

COMPOSITION

FIELD

This invention relates to the fields of medicine, cell biology, molecular biology and genetics. This invention relates to the field of medicine.

5 **BACKGROUND**

Unmet Need for Novel Treatment Strategies for Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with a reported global incidence of 854,000 in 2015.

10 More than half of the HCC cases worldwide occur in developing countries due to prevalent chronic Hepatitis B Virus (HBV) infections. HCC is also the fourth leading cause of cancer-associated mortality.

A great majority of patients are ineligible for curative therapies upon diagnosis as they are often in the late stages of the disease and the outcome of systemic therapy for HCC has been disappointing.

15 The dominant targeted therapy drug for HCC is Nexavar (Sorafenib, Bayer), a small molecule inhibitor for multiple protein kinases. However, even with its substantial usage in HCC treatment, Sorafenib only shows marginal clinical benefits with a low response rate and provides very limited improvement for overall survival. Since its launch in 2007, no new drugs have entered the market, indicating that development of new treatments for HCC is very
20 challenging.

Hence, there is still an urgent medical need to discover novel treatment strategies for HCC.

SUMMARY

25 According to a 1st aspect of the present invention, we provide 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. The antibody or antigen-binding fragment thereof may be capable of specifically binding to glypican-3 (GPC-3) (GenBank Accession Number: NP_004475.1). It

may be a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to such a sequence.

The antibody or antigen-binding fragment may be such that 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.

The antibody or antigen-binding fragment may be 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. The affinity may be measured as a EC₅₀ as determined by ELISA.

The antibody or antigen-binding fragment may be capable of binding to a cell line selected from the group consisting of: HepG2 (GPC-3^{High}) and Hep3B (GPC-3^{Medium}).

The antibody or antigen-binding fragment may be capable of inducing Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) of a GPC-3 expressing cell in the presence of natural killer (NK) cells.

The antibody or antigen-binding fragment may be a humanised antibody comprising a human constant region.

The antibody or antigen-binding fragment may comprise a monoclonal antibody, a humanised monoclonal antibody, an Fv, F(ab'), F(ab')₂ or single-chain Fv (scFv) fragment. It may comprise a single chain Fv fragment comprising a V_H sequence and V_L sequence.

94; and SEQ ID NO: 95 and SEQ ID NO: 96. The nucleic acid may be comprised in an expression vector.

As a 4th aspect of the present invention, there is provided a host cell comprising such a nucleic acid. The host cell may comprise a Chinese Hamster Ovary (CHO) or a HEK293 cell.

5 We provide, according to a 5th aspect of the present invention, a pharmaceutical composition comprising an antibody or antigen-binding fragment, CAR, CAR T, nucleic acid or host cell as set out above, together with a pharmaceutically acceptable excipient, diluent or carrier.

10 The present invention, in a 6th aspect, provides a compound comprising an antibody or antigen-binding fragment thereof, as shown above, linked to a cytotoxic agent. The linkage may be by means of a cleavable linker.

The compound may comprise an antibody-drug conjugate.

15 In a 7th aspect of the present invention, there is provided an antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, host cell, pharmaceutical composition, compound or antibody-drug conjugate as described above for use in a method of treatment, prevention or alleviation of a cancer, such as hepatocellular carcinoma (HCC).

20 According to an 8th aspect of the present invention, we provide a method of producing an antibody or antigen-binding fragment, the method comprising expressing a nucleic acid as described above in a host cell, such as a host cell as set out above and optionally isolating the expressed antibody or antigen-binding fragment.

We provide, according to a 9th aspect of the invention, use of such an antibody, antigen-binding fragment, CAR, CAR T, nucleic acid, pharmaceutical composition, compound or antibody-drug conjugate in the preparation of a medicament for the treatment, prevention or alleviation of a cancer, such as hepatocellular carcinoma (HCC).

25 There is provided, in accordance with a 10th aspect of the present invention, 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 set out above.

As an 11th aspect of the invention, we provide 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 as described above; 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).

We provide, according to a 12th aspect of the invention, there is provided a diagnostic kit for hepatocellular carcinoma (HCC), the kit comprising an antibody or antigen-binding fragment or a nucleic acid as set out above together with instructions for use.

The practice of this invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA Methods in Enzymology*, Academic Press; *Using Antibodies: A Laboratory Manual: Portable Protocol NO. 1* by Edward Harlow, David Lane, Ed Harlow (1999, Cold Spring Harbor Laboratory Press, ISBN 0-87969-544-7); *Antibodies: A Laboratory Manual* by Ed Harlow (Editor), David Lane (Editor) (1988, Cold Spring Harbor Laboratory Press, ISBN 0-87969-314-2), 1855. *Handbook of Drug Screening*, edited by Ramakrishna Seethala, Prabhavathi B. Fernandes (2001, New York, NY, Marcel Dekker, ISBN 0-8247-0562-9); and *Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench*, Edited Jane Roskams and Linda Rodgers, 2002, Cold Spring Harbor Laboratory, ISBN 0-87969-630-3. Each of these general texts is herein incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A to 1D are drawings showing binding ELISA of 24 anti-GPC-3 IgG₁ clones. Binding ELISA experiments were performed in 3 batches (Figure 1A, Figure 1B, Figure 1C) with humanized GC33 IgG₁ as the reference control. Calculated EC₅₀ values were shown in a table (Figure 1D).

Figures 2A to 2D are drawings showing binding curves of 24 anti-GPC-3 clones onto GPC-3^{High} HepG2 cells, GPC-3^{Medium} Hep3B cells and GPC-3^{Negative} SK-Hep1 cells at different antibody concentrations. Flow cytometry analyses were performed in 4 batches (Figure 2A, Figure 2B, Figure 2C, Figure 2D) with humanized GC33 IgG₁ as the reference control.

Figure 3 is a drawing showing cross-reactivity binding ELISA of anti-GPC-3 IgG₁ clones to human, cynomolgus and mouse GPC-3 recombinant proteins. The humanized GC33 IgG₁ was used as a drawing control.

Figures 4A and 4B are drawings showing binding of 24 anti-GPC-3 clones onto the mouse GPC-3 transduced Hepa1-6 cells (Hepa1-6-mGPC-3), and the parental Hepa1-6 cells at the antibody concentration of 20nM, with humanized GC33 IgG₁ as the reference control.

Figure 4A is a drawing of a histogram of fluorescence intensity showing the mGPC-3 expression level on Hepa1-6-mGPC-3 cells and the parental Hepa1-6 cells.

Figure 4B is a drawing of cells positively stained with different anti-GPC-3 antibodies were gated and the percentages (%) were calculated.

Figure 5 is a drawing showing specific killing of GPC-3^{High} HepG2 cells in ADCC assay in the presence of different anti-GPC-3 IgG₁ clones. Shown are mean cytotoxicity $\pm$ SD in triplicates. The humanized GC33 IgG₁ was used as a reference control.

Figures 6A to 6C are drawings showing specific cytolysis of GPC-3^{High} HepG2 cells and GPC-3^{Negative} SK-Hep1 cells by anti-GPC-3 CAR-T cells with time-course measurement using xCelligence impedance assay for 48 hours. Assays were performed in 3 batches (Figure 6A, Figure 6B, Figure 6C) using CAR-T cells generated from different donors. Shown are mean % cytolysis $\pm$ SD in triplicates. Anti-GPC-3 CAR-T cells generated from scFv sequence using the mouse GC33 clone were used as a reference control.

Figures 7A to 7C are drawings showing ELISA measurement of interferon- γ and IL-2 secretion from anti-GPC-3 CAR-T cells after 24 and 48 hours of co-culture with either GPC-3^{High} HepG2 cells or GPC-3^{Negative} SK-Hep1 cells. Assays were performed in 3 batches (Figure 7A, Figure 7B, Figure 7C) using CAR-T cells generated from different donors. Anti-GPC-3 CAR-T cells generated from scFv sequence using the mouse GC33 clone were used as a reference control.

Figures 8A to 8C are drawings showing binding curves of 5 anti-GPC-3 bi-specific T cell-engaging antibodies to (Figure 8A) GPC-3^{Positive} HepG2 cells, (Figure 8B) GPC-3^{Negative} SK-Hep1 cells and (Figure 8C) CD3^{Positive} human T cells at different antibody concentrations. Top panels show the mean fluorescence intensity of anti-GPC-3 bi-specific T cell-engaging antibodies binding to different cells, while bottom panels show the percentage of cells positively stained by the anti-GPC-3 bi-specific T cell-engaging antibodies.

Figures 9A to 9H are drawings showing anti-GPC-3 bi-specific T cell-engaging antibodies activate human T cells when co-cultured with GPC-3^{Positive} HepG2 cells. 24 hours post co-culture of naïve human T cells with either (Figure 9A) HepG2 cells or (Figure 9B) SK-Hep1 cells in the presence of different anti-GPC-3 bi-specific T cell-engaging antibodies at 3 different concentrations, T cells were harvested and stained with antibodies against T cell activation marker CD25 and CD69 for flow cytometry analysis. T cells treated with T cell activator, TransActTM (Miltenyi Biotec), was included as a positive control.

Figure 9C and Figure 9D are drawings showing percentage of T cells activated by different anti-GPC-3 bi-specific T cell-engaging antibodies at 3 different concentrations were calculated and presented as the total percentage of CD25+CD69- cells, CD25-CD69+ cells and CD25+CD69+ cells, after co-culture with (Figure 9C) GPC-3^{Positive} HepG2 cells or (Figure 9D) GPC-3^{Negative} SK-Hep1 cells.

Figure 9E and Figure 9F are drawings showing ELISA measurement of interferon- γ secretion from human T cells after 24 hours of co-culture with either (Figure 9E) GPC-3^{Positive} HepG2 cells or (Figure 9F) GPC-3^{Negative} SK-Hep1 cells with addition of different anti-GPC-3 bi-specific T cell-engaging antibodies at 3 different concentrations.

Figure 9G and Figure 9H are drawings showing ELISA measurement of IL-2 secretion from human T cells after 24 hours of co-culture with either (Figure 9G) GPC-3^{Positive} HepG2

cells or (Figure 9H) GPC-3^{Negative} SK-Hep1 cells with addition of different anti-GPC-3 bi-specific T cell-engaging antibodies at 3 different concentrations.

Figures 10A and 10B are drawings showing T cell mediated cytotoxicity of GPC-3^{Positive} HepG2 cells by anti-GPC-3 bi-specific T cell-engaging antibodies.

5 Figure 10A is a drawing showing percentage of cytolysis of GPC-3^{Positive} HepG2 cells mediated by activated human T cells with time-course measurement using xCelligence impedance assay up to 72 hours, upon treatment with 5 different anti-GPC-3 bi-specific T cell-engaging antibodies (clone 1C4, 1D2, 2B5, 4H1, and 5C4). Shown are mean % cytolysis $\pm$ SD in duplicate wells.

10 Figure 10B is a drawing showing percentage of cytolysis of 5 different anti-GPC-3 bi-specific T cell-engaging antibodies at different concentrations at the assay end point (72 hours).

Figures 11A to 11C are drawings showing that anti-GPC-3 CAR T cells prevented GPC-3^{Positive} HCC tumor formation in xenograft models. Two million of GPC-3^{Positive} Hep3B
15 cells were subcutaneously injected into the right flanks of NSG mice. Four days later, mice were randomly grouped (4-5 mice per group) and ten million of anti-GPC-3 CAR T cells (clone 5C4 scFv) or Mock T cells were intravenously injected into these mice via tail vein. Eighty-four days post initial tumor inoculation, all tumor-free mice from the CAR T group were re-challenged with one million of Hep3B cells via subcutaneous injection. Tumor size
20 (Figure 11A) and weight of each mouse (Figure 11B) were measured and recorded every 3-7 days. (Figure 11C) Six days after CAR T cell injection, blood serum from mice of Mock T group and CAR T group were analysed by Luminex assay and levels of representative cytokines are shown.

Figures 12A to 12 D are drawings showing that anti-GPC-3 CAR T cells suppressed
25 the growth of Hep3B xenografts *in vivo*.

Figure 12A is a drawing showing percentage of CAR expression on different anti-GPC-3 CAR T cells detected by flow cytometry analysis.

Figure 12B is a drawing showing percentage of cytolysis of GPC-3^{Positive} Hep3B cells and GPC-3^{Negative} SK-Hep1 cells mediated by anti-GPC-3 CAR T cells with time-course
30 measurement using xCelligence impedance assay up to 72 hours.

Figure 12C and Figure 12D are drawings showing two million of GPC-3^{Positive} Hep3B cells were subcutaneously injected into the right flanks of NSG mice. Twenty-six days later, mice were grouped according to the tumor size (3-4 mice per group). Ten million of different anti-GPC-3 CAR T cells (clone 1D2, clone 4H1, and clone 5C4) or Mock T cells were
 5 intravenously injected into these mice via tail vein. Tumor size (Figure 12C) and weight of each mouse (Figure 12D) were measured and recorded every 3-4 days.

Figures 13A to 13F are drawings showing anti-GPC-3 CAR T cells eradicated large Hep3B tumor xenografts with a combinatorial use of anti-PD1 antibodies *in vivo*.

On Day 0, two million of GPC-3^{Positive} Hep3B cells were subcutaneously injected into
 10 the right flank of NSG mice. Twenty-four days later, mice were grouped according to the measurable tumor size (4 mice per group). Ten million of anti-GPC-3 CAR T cells (clone 5C4) or Mock T cells were intravenously injected into these mice via tail vein. Ten days after CAR T cell injection (Day 34), all mice were intraperitoneal injected with anti-PD1 IgG₄ antibodies for a total of 5 times with an interval of 3- 5 days. Tumor size (Figure 13A) and
 15 weight of each mouse (Figure 13B) were measured and recorded every 3-4 days.

Figure 13C is a drawing showing survival curves of the mice from both Mock T group (dotted line) and CAR T group. Death was defined either when autonomous death of the mice was observed or when the bearing tumor size of the mice exceeds 2000mm³.

Figure 13D is a drawing showing that sixteen days after CAR T cell injection,
 20 peripheral immune cells were analysed by flow cytometry analysis and the population of the human T cells were boxed.

Figure 13E is a drawing showing the circulating concentrations of peripheral total T cells (CD3+), CAR T cells (CAR+CD3+), CD4 T cells (CD4+CD3+) and CD8 T cells (CD8+CD3+) were calculated and compared between Mock T and CAR T group of mice.

Figure 13F is a drawing showing blood serum from mice of Mock T group and CAR T
 25 group were analysed by Luminex assay and levels of representative cytokines are shown.

Figures 14A to 14K are drawings showing that anti-GPC-3 CAR T cells did not induce toxicity *in vivo*. Nine different anti-GPC-3 antibody scFv sequences were used to generate anti-GPC-3 CAR T cells. NSG mice with mixed gender (M: male, F: female) were
 30 intravenously injected with ten million of Mock T cells (Figure 14B) or different anti-GPC-3

CAR T cells (Figure 14C to Figure 14K) on Day 0. A group of mice without any treatment (Figure 14A) was also included as healthy control. Body weight of each mouse was measured and recorded every 3-7 days.

Figures 15A to 15F are drawings showing anti-GPC-3 mouse CAR T cells suppressed the growth of MC38-mGPC-3 xenografts *in vivo*.

Figure 15A is a drawing showing MC38 cells stably expressing mouse GPC-3 protein (MC38-mGPC-3) were established by transducing MC38 parental cells with lentivirus-based vectors. Expression of mouse GPC-3 protein was verified by staining with anti-GPC-3 IgG (clone 5C4) followed by flow cytometry analysis.

Figure 15B is a drawing showing percentage of mouse CAR expression on anti-GPC-3 mouse CAR T cells detected by flow cytometry analysis.

Figure 15C is a drawing showing percentage of cytolysis of MC38-mGPC-3 cells mediated by anti-GPC-3 mouse CAR T cells (clone 5C4) with time-course measurement using xCelligence impedance assay up to 96 hours.

Figure 15D and Figure 15E are drawings showing 0.8 million of MC38-mGPC-3 cells were subcutaneously injected into the right flank of WT C57BL/6 mice. Twelve days later, mice were grouped according to the measurable tumor size (4-5 mice per group). Eight million of anti-GPC-3 mouse CAR T cells (clone 5C4) or Mock T cells were intravenously injected into these mice via tail vein. Tumor size (Figure 15D) and weight of each mouse (Figure 15E) were measured and recorded every 3-4 days.

Figure 15F is a drawing showing anti-GPC-3 mouse CAR T cells did not induce toxicity in the WT mice. WT C57BL/6 mice were intravenously injected with eight million of anti-GPC-3 mouse CAR T cells (clone 5C4) or Mock T cells on Day 0. Body weight of each mouse was measured and recorded every 3-7 days.

SEQUENCES

SEQ ID NO: 1 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 5C4. SEQ ID NO: 2 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 5C4.

SEQ ID NO: 3 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4H1. SEQ ID NO: 4 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4H1.

5 SEQ ID NO: 5 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1D2. SEQ ID NO: 6 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1D2.

SEQ ID NO: 7 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1C4. SEQ ID NO: 8 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1C4.

10 SEQ ID NO: 9 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 2B5. SEQ ID NO: 10 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 2B5.

15 SEQ ID NO: 11 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1F1. SEQ ID NO: 12 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1F1.

SEQ ID NO: 13 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1H1. SEQ ID NO: 14 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1H1.

20 SEQ ID NO: 15 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4A5. SEQ ID NO: 16 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4A5.

SEQ ID NO: 17 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1D8. SEQ ID NO: 18 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1D8.

25 SEQ ID NO: 19 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1D3. SEQ ID NO: 20 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1D3.

SEQ ID NO: 21 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 2F1. SEQ ID NO: 22 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 2F1.

5 SEQ ID NO: 23 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 3C6. SEQ ID NO: 24 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 3C6.

SEQ ID NO: 25 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 3D12. SEQ ID NO: 26 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 3D12.

