

## Claims

1. An antibody that specifically recognizes glypican-3, wherein the antibody comprises:  
antibody (a), and its light chain variable region has CDR1 of SEQ ID NO: 16, CDR2 of  
5 SEQ ID NO: 18 and CDR3 of SEQ ID NO: 20, or its heavy chain variable region has CDR1  
of SEQ ID NO: 10, CDR2 of SEQ ID NO: 12 and CDR3 of SEQ ID NO: 14;

antibody (b), and its light chain variable region has CDR1 of SEQ ID NO: 16, CDR2 of  
SEQ ID NO: 18 and CDR3 of SEQ ID NO: 20; or its heavy chain variable region has CDR1  
of SEQ ID NO: 60, CDR2 of SEQ ID NO: 61 and CDR3 of SEQ ID NO: 14;

10 antibody (c), and its light chain variable region has CDR1 of SEQ ID NO: 16, CDR2 of  
SEQ ID NO: 18 and CDR3 of SEQ ID NO: 20, or its heavy chain variable region has CDR1  
of SEQ ID NO: 62, CDR2 of SEQ ID NO: 63 and CDR3 of SEQ ID NO: 14;

antibody (d), and its light chain variable region has CDR1 of SEQ ID NO: 16, CDR2 of  
SEQ ID NO: 18 and CDR3 of SEQ ID NO: 20; or its heavy chain variable region has CDR1  
15 of SEQ ID NO: 64, CDR2 of SEQ ID NO: 65 and CDR3 of SEQ ID NO: 14;

antibody (e), and its light chain variable region has CDR1 of SEQ ID NO: 67, CDR2 of  
SEQ ID NO: 68 and CDR3 of SEQ ID NO: 20; or its heavy chain variable region has CDR1  
of SEQ ID NO: 10, CDR2 of SEQ ID NO: 66 and CDR3 of SEQ ID NO: 14;

antibody (f), and its light chain variable region has CDR1 of SEQ ID NO: 69, CDR2 of  
20 SEQ ID NO: 70 and CDR3 of SEQ ID NO: 20, or its heavy chain variable region has CDR1  
of SEQ ID NO: 10, CDR2 of SEQ ID NO: 66 and CDR3 of SEQ ID NO: 14;

antibody (g), and its light chain variable region has CDR1 of SEQ ID NO: 16, CDR2 of  
SEQ ID NO: 70 and CDR3 of SEQ ID NO: 20, or its heavy chain variable region has CDR1  
of SEQ ID NO: 10, CDR2 of SEQ ID NO: 71 and CDR3 of SEQ ID NO: 72;

25 antibody (h), which recognizes the same antigenic determinant as that recognized by the  
antibody according to any one of (a) to (g).

2. The antibody of claim 1, wherein the amino acid sequence of the heavy chain  
variable region of antibody (a) is shown in positions 1-121 of SEQ ID NO: 4; or the amino  
acid sequence of the light chain variable region of the antibody is shown in positions  
30 137-247 of SEQ ID NO: 4;

The amino acid sequence of the heavy chain variable region of antibody (b) is shown in  
positions 1-121 of SEQ ID NO: 25; or the amino acid sequence of the light chain variable  
region of the antibody is shown in positions 137-247 of SEQ ID NO: 25;

The amino acid sequence of the heavy chain variable region of antibody (c) is shown in

positions 1-121 of SEQ ID NO: 27; or the amino acid sequence of the light chain variable region of the antibody is shown in positions 137-247 of SEQ ID NO: 27;

The amino acid sequence of the heavy chain variable region of the antibody (d) is shown in positions 1-121 of SEQ ID NO: 29; or the amino acid sequence of the light chain variable region of the antibody is shown in positions 137-247 of SEQ ID NO: 29;

The amino acid sequence of the heavy chain variable region of the antibody (e) is shown in positions 1-121 of SEQ ID NO: 31; or the amino acid sequence of the light chain variable region of the antibody is shown in positions 137-247 of SEQ ID NO: 31;

The amino acid sequence of the heavy chain variable region of the antibody (f) is shown in positions 1-121 of SEQ ID NO: 33; or the amino acid sequence of the light chain variable region of the antibody is shown in positions 137-247 of SEQ ID NO: 33; or

The amino acid sequence of the heavy chain variable region of antibody (g) is shown in positions 1-121 of SEQ ID NO: 35; or the amino acid sequence of the light chain variable region of the antibody is shown in positions 137-247 of SEQ ID NO: 35.

