue at position 228 is P, as numbered according to the EU index.

6. The antigen-binding molecule according to any one of claims 1 to 5, comprising a first chain of the structure of formula (I) and a second chain of the structure of formula (II),

VHa-linker 1-VHb-CH1-one subunit of Fc region

formula (I);

VLa-linker 2-VLb-CL

formula (II);

wherein:

5 VH_a and VH_b are heavy chain variable regions, and VL_a and VL_b are light chain variable regions;

VH_a and VL_a form the first antigen-binding domain that specifically binds to RANKL, and VH_b and VL_b form the second antigen-binding domain that specifically binds to NGF; or

10 VH_a and VL_a form the second antigen-binding domain that specifically binds to NGF, and VH_b and VL_b form the first antigen-binding domain that specifically binds to RANKL; and

linker 1 and linker 2 are peptide linkers identical or different in sequence;

preferably,

15 the VH_a comprises the amino acid sequence of SEQ ID NO: 37, 36 or 38, and the VL_a comprises the amino acid sequence of SEQ ID NO: 40; and/or

the VH_b comprises the amino acid sequence of SEQ ID NO: 64, and the VL_b comprises the amino acid sequence of SEQ ID NO: 65.

20 7. The antigen-binding molecule according to claim 6, wherein the linker 1 or linker 2 comprises the amino acid sequence set forth in any one selected from the group consisting of SEQ ID NOs: 70-97;

preferably, the linker 1 comprises the amino acid sequence of SEQ ID NO: 70, and/or the linker 2 comprises the amino acid sequence of SEQ ID NO: 85.

25 8. The antigen-binding molecule according to claim 6 or 7, wherein the CH1-Fc region comprises the amino acid sequence of SEQ ID NO: 51 or 66.

9. The antigen-binding molecule according to any one of claims 6 to 8, comprising:
30 a first chain, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 98, 100 or 101; and/or

a second chain, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 99;

preferably, the antigen-binding molecule comprises:

35 a first chain, comprising the amino acid sequence of SEQ ID NO: 98, 100 or 101; and
a second chain, comprising the amino acid sequence of SEQ ID NO: 99; more preferably, the antigen-binding molecule comprises two first chains identical in sequence and two

second chains identical in sequence.

10. The antigen-binding molecule according to any one of claims 1 to 9, wherein the antigen-binding molecule has at least one of the following properties:

- 5 i) an affinity of the first antigen-binding domain that specifically binds to RANKL for RANKL is higher than an affinity of the second antigen-binding domain that specifically binds to NGF for NGF;
- ii) an EC50 value with which the first antigen-binding domain that specifically binds to RANKL binds to human RANKL is less than an EC50 value with which the second
- 10 antigen-binding domain that specifically binds to NGF binds to human NGF; and
- iii) an IC50 value with which the first antigen-binding domain that specifically binds to RANKL blocks binding of RANKL to RANK is less than an IC50 value with which the second antigen-binding domain that specifically binds to NGF blocks binding of NGF to TrKA.

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11. An anti-RANKL antibody, comprising a heavy chain variable region and a light chain variable region, wherein:

- i) the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 7; a HCDR2 comprising the amino acid sequence of SEQ ID
- 20 NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; and the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
- ii) the heavy chain variable region comprises: a HCDR1 comprising the amino acid
- 25 sequence of SEQ ID NO: 7; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 15; and the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
- 30 iii) the heavy chain variable region comprises: a HCDR1 comprising the amino acid sequence of SEQ ID NO: 13; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3; and the light chain variable region comprises: a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5; and
- 35 a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6.

12. The anti-RANKL antibody according to claim 11, wherein:
the heavy chain variable region comprises an amino acid sequence having at least 90%
sequence identity to SEQ ID NO: 36, 37 or 38; and/or
the light chain variable region comprises an amino acid sequence having at least 90%
5 sequence identity to SEQ ID NO: 40;
preferably, the heavy chain variable region comprises the amino acid sequence of SEQ
ID NO: 36, 37 or 38; and/or the light chain variable region comprises the amino acid
sequence of SEQ ID NO: 40;
more preferably, the heavy chain variable region comprises the amino acid sequence of
10 SEQ ID NO: 37; and/or the light chain variable region comprises the amino acid sequence
of SEQ ID NO: 40.
13. The anti-RANKL antibody according to claim 11 or 12, comprising a heavy chain
constant region and a light chain constant region, wherein preferably, the heavy chain
15 constant region comprises the amino acid sequence of SEQ ID NO: 51 or 66, and/or the
light chain constant region comprises the amino acid sequence of SEQ ID NO: 52.
14. The anti-RANKL antibody according to any one of claims 11 to 13, comprising a
heavy chain and a light chain, wherein:
20 the heavy chain comprises an amino acid sequence having at least 85% sequence identity
to SEQ ID NO: 53, 55 or 56, and/or the light chain comprises an amino acid sequence
having at least 85% sequence identity to SEQ ID NO: 54;
preferably,
the heavy chain comprises the amino acid sequence of SEQ ID NO: 53, and/or the light
25 chain comprises the amino acid sequence of SEQ ID NO: 54; or
the heavy chain comprises the amino acid sequence of SEQ ID NO: 55, and/or the light
chain comprises the amino acid sequence of SEQ ID NO: 54; or
the heavy chain comprises the amino acid sequence of SEQ ID NO: 56, and/or the light
chain comprises the amino acid sequence of SEQ ID NO: 54.
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15. The anti-RANKL antibody according to any one of claims 11 to 13, wherein the anti-
RANKL antibody is an antibody fragment; preferably, the antibody fragment is selected
from the group consisting of Fab, Fab', F(ab')₂, Fd, Fv, scFv, dsFv and dAb.
- 35 16. The anti-RANKL antibody according to any one of claims 11 to 15, wherein:
the antibody binds to human RANKL with a K_D value of less than 5 nM; and/or
the antibody blocks binding of RANKL to RANK with an IC₅₀ value of less than 0.5 nM.