10 SEQ ID NO: 27 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 3A9. SEQ ID NO: 28 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 3A9.

15 SEQ ID NO: 29 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1F4. SEQ ID NO: 30 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1F4.

SEQ ID NO: 31 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1H10. SEQ ID NO: 32 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1H10.

20 SEQ ID NO: 33 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 3C12. SEQ ID NO: 34 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 3C12.

SEQ ID NO: 35 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4G11. SEQ ID NO: 36 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4G11.

25 SEQ ID NO: 37 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4A12. SEQ ID NO: 38 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4A12.

SEQ ID NO: 39 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1A12. SEQ ID NO: 40 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1A12.

5 SEQ ID NO: 41 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 3G12. SEQ ID NO: 42 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 3G12.

SEQ ID NO: 43 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4F9. SEQ ID NO: 44 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4F9.

10 SEQ ID NO: 45 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 4G4. SEQ ID NO: 46 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 4G4.

15 SEQ ID NO: 47 shows the amino acid sequence of the heavy chain (V_H) of monoclonal antibody 1E1. SEQ ID NO: 48 shows the amino acid sequence of the light chain (V_L) of monoclonal antibody 1E1.

DETAILED DESCRIPTION

Glypican-3, an Excellent Target for HCC Immunotherapy

20 Glypican-3 (GPC-3), a member of the heparin sulfate proteoglycan family, is a molecule attached to the cell surface by a glycosyl-phosphatidylinositol (GPI) anchor. In recent years, GPC-3 was found to be a highly specific biomarker for HCC diagnosis and progression, as it is almost exclusively overexpressed on HCC cells but not on normal adult hepatocytes or other normal tissues.

25 In addition, GPC-3 was also demonstrated to act as a co-receptor for several growth factors, which in turn function to stimulate oncogenic signaling pathways to promote HCC growth.

Hence, as a specific HCC surface marker, GPC-3 is an excellent target for antibody-based immunotherapies for HCC.

We disclose the nucleotide and amino-acid sequences of 24 antibodies, or the antigen-binding portions thereof, that specifically target glypican-3, both in solution and on the surface of cells.

We describe monoclonal antibodies raised against glypican-3 (GPC-3). We refer to these monoclonal antibodies as 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.

These antibodies are capable of specifically binding glypican-3 (GPC-3), both in solution as well as expressed on the cell surface. The antibodies are also capable of cross-reactivity with glypican-3 (GPC-3) from a number of other species. The antibodies are able to induce antibody dependent cell mediated cytotoxicity. They may be used to construct anti-GPC-3 CAR T cells, which are highly efficient in killing tumour cells.

We disclose the heavy chain variable region (V_H) sequences and a light chain variable region (V_L) sequences of each of the clones 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.

We show that Fab fragments, as well as single chain Fv fragments, comprising these variable regions, have the same cross-reactive and strongly neutralizing activities.

Accordingly, we provide for polypeptides comprising these variable regions for the treatment and detection of cancers such as hepatocellular carcinoma.

ANTI-GLYPICAN-3 (GPC-3) ANTIBODIES

The Examples describe the generation and production of antibodies generated from, and which have reactivity against, GPC-3 proteins.

We disclose the functional and structural characterization of monoclonal antibodies 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, which are mouse monoclonal antibodies raised against a glypican-3 (GPC-3). We show that these anti-GPC-3 antibodies are capable of killing tumour cells.

We therefore provide broadly for anti-GPC-3 antibodies. The anti-GPC-3 antibodies may comprise the heavy and light chain variable regions of 5C4, 4H1, 1D2, 1C4, 2B5, 1F1,

1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1.

The anti-GPC-3 antibodies may be used for targeting hepatocellular carcinoma.

5 The anti-tumour antibody may be capable of binding to GPC-3 polypeptides from a number of different species.

The antibody may be capable of cross-reactivity, i.e., able to bind to more than one polypeptide. For example, the antibody may be capable of binding to two or more variants of a particular polypeptide within a defined group of polypeptides. The two or more variants may therefore comprise cognate or homologous polypeptides from different types of polypeptides
10 in the group. The antibody may be capable of binding to substantially all of the variants of a particular polypeptide within that group.

The disclosure of this document enables the production of these antibodies as well as fragments and variants thereof, including humanised and chimeric antibodies, which have one or more similar or identical properties of anti-GPC-3 antibodies such as 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.
15

Such properties may include binding affinity, binding specificity, cross-reactivity, binding avidity, neutralizing activity, etc, as described in further detail below. The specific antibodies and variants thereof described in this document may be produced by a person
20 skilled in the art from the information disclosed in this document, and employing molecular biology techniques which we also describe in detail.

ANTI-GPC-3 ANTIBODIES

The polypeptide bound by the anti-GPC-3 antibody may comprise a glypican protein. We specifically disclose anti-GPC-3 antibodies.

25 Thus, the term “anti-GPC-3 antibody” should be taken to include each of the monoclonal antibodies 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 (as well as their humanised counterparts). Also included are polypeptides comprising the variable regions of any of antibodies 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 variants,
30

homologues, fragments and derivatives thereof. This term should also be taken to include reference to variants, homologues, fragments and derivatives of the anti-GPC-3 antibodies, as described below, where the context permits.

The anti-GPC-3 antibodies may be generated against glypican-3 from any species, such as human glypican-3. They may be used to bind to or target a tumour cell, such as a hepatocellular carcinoma cell.

For this reason, the anti-GPC-3 antibodies may also be considered anti-tumour antibodies or anti-liver cancer antibodies.

Monoclonal antibodies and variants thereof including Fab, scFv etc and humanised monoclonal antibodies as well as their properties are disclosed, such as in the Examples. The Examples also describe Fab fragments from the monoclonal antibodies, as well as single chain Fv,. Other variants, including humanised versions of each of these antibodies, are also disclosed.

We disclose the sequences of the variable regions of monoclonal antibodies 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. We further disclose variants, homologues, fragments and derivatives of these variable regions. Using this sequence information, a skilled person may produce antibodies comprising these variable regions or their variants, homologues, fragments and derivatives.

We further disclose the sequences of nucleic acid constructs for expressing these monoclonal antibodies. The sequences of these constructs enable the production of monoclonal antibodies which have identical sequences to antibodies 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. We further disclose variants, homologues, fragments and derivatives of antibodies 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.

We disclose the sequences of constructs capable of expressing humanised monoclonal antibodies 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. We describe methods of

expressing the antibodies of interest from cells transfected with the constructs, as well as variants, homologues, fragments and derivatives of these humanised constructs.

Using such sequences and the expression methods, the skilled person may readily transfect relevant host cells and cause them to express the whole monoclonal or humanised anti-GPC-3 antibodies, or variants, homologues, fragments and derivatives thereof.

The monoclonal antibodies and variants thereof may comprise the variable region of antibodies 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, as well as further comprising variable regions from other anti-GPC-3 antibodies known in the art. The anti-GPC-3 antibody may comprise the same or different variable regions in a single antibody molecule. They may comprise one variable region, or more than one variable region. Accordingly, we provide the skilled person with the ability to produce any number of antibodies which comprise the same or similar binding reactivity as antibodies 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 or other anti-GPC-3 antibodies.

Such antibodies may comprise the full or substantially complete sequences of an antibody (i.e., heavy chain and light chain), or they may comprise a fragment of a whole antibody (such as Fv, F(ab') and F(ab')₂ fragments or single chain antibodies (scFv)). The antibodies may further comprise fusion proteins or synthetic proteins which comprise the antigen-binding site of the antibody, as described in detail below.

It will also be evident that such antibodies may be engineered for desirable properties, such as lowered host reactivity, reduced rejection, etc.

The engineering could include "humanisation", by which term we mean the inclusion of (or substitution with) one or more human residues or sequences in an antibody sequence such as a mouse antibody sequence. "Humanisation" in the context of this document includes "chimeric" antibodies, in which the antibody comprises discrete sections of mouse and human sequences, e.g., where one or both of the variable regions comprise mouse sequences, and the remainder of the antibody molecule (such as the constant region) comprises human sequences. In such chimeric antibodies, the whole of the variable regions of, for example, a mouse or rat antibody may be expressed along with human constant regions. This provides such a chimeric

antibody with human effector functions and also reduces immunogenicity (HAMA) caused by the murine Fc region.

Humanisation 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 antibodies may
5 be carried out by any suitable means, such as the method described in Hanson BJ, Boon AC, Lim AP, Webb A, Ooi EE, Webby RJ. *Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice*. Respir Res 17:126, 2006.

Generally, a “chimeric antibody” may refer to an antibody having either a heavy and
10 light chain encoded by a nucleotide sequence derived from a murine immunoglobulin gene and either a heavy and light chain encoded by a nucleotide sequence derived from a human immunoglobulin gene.

“Humanisation” also includes CDR grafted or reshaped antibodies. It thus includes engineering at a more discrete level, e.g., antibodies in which the mouse variable region has
15 been mutated to include human residues to reduce immunogenicity. In such an antibody, only the complementarity determining regions from the rodent antibody V-regions may be combined with framework regions from human V-regions. Such antibodies should be more human and less immunogenic than chimaeric antibodies.

For the avoidance of doubt, where a specific antibody designation is referred to in this
20 document, this should be taken to include a reference to both the mouse monoclonal antibody (as secreted by a hybridoma), as well as to the humanised version of it, unless the context dictates otherwise. Thus, for example, where antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1 is referred to, this includes both the monoclonal antibodies 5C4, 4H1, 1D2, 1C4,
25 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1 (i.e., as derived from a mouse), as well as a humanised monoclonal antibodies 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.

GPC-3 BINDING POLYPEPTIDES

30 We further provide for polypeptides in general having GPC-3 protein binding activity. Such polypeptides include anti-GPC-3 antibodies. The GPC-3-binding polypeptides may

comprise one or more of the same or similar properties as the monoclonal antibodies 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. The polypeptides may be referred to for convenience generally as “anti-GPC-3 antibodies”.

5 It is within the skills of a reader to construct binding molecules which may not be (or may not be described as) antibodies or immunoglobulins but which comprise anti-GPC-3 binding activity as described here. Accordingly, and where the context allows the term “anti-GPC-3 antibodies” should be taken to include any molecule so long as it is capable of binding GPC-3. Such molecules may include polypeptides, small molecules, as well as antibodies and
10 immunoglobulins, and may be identified through various means known in the art, for example by screening a suitable library for GPC-3 binding activity.

 The GPC-3 binding polypeptides (which include anti-GPC-3 antibodies) may comprise similar or identical properties as the monoclonal antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12,
15 4F9, 4G4 and 1E1. Such similar or identical properties may in particular include binding properties. The GPC-3 binding polypeptides may in general be capable of binding to GPC-3 polypeptides, e.g., GPC-3 from human, GPC-3 from mouse, etc.

GPC-3 EPITOPES

 The anti-GPC-3 antibodies may have the same or similar binding specificity, binding
20 affinity and/or binding affinity as antibodies 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. The anti-GPC-3 antibodies may specifically bind to an epitope bound by antibodies 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.

25 Methods are known in the art to determine an epitope that is bound by a particular antibody. Such epitope mapping methods are described for example in Hanson et al., (2006). Respiratory Research, 7:126. Furthermore, a skilled person will be able to generate antibodies and screen them for particular properties.

 Accordingly, a skilled person will readily be able to identify anti-GPC-3 antibodies
30 which bind to the same epitope as monoclonal antibodies 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.

ANTIBODY BINDING

5 The anti-GPC-3 antibody may be capable of binding to glypican-3 from humans. It may further be capable of binding to glypican-3 from mouse.

The binding between the anti-GPC-3 antibody and its target may be more or less strong or weak, transient, semi-permanent or permanent. The antibody may bind to its target at an EC₅₀ binding affinity of 1µm or below, such as 100 nm or below.

10 It may bind with an affinity of 90 nm or below or 80 nm or below. It may bind with an affinity of 70 nm or below, 60 nm or below, 50 nm or below, 40 nm or below, 30 nm or below, 20 nm or below, 10 nm or below, 5 nm or below, 4 nm or below, 3 nm or below, 2 nm or below, 1 nm or below. It may bind with an affinity of 0.5 nm or below, 0.4 nm or below, 0.3 nm or below or 0.2 nm or below.

15 The antibody may bind to its target with a K_d of micromolar or nanomolar range. It may bind with a K_d of 10⁻⁷ M or less, 10⁻⁷ M or less, 10⁻⁸ M or less, 10⁻⁹ M or less or 10⁻¹⁰ M or less.

The binding may be measured by any means known in the art, such as ELISA or Surface Plasmon Resonance, both of which are described in detail in the Examples.

20 Binding of the anti-GPC-3 antibody to the GPC-3 polypeptide may take place within or outside the cell. Such binding may inactivate, inhibit or lower an activity of the GPC-3 polypeptide. The binding may neutralise a GPC-3 activity.

25 The activity may comprise any biological activity caused by or associated with the GPC-3 polypeptide. The activity may comprise binding to another protein, for example a receptor, a downstream protein or factor. The another protein may comprise GPC-3 itself. The activity may comprise multimerisation activity, such as trimerisation activity or homotrimerisation activity. Binding of anti-GPC-3 antibody to GPC-3 polypeptide may inactivate, inhibit or lower an activity of a receptor, downstream protein or factor. The activity may comprise a biochemical activity or a pathogenic activity.

The binding may inactivate, inhibit or lower an activity of a cell expressing GPC-3. It may inactivate or neutralise or kill the cell expressing GPC-3. The binding between the anti-GPC-3 antibody and the GPC-3 may kill a tumour cell expressing GPC-3.

ANTIBODIES

5 The terms “antibody” and “immunoglobulin”, as used in this document, may be employed interchangeably where the context permits. These term include fragments of proteolytically-cleaved or recombinantly-prepared portions of an antibody molecule that are capable of selectively reacting with or recognising GPC-3 or an epitope thereof, such as an epitope of GPC-3 bound by antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3,
10 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1.

Non limiting examples of such proteolytic and/or recombinant fragments include Fab, F(ab')₂, Fab', Fv fragments, and single chain antibodies(scFv) containing a V_L and V_H domain joined by a peptide linker. These Fvs may be covalently or non-covalently linked to form antibodies having two or more binding sites.

15 By “ScFv molecules” we mean molecules wherein the V_H and V_L partner domains are linked via a flexible oligopeptide. A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein(1991) Nature 349, 293-299.

Whole antibodies, and F(ab')₂ fragments are “bivalent”. By “bivalent” we mean that
20 the said antibodies and F(ab') fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent having only one antigen combining site.

The anti-GPC-3 antibody may comprise a high affinity antibody with an binding affinity of 1μm or below, such as 100 nm or below, for example, 0.17 nM to 84 nM.

25 The term “binding affinity” as used in this document refers to the EC₅₀ binding affinity an antibody such as an anti-GPC-3 antibody disclosed here. It may be measured using ELISA or Surface Plasmon Resonance. A high EC₅₀ binding affinity is desirable as it reflects the affinity of an Fab fragment for an antigen.

30 The term “affinity” may also be defined in terms of the dissociation rate or off-rate (k_{off}) of a an antibody such as an anti-GPC-3 antibody. The lower the off-rate the higher the affinity that an antibody such as an anti-GPC-3 antibody has for an antigen.

The anti-GPC-3 antibody may comprise a peptide *per se* or form part of a fusion protein.

The anti-GPC-3 antibodies described here include any antibody that comprises GPC-3 binding activity, such as binding ability to GPC-3 or binding to the same epitope bound by antibodies 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.

The anti-GPC-3 antibodies also include the entire or whole antibody, whether mouse, humanised or human, such antibody derivatives and biologically-active fragments. These may include antibody fragments with GPC-3 binding activity that have amino acid substitutions or have sugars or other molecules attached to amino acid functional groups, etc.

The anti-GPC-3 antibody may comprise isolated antibody or purified antibody. It may be obtainable from or produced by any suitable source, whether natural or not, or it may be a synthetic anti-GPC-3 antibody, a semi-synthetic anti-GPC-3 antibody, a derivatised anti-GPC-3 antibody or a recombinant anti-GPC-3 antibody.

Where the anti-GPC-3 antibody is a non-native anti-GPC-3 antibody, it may include at least a portion of which has been prepared by recombinant DNA techniques or an anti-GPC-3 antibody produced by chemical synthesis techniques or combinations thereof.

The term “derivative” as used in this document includes chemical modification of an anti-GPC-3 antibody. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group, for example. The sequence of the anti-GPC-3 antibody may be the same as that of the naturally occurring form or it may be a variant, homologue, fragment or derivative thereof.

ANTIBODY VARIABLE REGIONS

The term “variable region”, as used in this document, refers to the variable regions, or domains, of the light chains (V_L) and heavy chains (V_H) which contain the determinants for binding recognition specificity and for the overall affinity of the antibody against GPC-3 (or variant, homologue, fragment or derivative), as the case may be.

The variable domains of each pair of light (V_L) and heavy chains (V_H) are involved in antigen recognition and form the antigen binding site. The domains of the light and heavy chains have the same general structure and each domain has four framework (FR) regions,

whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The FR regions maintain the structural integrity of the variable domain. The CDRs are the polypeptide segments within the variable domain that mediate binding of the antigen.

5 The term “constant region”, as used in this document, refers to the domains of the light (CL) and heavy (CH) chain of the antibody (or variant, homologue, fragment or derivative) which provide structural stability and other biological functions such as antibody chain association, secretion, transplacental mobility, and complement binding, but which are not involved with binding a GPC-3 epitope. The amino acid sequence and corresponding exon
10 sequences in the genes of the constant region will be dependent upon the species from which it is derived. However, variations in the amino acid sequence leading to allotypes are relatively limited for particular constant regions within a species. An “allotype” is an antigenic determinant (or epitope) that distinguishes allelic genes.

 The variable region of each chain is joined to the constant region by a linking
15 polypeptide sequence. The linkage sequence is coded by a “J” sequence in the light chain gene, and a combination of a “D” sequence and a “J” sequence in the heavy chain gene.