3. An antibody that specifically recognizes glypican-3 (GPC3), wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises: CDR1 of the amino acid sequence of SEQ ID NO: 73, CDR2 of the amino acid sequence of SEQ ID NO: 74 and CDR3 of the amino acid sequence of SEQ ID NO: 75; or the light chain variable region comprises CDR1 of the amino acid sequence of SEQ ID NO: 76, CDR2 of the amino acid sequence of SEQ ID NO: 77 and CDR3 of the amino acid sequence of SEQ ID NO: 78.

4. The antibody of claim 3, wherein the antibody is a humanized monoclonal antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region thereof comprises: CDR1 of the amino acid sequence of SEQ ID NO: 73, CDR2 of the amino acid sequence of SEQ ID NO: 74 and CDR3 of the amino acid sequence of SEQ ID NO: 75; or the light chain variable region thereof comprises CDR1 of the amino acid sequence of SEQ ID NO: 76, CDR2 of the amino acid sequence of SEQ ID NO: 77 and CDR3 of the amino acid sequence of SEQ ID NO: 78.

5. The antibody of claim 4, wherein the antibody has a heavy chain variable region of the amino acid sequence of SEQ ID NO: 58 or a light chain variable region of the amino acid sequence of SEQ ID NO: 59.

6. The antibody of claim 4, wherein the antibody has a heavy chain variable region of the amino acid sequence of SEQ ID NO: 81 or a light chain variable region of the amino acid sequence of SEQ ID NO: 82.

7. A nucleic acid encoding the antibody of any one of claims 1-6.

8. An expression vector comprising the nucleic acid of claim 7.

9. A host cell comprising the expression vector of claim 8 or having the nucleic acid of claim 7 integrated into its genome.

10. Use of the antibody of any one of claims 1-6 for the preparation of a targeted drug, antibody-drug conjugate, or a polyfunctional antibody that specifically targets tumor cells expressing glypican-3; or

for the preparation of a reagent for diagnosing a tumor expressing Glypican-3; or

for the preparation of chimeric antigen receptor-modified immune cells.

11. A multi-functional immunoconjugate, comprising:

the antibody of any one of claims 1-6; and

a functional molecule linked thereto; and the functional molecule is selected from a group consisting of a molecule targeting a tumor surface marker, a tumor-suppressing molecule, a molecule targeting a surface marker of an immune cell, or a detectable label.

12. The multifunctional immunoconjugate of claim 11, wherein the molecule that targets a tumor surface marker is an antibody or ligand that binds to a tumor surface marker; or

the tumor-suppressing molecule is an anti-tumor cytokine or anti-tumor toxin; and preferably, the cytokine includes: IL-12, IL-15, IFN-beta and TNF-alpha.

13. The multifunctional immunoconjugate of claim 11, wherein the detectable label includes a fluorescent label or a chromogenic label.

14. The multifunctional immunoconjugate of claim 12, wherein the antibody that binds to a tumor surface marker refers to an antibody that recognizes antigens other than glypican-3, wherein the other antigens include: EGFR, EGFRvIII, mesothelin, HER2, EphA2, Her3, EpCAM, MUC1, MUC16, CEA, Claudin 18.2, folate receptor, Claudin 6, WT1, NY-ESO-1, MAGE 3, ASGPR1 or CDH16.

15. The multifunctional immunoconjugate of claim 11, wherein the molecule that targets the surface marker of the immune cell is an antibody that binds to T cell surface marker, which can form a T-cell-engaging bifunctional antibody with the antibody of any one of claims 1-7.

16. The multifunctional immunoconjugate of claim 15, wherein the antibody that binds to the immune cell surface marker is an anti-CD3 antibody.

17. The multifunctional immunoconjugate of claim 16, wherein the multifunctional immunoconjugate is a fusion peptide, and further comprises a linker peptide between the

antibody of any one of claims 1-5 and the functional molecule linked thereto.

18. A nucleic acid encoding the multi-functional immunoconjugate of any one of claims 9-17.

19. Use of the multifunctional immunoconjugate of any one of claims 11-17, for the preparation of an antineoplastic agent, or

for the preparation of an agent for diagnosis of tumors that express Glypican-3; or  
for the preparation of chimeric antigen receptor-modified immune cells; preferably, the immune cells include T lymphocyte, NK cell or NKT lymphocyte.

