

CLAIMS

1. An antibody or antigen-binding fragment thereof comprising a heavy chain variable region (V_H) sequence and a light chain variable region (V_L) sequence of a clone selected from: 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1 and which is capable of specifically binding to glypican-3 (GPC-3) (GenBank Accession Number: NP_004475.1), or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.
2. An antibody or antigen-binding fragment according to Claim 1, in which the V_H and V_L sequence are selected from: SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; and SEQ ID NO: 47 and SEQ ID NO: 48.
3. An antibody or antigen-binding fragment according to Claim 1 or 2, which is capable of binding to an epitope of human glypican-3 (GPC-3) with an affinity of 55 nM or less, 50 nM or less, 45 nM or less, 40 nM or less, 35 nM or less, 30 nM or less, 25 nM or less, 20 nM or less, 15 nM or less, 10 nM or less, 5 nM or less, 1 nM or less, 0.9 nM or less, 0.8 nM or less, 0.7 nM or less, 0.6 nM or less, 0.5 nM or less, 0.4 nM or less, 0.3 nM or less, 0.2 nM or less or 0.1 nM or less, measured as a EC50 as determined by ELISA.
4. An antibody or antigen-binding fragment according to Claim 1, 2 or 3, which is capable of binding to a cell line selected from the group consisting of: HepG2 (GPC-3^{High}) and Hep3B (GPC-3^{Medium}).
5. An antibody or antigen-binding fragment according to any preceding claim, which is capable of inducing Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) of a GPC-3 expressing cell in the presence of natural killer (NK) cells.

6. An antibody or antigen-binding fragment according to any preceding claim, which is a humanised antibody comprising a human constant region.
7. An antibody or antigen-binding fragment according to any preceding claim, which comprises a monoclonal antibody, a humanised monoclonal antibody, an Fv, F(ab'), F(ab')₂ or single-chain Fv (scFv) fragment, preferably a single chain Fv fragment.
8. An antibody or antigen-binding fragment according to any preceding claim, which is a monoclonal antibody selected from the group consisting of: 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1.
9. An antibody or antigen-binding fragment according to any preceding claim, which further comprises a heavy chain variable region (V_H) sequence and a light chain variable region (V_L) sequence capable of binding to CD3 (GenBank Accession Number: NM_000733.4).
10. An antibody or antigen-binding fragment according to Claim 9, which is further capable of specifically binding to CD-3 or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.
11. An antibody or antigen-binding fragment according to Claim 9 or 10, in which the heavy chain variable region (V_H) sequence and a light chain variable region (V_L) sequence capable of binding to CD3 comprise the sequences set out in SEQ ID NO: 97 and SEQ ID NO: 98.
12. An antibody or antigen-binding fragment according to Claim 9, 10 or 11, which is capable of activating T-cells.
13. A chimeric antigen receptor (CAR) comprising: (a) an antigen-binding fragment according to any preceding claim; (b) a transmembrane domain; and (c) a co-stimulatory intracellular signalling domain; or a chimeric antigen receptor T Cell (CAR T) expressing such a chimeric antigen receptor.
14. A nucleic acid capable of encoding an antibody or antigen-binding fragment according to any preceding claim, preferably comprising sequences selected from the group consisting of: SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO:

53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 57 and SEQ ID NO: 58; SEQ ID NO: 59 and SEQ ID NO: 60; SEQ ID NO: 61 and SEQ ID NO: 62; SEQ ID NO: 63 and SEQ ID NO: 64; SEQ ID NO: 65 and SEQ ID NO: 66; SEQ ID NO: 67 and SEQ ID NO: 68; SEQ ID NO: 69 and SEQ ID NO: 70; SEQ ID NO: 71 and SEQ ID NO: 72; SEQ ID NO: 73 and SEQ ID NO: 74; SEQ ID NO: 75 and SEQ ID NO: 76; SEQ ID NO: 77 and SEQ ID NO: 78; SEQ ID NO: 79 and SEQ ID NO: 80; SEQ ID NO: 81 and SEQ ID NO: 82; SEQ ID NO: 83 and SEQ ID NO: 84; SEQ ID NO: 85 and SEQ ID NO: 86; SEQ ID NO: 87 and SEQ ID NO: 88; SEQ ID NO: 89 and SEQ ID NO: 90; SEQ ID NO: 91 and SEQ ID NO: 92; SEQ ID NO: 93 and SEQ ID NO: 94; and SEQ ID NO: 95 and SEQ ID NO: 96, for example as comprised in an expression vector.