ANTIBODY VARIABLE REGION SEQUENCES

 Anti-GPC-3 antibodies, according to the methods and compositions described here, may be generated from these variable region sequences by methods known in the art.

20 For example, the heavy and light chain sequences may be recombined into a constant sequence for a chosen antibody, through recombinant genetic engineering techniques which are known to the skilled person.

 Constant region sequences are known in the art, and are available from a number of databases, such as the IMGT/LIGM-DB database (described in Giudicelli et al, 2006, Nucleic
25 Acids Research 34(Database Issue):D781-D784 and LeFranc et al (1995) *LIGM-DB/IMGT: An Integrated Database of Ig and TcR, Part of the Immunogenetics Database*. Annals of the New York Academy of Sciences 764 (1) , 47–47 doi:10.1111/j.1749-6632.1995.tb55805.x) and the IMGT/GENE-DB database (described in Giudicelli et al, 2005, Nucleic Acids Res. 2005 Jan 1;33(Database issue):D256-61). IMGT/LIGM-DB and IMGT/GENE-DB are part of
30 the ImMunoGeneTics Database located at www.ebi.ac.uk/imgt/.

Methods for combining variable regions with given sequences and constant regions to produce whole antibodies are known in the art and are described in Hanson et al., (2006). *Respiratory Research*, 7:126.

ANTIBODY SINGLE CHAIN ANTIBODY SEQUENCES

5 Fragments of whole antibodies such as Fv, F(ab') and F(ab')₂ fragments or single chain antibodies (scFv) may be produced by means known in the art.

Using the disclosed sequences and the methods described in the literature, for example, the heavy and light chains of the variable region of antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 10 3G12, 4F9, 4G4 and 1E1, having the sequences shown in this document, may be transgenically fused to a mouse IgG constant region sequence to produce a mouse monoclonal anti-GPC-3 antibody. The variable region of antibodies 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 may be engineered with mouse or human IgG constant regions to produce 15 mouse monoclonal or humanized antibodies capable of binding to GPC-3 polypeptide.

POLYPEPTIDE SEQUENCES

It will be understood that polypeptide sequences disclosed here are not limited to the particular sequences set forth in this document, but also include homologous sequences obtained from any source, for example related cellular homologues, homologues from other 20 species and variants or derivatives thereof, provided that they have at least one of the biological activities of an anti-GPC-3 antibody, as the case may be.

This disclosure therefore encompasses variants, homologues or derivatives of the amino acid sequences set forth in this document, as well as variants, homologues or derivatives of the amino acid sequences encoded by the nucleotide sequences disclosed here. 25 Such sequences are generally referred to as a "anti-GPC-3 antibody" sequence.

BIOLOGICAL ACTIVITIES

In some embodiments, the sequences comprise at least one biological activity of an anti-GPC-3 antibody, as the case may be.

The biological activity may comprise an immunological activity. The anti-GPC-3 30 antibody may comprise an identical or similar immunological activity as compared to

antibodies 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, or its humanised versions. By “immunological activity” we mean the capability of the anti-GPC-3 antibody to induce a specific immune response in appropriate animals or cells on binding with a GPC-3 antigen.

5 The biological activity may comprise antigen binding activity. The anti-GPC-3 antibody may bind to GPC-3 or an epitope thereof. The anti-GPC-3 antibody may bind to the same epitope bound by antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1.

 The anti-GPC-3 antibody may bind to the antigen or epitope with the same, a reduced
10 or elevated affinity or avidity. For example, the anti-GPC-3 antibody may bind to the antigen or epitope with at least 10%, such as 20%, such as 30%, 40% 50%, 60%, 70%, 80%, 90% or more, affinity or avidity compared to the cognate antibody or its humanised counterparts, as the case may be.

 The activity may include inhibition of glypican-3 activity. Glypican-3 acts as a co-
15 receptor for growth factors, such as BMP, FGF, hedgehogs, which was reported to be associated with HCC development, progression and metastasis.

 The anti-GPC-3 antibody may have such inhibition activity that is the same as, reduced from, or elevated from, the cognate antibody. For example, the anti-GPC-3 antibody may be at least 10%, such as 20%, such as 30%, 40% 50%, 60%, 70%, 80%, 90% or more,
20 effective compared to the cognate antibody, e.g., antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1 or its humanised counterparts, as the case may be. By this we mean that, say, if the cognate antibody is capable of reducing glypican-3 activity, etc by for example 90%, the anti-GPC-3 antibody may be capable of doing so by below 90%, 85%, 80%, 75%, 70%, 65%,
25 60%, 55%, 50%, 45%, 40%, 35%, 30%, etc, as compared to an untreated animal or cell.

 Other assays that detect antibody events can also be used, instead of, or in addition to, the assays described.

HOMOLOGUES

 The anti-GPC-3 antibody polypeptides disclosed include homologous sequences
30 obtained from any source, for example related viral/bacterial proteins, cellular homologues

and synthetic peptides, as well as variants or derivatives thereof. Thus polypeptides also include those encoding homologues of anti-GPC-3 antibody from other species including other animals such as mammals (e.g. mice, rats or rabbits) or humans.

In the context of the present document, a homologous sequence or homologue is taken to include an amino acid sequence which is at least 60, 70, 80 or 90% identical, such as at least 95 or 98% identical at the amino acid level over at least 30, such as 50, 70, 90 or 100 amino acids with a relevant polypeptide sequence, for example as shown in the sequence listing herein. In the context of this document, a homologous sequence is taken to include an amino acid sequence which is at least 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% identical, such as at least 95 or 98% identical at the amino acid level, such as over at least 15, 25, 35, 50 or 100, such as 200, 300, 400 or 500 amino acids with the sequence of a relevant polypeptide. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present document homology may be expressed in terms of sequence identity. The sequence identity may be determined relative to the entirety of the length the relevant sequence, i.e., over the entire length or full length sequence of the relevant gene, for example.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues (for example less than 50 contiguous amino acids).

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting “gaps” in the sequence alignment to try to maximise local homology.

However, these more complex methods assign “gap penalties” to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. “Affine gap costs” are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, the default values may be used when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.*, 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60). The GCG Bestfit program may be used.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). The public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62, may be used.

Once the software has produced an optimal alignment, it is possible to calculate % homology, such as % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

VARIANTS AND DERIVATIVES

The terms “variant” or “derivative” in relation to the amino acid sequences as described here includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acids from or to the sequence. The resultant amino acid sequence may retain substantially the same activity as the unmodified sequence, such as having at least the same activity as the anti-GPC-3 antibody polypeptides shown in this document, for example in the sequence listings. Thus, the key feature of the sequences – namely ability to bind to GPC-3 polypeptides or reduction in viral infectivity, homotrimerization, viral absorption, etc, as described elsewhere – may be retained.

Polypeptides having the amino acid sequence shown in the Examples, or fragments or homologues thereof may be modified for use in the methods and compositions described here. Typically, modifications are made that maintain the biological activity of the sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions provided that the modified sequence retains the biological activity of the unmodified sequence. Amino acid substitutions may include the use of non-naturally occurring analogues, for example to increase blood plasma half-life of a therapeutically administered polypeptide.

Natural variants of an anti-GPC-3 antibody are likely to comprise conservative amino acid substitutions. Conservative substitutions may be defined, for example according to the Table below. Amino acids in the same block in the second column such as those in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

FRAGMENTS

Polypeptides disclosed here and useful as markers also include fragments of the above mentioned full length polypeptides and variants thereof, including fragments of the sequences set out in the sequence listings.

- 5 Polypeptides also include fragments of the full length sequence of any of the anti-GPC-3 antibody polypeptides. Fragments may comprise at least one epitope. Methods of identifying epitopes are well known in the art. Fragments will typically comprise at least 6 amino acids, such as at least 10, 20, 30, 50 or 100 or more amino acids.

- 10 Polypeptide fragments of the anti-GPC-3 antibody proteins and allelic and species variants thereof may contain one or more (e.g. 5, 10, 15, or 20) substitutions, deletions or insertions, including conserved substitutions. Where substitutions, deletion and/or insertions occur, for example in different species, such as less than 50%, 40% or 20% of the amino acid residues depicted in the sequence listings are altered.

- 15 anti-GPC-3 antibody and their fragments, homologues, variants and derivatives, may be made by recombinant means. However, they may also be made by synthetic means using techniques well known to skilled persons such as solid phase synthesis. The proteins may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and β -galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. The fusion protein may be such that
- 20

it will not hinder the function of the protein of interest sequence. Proteins may also be obtained by purification of cell extracts from animal cells.

5 The anti-GPC-3 antibody polypeptides, variants, homologues, fragments and derivatives disclosed here may be in a substantially isolated form. It will be understood that such polypeptides may be mixed with carriers or diluents which will not interfere with the intended purpose of the protein and still be regarded as substantially isolated. A anti-GPC-3 antibody variant, homologue, fragment or derivative may also be in a substantially purified form, in which case it will generally comprise the protein in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the protein in the preparation is a protein.

10 The anti-GPC-3 antibody polypeptides, variants, homologues, fragments and derivatives disclosed here may be labelled with a revealing label. The revealing label may be any suitable label which allows the polypeptide, etc to be detected. Suitable labels include radioisotopes, e.g. ^{125}I , enzymes, antibodies, polynucleotides and linkers such as biotin. Labelled polypeptides may be used in diagnostic procedures such as immunoassays to determine the amount of a polypeptide in a sample. Polypeptides or labelled polypeptides may also be used in serological or cell-mediated immune assays for the detection of immune reactivity to said polypeptides in animals and humans using standard protocols.

20 The anti-GPC-3 antibody polypeptides, variants, homologues, fragments and derivatives disclosed here, optionally labelled, may also be fixed to a solid phase, for example the surface of an immunoassay well or dipstick. Such labelled and/or immobilised polypeptides may be packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like. Such polypeptides and kits may be used in methods of detection of antibodies to the polypeptides or their allelic or species variants by immunoassay.

25 Immunoassay methods are well known in the art and will generally comprise: (a) providing a polypeptide comprising an epitope bindable by an antibody against said protein; (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

30 The anti-GPC-3 antibody polypeptides, variants, homologues, fragments and derivatives disclosed here may be used in *in vitro* or *in vivo* cell culture systems to study the role of their corresponding genes and homologues thereof in cell function, including their

function in disease. For example, truncated or modified polypeptides may be introduced into a cell to disrupt the normal functions which occur in the cell. The polypeptides may be introduced into the cell by *in situ* expression of the polypeptide from a recombinant expression vector (see below). The expression vector optionally carries an inducible promoter to control the expression of the polypeptide.

The use of appropriate host cells, such as insect cells or mammalian cells, is expected to provide for such post-translational modifications (e.g. myristolation, glycosylation, truncation, lipidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products. Such cell culture systems in which the anti-GPC-3 antibody polypeptides, variants, homologues, fragments and derivatives disclosed here are expressed may be used in assay systems to identify candidate substances which interfere with or enhance the functions of the polypeptides in the cell.

POLYNUCLEOTIDE SEQUENCES

The variable regions, monoclonal antibody sequences and humanised antibody sequences may comprise polynucleotides. These may comprise DNA or RNA.

They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present document, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides.

Where the polynucleotide is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the methods and compositions described here. Where the polynucleotide is single-stranded, it is to be understood that the complementary sequence of that polynucleotide is also included.

VARIANTS, DERIVATIVES AND HOMOLOGUES

The terms "variant", "homologue" or "derivative" in relation to a nucleotide sequence described in this document include any substitution of, variation of, modification of, replacement

of, deletion of or addition of one (or more) nucleotides from or to the sequence. The resulting sequence may be capable of encoding a polypeptide which has GPC-3 binding activity as described elsewhere in this document.

As indicated above, with respect to sequence identity, a “homologue” has such as at
 5 least 5% identity, at least 10% identity, at least 15% identity, at least 20% identity, at least 25% identity, at least 30% identity, at least 35% identity, at least 40% identity, at least 45% identity, at least 50% identity, at least 55% identity, at least 60% identity, at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, or at least 95% identity to a relevant sequence.

10 There may be at least 95% identity, such as at least 96% identity, such as at least 97% identity, such as at least 98% identity, such as at least 99% identity. Nucleotide homology comparisons may be conducted as described above. A sequence comparison program such as the GCG Wisconsin Bestfit program described above may be used for this purpose. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch.
 15 The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

HYBRIDISATION

We further describe nucleotide sequences that are capable of hybridising selectively to any of the sequences presented herein, such as a 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5,
 20 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1 variable region, antibody and humanised antibody or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences may be at least 15 nucleotides in length, such as at least 20, 30, 40 or 50 nucleotides in length.

The term “hybridisation” as used herein shall include “the process by which a strand of
 25 nucleic acid joins with a complementary strand through base pairing” as well as the process of amplification as carried out in polymerase chain reaction technologies.

Polynucleotides capable of selectively hybridising to the nucleotide sequences presented herein, or to their complement, will be generally at least 70%, such as at least 80 or 90% and such as at least 95% or 98% homologous to the corresponding nucleotide sequences presented
 30 herein over a region of at least 20, such as at least 25 or 30, for instance at least 40, 60 or 100 or more contiguous nucleotides.

The term “selectively hybridisable” means that the polynucleotide used as a probe is used under conditions where a target polynucleotide is found to hybridize to the probe at a level significantly above background. The background hybridization may occur because of other polynucleotides present, for example, in the cDNA or genomic DNA library being screened. In this event, background implies a level of signal generated by interaction between the probe and a non-specific DNA member of the library which is less than 10 fold, such as less than 100 fold as intense as the specific interaction observed with the target DNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P .

Hybridisation conditions are based on the melting temperature (T_m) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined “stringency” as explained below.

Maximum stringency typically occurs at about $T_m - 5^\circ\text{C}$ (5°C below the T_m of the probe); high stringency at about 5°C to 10°C below T_m ; intermediate stringency at about 10°C to 20°C below T_m ; and low stringency at about 20°C to 25°C below T_m . As will be understood by those of skill in the art, a maximum stringency hybridisation can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridisation can be used to identify or detect similar or related polynucleotide sequences.

We disclose nucleotide sequences that can hybridise to a nucleic acid, or a fragment, homologue, variant or derivative thereof, under stringent conditions (e.g. 65°C and $0.1\times\text{SSC}$ { $1\times\text{SSC} = 0.15\text{ M NaCl}$, $0.015\text{ M Na}_3\text{ Citrate pH } 7.0$ }).

Where a polynucleotide is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present disclosure. Where the polynucleotide is single-stranded, it is to be understood that the complementary sequence of that polynucleotide is also disclosed and encompassed.

Polynucleotides which are not 100% homologous to the sequences disclosed here but fall within the disclosure can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of individuals, for example individuals from different populations. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat,

mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes
5 comprising all or part of the disclosed sequences under conditions of medium to high stringency.

The polynucleotides described here may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, such as at least
10 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides as used herein. Fragments may be less than 500, 200, 100, 50 or 20 nucleotides in length.

Polynucleotides such as a DNA polynucleotides and probes may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also
15 be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for
20 example using PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the
25 reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

GLYPICAN-3

Glypican-3 is also known as GPC3 and rat homologue of OCI-5. Its cytogenetic
30 location is Xq26.2 and its genomic coordinates (GRCh38) are X:133,535,744-133,985,615

Members of the glypican family, including GPC3, are heparan sulfate proteoglycans that bind to the exocytosomal surface of the plasma membrane through a covalent glycosylphosphatidylinositol (GPI) linkage. The main function of membrane-attached glypicans is to regulate the signaling of WNTs, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins (Filmus et al., 2008).

To identify the molecular basis for Simpson-Golabi-Behmel syndrome (SGBS; see 312870), also called Simpson dysmorphia syndrome (SDYS), Pilia et al. (1996) adopted a positional cloning approach, using an X/autosome translocation. They used the cell line GM0097, which was deposited in the NIGMS repository in 1974. This cell line originated from a woman diagnosed with Beckwith-Wiedemann syndrome (BWS; 130650) and showed a karyotype with a de novo X;1 translocation. The karyotype suggested that she was affected by the X-linked SGBS rather than BWS, which is determined by a mutation on 11p. They mapped the breakpoint in an existing contig assembled across Xq26 and found a gene, called GPC3 by them, which was interrupted by this translocation. The gene was interrupted in another female patient with overgrowth and an X;16 translocation and exhibited deletions in 3 different SGBS families. The 2,130-bp cDNA encodes a deduced protein of 580 amino acids beginning 151 bp from the start of the sequence. GPC3 shares a number of features with the GPC1 gene (600395).

Filmus et al., 1988 isolated rat Gpc3 as a transcript developmentally regulated in intestine, and Filmus et al., 1995 showed that Gpc3, which they called Oci-5, is GPI-linked heparan sulfate proteoglycan.

Pilia et al. (1996) determined that the GPC3 gene contains 8 exons and encompasses approximately 500 kb.

By fluorescence in situ hybridization, Shen et al. (1997) mapped the GPC3 gene to human Xq26 and rat Xq36.

Using DNA microarrays to compare gene expression patterns in normal human placenta with those in other tissues, Sood et al. (2006) found that several genes involved in growth and tissue remodeling were expressed at relatively higher levels in the villus sections of placenta compared with other tissues. These included GPC3, CDKN1C (600856), and IGF2 (147470). The GPC3 and CDKN1C genes are mutated in patients with Simpson-Golabi-Behmel syndrome and Beckwith-Wiedemann syndrome (130650), respectively, both fetal-

placental overgrowth syndromes. In contrast, loss of IGF2 is associated with fetal growth restriction in mice. The relatively higher expression of genes that both promote and suppress growth suggested to Sood et al. (2006) tight and local regulation of the pathways that control placental development.

