CLAIMS

1. An antigen-binding construct, comprising a first antigen-binding fragment that is monovalent and specifically binds to ECD4 (extracellular domain IV) antigen of HER2 on an HER2-expressing cell and a second antigen-binding fragment that is monovalent and specifically binds to ECD2 (extracellular domain II) antigen of HER2 on an HER2-expressing cell,

wherein the first antigen-binding fragment is an scFv, and comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 43, 28 and 29, respectively, and a light chain CDR1, a light chain CDR2 and a light chain CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 30, 31 and 32, respectively; and

wherein the second antigen-binding fragment comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 45, 46 and 47, respectively, and a light chain CDR1, a light chain CDR2 and a light chain CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 48, 49 and 50, respectively.

2. The antigen-binding construct according to claim 1, wherein the antigen-binding construct is an antibody.

3. The antigen-binding construct according to claim 1 or 2, wherein the first antigen-binding fragment is selected from:
the first antigen-binding fragment comprising a heavy chain variable region and a light chain variable region, which comprise the amino acid sequences set forth in SEQ ID NOs: 35 and 36, respectively; or
the first antigen-binding fragment comprising a heavy chain variable region and a light chain variable region consisted of the amino acid sequences set forth in SEQ ID NOs: 35 and 36, respectively.

4. The antigen-binding construct according to any one of claims 1–3, wherein a VH and a VL of the first antigen-binding fragment are arranged from N-terminus to C-terminus in the following order: VH-linker-VL.

5. The antigen-binding construct according to any one of claims 1-4, wherein the second antigen-binding fragment is an Fab.

6. The antigen-binding construct according to any one of claims 1-5, wherein the second antigen-binding fragment comprises a heavy chain variable region with the amino acid sequence set forth in SEQ ID NO: 37 and a light chain variable region with the amino acid sequence set forth in SEQ ID NO: 38.

7. The antigen-binding construct according to any one of claims 1–6, wherein the antigen-binding construct comprises an immunoglobulin functional domain operably linked to the first antigen-binding fragment or/and the second antigen-binding fragment, the immunoglobulin functional domain comprising: i. one or more of a CL, a CH1, a CH2 and a CH3; or ii. an Fc.

8. The antigen-binding construct according to claim 7, wherein the CL, CH1, CH2, CH3 and Fc are derived from CL, CH1, CH2, CH3 and Fc of human IgG, respectively.

9. The antigen-binding construct according to claim 7, wherein the CL, CH1, CH2, CH3 or Fc has a modification.

10. The antigen-binding construct according to claim 9, wherein the modification the CH3 or Fc has is: (1) an amino acid substitution at position 435 or/and position 436 according to the EU numbering system; and/or (2) mutation sites of knob-into-hole.

11. The antigen-binding construct according to any one claims 7-10, wherein the Fc is a dimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide; the first antigen-binding fragment is operably linked to the first Fc polypeptide, and the second antigen-binding fragment is operably linked to the second Fc polypeptide.

12. The antigen-binding construct according to any one of claims 7-11, wherein the Fc is derived from Fc of human IgG1 or human IgG4.

13. The antigen-binding construct according to any one of claims 1–12, wherein the antigen-binding construct is a bispecific antibody or a multispecific antibody.

14. The antigen-binding construct according to any one of claims 1–12, wherein the antigen-binding construct is bivalent or polyvalent.

15. The antigen-binding construct according to claim 1, wherein the antigen-binding construct is a bivalent bispecific antibody, comprising: a heavy chain comprising SEQ ID NO: 11, a heavy chain comprising SEQ ID NO: 13, and a light chain comprising SEQ ID NO: 15; or
the antigen-binding construct is a bivalent bispecific antibody, comprising: a heavy chain of SEQ ID NO: 11, a heavy chain of SEQ ID NO: 13, and a light chain of SEQ ID NO: 15.

16. The antigen-binding construct according to any one of claims 1–15, wherein the antigen-binding construct has reduced aggregation compared to antigen-binding constructs in which the amino acid at position 30 is not E or/and the amino acid at position 53 is not Y; or/and the antigen-binding construct does not have significantly reduced binding affinity for ECD4 antigen or/and ECD2 antigen of HER2 compared to antigen-binding constructs in which the amino acid at position 30 is not E or/and the amino acid at position 53 is not Y.

17. The antigen-binding construct according to any one of claims 1–16, wherein the antigen-binding construct is afucosylated.

18. A pharmaceutical composition, comprising the antigen-binding construct according to any one of claims 1–17 and a pharmaceutically acceptable carrier.

19. An isolated nucleic acid or a collection of isolated nucleic acids, comprising at least one nucleic acid sequence encoding at least one antigen-binding fragment of the antigen-binding construct according to any one of claims 1–17.

20. A vector or a collection of vectors, comprising one or more of the nucleic acid and the collection of nucleic acids according to claim 19.

21. An isolated cell, comprising the nucleic acid or the collection of nucleic acids according to claim 19, or the vector or the collection of vectors according to claim 20.

22. A method for preparing the antigen-binding construct according to any one of claims 1–17, comprising: culturing a host cell under conditions suitable for expression of the antigen-binding construct, wherein the host cell comprises a nucleic acid encoding the antigen-binding construct, or the vector or the collection of vectors according to claim 20; and purifying the construct.

23. The antigen-binding construct according to any one of claims 1–17 or the pharmaceutical composition according to claim 18 for use in treating an HER2-expressing tumor in a subject.

24. The antigen-binding construct or the pharmaceutical composition for use according to claim 23, comprising contacting a tumor cell with the antigen-binding construct so that an HER2-expressing tumor cell is killed or growth of an HER2-expressing tumor cell is inhibited.

25. The antigen-binding construct or the pharmaceutical composition for use according to claim 23 or 24, wherein the tumor is biliary tract cancer, carcinosarcoma, esophageal cancer, gastroesophageal junction cancer, breast cancer, gastric cancer, pancreatic cancer, head and neck cancer, colorectal cancer, renal cancer, cervical cancer, ovarian cancer, endometrial cancer, uterine cancer, malignant melanoma, pharyngeal cancer, oral cancer, or skin cancer.

26. The antigen-binding construct or the pharmaceutical composition for use according to any one of claims 23–25, comprising administering to a cell an effective amount of the antigen-binding construct or the pharmaceutical composition so that HER2 signaling in the cell is inhibited, reduced or blocked.