15. A host cell comprising a nucleic acid according to Claim 14, in which the host cell preferably comprises a Chinese Hamster Ovary (CHO) or a HEK293 cell.

16. A pharmaceutical composition comprising an antibody or antigen-binding fragment, CAR, CAR T, nucleic acid or host cell according to any preceding claim, together with a pharmaceutically acceptable excipient, diluent or carrier.

17. A compound comprising an antibody or antigen-binding fragment thereof according to any of Claims 1 to 12 linked to a cytotoxic agent, such as with a cleavable linker.

18. A compound according to Claim 17, in which the compound comprises an antibody-drug conjugate.

19. An antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, host cell, pharmaceutical composition, compound or antibody-drug conjugate according to any preceding claim for use in a method of treatment, prevention or alleviation of a cancer, such as hepatocellular carcinoma (HCC).

20. A method of producing an antibody or antigen-binding fragment, the method comprising expressing a nucleic acid according to Claim 14 in a host cell, preferably a host cell according to Claim 15 and optionally isolating the expressed antibody or antigen-binding fragment.

21. Use of an antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, pharmaceutical composition, compound or antibody-drug conjugate according to any of

Claims 1 to 19 in the preparation of a medicament for the treatment, prevention or alleviation of a cancer, such as hepatocellular carcinoma (HCC).

22. A method of detecting a hepatocellular carcinoma (HCC) cell, the method comprising detecting modulation of expression, amount or activity of glypican-3 (GPC-3) in or of the cell,
5 by means of an antibody or antigen-binding fragment according to any of Claims 1 to 12.

23. A method of diagnosing hepatocellular carcinoma (HCC) in an individual, in which the method comprises detecting, in a cell of the individual:

a modulated expression level of glypican-3 (GPC-3) by contacting the cell with an antibody or antigen-binding fragment according to any of Claims 1 to 12;

10 as compared to the expression level of glypican-3 in a cell of an individual known not to be suffering from hepatocellular carcinoma (HCC);

in which an increased expression level of the glypican-3 indicates that the individual is suffering, or is likely to be suffering, from hepatocellular carcinoma (HCC).

24. A diagnostic kit for hepatocellular carcinoma (HCC), the kit comprising an antibody or
15 antigen-binding fragment according to Claim 1 to 12 or a nucleic acid according to Claim 14 together with instructions for use.

25. An antibody, antigen-binding fragment, nucleic acid, pharmaceutical composition, compound, antibody-drug conjugate, use, method or diagnostic kit as hereinbefore described with reference to and as shown in Figures 1 to 15 of the accompanying drawings.

CLAIMS

1. An antibody or antigen-binding fragment thereof comprising a heavy chain variable region (V_H) sequence of SEQ ID NO: 1 and a light chain variable region (V_L) sequence of SEQ ID NO: 2, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or
5 99% sequence identity to the sequence of such an antibody or antigen-binding fragment, and which is capable of specifically binding to glypican-3 (GPC-3) (GenBank Accession Number: NP_004475.1).
2. An antibody or antigen-binding fragment according to Claim 1, which is capable of binding to an epitope of human glypican-3 (GPC-3) with an affinity of 55 nM or less, 50 nM
10 or less, 45 nM or less, 40 nM or less, 35 nM or less, 30 nM or less, 25 nM or less, 20 nM or less, 15 nM or less, 10 nM or less, 5 nM or less, 1 nM or less, 0.9 nM or less, 0.8 nM or less, 0.7 nM or less, 0.6 nM or less, 0.5 nM or less, 0.4 nM or less, 0.3 nM or less, 0.2 nM or less or 0.1 nM or less, measured as a EC50 as determined by ELISA.
3. An antibody or antigen-binding fragment according to Claim 1 or 2, which is (a) capable
15 of binding to a cell line selected from the group consisting of: HepG2 (GPC-3^{High}) and Hep3B (GPC-3^{Medium}); or (b) capable of inducing Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) of a GPC-3 expressing cell in the presence of natural killer (NK) cells, or both.
4. An antibody or antigen-binding fragment according to Claim 1, 2 or 3, which is a
20 humanised antibody comprising a human constant region, or which comprises a monoclonal antibody, a humanised monoclonal antibody, an Fv, F(ab'), F(ab')₂ or single-chain Fv (scFv) fragment, preferably a single chain Fv fragment.
5. An antibody or antigen-binding fragment according to any preceding claim, which is a monoclonal IgG1 antibody.
6. An antibody or antigen-binding fragment according to any preceding claim, which
25 further comprises a heavy chain variable region (V_H) sequence and a light chain variable region (V_L) sequence capable of binding to a second antigen such as a T-cell surface antigen, preferably a CD protein, more preferably CD3 (GenBank Accession Number: NM_000733.4) or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