5 Capurro et al. (2008) found that GPC3 inhibited soluble hedgehog activity in the medium of SHH (600725)-expressing mouse embryonic fibroblasts and IHH (600726)-expressing human embryonic kidney cells. GPC3 interacted with SHH, but not with the SHH receptor Patched (PTCH1; 601309), and it competed with Patched for SHH binding. Furthermore, GPC3 induced SHH endocytosis and degradation. The heparan sulfate chains of
10 GPC3 were not required for its interaction with SHH, but membrane attachment via the GPI anchor was required.

 Maurel et al. (2013) observed that expression of both microRNA-1291 (MIR1291; 615487) and GPC3 was upregulated in hepatocellular carcinoma. They found that MIR1291 did not directly bind to GPC3 mRNA, but rather enhanced its stability by binding to
15 and directing degradation of IRE1A (ERN1; 604033), an endoribonuclease that functions in the endoplasmic reticulum unfolded protein response. In the absence of MIR1291, IRE1A bound a canonical site in the 3-prime UTR of GPC3 and cleaved the mRNA, causing its degradation via the unfolded protein response. Unlike most miRNAs, which typically bind complementary sequences in the 3-prime UTRs of target mRNAs, MIR1291 bound a
20 complementary site in the 5-prime UTR of IRE1A to direct its degradation.

 In the initial studies of Pilia et al. (1996) in which 6 of the 8 exons of the GPC3 gene were examined, deletions were identified in 3 of 6 patients with SGBS. This suggested that large scale deletions may be responsible for a considerable proportion of cases of Simpson-Golabi-Behmel syndrome. This might not be unexpected given the large region of genomic
25 DNA covered by the GPC3 gene (approximately 500 kb) and the high proportion of deletions found in some other disorders involving large genes, for example, in the dystrophin gene (300377) in patients with Duchenne muscular dystrophy (310200). Lindsay et al. (1997) carried out studies to determine the proportion and type of deletions present in the GPC3 gene in 18 families with SGBS (approximately half of reported cases). Deletions were
30 detected in only 5 families (1 of which had previously been reported). PCR analysis was carried out using primer pairs that amplified fragments from each of the 8 exons of the GPC3 gene and deletions were found in all exons of the gene except exon 3. The results suggested

that large scale deletions may be less common in SGBS than was originally thought. One patient, with an exon 4 and 5 deletion, lacked the characteristic facial dysmorphic features. This raised the possibility of involvement of GPC3 gene defects in a wider range of overgrowth disorders.

5 *Simpson-Golabi-Behmel Syndrome, Type 1*

Veugelers et al. (2000) identified 1 SGBS patient with a deletion of GPC3 exon 7 (300037.0002). Six SGBS patients showed point mutations in GPC3: 1 frameshift, 3 nonsense, and 1 splice mutation (300037.0004) predicted a loss of function of the glypican-3 protein. One missense mutation, W296R (300037.0003), altered a conserved amino acid found
10 in all glypicans identified to that time. A GPC3 protein that reproduced this mutation was poorly processed and failed to increase the cell surface expression of heparan sulfate, suggesting that this missense mutation is also a loss-of-function mutation.

Sakazume et al. (2007) identified mutations in the GPC3 gene in 7 Japanese boys with SGBS1. One of the boys had an affected younger brother. All the mutations were predicted to
15 result in complete loss of function. Only 1 patient had a large deletion, and there were 5 nonsense and 1 frameshift mutations. There were no apparent genotype/phenotype correlations.

Wilms Tumor, Somatic

White et al. (2002) identified 2 nonconservative single base changes in the GPC3 gene
20 in Wilms tumor (194070) tissue only (300037.0006-300037.0007), implying a possible role of GPC3 in Wilms tumor development. They pointed out that Wilms tumor has been found in a number of patients with Simpson-Golabi-Behmel syndrome (Hughes-Benzie et al., 1996; Xuan et al., 1999).

Capurro et al. (2008) stated that Gpc3-null mouse embryos show significant
25 overgrowth by embryonic day 12.5. They found that embryos between days 10.5 and 13.5 displayed increased hedgehog signaling, as measured by elevated Patched and Gli1 (165220) mRNA levels.

The above text is adapted from the OMIM entry for Glypican-3
(<https://www.omim.org/entry/300037>), contributors Patricia A. Hartz, Cassandra L. Kniffin,
30 Victor A. McKusick, Anne M. Stumpf, Ada Hamosh, George E. Tiller and Michael J. Wright.

GPC-3 POLYPEPTIDES AND NUCLEIC ACIDS

GPC-3 polypeptide homologues, variants, derivatives and fragments may be defined similarly, as set out in the previous paragraphs.

Where the context permits, a reference to GPC-3 polypeptide should be taken to include reference to a GPC-3 polypeptide homologue, variant, derivative or fragment. Similarly, where the context permits, a reference to an GPC-3 nucleic acid should be taken to include reference to an GPC-3 nucleic acid homologue, variant, derivative or fragment.

An example of a glypican-3 nucleic acid is the sequence having GenBank Accession Number NM_004484.4.

10 An example of a glypican-3 polypeptide is the sequence having GenBank Accession Number NP_004475.1.

ANTI-GPC-3 ANTIBODY PRODUCTION

The anti-GPC-3 antibody can be produced by recombinant DNA methods or synthetic peptide chemical methods that are well known to those of ordinary skill in the art.

15 By way of example, the anti-GPC-3 antibody may be synthesized by techniques well known in the art, as exemplified by “Solid Phase Peptide Synthesis: A Practical Approach” E. Atherton and R. C. Sheppard, IRL Press, Oxford England. Similarly, multiple fragments can be synthesized which are subsequently linked together to form larger fragments. These synthetic peptide fragments can also be made with amino acid substitutions at specific
20 locations in order to test for activity *in vitro* and *in vivo*.

The anti-GPC-3 antibody can be synthesized in a standard microchemical facility and purity checked with HPLC and mass spectrophotometry. Methods of peptide synthesis, HPLC purification and mass spectrophotometry are commonly known to those skilled in these arts.

The anti-GPC-3 antibody may also be expressed under *in vitro* and *in vivo* conditions
25 in a transformed host cell into which has been incorporated the DNA sequences described here (such as variable sequences) or allelic variations thereof and which can be used in the prevention and/or treatment of cancers such as hepatocellular carcinoma.

The term “vector” includes expression vectors and transformation vectors. The term “expression vector” means a construct capable of *in vivo* or *in vitro* expression. The term

“transformation vector” means a construct capable of being transferred from one species to another.

Vectors which may be used for expression include recombinant viral vectors, in particular recombinant retroviral vectors (RRV) such as lentiviral vectors, adenoviral vectors
5 including a combination of retroviral vectors.

The term ‘recombinant retroviral vector’ (RRV) refers to a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell includes reverse transcription and integration into the target cell genome. The RRV
10 carries non-viral coding sequences which are to be delivered by the vector to the target cell. An RRV is incapable of independent replication to produce infectious retroviral particles within the final target cell. Usually the RRV lacks a functional gag pol and/or env gene and/or other genes essential for replication. Vectors which may be used include recombinant pox viral vectors such as fowl pox virus (FPV), entomopox virus, vaccinia virus such as NYVAC,
15 canarypox virus, MVA or other non-replicating viral vector systems such as those described for example in WO9530018.

Pox viruses may be engineered for recombinant gene expression and for the use as recombinant live vaccines in a dual immunotherapeutic approach. The principal rationale for using live attenuated viruses, such as viruses, as delivery vehicles and/or vector based vaccine
20 candidates, stems from their ability to elicit cell mediated immune responses. The viral vectors, as outlined above, are capable of being employed as delivery vehicles and as vector based vaccine candidates because of the immunogenicity of their constitutive proteins, which act as adjuvants to enhance the immune response, thus rendering a nucleotide sequence of interest (NOI) such as a nucleotide sequence encoding an anti-GPC-3 antibody more
25 immunogenic.

The pox virus vaccination strategies have used recombinant techniques to introduce NOIs into the genome of the pox virus. If the NOI is integrated at a site in the viral DNA which is non-essential for the life cycle of the virus, it is possible for the newly produced recombinant pox virus to be infectious, that is to say to infect foreign cells and thus to express
30 the integrated NOI. The recombinant pox virus prepared in this way can be used as live vaccines for the prophylaxis and/or treatment of disease.

Other requirements for pox viral vector delivery systems include good immunogenicity and safety. MVA is a replication-impaired vaccinia strain with a good safety record. In most cell types and normal human tissue, MVA does not replicate. Limited replication of MVA is observed in a few transformed cell types such as BHK21 cells. Carroll et al (1997 Vaccine 15 : 387-394) have shown that the recombinant MVA is equally as good as conventional recombinant vaccinia vectors at generating a protective CD8+T cell response and is an efficacious alternative to the more commonly used replication competent vaccinia virus. The vaccinia virus strains derived from MVA, or independently developed strains having the features of MVA which make MVA particularly suitable for use in a vaccine, are also suitable for use as a delivery vehicle.

The nucleotide sequence of interest, and of which expression is desired, may operably linked to a transcription unit. The term "transcription unit" as described herein are regions of nucleic acid containing coding sequences and the signals for achieving expression of those coding sequences independently of any other coding sequences. Thus, each transcription unit generally comprises at least a promoter, an optional enhancer and a polyadenylation signal. The term "promoter" is used in the normal sense of the art, e. g. an RNA polymerase binding site. The promoter may contain an enhancer element. The term "enhancer" includes a DNA sequence which binds to other protein components of the transcription initiation complex and thus facilitates the initiation of transcription directed by its associated promoter. The term "cell" includes any suitable organism. The cell may comprise a mammalian cell, such as a human cell.

The term "transformed cell" means a cell having a modified genetic structure. For example, as described here, a cell has a modified genetic structure when a vector such as an expression vector has been introduced into the cell. The term "organism" includes any suitable organism. The organism may comprise a mammal such as a human.

Here the term "transgenic organism" means an organism comprising a modified genetic structure. For example, the organism may have a modified genetic structure if a vector such as an expression vector has been introduced into the organism.

ANTIBODY EXPRESSION

We further describe a method comprising transforming a host cell with a or the nucleotide sequences described in this document, such as 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 variable regions, antibody sequences or humanized antibody sequences.

We also provide a method comprising culturing a transformed host cell-which cell has been transformed with a or the such nucleotide sequences under conditions suitable for the expression of the anti-GPC-3 antibody encoded by said nucleotide sequences.

We further provide a method comprising culturing a transformed host cell-which cell has been transformed with a or the such nucleotide sequences under conditions suitable for the expression of the anti-GPC-3 antibody encoded by said nucleotide sequences; and then recovering said anti-GPC-3 antibody from the transformed host cell culture.

Thus, anti-GPC-3 antibody encoding nucleotide sequences, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression thereof in appropriate host cells.

By way of example, anti-GPC-3 antibody may be produced in recombinant *E. coli*, yeast or mammalian expression systems, and purified with column chromatography.

In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved neutralization of viral activity, infection, progression, etc. Fab, Fv, ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the production of large amounts of the such fragments.

The nucleotide sequences encoding the anti-GPC-3 antibody may be operably linked to a promoter sequence capable of directing expression of the anti-GPC-3 antibody encoding nucleotide sequences in a suitable host cell. When inserted into the host cell, the transformed host cell may be cultured under suitable conditions until sufficient levels of the anti-GPC-3 antibody are achieved after which the cells may be lysed and the anti-GPC-3 antibody is isolated.

Host cells transformed with the anti-GPC-3 antibody encoding nucleotide sequences may be cultured under conditions suitable for the expression and recovery of the anti-GPC-3 antibody from cell culture. The protein produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing the

anti-GPC-3 antibody encoding nucleotide sequences can be designed with signal sequences which direct secretion of the anti-GPC-3 antibody encoding nucleotide sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the anti-GPC-3 antibody encoding nucleotide sequence to a nucleotide
5 sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al(1993) DNA Cell Biol 12:441- 5 3', see also the discussion below on vectors containing fusion proteins).

The anti-GPC-3 antibody may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification
10 facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath J (1992) Protein Expr Purif 3-26328 1), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor
15 XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the anti-GPC-3 antibody is useful to facilitate purification.

The nucleotide sequences described here may be engineered in order to alter a the anti-GPC-3 antibody encoding sequences for a variety of reasons, including but not limited to alterations which modify the cloning, processing and/or expression of the gene product. For
20 example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns or to change codon preference.

In another embodiment, a or the natural, modified or recombinant anti-GPC-3 antibody encoding nucleotide sequences may be ligated to a heterologous sequence to encode a fusion
25 protein. By way of example, fusion proteins comprising the anti-GPC-3 antibody or an enzymatically active fragment or derivative thereof linked to an affinity tag such as glutathione-S-transferase (GST), biotin, His6, ac-myc tag (see Emrich et al 1993 BiocemBiophys Res Commun 197(1): 21220), hemagglutinin (HA) (as described in Wilson et al (1984 Cell 37 767) or a FLAG epitope (Ford et al 1991 Protein Expr Purif Apr; 2 (2):95-
30 107).

The fused recombinant protein may comprise an antigenic coprotein such as GST, beta-galactosidase or the lipoprotein D from *Haemophilus influenzae* which are relatively large co-proteins, which solubilise and facilitate production and purification thereof. Alternatively, the fused protein may comprise a carrier protein such as bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). In certain embodiments, the marker sequence may comprise a hexa-histidine peptide, as provided in the pQE vector (Qiagen Inc) and described in Gentz et al (1989 PNAS 86: 821-824). Such fusion proteins are readily expressible in yeast culture (as described in Mitchell et al 1993 Yeast 5:715-723) and are easily purified by affinity chromatography. A fusion protein may also be engineered to contain a cleavage site located between the nucleotide sequence encoding the anti-GPC-3 antibody and the heterologous protein sequence, so that the anti-GPC-3 antibody may be cleaved and purified away from the heterologous moiety. In another embodiment, an assay for the target protein may be conducted using the entire, bound fusion protein. Alternatively, the co-protein may act as an adjuvant in the sense of providing a generalised stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Although the presence/absence of marker gene expression suggests that the nucleotide sequence for anti-GPC-3 antibody is also present, its presence and expression should be confirmed. For example, if the anti-GPC-3 antibody encoding nucleotide sequence is inserted within a marker gene sequence, recombinant cells containing the anti-GPC-3 antibody coding regions may be identified by the absence of the marker gene function. Alternatively, a marker gene may be placed in tandem with a anti-GPC-3 antibody encoding nucleotide sequence under the control of a single promoter.

Expression of the marker gene in response to induction or selection usually indicates expression of the anti-GPC-3 antibody as well.

Additional methods to quantitate the expression of a particular molecule include radiolabeling (Melby PC et al 1993 J Immunol Methods 159:235-44) or biotinylating (Duplaa C et al 1993 Anal Biochem 229:36) nucleotides, co amplification of a control nucleic acid. and standard curves onto which the experimental results are interpolated.

Quantitation of multiple samples may be speeded up by running the assay in an ELISA format where the anti-GPC-3 antibody of interest is presented in various dilutions and a spectrophotometric or calorimetric response gives rapid quantitation.

Altered anti-GPC-3 antibody nucleotide sequences which may be made or used
5 include deletions, insertions or substitutions of different nucleotide residues resulting in a nucleotide sequence that encodes the same or a functionally equivalent anti-GPC-3 antibody. By way of example, the expressed anti-GPC-3 antibody may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent anti-GPC-3 antibody. Deliberate amino acid substitutions may be made on the
10 basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the binding affinity of the anti-GPC-3 antibody is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine,
15 valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

BI-SPECIFIC ANTIBODIES

We disclose bi-specific antibodies or antigen-binding fragments capable of binding both GPC-3 as well as another antigen.

The second antigen may comprise a T-cell surface antigen. An example of a T-cell
20 surface antigen is a CD protein, such as CD3 (GenBank Accession Number: NM_000733.4).

We specifically disclose an anti-GPC-3 bi-specific T cell-engaging antibody, which is capable of specifically recognising and binding to both GPC-3 and human CD3 molecules on the cell surface.

Such a bi-specific antibody may be constructed by replacing the Fab region of one arm
25 of the antibody with sequences capable of binding to a T-cell surface antigen such as CD3. Such T-cell surface antigen binding sequences may for example comprise V_H and V_L regions of a known antibody against the T-cell surface antigen. They may be provided in the form of an anti-CD3 scFv fragment, for example.

A detailed protocol for constructing a bi-specific antibody capable of binding CD3 is
30 set out in the Examples.

A bi-specific antibody or antigen-binding fragment may be capable of activating naïve T-cells, inducing cytokine release (such as of interferon- γ and IL-2) and/or of inducing cell death of GPC-3 expressing cells.

CHIMERIC ANTIGEN RECEPTORS (CARS)

5 Chimeric antigen receptors (CARs), also known as chimeric T cell receptors, artificial T cell receptors and chimeric immunoreceptors, are engineered receptors which graft an arbitrary specificity onto an immune effector cell.

In a classical CAR, the specificity of a monoclonal antibody is grafted on to a T cell. CAR-encoding nucleic acids may be transferred to T cells using, for example, retroviral
10 vectors. In this way, a large number of cancer-specific T cells can be generated for adoptive cell transfer. Phase I clinical studies of this approach show efficacy.

The target-antigen binding domain of a CAR is commonly fused via a spacer and transmembrane domain to a signaling endodomain. When the CAR binds the target-antigen, this results in the transmission of an activating signal to the T-cell it is expressed on.

15 We disclose a CAR which comprises a glypican-3 binding domain which is based on any of the antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1.

The CAR may comprise a glypican-3-binding domain which comprises

- 20 (a) a heavy chain variable region (V_H) having complementarity determining regions (CDRs) of any of the above antibodies, as set out in this document;
- (b) a light chain variable region (V_L) having CDRs of any of the above antibodies, as set out in this document.

It may be possible to introduce one or more mutations (substitutions, additions or deletions) into the or each CDR without negatively affecting glypican-3-binding activity. Each
25 CDR may, for example, have one, two or three amino acid mutations.

The CAR may comprise one of the following V_H sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35,

SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47.

