

CLAIMS

1. A heterodimeric bispecific antibody comprising a first antigen-binding functional domain capable of specifically binding to PD-1 and a second antigen-binding functional domain capable of specifically binding to HER2, wherein the heterodimeric bispecific antibody comprises a first Fc chain and a second Fc chain which are linked to each other via one or more disulfide bonds, the first Fc chain and the second Fc chain being respectively linked to the PD-1 antigen-binding functional domain and the HER2 antigen-binding functional domain via a covalent bond or a linker, or the first Fc chain and the second Fc chain being respectively linked to the HER2 antigen-binding functional domain and the PD-1 antigen-binding functional domain via a covalent bond or a linker, and the first Fc chain and the second Fc chain comprise 5 amino acid substitutions at the following positions:

amino acid substitutions at positions T366 and D399 of the first Fc chain and amino acid substitutions at positions L351, Y407, and K409 of the second Fc chain;

wherein the amino acid substitutions of the first Fc chain are T366L and D399R, and the amino acid substitutions of the second Fc chain are L351E, Y407L, and K409V, and

wherein the first and second Fc chains comprising the above amino acid substitutions have a tendency to undergo heterodimerization rather than homodimerization, and the amino acid positions are numbered according to the Kabat EU numbering system.

2. The heterodimeric bispecific antibody of claim 1, wherein the Fc chain is derived from IgG.

3. The heterodimeric bispecific antibody of claim 1, wherein the PD-1 and HER2 antigen-binding functional domains are Fab fragments or scFv fragments.
- 5 4. The heterodimeric bispecific antibody of claim 1, wherein the PD-1 and HER2 antigen-binding functional domains are all Fab fragments.
5. The heterodimeric bispecific antibody of claim 1, wherein one of the PD-1 and HER2 antigen-binding functional domains is a Fab fragment and
10 the other is a scFv fragment.
6. The heterodimeric bispecific antibody of any one of claims 3 to 5, wherein the Fab fragment comprises a first heavy chain variable region and a second heavy chain variable region that are different from each other and a first
15 light chain variable region and a second light chain variable region that are different from each other.
7. The heterodimeric bispecific antibody of claim 1, wherein, when each of the first Fc chain covalently bonded to the PD-1 antigen-binding region and the second Fc chain covalently bonded to the HER2 antigen-binding region,
20 or each of the first Fc chain covalently bonded to the HER2 antigen-binding region and the second Fc chain covalently bonded to the PD-1 antigen-binding region, is present alone in the presence of a reducing agent, the weight ratio of homodimer formation is less than 50%.
- 25 8. The heterodimeric bispecific antibody of claim 1, wherein an amino acid sequence of the bispecific antibody comprising at least one of SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16, and 18.

9. An isolated polynucleotide encoding the heterodimeric bispecific antibody of claim 1.

10. The isolated polynucleotide of claim 9, comprising a sequence
5 selected from SEQ ID NOs. 1, 3, 5, 7, 9, 11, 13, 15 and 17.

11. A recombinant expression vector comprising the isolated polynucleotide of claim 9.

10 12. The recombinant expression vector of claim 11, wherein the expression vector is a plasmid vector X0GC obtained by modifying pcDNA.

13. A host cell comprising the recombinant expression vector of claim
11.

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14. The host cell of claim 13, wherein the host cell is selected from a human embryonic kidney cell HEK293, or HEK293T, HEK293E, or HEK293F derived from the HEK293 cell; and a Chinese hamster ovary cell CHO, or CHO-S, CHO-dhfr, CHO/DG44, or ExpiCHO derived from the CHO cell.

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15. A pharmaceutical composition for use in the prevention or treatment of a disease or disorder comprising: the heterodimeric bispecific antibody of claim 1, the isolated polynucleotide of claim 9, the recombinant expression vector of claim 11, or the host cell of claim 13, and a
25 pharmaceutically acceptable carrier,

wherein the disease or disorder comprises one or more of is selected from leukemia, lymphoma, myeloma, brain tumor, head and neck squamous cell carcinoma, non-small cell lung cancer, nasopharyngeal carcinoma, esophageal cancer, stomach cancer, pancreatic cancer, gallbladder cancer,
30 liver cancer, colon cancer, breast cancer, ovarian cancer, cervical cancer,

endometrial cancer, uterine sarcoma, prostate cancer, bladder cancer, renal cell carcinoma, and melanoma.

16. A method of producing the heterodimeric bispecific antibody of
5 claim 1, the method comprising:

- 1) expressing the isolated polynucleotide of claim 9 or the recombinant expression vector of claim 11 in a host cell;
- 2) purifying and reducing each protein expressed in the host cell; and
- 3) mixing the reduced proteins and then oxidizing the mixture.

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17. The method of claim 16, wherein the host cell is selected from a human embryonic kidney cell HEK293, or HEK293T, HEK293F, or HEK293E derived from the HEK293 cell; and a Chinese hamster ovary cell CHO, or CHO-S, CHO-dhfr, CHO/DG44, or ExpiCHO derived from the CHO cell.

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18. The method of claim 16, wherein the reducing comprises:

- 1) adding a reducing agent, wherein the reducing agent is selected from 2-mercaptoethylamine, dithiothreitol, tri(2-carboxyethyl)phosphine, other chemical derivatives, and a combination thereof,
- 20 2) performing a reduction reaction in the presence of dithiothreitol at a concentration of 0.1 mM or more at 4°C for 3 hours, and
- 3) removing the reducing agent by desalting, etc.

19. The method of claim 16, wherein the oxidizing comprises:

- 25 1) oxidizing in air or adding an oxidizing agent, wherein the oxidizing agent is selected from L-dehydroascorbic acid and other chemical derivatives, and
- 2) performing an oxidization reaction in the presence of L-dehydroascorbic acid at a concentration of 0.5 mM or more at 4 °C for 5 hours.

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20. The method of any one of claims 16, further comprising isolating and purifying.

21. Use of the heterodimeric bispecific antibody of any one of claims 1
5 to 8, the isolated polynucleotide of claim 9 or 10, the recombinant expression
vector of claim 11 or 12, or the host cell of claim 13 or 14 in the preparation of a
medicament for the prevention or treatment of a disease or disorder,

wherein the disease or disorder comprises one or more of is selected
from leukemia, lymphoma, myeloma, brain tumor, head and neck squamous
10 cell carcinoma, non-small cell lung cancer, nasopharyngeal carcinoma,
esophageal cancer, stomach cancer, pancreatic cancer, gallbladder cancer,
liver cancer, colon cancer, breast cancer, ovarian cancer, cervical cancer,
endometrial cancer, uterine sarcoma, prostate cancer, bladder cancer, renal cell
carcinoma, and melanoma.