7. An antibody or antigen-binding fragment according to Claim 6, in which the heavy chain variable region (V_H) sequence and a light chain variable region (V_L) sequence capable of binding to CD3 comprise the sequences set out in SEQ ID NO: 97 and SEQ ID NO: 98.
8. An antibody or antigen-binding fragment according to Claim 6 or 7, which is capable of activating T-cells.
9. A chimeric antigen receptor (CAR) comprising: (a) an antigen-binding fragment according to any preceding claim; (b) a transmembrane domain; and (c) a co-stimulatory intracellular signalling domain; or a chimeric antigen receptor T Cell (CAR T) expressing such a chimeric antigen receptor.
10. A nucleic acid capable of encoding an antibody or antigen-binding fragment according to any preceding claim, preferably comprising sequences selected from the group consisting of: SEQ ID NO: 49 and SEQ ID NO: 50, for example as comprised in an expression vector.
11. A host cell comprising a nucleic acid according to Claim 10, in which the host cell preferably comprises a Chinese Hamster Ovary (CHO) or a HEK293 cell.
12. A pharmaceutical composition comprising an antibody or antigen-binding fragment, CAR, CAR T, nucleic acid or host cell according to any preceding claim, together with a pharmaceutically acceptable excipient, diluent or carrier.
13. A compound comprising an antibody or antigen-binding fragment thereof according to any of Claims 1 to 8 linked to a cytotoxic agent, such as with a cleavable linker.
14. A compound according to Claim 13, in which the compound comprises an antibody-drug conjugate.
15. An antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, host cell, pharmaceutical composition, compound or antibody-drug conjugate according to any preceding claim for use in a method of treatment or alleviation of a cancer, such as hepatocellular carcinoma (HCC).
16. A method of producing an antibody or antigen-binding fragment, the method comprising expressing a nucleic acid according to Claim 10 in a host cell, preferably a host cell according to Claim 11 and optionally isolating the expressed antibody or antigen-binding fragment.

17. Use of an antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, pharmaceutical composition, compound or antibody-drug conjugate according to any of Claims 1 to 15 in the preparation of a medicament for the treatment or alleviation of a cancer, such as hepatocellular carcinoma (HCC).
- 5 18. A method of detecting a hepatocellular carcinoma (HCC) cell, the method comprising detecting modulation of expression, amount or activity of glypican-3 (GPC-3) in or of the cell, by means of an antibody or antigen-binding fragment according to any of Claims 1 to 8.
19. A method of diagnosing hepatocellular carcinoma (HCC) in an individual, in which the method comprises detecting, in a cell of the individual:
- 10 a modulated expression level of glypican-3 (GPC-3) by contacting the cell with an antibody or antigen-binding fragment according to any of Claims 1 to 8;
- as compared to the expression level of glypican-3 in a cell of an individual known not to be suffering from hepatocellular carcinoma (HCC);
- in which an increased expression level of the glypican-3 indicates that the individual is
- 15 suffering, or is likely to be suffering, from hepatocellular carcinoma (HCC).
20. A diagnostic kit for hepatocellular carcinoma (HCC), the kit comprising an antibody or antigen-binding fragment according to Claim 1 to 8 or a nucleic acid according to Claim 10 together with instructions for use.