The CAR may comprise one of the following V_L sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14,
5 SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48.

The CAR may comprise a variant of any of these sequences having at least 80, 85, 90,
10 95, 98 or 99% sequence identity, provided that the variant sequence retain the capacity to bind glypican-3 (when in conjunction with a complementary V_L or V_H domain, if appropriate).

The percentage identity between two polypeptide sequences may be readily determined by programs such as BLAST which is freely available at <http://blast.ncbi.nlm.nih.gov>.

15 *Transmembrane Domain*

The CAR may also comprise a transmembrane domain which spans the membrane. It may comprise a hydrophobic alpha helix. The transmembrane domain may be derived from CD28, which gives good receptor stability.

Intracellular T Cell Signaling Domain (ENDODOMAIN)

20 The endodomain is the signal-transmission portion of the CAR. After antigen recognition, receptors cluster and a signal is transmitted to the cell. The most commonly used endodomain component is that of CD3-zeta which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling may be needed. For
25 example, chimeric CD28 and OX40 can be used with CD3-Zeta to transmit a proliferative / survival signal, or all three can be used together.

The endodomain of the CAR may comprise the CD28 endodomain and OX40 and CD3-Zeta endodomain.

A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to such an endodomain, provided that the sequence provides an effective transmembrane domain/intracellular T cell signaling domain.

Signal Peptide

- 5 The CAR may comprise a signal peptide so that when the CAR is expressed inside a cell, such as a T-cell, the nascent protein is directed to the endoplasmic reticulum and subsequently to the cell surface, where it is expressed.

10 The core of the signal peptide may contain a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognized and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

- 15 The signal peptide may be at the amino terminus of the molecule.

The CAR may have the general formula:

Signal peptide – glypican-3-binding domain – spacer domain - transmembrane domain - intracellular T cell signaling domain.

Spacer

- 20 The CAR may comprise a spacer sequence to connect the glypican-3-binding domain with the transmembrane domain and spatially separate the glypican-3-binding domain from the endodomain. A flexible spacer allows to the glypican-3-binding domain to orient in different directions to enable glypican-3 binding.

- 25 The spacer sequence may, for example, comprise an IgG1 Fc region, an IgG1 hinge or a CD8 stalk, or a combination thereof. The spacer may alternatively comprise an alternative sequence which has similar length and/or domain spacing properties as an IgG1 Fc region, an IgG1 hinge or a CD8 stalk.

A human IgG1 spacer may be altered to remove Fc binding motifs.

Examples of amino acid sequences for these spacers are given below: hinge-CH2CH3 of human IgG1, human CD8 stalk, human IgG1 hinge, IgG1 Hinge-Fc and IgG1 Hinge – Fc modified to remove Fc receptor recognition motifs.

5 DIAGNOSTIC KITS

We also provide diagnostic kits for detecting cancer, including hepatocellular carcinoma in an individual, or susceptibility to such in an individual.

The diagnostic kit may comprise means for detecting expression, amount or activity of GPC-3 in the individual, by any means as described in this document. The diagnostic kit may therefore comprise any one or more of the following: an anti-GPC-3 antibody, an antibody capable of binding to the same epitope as monoclonal antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, monoclonal antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, Fab from 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, scFv from 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, an antibody comprising a variable region of antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, or a humanised monoclonal antibody 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 or 1E1, etc

The diagnostic kit may comprise instructions for use, or other indicia. The diagnostic kit may further comprise means for treatment or prophylaxis of cancer (for example hepatocellular carcinoma), such as any of the compositions described in this document, or any means known in the art for treating cancer (such as hepatocellular carcinoma). In particular, the diagnostic kit may comprise an anti-GPC-3 antibody as described, for example obtained by screening.

HEPATOCELLULAR CARCINOMA / LIVER CANCER

The anti-GPC-3 antibodies and binding agents disclosed in this document may be used for the treatment, prevention or alleviation of a cancer such as liver cancer.

5 Carcinoma of the liver (hepatocellular carcinoma, HCC) is the fifth most common malignancy worldwide and third highest cause of global cancer mortality (Ferley J, Bray F. Pisani P, Parkin DM. GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC CancerBase No5 version 20. IARC Press, Lyon, France; 2004).

10 Hepatocellular carcinoma (HCC) has replaced stomach cancer as the 3rd highest ranking cancer among Singaporean males, accounting for 8.1% of all cancers diagnosed in 1998-2002 (Seow A, K. W., Chia KS, Shi LM, Lee HP, Shanmugaratnam K (2004). "Trends in Cancer Incidence in Singapore 1968-2002." Singapore Cancer Registry (Report no. 6)). In Asia, where about 250,000 new cases per year are reported in China alone, HCC is endemic. Worldwide, it is the leading cause of cancer mortality (Bosch, F. X., J. Ribes, et al. (2005). "Epidemiology of hepatocellular carcinoma." Clin Liver Dis 9(2): 191-211, v.). Even in
15 countries like the USA and Europe where the prevalence of HCC is lower, the incidence of HCC is on the rise (El-Serag, H. B. and A. C. Mason (1999). "Rising incidence of hepatocellular carcinoma in the United States." N Engl J Med 340(10): 745-50).

20 More than 90% of HCCs are diagnosed at an advanced stage and are often associated with liver cirrhosis. HCCs typically exhibit highly aggressive clinical behaviour with the majority of patients dying within 12 months of diagnosis (El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. J Clin Gastroenterol 2002; 35(5 Suppl 2):S72-8).

25 Often, patients present at such advanced disease stages that surgery is not an option; in cases where surgical resections are performed the two-year recurrence rate is still as high as 50% (Nagasue N, Kohno H, Chang YC, Taniura H, Yamanoi A, Uchida M, et al. Liver resection for hepatocellular carcinoma. Results of 229 consecutive patients during 11 years. Ann Surg 1993; 217:375-84; Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, et al. Recurrence of hepatocellular carcinoma after surgery. Br J Surg 1996; 83:1219-22.).

30 HCC is a relatively chemotherapy refractory cancer. There is no single agent or multi-agent chemotherapy that is particularly effective. Doxorubicin is the most commonly used chemotherapy agent in metastatic HCC with response rates of less than 20% (Johnson, P. J.,

- R. Williams, et al. (1978). "Induction of remission in hepatocellular carcinoma with doxorubicin." Lancet **1** (8072): 1006-9) and statistically insignificant survival advantage. More recent results of a trial using 3-drug combination chemotherapy and interferon showed response rates of 20.9% and median survival of 8.67 months (Yeo, W., T. S. Mok, et al. (2005). "A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma." J Natl Cancer Inst **97**(20): 1532-8). This regimen did not show better survival compared to doxorubicin alone and was associated with more toxicities.

Recent results from randomized clinical trials have shown that standard
10 chemotherapeutic regimens are minimally effective in prolonging HCC patient survival (Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, et al. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst 2005; 97:1532-38).

15 Besides tumor recurrence and metastasis, peritoneal ascites is another significant cause of morbidity in advanced stage HCC patients, often arising as a result of compromised liver function, portal vein blockage, and increased endothelial cell permeability.

PROPHYLACTIC AND THERAPEUTIC METHODS

The monoclonal antibodies 5C4, 4H1, 1D2, 1C4, 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1,
20 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1 may be used for treatment of disease in humans or other animals.

We show in the Examples that such anti-GPC-3 antibodies have anti-tumour activity. Specifically, the Examples indicate that the anti-GPC-3 antibodies may be used for treating and preventing hepatocellular carcinoma.

25 We disclose methods of treating cancers, including hepatocellular carcinoma. Methods of preventing cancer such as hepatocellular carcinoma (i.e., prophylaxis) also suitably employ the same or similar approaches.

Accordingly, we provide for the use of anti-GPC-3 antibodies in the treatment or prevention of a proliferative disease. The proliferative disease may comprise cancer. The anti-

GPC-3 antibodies may be used as drugs or therapies to treat cancer such as hepatocellular carcinoma. They may be used to prevent such infection or progress of the disease.

5 In general terms, our methods involve manipulation of cells, by modulating (such as down-regulating) the expression, amount or activity of GPC-3. The treatment may comprise generally contacting a tumour cell, or a cell suspected of being a tumour cell, with an anti-GPC-3 antibody. The methods may involve exposing a patient to an anti-GPC-3 antibody or variant thereof as described here.

10 It may or in addition be exposed to an anti-cancer agent such as an antibody or other molecule known to have effect in preventing or treating cancer such as liver cancer. Where this is so, the cell may be exposed to both the antibody and the agent together, or individually in sequence. The exposure may be repeated a number of times. Any combination of anti-GPC-3 antibody and an other agent antibody in whatever amount or relative amount, in whatever timing of exposure, may be used.

15 We therefore provide for the use of combinations of anti-GPC-3 antibodies and anti-cancer agents, as described above, in the treatment of a cancer such as liver cancer.

20 The cell may be an individual cell, or it may be in a cell mass. The cell may be inside the body of an organism. The organism may be one which is known to be suffering from cancer, or it could be one in which cancer is suspected, or it could be one which is susceptible to cancer. The treatment may comprise administering the antibody or antibodies to the organism. As above, a single antibody may be administered, or a combination of anti-GPC-3 antibody and an anti-cancer agent may be administered. The administration may be simultaneous or sequential, as described above. Thus, the treatment may comprise administering an anti-GPC-3 antibody simultaneously or sequentially with an anti-cancer agent to the individual.

25 For this purpose, a number of criteria may be designated, which reflect the progress of treatment or prophylaxis or the well-being of the patient. Useful criteria in the case of cancer may include TNM staging, the Okuda system, CLIP score, BCLC staging as known the art. For example the measurement of tumor size and severity of cirrhosis, for example as measured by the amount of ascites, serum albumin, and bilirubin levels may be used in the
30 assessment of treatment.

Treatment of liver cancer is described in detail in Liu et al (2015), *Cold Spring Harb Perspect Med.* 2015 Sep; 5(9).

Thus, as an example, a treated individual may show a decrease in such a symptom as measured by an appropriate assay or test. A treated individual may for example show a 1%,
5 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more decrease in one or more symptoms, compared to an individual who has not been treated.

For example, a patient disease may be defined as being “treated” if a condition associated with the disease is significantly inhibited (*i.e.*, by 50% or more) relative to controls.
10 The inhibition may be by at least 75% relative to controls, such as by 90%, by 95% or 100% relative to controls. By the term “treatment” we mean to also include prophylaxis or alleviation of cancer such as liver cancer.

The antibody approach to therapy involving use of anti-GPC-3 antibodies may be combined with other approaches for therapy of such disorders including conventional drug
15 based approaches.

TREATMENT

The mainstay of treatment is supportive therapy. The patient is encouraged to keep up oral intake, especially of oral fluids. If the patient is unable to maintain oral intake, supplementation with intravenous fluids may be necessary to prevent dehydration and
20 significant hemoconcentration. A platelet transfusion is indicated if the platelet level drops significantly.

PHARMACEUTICAL COMPOSITIONS

As disclosed herein, anti-GPC-3 antibodies may be used to treat or prevent cancer such as liver cancer.

25 Anti-GPC-3 antibodies can be administered in a variety of ways including enteral, parenteral and topical routes of administration. For example, suitable modes of administration include oral, subcutaneous, transdermal, transmucosal, iontophoretic, intravenous, intramuscular, intraperitoneal, intranasal, subdural, rectal, and the like.

In accordance with other embodiments, there is provided a composition comprising an anti-GPC-3 antibody, together with a pharmaceutically acceptable carrier or excipient for the treatment or prevention of cancer such as liver cancer.

Suitable pharmaceutically acceptable excipients include processing agents and drug
5 delivery modifiers and enhancers, such as, for example, calcium phosphate, magnesium stearate, talc, monosaccharides, disaccharides, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, dextrose, hydroxypropyl- β -cyclodextrin, polyvinylpyrrolidinone, low melting waxes, ion exchange resins, and the like, as well as combinations of any two or more thereof. Other suitable pharmaceutically acceptable
10 excipients are described in "Remington's Pharmaceutical Sciences," Mack Pub. Co., New Jersey (1991), incorporated herein by reference.

Pharmaceutical compositions containing an anti-GPC-3 antibody may be in any form suitable for the intended method of administration, including, for example, a solution, a suspension, or an emulsion. Liquid carriers are typically used in preparing solutions,
15 suspensions, and emulsions. Liquid carriers contemplated for use in the practice include, for example, water, saline, pharmaceutically acceptable organic solvent (s), pharmaceutically acceptable oils or fats, and the like, as well as mixtures of two or more thereof. The liquid carrier may contain other suitable pharmaceutically acceptable additives such as solubilizers, emulsifiers, nutrients, buffers, preservatives, suspending agents, thickening agents, viscosity
20 regulators, stabilizers, and the like. Suitable organic solvents include, for example, monohydric alcohols, such as ethanol, and polyhydric alcohols, such as glycols.

Suitable oils include, for example, soybean oil, coconut oil, olive oil, safflower oil, cottonseed oil, and the like. For parenteral administration, the carrier can also be an oily ester such as ethyl oleate, isopropyl myristate, and the like. Compositions may also be in the form
25 of microparticles, microcapsules, liposomal encapsulates, and the like, as well as combinations of any two or more thereof.

The anti-GPC-3 antibody may be administered orally, parenterally, sublingually, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical
30 administration may also involve the use of transdermal administration such as transdermal

patches or ionophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrastemal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-propanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e. g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavoring, and perfuming agents.

In accordance with yet other embodiments, we provide methods for inhibiting any activity of GPC-3, in a human or animal subject, the method comprising administering to a subject an amount of an anti-GPC-3 antibody (or composition comprising such compound)

effective to inhibit the relevant activity in the subject. Other embodiments provide methods for treating cancer such as liver cancer, in a human or animal subject, comprising administering to the cell or to the human or animal subject an amount of a compound or composition as described here effective to inhibit a GPC-3 activity in the cell or subject. The
5 subject may be a human or non-human animal subject. Inhibition of protein activity includes detectable suppression of the relevant protein activity either as compared to a control or as compared to expected protein activity.

Effective amounts of the anti-GPC-3 antibody generally include any amount sufficient to detectably inhibit the relevant protein activity by any of the assays described herein, by
10 other assays known to those having ordinary skill in the art or by detecting an alleviation of symptoms in a subject afflicted with cancer such as liver cancer.

Successful treatment of a subject in accordance may result in the inducement of a reduction or alleviation of symptoms in a subject afflicted with a medical or biological disorder to, for example, halt the further progression of the disorder, or the prevention of the
15 disorder. Thus, for example, treatment cancer such as liver cancer can result in a reduction in symptoms as described above.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be understood, however, that the specific dose level for any
20 particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. The therapeutically effective amount for a given situation can be readily determined by routine experimentation and is within the skill and judgment of the
25 ordinary clinician.

A therapeutically effective dose will generally be from about 10 μ g/kg/day to 100mg/kg/day, for example from about 25 μ g/kg/day to about 20 mg/kg/day or from about 50 μ g/kg/day to about 2mg/kg/day of an anti-GPC-3 antibody, which may be administered in one or multiple doses.

30 The anti-GPC-3 antibody can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid

substances. Liposomes are formed by mono-or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound, stabilizers, preservatives, excipients, and the like. Lipids which may be used include the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N. W., p. 33 et seq (1976).

While the anti-GPC-3 antibody can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other agents used in the treatment of disorders. Representative agents useful in combination with the anti-GPC-3 antibody for the treatment of cancer such as liver cancer include, for example, Sorafenib, Lenvatinib and Regorafenib.

When additional active agents are used in combination with the anti-GPC-3 antibody, the additional active agents may generally be employed in therapeutic amounts as indicated in the PHYSICIANS' DESK REFERENCE (PDR) 53rd Edition (1999), which is incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art.

The anti-GPC-3 antibody and the other therapeutically active agents can be administered at the recommended maximum clinical dosage or at lower doses. Dosage levels of the active anti-GPC-3 antibody in the compositions may be varied so as to obtain a desired therapeutic response depending on the route of administration, severity of the disease and the response of the patient. The combination can be administered as separate compositions or as a single dosage form containing both agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Bioavailability

The compounds disclosed here (and combinations) are in some embodiments orally bioavailable. Oral bioavailability refers to the proportion of an orally administered drug that reaches the systemic circulation. The factors that determine oral bioavailability of a drug are

dissolution, membrane permeability and metabolic stability. Typically, a screening cascade of firstly in vitro and then in vivo techniques is used to determine oral bioavailability.

Dissolution, the solubilisation of the drug by the aqueous contents of the gastrointestinal tract (GIT), can be predicted from in vitro solubility experiments conducted at appropriate pH to mimic the GIT. The anti-GPC-3 antibody may in some embodiments have a minimum solubility of 50 mg/ml. Solubility can be determined by standard procedures known in the art such as described in Adv. Drug Deliv. Rev. 23, 3-25, 1997.

Membrane permeability refers to the passage of the compound through the cells of the GIT. Lipophilicity is a key property in predicting this and is defined by in vitro Log $D_{7.4}$ measurements using organic solvents and buffer. The anti-GPC-3 antibody may have a Log $D_{7.4}$ of -2 to +4 or -1 to +2. The log D can be determined by standard procedures known in the art such as described in J. Pharm. Pharmacol. 1990, 42:144.

Cell monolayer assays such as CaCO₂ add substantially to prediction of favourable membrane permeability in the presence of efflux transporters such as p-glycoprotein, so-called caco-2 flux. The anti-GPC-3 antibody may have a caco-2 flux of greater than $2 \times 10^{-6} \text{ cm s}^{-1}$, for example greater than $5 \times 10^{-6} \text{ cm s}^{-1}$. The caco flux value can be determined by standard procedures known in the art such as described in J. Pharm. Sci, 1990, 79, 595-600.