17. A bispecific antibody, comprising the anti-RANKL antibody according to any one of claims 11 to 16.
- 5 18. An isolated nucleic acid, encoding the antigen-binding molecule according to any one of claims 1 to 10, or the anti-RANKL antibody according to any one of claims 11 to 16, or the bispecific antibody according to claim 17.
19. A vector, comprising the isolated nucleic acid according to claim 18.
- 10 20. A host cell, comprising the vector according to claim 19.
21. A method for preparing an antigen-binding molecule, an anti-RANKL antibody or a bispecific antibody, comprising culturing the host cell according to claim 20 under
15 conditions suitable for expression of the antigen-binding molecule or antibody.
22. A pharmaceutical composition, comprising:
a therapeutically effective amount of the antigen-binding molecule according to any one of claims 1 to 10, or the anti-RANKL antibody according to any one of claims 11 to 16,
20 or the bispecific antibody according to claim 17; and
one or more pharmaceutically acceptable carriers, diluents, buffers or excipients.
23. Use of the antigen-binding molecule according to any one of claims 1 to 10, or the anti-RANKL antibody according to any one of claims 11 to 16, or the bispecific antibody
25 according to claim 17, or the pharmaceutical composition according to claim 22 in the manufacture of a medicament for treating or preventing a disease, wherein the disease is pain, joint stiffness or bone loss;
preferably, the pain is selected from the group consisting of osteoarticular pain, rheumatoid arthritis pain, gout, bone cancer pain, fracture pain, post-surgical pain, cancer
30 pain, painful bladder syndrome, musculoskeletal pain, prostatitis, pelvic pain, interstitial cystitis, lower back pain, dysmenorrhea, pain associated with bone disease, trigeminal neuralgia, post-herpetic neuralgia, herpes zoster infection, sciatica, migraine, diabetic neuropathy, and pain associated with peripheral nerves; and
wherein the bone loss is associated with at least one condition selected from the group
35 consisting of osteoporosis, Paget's disease, osteomyelitis, hypercalcemia, osteopenia, osteoporosis, osteonecrosis, bone injury, bone resorption, osteogenesis imperfecta, inflammation, autoimmune disease, enteritis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, periodontal bone resorption, osteolytic metastasis, and cancer.
- 40 24. The use according to claim 23, wherein the cancer is selected from the group

consisting of breast cancer, prostate cancer, thyroid cancer, kidney cancer, lung cancer, esophageal cancer, rectal cancer, bladder cancer, cervical cancer, ovarian cancer, liver cancer, gastrointestinal cancer, melanoma, multiple myeloma, osteosarcoma, lymphoma, non-small cell lung cancer, bone tumor, and Hodgkin's disease.

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25. Use of the antigen-binding molecule according to any one of claims 1 to 10, or the anti-RANKL antibody according to any one of claims 11 to 16, or the bispecific antibody according to claim 17, or the pharmaceutical composition according to claim 22 in the manufacture of a medicament for treating or preventing an NGF- or RANKL-associated disease;

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preferably, wherein the NGF- or RANKL-associated disease is pain, joint stiffness or bone loss;

more preferably, wherein the pain is selected from the group consisting of osteoarticular pain, rheumatoid arthritis pain, gout, bone cancer pain, fracture pain, post-surgical pain, cancer pain, painful bladder syndrome, musculoskeletal pain, prostatitis, pelvic pain, interstitial cystitis, lower back pain, dysmenorrhea, pain associated with bone disease, trigeminal neuralgia, post-herpetic neuralgia, herpes zoster infection, sciatica, migraine, diabetic neuropathy, and pain associated with peripheral nerves; and

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wherein the bone loss is associated with at least one condition selected from the group consisting of osteoporosis, Paget's disease, osteomyelitis, hypercalcemia, osteopenia, osteoporosis, osteonecrosis, bone injury, bone resorption, osteogenesis imperfecta, inflammation, autoimmune disease, enteritis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, periodontal bone resorption, osteolytic metastasis, and cancer.

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26. Use of an NGF antagonist and a RANKL antagonist in the manufacture of a medicament for treating pain or inhibiting bone loss;

wherein the NGF antagonist and the RANKL antagonist are to be administered simultaneously or sequentially;

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preferably, wherein the NGF antagonist is an anti-NGF antibody and/or the RANKL antagonist is an anti-RANKL antibody.

27. The use according to claim 26, wherein:

the anti-NGF antibody is tanezumab, and/or

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the anti-RANKL antibody is denosumab.