20. A chimeric antigen receptor comprising the antibody of any one of claims 1-6, a transmembrane region and an intracellular signal region, which are sequentially linked.

21. The chimeric antigen receptor of claim 20, wherein the intracellular signal region is selected from a group consisting of intracellular signal region sequences of CD3 $\zeta$ , Fc $\epsilon$ RI $\gamma$ , CD27, CD28, CD137, CD134, MyD88, CD40 or a combination thereof.

22. The chimeric antigen receptor of claim 20, wherein the transmembrane region comprises a transmembrane region of CD8 or CD28.

23. The chimeric antigen receptor of claim 20, wherein the chimeric antigen receptor comprises the following sequentially connected antibody, a transmembrane region and an intracellular signal region:

The antibody of any one of claims 1-6, CD8, and CD3 $\zeta$ ;

The antibody of any one of claims 1-6, CD8, CD137 and CD3 $\zeta$ ;

The antibody of any one of claims 1-6, the transmembrane region of CD28 molecule, the intracellular signaling region of CD28 molecule and CD3 $\zeta$ ; or

The antibody of any one of claims 1-6, the transmembrane region of CD28 molecule, the intracellular signaling region of CD28 molecule, CD137 and CD3 $\zeta$ .

24. The chimeric antigen receptor of claim 20, wherein the antibody is a single chain antibody or a domain antibody.

25. The chimeric antigen receptor of claim 20, wherein the chimeric antigen receptor comprises:

SEQ ID NO: 49 or the amino acid sequence shown in positions 22-346 thereof;

SEQ ID NO: 50 or the amino acid sequence shown in positions 22-447 thereof;

SEQ ID NO: 51 or the amino acid sequence shown in positions 22-491 thereof;

SEQ ID NO: 52 or the amino acid sequence shown in positions 22-494 thereof;

SEQ ID NO: 53 or the amino acid sequence shown in positions 22-536 thereof;

The amino acid sequence of SEQ ID NO: 86;

The amino acid sequence of SEQ ID NO: 88; or

The amino acid sequence of SEQ ID NO: 90.

26. A nucleic acid encoding the chimeric antigen receptor of any one of claims 20-25.

27. An expression vector comprising the nucleic acid of claim 22.

5 28. A virus comprising the vector of claim 27.

29. Use of the chimeric antigen receptor of any one of claims 20-25, or the nucleic acid of claim 26, or the expression vector of claim 27 or the virus of claim 28 for the preparation of genetically modified immune cells targeting the tumor that expresses Glypican-3.

10 30. The use of claim 29, wherein the tumor expressing glypican-3 includes (but is not limited to) liver cancer, melanoma, ovarian clear cell carcinoma, yolk sac tumor and neuroblastoma.

31. A genetically modified immune cell, which is transduced with the nucleic acid of claim 26 or the expression vector of claim 27 or the virus of claim 28; or

has the chimeric antigen receptor of any one of claims 20-25 expressed on its surface.

15 32. The immune cell of claim 31, wherein it further carry an exogenous encoding sequence for cytokines; and preferably, the cytokines include IL-12, IL-15 or IL-21.

33. The immune cell of claim 31, wherein it also expresses another chimeric antigen receptor which does not contain CD3 $\zeta$  but contains the intracellular signaling domain of CD28, the intracellular signaling domain of CD137, or a combination of both.

20 34. The immune cell of claim 31, wherein it further expresses a chemokine receptor; and preferably, the chemokine receptor includes: CCR2.

35. The immune cell of claim 31, wherein it further expresses siRNA which can reduce expression of PD-1 or a protein which blocks PD-L1.

25 36. The immune cell of claim 31, wherein it further expresses a safety switch; and preferably, the safety switch includes iCaspase-9, Truncated EGFR or RQR8.

37. The immune cell of claim 31, wherein the immune cells include T lymphocytes, NK cells or NKT cells.

38. Use of the genetically modified immune cell of any one of claims 31-37 for the preparation of a tumor-inhibiting drug, and the tumor is a tumor expressing glypican-3.

30 39. A pharmaceutical composition comprising:

The antibody of any one of claims 1-6 or a nucleic acid encoding the antibody; or

The immunoconjugate of any one of claims 11-17; or

The chimeric antigen receptor of any one of claims 20-21; or

The genetically modified immune cell of any one of claims 31-37.