Metabolic stability addresses the ability of the GIT or the liver to metabolise compounds during the absorption process: the first pass effect. Assay systems such as microsomes, hepatocytes etc are predictive of metabolic liability. The compounds of the Examples may in some embodiments show metabolic stability in the assay system that is commensurate with an hepatic extraction of less than 0.5. Examples of assay systems and data manipulation are described in Curr. Opin. Drug Disc. Devel., 201, 4, 36-44, Drug Met. Disp., 2000, 28, 1518-1523.

Because of the interplay of the above processes further support that a drug will be orally bioavailable in humans can be gained by in vivo experiments in animals. Absolute bioavailability is determined in these studies by administering the compound separately or in mixtures by the oral route. For absolute determinations (% absorbed) the intravenous route is also employed. Examples of the assessment of oral bioavailability in animals can be found in Drug Met. Disp., 2001, 29, 82-87; J. Med Chem, 1997, 40, 827-829, Drug Met. Disp., 1999, 27, 221-226.

The term “pharmaceutically acceptable carrier” as used herein generally refers to organic or inorganic materials, which cannot react with active ingredients. The carriers include but are not limited to sugars, such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium
5 carboxymethylcellulose, ethylcellulose and cellulose acetates; powdered tragacanth; malt; gelatin; talc; stearic acids; magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cotton seed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; agar; alginic acids; pyrogen-free water; isotonic saline; and phosphate buffer solution; skim milk powder; as well
10 as other non-toxic compatible substances used in pharmaceutical formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well as coloring agents, flavoring agents, lubricants, excipients, tableting agents, stabilizers, anti-oxidants and preservatives, can also be present.

The term “therapeutically effective amount” as used herein generally refers to an
15 amount of an agent, for example the amount of a compound as an active ingredient, that is sufficient to effect treatment as defined herein when administered to a subject in need of such treatment. A therapeutically effective amount of a compound, salt, derivative, isomer or enantiomer of the present invention will depend upon a number of factors including, for example, the age and weight of the subject, the precise condition requiring treatment and its
20 severity, the nature of the formulation, and the route of administration, and will ultimately be at the discretion of the attendant physician or veterinarian. However, an effective amount of a compound of the present invention for the treatment of disorders associated with bacterial or viral infection, in particular bacterial meningitis, will generally be in the range of about 10 to about 40 mg/kg body weight of recipient (mammal) per day and more usually about 40 mg/kg
25 body weight per day. Thus, for a 70 kg adult subject, the actual amount per day would typically be about 2,800 mg, and this amount may be given in a single dose per day or more usually in a number (such as two, three, four, five or six) of sub-doses per day such that the total daily dose is the same. An effective amount of a salt of the present invention may be determined as a proportion of the effective amount of the compound per se.

30 The term “treatment” as used herein refers to any treatment of a condition or disease in an animal, particularly a mammal, more particularly a human, and includes: preventing the disease or condition from occurring in a subject which may be predisposed to the disease but

has not yet been diagnosed as having it; inhibiting the disease or condition, i.e. arresting its development; relieving the disease or condition, i.e. causing regression of the condition; or relieving the conditions caused by the disease, i.e. symptoms of the disease.

Chemical Derivative

- 5 The term “derivative” or “derivatised” as used herein includes chemical modification of a compound. Illustrative of such chemical modifications would be replacement of hydrogen by a halo group, an alkyl group, an acyl group or an amino group.

Chemical Modification

In one embodiment, the compound may be a chemically modified compound.

- 10 The chemical modification of a compound may either enhance or reduce hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the compound and the target.

In one aspect, the identified compound may act as a model (for example, a template) for the development of other compounds.

15 **INDIVIDUAL**

The compounds are delivered to individuals. As used herein, the term “individual” refers to vertebrates, particularly members of the mammalian species. The term includes but is not limited to domestic animals, sports animals, primates and humans.

Examples

20 **Example 1. Discovery**

Recombinant human GPC-3 protein was used to isolate binders from a library of Fab sequences constructed by SIgN, using phage display technology.

- Out of 570 clones screened, 24 clones showing the highest binding to GPC-3 in ELISA were isolated and cloned into IgG₁ format for further characterisation: 5C4, 4H1, 1D2, 1C4,
25 2B5, 1F1, 1H1, 4A5, 1D8, 1D3, 2F1, 3C6, 3D12, 3A9, 1F4, 1H10, 3C12, 4G11, 4A12, 1A12, 3G12, 4F9, 4G4 and 1E1.

Example 2. Binding Affinity for GPC-3

The 24 antibody clones were tested in ELISA against recombinant human GPC-3 protein in 3 batches to assess binding affinity for the target, with the humanized GC33 clone (Chugai pharmaceutical, Roche) used as a reference control antibody (Figure 1A, 1B and 1C).

5 With the exception of clone 1E1 and 3D12, all other 22 clones showed medium to high affinity for human GPC-3 (Figure 1D).

Example 3. Specific Binding of Anti-GPC-3 Antibodies to GPC-3 Expressing Cells

The ability of anti-GPC-3 antibodies to specifically recognize and bind GPC-3 on the surface of cells was measured using HepG2 (GPC-3^{High}), Hep3B (GPC-3^{Medium}) and SK-Hep1
10 (GPC-3^{Negative}) cells. HepG2 and Hep3B cells constitutively express GPC-3, while SK-Hep1 cells do not express GPC-3.

Briefly, HepG2 (GPC-3^{High}), Hep3B (GPC-3^{Medium}) and SK-Hep1 (GPC-3^{Negative}) cells were seeded in cell suspension at 50000 per well in 96-well round-bottom plate before incubation with different concentrations of anti-GPC-3 antibodies. Binding was measured by
15 flow cytometry using a secondary labelled antibody.

In this assay, most anti-GPC-3 antibodies bound strongly to the GPC-3 expressing HepG2 and Hep3B cells, but did not bind or weakly bound to GPC-3 negative SK-Hep1 cells (Figure 2). Clone 1C4, 1D2, 1D3, 1D8, 1F4, 1H1, 1H10, 2B5, 2F1, 3A9, 3D12, 4A5, 4H1, and 5C4 showed highly specific binding to GPC-3 as they did not bind to SK-Hep1 cells even
20 at the highest antibody concentration (20nM). This suggests that these antibodies bind to surface of the cells in a highly specific manner dependent on GPC-3 expression.

Example 4. Cross Species Reactivity of Anti-GPC-3 Antibodies to Cynomolgus and Mouse GPC-3 Proteins

As glypican-3 is a highly conserved gene, we performed cross-reactivity ELISA to
25 assess the cross species reactivity of our anti-GPC-3 antibodies, where recombinant human, cynomolgus and mouse GPC-3 proteins were each biotinylated and used in a binding ELISA to test their bindings to each of the anti-GPC-3 IgG₁ clones.

Except for clone 1D8 and the humanized GC33, all the other anti-GPC-3 IgG₁ clones exhibited cross-reactivity to cynomolgus GPC-3 and mouse GPC-3 (Figure 3), indicating that

these antibodies recognize common epitopes of GPC-3 protein across human, cynomolgus and mouse antigens.

Next, the ability of anti-GPC-3 antibodies to specifically recognize and bind mouse GPC-3 on the surface of cells was measured using mouse GPC-3 stably transduced Hepa1-6 cells (named as “Hepa1-6-mGPC-3”) and the parental Hepa1-6 cells.

At the staining concentration of 20nM, most anti-GPC-3 antibodies bound strongly to the Hepa1-6-mGPC-3 cells, but did not bind to the parental Hepa1-6 cells (Figure 4A).

Clone 1C4, 1D2, 1D3, 1F1, 1H1, 1H10, 2B5, 2F1, 3A9, 3C6, 3G12, 4A12, 4G11, 4H1, and 5C4 showed highly specific binding to mouse GPC-3 as they did not show high percentage of cell binding to the parental Hepa1-6 cells (Figure 4B).

Interestingly, although clone 1F4, 3D12, and 4F9 exhibited positive binding signal in the cross-reactivity binding ELISA (Figure 3), they could not efficiently stain the mouse GPC-3 antigen expressed on the cell surface (Figure 4).

Example 5. *In vitro* Activity: Induction of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

To assess the ability of the anti-GPC-3 antibodies to induce ADCC, GPC-3 positive HepG2 cells were mixed with naïve natural killer (NK) cells at a ratio of 1:2.5 (E:T ratio = 2.5:1) in the absence or presence of each different anti-GPC-3 IgG₁ clones (2.5µg/ml).

The cell index values were then measured continuously using xCelligence RTCA system at an interval of 15 minutes for 44 hours. In this assay, cell index values of tumour cells are measured by the impedance of current across the transistor plate caused by tumour cell adherence. The end-point cytotoxicity was calculated and shown in Figure 5.

Compared to the cytotoxicity induced by NK cells alone, significant increase in killing was observed in more than half of the anti-GPC-3 antibody clones, with clone 1C4, 1D2 and 4H1 showing the highest antibody-dependent cell-mediated cytotoxicity.

Example 6. Use in a Chimeric Antigen Receptor (CAR) T Cell Construct

The sequences of the antibodies can also be used to construct anti-GPC-3 CAR T cells.

15 clones were chosen to be expressed as CAR in a second-generation CAR format (CD3zeta plus 4-1BB intracellular domain).

To assess the function of these anti-GPC-3 CAR T cells, T cells from healthy donors were activated by TransAct™ (CD3/CD28 agonists) in the presence of IL-2 (50 unit/ml) for 24 hours and CAR gene was introduced by lentiviral based transduction process. 72 hours post transduction, the potency of these anti-GPC-3 CAR T cells were subjected to T cell-mediated cytotoxicity assay using xCELLigence RTCA system.

The CAR T cells were proved to be highly efficient in killing tumour cells.

While mock transduced T cells showed 10-15% cytolysis of GPC-3 expressing HepG2 cells, anti-GPC-3 CAR T cells were able to kill 30-100% of HepG2 cells (Figure 6). Within 48 hours of co-culture, clone 1C4, 1D3, 1F1, 2F1, 3C6, 1D2, 1H1, 2B5, 1D8, 4A5, 4H1, and 5C4 based anti-GPC-3 CAR T cells could lyse 90-100% of GPC-3^{High} HepG2 cells. The killing by these CAR T cells was highly specific to antigen expression, as the same CAR T cells only showed very minimal background killing of GPC-3^{Negative} SK-Hep1 cells (10-30%).

In addition, 24 and 48 hours post co-culture of anti-GPC-3 CAR T cells with GPC-3^{High} HepG2 cells or GPC-3^{Negative} SK-Hep1 cells, cell culture supernatants were collected and subjected to ELISA to assess the cytokine release of interferon- γ and IL-2 from anti-GPC-3 CAR T cells. When co-cultured with GPC-3^{High} HepG2 cells, anti-GPC-3 CAR T cells based on clone 1C4, 1D3, 1F1, 2F1, 3C6, 1D2, 1H1, 2B5, 1D8, 4A5, 4H1 and 5C4 secreted a large amount of interferon- γ and IL-2. However, levels of interferon- γ and IL-2 secretion were minimal when the anti-GPC-3 CAR T cells were added to GPC-3^{Negative} SK-Hep1 cells, indicating the superior specificity of these anti-GPC-3 CAR T cells in responding to and killing of GPC-3 expressing cells (Figure 7).

Interestingly, both Figure 6 and Figure 7 showed a moderate degree of non-specific off-target reactivity of anti-GPC-3 CAR T cells expressing mouse GC33 clone.

Example 7. Nucleotide and Amino Acid Sequences of Antibodies

Amino acid and nucleotide sequences for 24 fully human monoclonal antibodies and their derivatives, able to specifically bind glypican-3 and trigger killing of the cells expressing the antigen are set out below.

The antibodies are 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.

SEQUENCES

AMINO-ACID SEQUENCES

- 5 CDRs are in bold and highlighted in **bold** (CDR1), *italic* (CDR2) or underline (CDR3).

Clone	Heavy Chain (V_H) Amino Acid Sequence	Light Chain (V_L) Amino Acid Sequence
5C4	SEQ ID NO: 1	SEQ ID NO: 2
4H1	SEQ ID NO: 3	SEQ ID NO: 4
1D2	SEQ ID NO: 5	SEQ ID NO: 6
1C4	SEQ ID NO: 7	SEQ ID NO: 8
2B5	SEQ ID NO: 9	SEQ ID NO: 10
1F1	SEQ ID NO: 11	SEQ ID NO: 12
1H1	SEQ ID NO: 13	SEQ ID NO: 14
4A5	SEQ ID NO: 15	SEQ ID NO: 16
1D8	SEQ ID NO: 17	SEQ ID NO: 18
1D3	SEQ ID NO: 19	SEQ ID NO: 20
2F1	SEQ ID NO: 21	SEQ ID NO: 22
3C6	SEQ ID NO: 23	SEQ ID NO: 24
3D12	SEQ ID NO: 25	SEQ ID NO: 26
3A9	SEQ ID NO: 27	SEQ ID NO: 28
1F4	SEQ ID NO: 29	SEQ ID NO: 30
1H10	SEQ ID NO: 31	SEQ ID NO: 32
3C12	SEQ ID NO: 33	SEQ ID NO: 34
4G11	SEQ ID NO: 35	SEQ ID NO: 36
4A12	SEQ ID NO: 37	SEQ ID NO: 38
1A12	SEQ ID NO: 39	SEQ ID NO: 40

Clone	Heavy Chain (V _H) Amino Acid Sequence	Light Chain (V _L) Amino Acid Sequence
3G12	SEQ ID NO: 41	SEQ ID NO: 42
4F9	SEQ ID NO: 43	SEQ ID NO: 44
4G4	SEQ ID NO: 45	SEQ ID NO: 46
1E1	SEQ ID NO: 47	SEQ ID NO: 48

Table D1. Heavy Chain and Light Chain Amino Acid Sequence ID Numbers
(sequences set out below)

HEAVY CHAIN VARIABLE DOMAIN AMINO ACID SEQUENCES FOR HUMAN IGG1 (V_H)

SEQ ID NO: 1: Heavy Chain Variable Domain Amino Acid Sequence of 5C4

5 QVQLQESGGGLVKPGGSLRLSCAAS**GF****TFSSY**SMNWVRQAPGKGLEWVAVI**SYDGSNK**
YYADSVKGRFTISRDN SKNTLYLQMN SLRTEDTAVYYCARGAGPFTGFNWFDPWGQGT

SEQ ID NO: 3: Heavy Chain Variable Domain Amino Acid Sequence of 4H1

EVQLVQSGGGVVPGRSLRLSCAAS**GF****TFSSY**AMHWVRQAPGKGLEWVAVI**SYDGSNK**
YYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARAPGLEDYWGQGT

SEQ ID NO: 5: Heavy Chain Variable Domain Amino Acid Sequence of 1D2

10 EVQLVQSGAEVKKPGSSVKVSKAS**GG****TFSSY**AISWVRQAPGQGLEWMGGI**IPIFGTA**
NYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAREWQQQLVPLGYMDVWGKGT

SEQ ID NO: 7: Heavy Chain Variable Domain Amino Acid Sequence of 1C4

15 QVQLVQSGGGLVKPGRSLRLSCTAS**GF****TFGDY**AMHWVRQAPGKGLEWVAVI**SYDGSNK**
YYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDYGDYRYPYGM DVWGQGT

SEQ ID NO: 9: Heavy Chain Variable Domain Amino Acid Sequence of 2B5

QVQLQESGGGVVPGRSLRLSCAAS**GF****TFSSY**AMHWVRQAPGKGLEWVAVI**SYDGSNK**
YYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKEGTYYYDSSGYWDYYYGMDVWGQ
GTT

SEQ ID NO: 11: Heavy Chain Variable Domain Amino Acid Sequence of 1F1

20 QVQLVQSGAEVKKPGASVKVSKAS**GY****TF****TSYGIS**WVRQAPGQRLEWMGW**INAGNGNT**
KYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCATVVYWGQGT

SEQ ID NO: 13: Heavy Chain Variable Domain Amino Acid Sequence of 1H1

QVQLQESGAGLLRPSETLSLTCAVY**GGSFSGY**YWSWIRQPPGKGLEWLGEI**SHSGSTN**
 YNPSLKSRVTISVDTSKNQFSLKLSSVTAADAAVYYCARWVWSGYNGEGYFDLWGRGTL

SEQ ID NO: 15: Heavy Chain Variable Domain Amino Acid Sequence of 4A5

5 EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSY**GISWVRQAPGQGLEWMGWI**SAYNGNT**
 NYAQKLQGRVTMTTDTSTSTAYMELRSLRSDTAVYYCARDGLSSSWFY⁵YGGMDVWGQGT

SEQ ID NO: 17: Heavy Chain Variable Domain Amino Acid Sequence of 1D8

QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSY**GISWVRQAPGQGLEWMGWI**SAYNGNT**
 NYAQKLQGRVTMTTDTSTSTAYMELRSLRSDTAVYYCARDGGLIAA**AFGY**YGGMDVWGQGT
 10 T

SEQ ID NO: 19: Heavy Chain Variable Domain Amino Acid Sequence of 1D3

QVQLVQSGAEVKKPGASVKVSCKAS**GYTF****TGY**YMHVVRQAPGQGLEWMGWI**INPNSGGT**
 NYAQNFQGRVTITRDTASTAYMELSSLRSEDTAVYYCARGSWQTGSFDIWGQGT

SEQ ID NO: 21: Heavy Chain Variable Domain Amino Acid Sequence of 2F1

15 EVQLVQSGAEVKKPGSSVKVSCKAS**GGTFSSY**AISWVRQAPGQGLEWMGGI**IPIFGTA**
 NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDSPYYYMDVWGKGT

SEQ ID NO: 23: Heavy Chain Variable Domain Amino Acid Sequence of 3C6

QVQLQESGGGVVQPGRSLRLSCAAS**GFTFSSY**AMHWVRQAPGKGLEWVAVI**SYDGSNK**
 YYADSVKGRFTISRDNKNTLYLQMNSLRSED**TAVYYCAYGRYNWNLYFDY**WGQGT

SEQ ID NO: 25: Heavy Chain Variable Domain Amino Acid Sequence of 3D12

20 EVQLVQSGAEVKKPGSSVKVSCKAS**GGTFSSY**AISWVRQAPGQGLEWMGGI**IPIFGTA**
 NYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGIAARPSYYYMDVWGKGT

SEQ ID NO: 27: Heavy Chain Variable Domain Amino Acid Sequence of 3A9

25 QVQLQQWGAGLLKPSETLSLTCAVY**GGSFSGY**YWSWIRQPPGKGLEWIGE**INHSGSTN**
 YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARLYKRRPFDYWGQGT

SEQ ID NO: 29: Heavy Chain Variable Domain Amino Acid Sequence of 1F4

QVQLQQWGAGLLKPSETLSLTCAVY**GGSFSGY**YWSWIRQPPGKGLEWIGE**INHSGSTN**
 YNPSLKSRVTISVDTSKNQFSLKLSSVTVADTAVYYCARLYKRRPFDYWGQGT

SEQ ID NO: 31: Heavy Chain Variable Domain Amino Acid Sequence of 1H10

QLQLQESGGGLVQPGGSLRLSCAAS**GF****TFSSY**VMSWVRQAPGKGLEWVSTISGSGGST
YYADSVKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCVMGWYFDLWGRGTL

SEQ ID NO: 33: Heavy Chain Variable Domain Amino Acid Sequence of 3C12

5 QVTLKESGPTLVKPTQTLTLTCTFS**GF****SLSTSG**VGVGWIRQPPGKALEWLALIYWND
KRYSPSLKSRLTITKDTSKNQVVLMTNMDPVDATYYCAHSRIAARRVLDYWGQGT

SEQ ID NO: 35: Heavy Chain Variable Domain Amino Acid Sequence of 4G11

QVQLQESGPGLVKPSGTLSTLCAVSG**GS****ISSN**WWSWVRQPPGKGLEWIGEIYHSGST
NYPNLSKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARDTSGVAFDYWGQGT

SEQ ID NO: 37: Heavy Chain Variable Domain Amino Acid Sequence of 4A12

10 DVQLVESGGGLVQPGGSLRLSCAAS**GF****TFSSY**AMSWVRQAPGKGLEWVSAISGSGGST
YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAEGRIQLDYWGQGT

SEQ ID NO: 39: Heavy Chain Variable Domain Amino Acid Sequence of 1A12

15 QVQLQQWGAGLLKPSETLSLTCAAY**GS****FSGY**YWSWIRQPPGKGLEWIGEINHSGSTN
YNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARLYKRRPFDYWGQGT

SEQ ID NO: 41: Heavy Chain Variable Domain Amino Acid Sequence of 3G12

QVQLQQWGAGLLKPSETLSLTCAVY**GS****FSGY**YWSWIRQPPGKGLEWIGEINHSGSTN
YNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARLYKRRPFDYWGQGT

SEQ ID NO: 43: Heavy Chain Variable Domain Amino Acid Sequence of 4F9

20 EVQLVQSGAEVKKPGASVKVSKAS**GY****TFRNY**GISWVRQAPGQGLEWMGWI SAYNGNT
NYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARADSSSWYVGTYYYYYGM DVWGQ
TT

SEQ ID NO: 45: Heavy Chain Variable Domain Amino Acid Sequence of 4G4

25 QVQLQQWGAGLLKPSETLSLTCAVY**GS****FSGY**YWSWIRQPPGKGLEWIGEINHSGSTN
YNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARLYKRRPFDYWGQGT

SEQ ID NO: 47: Heavy Chain Variable Domain Amino Acid Sequence of 1E1

QVQLQQWGAGLLKPSETLSLTCAVY**GS****FSGY**YWSWIRQPPGKGLEWIGEINHSGSTN
YNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARLYKRRPFDYWGQGT

LIGHT CHAIN VARIABLE DOMAIN AMINO ACID SEQUENCES FOR HUMAN IGG1 (V_L)*SEQ ID NO: 2: Light Chain Variable Domain Amino Acid Sequence of 5C4*

EIVLTQSPATLSVSPGERATLSC**RASQSVDSYLA**WYQQKPGQAPRLLIYSASTRATGI
 PARFSGTSGSGTEFTLTISSLQSEDFAVYYCQQYKRWPTWTFGLGTR

5 *SEQ ID NO: 4: Light Chain Variable Domain Amino Acid Sequence of 4H1*

DIQMTQSPSSLSASVGDRVTITC**RASQSISSYLN**WYQQKPGTAPKLLIYAASSSLQSG
 VPSRFSGSGSGTDFALTISSLQPEDFATYYCLDYNYPLTFGGGTK

SEQ ID NO: 6: Light Chain Variable Domain Amino Acid Sequence of 1D2

ETTLTQSPGTLSSLSPGERATLSC**RASHSVSSNFLA**WYQQKPGQAPRLLIYAASSRTTG
 10 VPDRFSGSGSGTDFTLTISRLEPEDSAVYYCQQYGYSPLYTFGQGTK

SEQ ID NO: 8: Light Chain Variable Domain Amino Acid Sequence of 1C4

DIQMTQSPSTLSASVGDRVTITC**RASQSIKWLA**WYQHKPGKAPKVLIIYKASTLQSGV
 PSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPLTFGGGTK

SEQ ID NO: 10: Light Chain Variable Domain Amino Acid Sequence of 2B5

15 DVVMTQSPLSLPVTPGEPASIS**RSSQSLLDS**DGYNFLDWYVQKPGQSPQRLIYLGS
 RASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMQALQTPWTFGQGTK

SEQ ID NO: 12: Light Chain Variable Domain Amino Acid Sequence of 1F1

EIVLTQSPSLSPVTPGEPASIS**RSSQSLLHSNG**NYLDWYLQKPGQSPQILIYLGSH
 RASGVPDRFSGSASGTDFTLKISRVEAEDVGVYYCMQALQTPTFGGGTK

20 *SEQ ID NO: 14: Light Chain Variable Domain Amino Acid Sequence of 1H1*

ETTLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIYGASSRATG
 IPDRFSGRSGSGTDFTLTITRLEPEDFAVYYCQYYGSSPRTFGQGTK

SEQ ID NO: 16: Light Chain Variable Domain Amino Acid Sequence of 4A5

DVVMTQSPLSLPVTPGEPASIS**RSSQSLLHSNG**NYLDWYLQKPGQSPQLLIYLGSN
 25 RASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTFGQGTK

SEQ ID NO: 18: Light Chain Variable Domain Amino Acid Sequence of 1D8

EIVLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIYGASSRATGI
 PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYERPPTFGGTK

SEQ ID NO: 20: Light Chain Variable Domain Amino Acid Sequence of 1D3

SYELTQPPSVSGTPGQSVTISC**SGSNSNLGSNYVY**WYQVVPGTAPKLVIYRNKYRPPG
VPDRFSGSKSGTSASLAISGLRSDDEAEYYCSGWDDSLSGRLFGGGTK

SEQ ID NO: 22: Light Chain Variable Domain Amino Acid Sequence of 2F1

5 DVVMTQSPFLFPLVTLGQPASIS**CRSSQSLVYSDGNTYLS**WFQQRPGQSPRRLVYKVSS
RDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVIYCMQGTHWPRTFGQGTR

SEQ ID NO: 24: Light Chain Variable Domain Amino Acid Sequence of 3C6

NFMLTQPHSVSESPGKTVTISC**TGSSGSIASNYVQ**WYQQRPGSVPTTVIYEDNQRPSPG
VPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSFDSSNYWVFGGGTK

10 *SEQ ID NO: 26: Light Chain Variable Domain Amino Acid Sequence of 3D12*

DIQMTQSPSSLSASVGDRVTITC**RASQNISSYLN**WYQKPGKAPKLLIYAASSLQSGV
PSRFSGSGSGTDFILTISSLPEDFATYYCQQSYSTPPTFGQGTR

SEQ ID NO: 28: Light Chain Variable Domain Amino Acid Sequence of 3A9

15 ETTLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQKPGQAPRLLIYGASRRATG
IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPITFGQGTR

SEQ ID NO: 30: Light Chain Variable Domain Amino Acid Sequence of 1F4

ETTLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQKPGQAPRLLIYGASRRATG
SPERFSGGGSGTDFTLTISRLEPEDFAVYYCQHYGNSPGTFGQGTR

SEQ ID NO: 32: Light Chain Variable Domain Amino Acid Sequence of 1H10

20 EIVLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQKPGQAPRLLIYDASNRATG
IPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPRATFGQGTR

SEQ ID NO: 34: Light Chain Variable Domain Amino Acid Sequence of 3C12

QSVLTQPPSASVAPGKTARITC**GGNNIGSKSVH**WYQKPGQSPVLVIYQDTKRPSGIP
ERFSGSNSGNTATLTISGAQPMDEADYYCQAWYSTIMVFGGGTK

25 *SEQ ID NO: 36: Light Chain Variable Domain Amino Acid Sequence of 4G11*

EIVLTQSPGTLSSLSPGERATLSC**RASQSVSSSYLA**WYQKPGQAPRLLIYGASSRATG
IPDRFSGGGSGTDFTLTISRLEPEDSAVYYCQQYDHSPTFGQGTR

SEQ ID NO: 38: Light Chain Variable Domain Amino Acid Sequence of 4A12

30 LPVLTQPPSVSVSPGQTARITC**SGYELGDKYAF**WYQKPGQSPVLVIYQDTKRPSGIP
ERFSGSSSGDTATLIISGTQILDEADYYCQAWDSSTHVVFGGGTK

SEQ ID NO: 40: Light Chain Variable Domain Amino Acid Sequence of 1A12

ETTLTQSPGTLSSLSPGERATLSC**RASQSISSIY**LAWYQQKPGQAPRLLIYGASRRATG
IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSLRTFGQGTK

SEQ ID NO: 42: Light Chain Variable Domain Amino Acid Sequence of 3G12

5 ETTLTQSPGTLSSLSPGERATLSC**RASQSVSSSY**LAWYQQKRGQAPRLLIYGASRRATG
IPDRFSGSGSGSDFTLTISRLEPEDFAVYYCQQYGSSPSTFGQGTK

SEQ ID NO: 44: Light Chain Variable Domain Amino Acid Sequence of 4F9

DVVMTQSPLSLPVTTPGESASISCR**SSQSL**HSNGYNYLDWYLQKPGQSPQLLIYLGSN
RASGVDPDRFSGSGSGTDFTLKISRVAEDVGVYYCMQVLQTPWTFGQGTK

10 *SEQ ID NO: 46: Light Chain Variable Domain Amino Acid Sequence of 4G4*

ETTLTQSPGTLSSLSPGERATLSC**RASQSVSSSY**LAWYQQKPGQAPRLLIYNASRRATG
IPDRFSGSGSGADFTLTISRDPEDFAVYFCQQYGSSPQTFGQGTK

SEQ ID NO: 48: Light Chain Variable Domain Amino Acid Sequence of 1E1

15 VTTLTQSPGTLSSLSPGERATLSC**RASESVSSRY**LAWYQQRPGQAPRLLIYGASRRATG
SPERFSGGGSGTDFTLTISRLEPEDFAVYYCQHYGNSPGTFGQGTK

NUCLEOTIDE SEQUENCES

CDRs are in bold and highlighted in **bold** (CDR1), *italic* (CDR2) or underline (CDR3).

Clone	Heavy Chain (V _H) Nucleic Acid Sequence	Light Chain (V _L) Nucleic Acid Sequence
5C4	SEQ ID NO: 49	SEQ ID NO: 50
4H1	SEQ ID NO: 51	SEQ ID NO: 52
1D2	SEQ ID NO: 53	SEQ ID NO: 54
1C4	SEQ ID NO: 55	SEQ ID NO: 56
2B5	SEQ ID NO: 57	SEQ ID NO: 58
1F1	SEQ ID NO: 59	SEQ ID NO: 60
1H1	SEQ ID NO: 61	SEQ ID NO: 62
4A5	SEQ ID NO: 63	SEQ ID NO: 64

Clone	Heavy Chain (V _H) Nucleic Acid Sequence	Light Chain (V _L) Nucleic Acid Sequence
1D8	SEQ ID NO: 65	SEQ ID NO: 66
1D3	SEQ ID NO: 67	SEQ ID NO: 68
2F1	SEQ ID NO: 69	SEQ ID NO: 70
3C6	SEQ ID NO: 71	SEQ ID NO: 72
3D12	SEQ ID NO: 73	SEQ ID NO: 74
3A9	SEQ ID NO: 75	SEQ ID NO: 76
1F4	SEQ ID NO: 77	SEQ ID NO: 78
1H10	SEQ ID NO: 79	SEQ ID NO: 80
3C12	SEQ ID NO: 81	SEQ ID NO: 82
4G11	SEQ ID NO: 83	SEQ ID NO: 84
4A12	SEQ ID NO: 85	SEQ ID NO: 86
1A12	SEQ ID NO: 87	SEQ ID NO: 88
3G12	SEQ ID NO: 89	SEQ ID NO: 90
4F9	SEQ ID NO: 91	SEQ ID NO: 92
4G4	SEQ ID NO: 93	SEQ ID NO: 94
1E1	SEQ ID NO: 95	SEQ ID NO: 96

Table D2. Heavy Chain and Light Chain Nucleic Acid Sequence ID Numbers
(sequences set out below)

HEAVY CHAIN VARIABLE DOMAIN NUCLEIC ACID SEQUENCES FOR HUMAN IGG1 (V_H)

SEQ ID NO: 49: Heavy Chain Variable Domain Nucleic Acid Sequence of 5C4

5 CAGGTGCAGCTGCAGGAGTCGGGGGAGGCCTGGTCAAGCCTGGGGGTCCCTGAGAC
TCTCCTGTGCAGCCTCTGGATTACCTTCAGTAGCTATAGCATGAACTGGGTCCGCCAGGCTC
CAGGGAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTATGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAACTGAGGACACGGCTGTGTATTACTGTGCGAGGGGCGCGGGGCCTTTTA
10 CGGGATTCAACTGGTTCGACCCCTGGGGCCAGGGAACCCTG

SEQ ID NO: 51: Heavy Chain Variable Domain Nucleic Acid Sequence of 4H1

GAGGTCCAGCTGGTACAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGAC
TCTCCTGTGCAGCCTCT**GGATTACCTTCAGTAGCTAT**GCTATGCACTGGGTCCGCCAGGCTC
CAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCAG
5 ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGCCCCCTGGGTGGAGG
ACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 53: Heavy Chain Variable Domain Nucleic Acid Sequence of 1D2

GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAAGG
10 TCTCCTGCAAGGCTTCT**GGAGGCACCTTCAGCAGCTAT**GCTATCAGCTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCAC
AGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGC
TGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGAGTGGCAGCAGCAGC
TGGTACCCCTCGGCTACATGGACGTCTGGGGCAAAGGGACCACG

SEQ ID NO: 55: Heavy Chain Variable Domain Nucleic Acid Sequence of 1C4

CAGGTCCAGCTGGTGCAGTCTGGGGGAGGCTTGGTAAAGCCAGGGCGGTCCCTGAGAC
TCTCCTGTACAGCTTCT**GGATTACCTTTGGTGATTAT**GCTATGCACTGGGTCCGCCAGGCTC
CAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTATGCAG
ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
20 TGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATTACGGTGATTACT
ATCGGCCCTACGGTATGGACGTCTGGGGCCAAGGGACCACG

SEQ ID NO: 57: Heavy Chain Variable Domain Nucleic Acid Sequence of 2B5

CAGGTGCAGCTGCAGGAGTCCGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGAC
TCTCCTGTGCAGCCTCT**GGATTACCTTCAGTAGCTAT**GCTATGCACTGGGTCCGCCAGGCTC
25 CAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCAG
ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAAAGAGGGGAACGTATTACT
ATGATAGTAGTGTTATTGGGACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG

SEQ ID NO: 59: Heavy Chain Variable Domain Nucleic Acid Sequence of 1F1

CAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGG
30 TCTCCTGCAAGGCTTCT**GGTTACACCTTACCAGCTAT**GGTATCAGCTGGGTGCGACAGGCCC
CCGGACAAAGGCTTGAGTGGATGGGATGGATCAACGCTGGCAATGGTAACACAAAATATTAC

AGAAGTTCCAGGGCAGAGTCACCATTACCAGGGACACATCCGCGAGCACAGCCTACATGGAGC
TGAGCAGCCTGAGATCTGAAGACACGGCTGTGTATTACTGTGCGACCGTTGTCTACTGGGGCC
AGGGCACCCCTG

SEQ ID NO: 61: Heavy Chain Variable Domain Nucleic Acid Sequence of 1H1

5 CAGGTGCAGCTGCAGGAGTCCGGCGCAGGACTGTTGAGGCCTTCGGAGACCCTGTCCC
TCACTTGCGCTGTCTATGGTGGGTCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCCC
CAGGGAAGGGCTGGAGTGGCTTGGGGAAATCAGTCATAGTGGGAAGCACCACCTACAACCCGT
CCCTCAAGAGTCGAGTCACCATATCAGTTGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACGCGGCTGTATATTACTGTGCGAGATGGGTTTGGAGTGGTTATT
10 ATAACGGTGAGGGGTACTTCGATCTCTGGGGCCGTGGCACCCCTG

SEQ ID NO: 63: Heavy Chain Variable Domain Nucleic Acid Sequence of 4A5

GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGG
TCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTACGGTATCAGCTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGCAC
15 AGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGC
TGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGACGGATTAAGCAGCA
GCTGGTTCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG

SEQ ID NO: 65: Heavy Chain Variable Domain Nucleic Acid Sequence of 1D8

CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGG
20 TCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTACGGTATCAGCTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGCAC
AGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGC
TGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATGGGGGACTTATAG
CAGCAGCTTTCGGTTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG

SEQ ID NO: 67: Heavy Chain Variable Domain Nucleic Acid Sequence of 1D3

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGG
TCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAACTATGCAC
AGAACTTTTCAGGGCAGAGTCACCATTACCAGGGACACATCCGCGAGCACAGCCTACATGGAGC
30 TGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGGGGATCGTGGCAAACGG
GTTCTTTTGATATCTGGGGCCAAGGAACCACG

SEQ ID NO: 69: Heavy Chain Variable Domain Nucleic Acid Sequence of 2F1

GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAAGG
TCTCCTGCAAGGCTTCT**GGAGGCACCTTCAGCAGCTAT**GCTATCAGCTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACCTACGCAC
5 AGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGGAGC
TGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGACAGCCCCTACTACT
ACTACATGGACGTCTGGGGCAAAGGGACCACG

SEQ ID NO: 71: Heavy Chain Variable Domain Nucleic Acid Sequence of 3C6

CAGGTGCAGCTGCAGGAGTCGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGAC
10 TCTCCTGTGCAGCCTCT**GGATTACCTTCAGTAGCTAT**GCTATGCACTGGGTCCGCCAGGCTC
CAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTACGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGTACGGTAGATACAACTGGA
ATCTTTACTTTGACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 73: Heavy Chain Variable Domain Nucleic Acid Sequence of 3D12

GAAGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAAGG
TCTCCTGCAAGGCTTCT**GGAGGCACCTTCAGCAGCTAT**GCTATCAGCTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACCTACGCAC
AGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGC
20 TGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCATAGCAGCTCGTC
CGAGCTACTACTACTACATGGACGTCTGGGGCAAAGGGACCACG

SEQ ID NO: 75: Heavy Chain Variable Domain Nucleic Acid Sequence of 3A9

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
TCACCTGCGCTGTCTAT**GGTGGGTCCCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
25 CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT
CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGCACCCCTG

SEQ ID NO: 77: Heavy Chain Variable Domain Nucleic Acid Sequence of 1F4

30 CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
TCACCTGCGCTGTCTAT**GGTGGGTCCCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT

CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGTCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 79: Heavy Chain Variable Domain Nucleic Acid Sequence of 1H10

5 CAGCTGCAGCTGCAGGAGTCCGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGAC
TCTCCTGTGCAGCCTCT**GGATTACCTTTAGCAGCTAT**GTTCATGAGCTGGGTCCGCCAGGCTC
CAGGGAAGGGGCTGGAGTGGGTCTCAACTATCAGTGGTAGTGGTGGGAGCACATACTACGCAG
ACTCCGTGAAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTTCAAA
TGAGCAGTCTGAGAGCTGAGGACACGGCTGTGTATTATTGTGTGATGGGCTGGTACTTCGATC
10 TCTGGGGCCGTGGAACCCTG

SEQ ID NO: 81: Heavy Chain Variable Domain Nucleic Acid Sequence of 3C12

CAGGTCACCTTGAAGGAGTCTGGTCCCTACGCTGGTGAAACCCACACAGACCCTCACGC
TGACCTGCACCTTCTCT**GGGTTCCTCACTCAGCACTAGTGGAGT**GGGTGTGGGCTGGATCCGTC
AGCCCCCAGGAAAGGCCCTGGAGTGGCTTGCACTCATT TATTGGAATGATGATAAGCGCTACA
15 GCCCATCTCTGAAGAGCAGGCTCACCATCACCAAGGACACCTCCAAAACCAGGTGGTCCTTA
CAATGACCAACATGGACCCTGTGGACACAGCCACATATTACTGTGCACACAGCCGTATAGCAG
CTCGTCGGGTGCTTGACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 83: Heavy Chain Variable Domain Nucleic Acid Sequence of 4G11

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCC
20 TCACCTGCGCTGTCTCT**GGTGGCTCCATCAGCAGTAGTAAC**TGGTGGAGTTGGGTCCGCCAGC
CCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATC TATCATAGTGGGAGCACCAACTACAACC
CGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAAGAACCAGTTCTCCCTGAAGC
TGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGATACTTCTGGGGTTG
CTTTTGACTACTGGGGCCAGGGCACCCCTG

SEQ ID NO: 85: Heavy Chain Variable Domain Nucleic Acid Sequence of 4A12

GACGTGCAGCTCGTGGAGTCTGGAGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGAC
TCTCCTGTGCAGCCTCT**GGATTACCTTTAGCAGCTAT**GCCATGAGCTGGGTCCGCCAGGCTC
CAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAG
ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
30 TGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGGAGGGGAGGATACAGCTTG
ACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 87: Heavy Chain Variable Domain Nucleic Acid Sequence of 1A12

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
TCACCTGCGCTGCCTAT**GGTGGGTCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT
5 CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGAACCCTG

SEQ ID NO: 89: Heavy Chain Variable Domain Nucleic Acid Sequence of 3G12

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
10 TCACCTGCGCTGTCTAT**GGTGGGTCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT
CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGCACCCCTG

SEQ ID NO: 91: Heavy Chain Variable Domain Nucleic Acid Sequence of 4F9

GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAAG
TCTCCTGCAAGGCTTCT**GGTTACACCTTTCGTA**ACTATGGTATCAGTTGGGTGCGACAGGCCC
CTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGCAC
AGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGC
20 TGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGCGGATAGCAGCAGCT
GGTACGTCGGGACTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG

SEQ ID NO: 93: Heavy Chain Variable Domain Nucleic Acid Sequence of 4G4

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
TCACCTGCGCTGTCTAT**GGTGGGTCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
25 CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT
CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGCACCCCTG

SEQ ID NO: 95: Heavy Chain Variable Domain Nucleic Acid Sequence of 1E1

30 CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCC
TCACCTGCGCTGTCTAT**GGTGGGTCTTCAGTGGTTACT**ACTGGAGCTGGATCCGCCAGCCCC
CAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGT

CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTTTATAAAAGGAGGCCCT
TTGACTACTGGGGCCAGGGCACCCCTG

LIGHT CHAIN VARIABLE DOMAIN NUCLEIC ACID SEQUENCES FOR HUMAN IgG1 (V_L)

5 *SEQ ID NO: 50: Light Chain Variable Domain Nucleic Acid Sequence of 5C4*
 GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAGAGAGCCA
CCCTCTCTCTGC**AGGGCCAGTCAGAGTGTTGACAGCTACTTAGCC**TGGTACCAGCAGAAACCTG
GCCAGGCTCCCCGGCTCCTCATCTATAGTGCGTCCACGAGGGCCACTGGTATCCCAGCCAGGT
TCAGTGGCACTGGGTCTGGGACAGAGTTCACTCTCACCATCAGCAGCCTGCAGTCTGAAGATT
10 TTGCAGTTTACTACTGTCAGCAGTATAAGAGGTGGCCACGTGGACGTTTCGGTCTAGGGACCA
 GG

SEQ ID NO: 52: Light Chain Variable Domain Nucleic Acid Sequence of 4H1
 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCA
CCATCACTTGCC**CGGGCAAGTCAGAGCATTAGCAGCTATTTAAAT**TGGTATCAGCAGAAACCAG
15 GGACAGCCCCTAAGCTCCTGATCTATGCTGCATCATCCAGTTTACAAAGTGGGGTCCCATCAA
GGTTCAGCGGCAGTGGATCTGGCACAGATTTGCTCTCACCATCAGCAGCCTGCAGCCTGAAG
ATTTTGCAACTTATTACTGTCTACAAGATTACAATTACCCTCTCACTTTTCGGCGGAGGGACCA
 AG

SEQ ID NO: 54: Light Chain Variable Domain Nucleic Acid Sequence of 1D2
20 GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCTCTGT**AGGGCCAGTCACAGTGTTAGCAGCAACTTCTTAGCC**TGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGCTGCATCCAGCAGGACCCTGGCGTCCCAGACA
GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAG
ATTCTGCAGTGTATTACTGTCAGCAATATGGTTACTCACCCCTGTACACTTTTGGCCAGGGGA
25 CCAAG

SEQ ID NO: 56: Light Chain Variable Domain Nucleic Acid Sequence of 1C4
 GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCCTCTGTAGGAGACAGAGTCA
CCATCACTTGTC**CGGGCCAGTCAGAGTATTAGTAAGTGGTTGGCC**TGGTATCAGCATAAACCCAG
GGAAAGCCCCCTAAAGTCCTGATCTATAAGGCGTCTACTTTACAAAGTGGGGTCCCGTCGAGGT
30 TCAGCGGCAGTGGATCTGGGACAGAATTCACCTCTACAATCAGCAGCCTGCAGCCTGAAGATT
TTGCAACTTATTACTGTCAACAGCTTAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAG

SEQ ID NO: 58: Light Chain Variable Domain Nucleic Acid Sequence of 2B5

GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAGAGCCGGCCT
CCATCTCCTGC**AGGTC****TAGCCAGAGCCTCCTGGATAGTGACGGATACAAC****TTTTTGGAT**TGGT
ATGTGCAGAAGCCAGGGCAGTCTCCACAGCGCCTGATCTATTTGGGCTCTATTCGGGCCTCCG
5 GGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAGAG
TGGAGGCTGACGATGTTGGGGTTTATTATTGCATGCAAGCTCTACAAACTCCTTGGACGTTTCG
GCCAAGGGACCAAG

SEQ ID NO: 60: Light Chain Variable Domain Nucleic Acid Sequence of 1F1

GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAGAGCCGGCCT
10 CCATCTCCTGC**AGGTC****TAGTCAGAGCCTCCTGCATAGTAATGGATACAAC****TATTTGGAT**TGGT
ACCTGCAGAAGCCAGGGCAGTCTCCACAGATCCTGATCTACTTGGGTTCTCATCGGGCCTCCG
GGGTCCCTGACAGGTTCACTGGCAGTGCATCAGGCACAGATTTTACACTGAAAATCAGCAGAG
TGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACTCCCCTCACTTTTCG
GCGGAGGGACCAAG

SEQ ID NO: 62: Light Chain Variable Domain Nucleic Acid Sequence of 1H1

GAAACGACACTCACGCAGTCTCCTGGCACCCCTGTCTTTGTCTCCAGGTGAAAGAGCCA
CCCTCTCCTGT**AGGGCCAGTCAGAGTGT****TAGCAGCAGCTACTTAGCCT**TGGTACCAGCAGAAGC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA
GGTTCAGTGGCCGTGGGTCTGGGACAGACTTCACTCTCACTATCACCAGACTGGAGCCTGAAG
20 ATTTTGCAGTGTATTACTGT**CAGTACTATGGTAGCTCACCTCGGACT**TTTTGGCCAGGGGACCA
AG

SEQ ID NO: 64: Light Chain Variable Domain Nucleic Acid Sequence of 4A5

GATGTTGTGATGACTCAGTCTCCACTATCCCTGCCCCGTCACCCCTGGAGAGCCGGCCT
CCATCTCCTGC**AGGTC****TAGTCAGAGCCTCCTGCATAGTAATGGATACAAC****TATTTGGAT**TGGT
25 ACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCG
GGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAGAG
TGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACTTTTGGCCAGGGGA
CCAAG

SEQ ID NO: 66: Light Chain Variable Domain Nucleic Acid Sequence of 1D8

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGC**AGGGCCAGTCAGAGTGT****TAGCAGCTACTTAGCCT**TGGTACCAGCAGAAACCTG
GCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGT

TCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATT
TTGCAGTGTACTACTGTCAACAGTATGAGAGGCCCCCACTTTCGGCGGGGGGACCAAG

SEQ ID NO: 68: Light Chain Variable Domain Nucleic Acid Sequence of 1D3

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCTGGGACCCCCGGGCAGAGTGTACCA
5 TCTCTTGT**TCTGGGAGCAACTCCAACCTCGGATCTAATTATGTGTACT**TGGTACCAGGTGGTCC
CAGGCACGGCCCCCAAACTCGTTCATCTATAGAAATAAATATCGGCCCCCAGGGGTCCCTGACC
GATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCCGACG
ATGAGGCTGAATATTATTGTTCCGGATGGGATGACAGCCTGAGTGGTCGACTATTTCGGCGGAG
GGACCAAG

10 *SEQ ID NO: 70: Light Chain Variable Domain Nucleic Acid Sequence of 2F1*

GATGTTGTGATGACTCAGTCTCCGCTCTTCCTGCCCGTCACCCTTGGACAGCCGGCCT
CCATCTCCTGC**AGGTCTAGTCAAAGCCTCGTATACAGTGATGGAAACACCTACTTGAGT**TGGT
TTCAGCAGAGGCCAGGCCAATCTCCAAGGCGCCTAGTTTATAAGGTTTCTAGCCGGGACTCTG
GGGTCCCAGACAGATTGAGCGGCAGTGGGTGAGGCACTGATTTACACTGAAAATCAGCAGGG
15 TGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGGTACACACTGGCCTCGGACGTTTCG
GCCAAGGGACCAAG

SEQ ID NO: 72: Light Chain Variable Domain Nucleic Acid Sequence of 3C6

AATTTTATGCTGACTCAGCCCCACTCTGTGTCTGGAGTCTCCGGGGAAGACGGTTACCA
TCTCCTGC**ACCGGCAGCAGTGGCAGCATTGCCAGCAACTATGTGCAGT**TGGTACCAGCAGCGCC
20 CGGGCAGTGTCCCCACCACTGTGATCTATGAGGATAACCAAAGACCCTCTGGGGTCCCTGATC
GGTTCTCTGGCTCCATCGACAGCTCCTCCAACCTCTGCCTCCCTCACCATCTCTGGACTGAAGA
CTGAGGACGAGGCTGACTACTACTGTCAGTCTTTTGATAGCAGCAATTATTGGGTGTTTCGGCG
GAGGGACCAAG

SEQ ID NO: 74: Light Chain Variable Domain Nucleic Acid Sequence of 3D12

25 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCA
CCATCACTTGCC**CGGGCAAGTCAGAACATTAGCAGCTATTTAAAT**TGGTATCAGCAGAAACCAG
GGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGAGTCCCATCAAGGT
TCAGTGGCAGTGGATCTGGGACAGATTTCAATTCTCACCATCAGCAGTCTGCAACCTGAAGATT
TTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCCCACCTTCGGCCAAGGGACACGA

SEQ ID NO: 76: Light Chain Variable Domain Nucleic Acid Sequence of 3A9

GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGAAGGGCCACTGGCATCCCAGACA
5 GGTTCAGCGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAG
ATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGATCACCTTCGGCCAAGGGACAC
GA

SEQ ID NO: 78: Light Chain Variable Domain Nucleic Acid Sequence of 1F4

GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
10 CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCACCTACTTAGCCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATTTACGGTGCATCCAGGCGGGCCACTGGCTCCCCAGAGA
GGTTCAGTGGCGGTGGGTCTGGGACAGACTTCACTCTCACCATCAGTAGACTGGAGCCCCGAAG
ATTTGCGAGTCTATTACTGTCAACATTATGGTAACTCACCGGGGACGTTTCGGCCAAGGGACCA
AG

SEQ ID NO: 80: Light Chain Variable Domain Nucleic Acid Sequence of 1H10

GAAATTGTGCTGACTCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA
GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAG
20 ATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCCCCGAGGGCCACCTTCGGCCAAG
GGACACGA

SEQ ID NO: 82: Light Chain Variable Domain Nucleic Acid Sequence of 3C12

CAGTCTGTGCTGACTCAGCCACCCTCGGCGTCAGTGGCCCCAGGAAAGACGGCCAGGA
TTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCC
25 AGTCCCCTGTGCTGGTCATCTATCAAGATACCAAGCGGCCCTCAGGGATCCCTGAGCGATTCT
CTGGCTCCAACCTCTGGGAACACAGCCACTCTGACCATCAGCGGAGCCCAGCCTATGGATGAGG
CTGACTATTACTGTCAGGCGTGGTACAGCACCATTATGGTATTTGGCGGAGGGACCAAG

SEQ ID NO: 84: Light Chain Variable Domain Nucleic Acid Sequence of 4G11

GAAATTGTGCTGACTCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
30 CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA

GGTTCAGTGGCGGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAG
ATTCTGCAGTGTATTACTGTCAGCAATATGATCATTACCCACTTTTGGCCAGGGGACCAAG

SEQ ID NO: 86: Light Chain Variable Domain Nucleic Acid Sequence of 4A12

CTGCCTGTGCTGACTCAGCCCCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCAGGA
5 TCACCTGCTCTGGATATGAATTGGGGGATAAATATGCTTTCTGGTATCAGCAGAAGCCAGGCC
AGTCCCCTGTTCTGGTCATTTATCAAGATACCAAGCGGCCCTCAGGGATCCCTGAGCGATTCT
CTGGCTCCAGCTCTGGGGACACAGCCACTCTGATCATCAGCGGGACCCAAATTTTGGATGAGG
CTGACTATTACTGTCAGGCGTGGGACAGCAGCACCCATGTGGTATTCGGCGGAGGGACCAAG

SEQ ID NO: 88: Light Chain Variable Domain Nucleic Acid Sequence of 1A12

10 GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTATTAGCAGCATCTACTTAGCCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATTTATGGTGCATCCAGGAGGGCCACTGGCATCCCAGACA
GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAG
ATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACTTCGGACGTTTCGGCCAAGGGACCA
15 AG

SEQ ID NO: 90: Light Chain Variable Domain Nucleic Acid Sequence of 3G12

GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAAC
GTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCCGCAGGGCCACTGGCATCCCAGACA
20 GGTTCAGTGGCAGTGGGTCTGGGTGAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAG
ATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCCCTCGACGTTTCGGCCAAGGGACCA
AG

SEQ ID NO: 92: Light Chain Variable Domain Nucleic Acid Sequence of 4F9

GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTACCCCTGGAGAGTCGGCCT
25 CCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAATGGATACAACCTATTTGGATTGGT
ACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCG
GGGTCCCTGACAGGTTCAAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAGAG
TGGGGGCTGAGGATGTTGGGGTTTATTACTGTATGCAAGTTCTACAAACTCCCTGGACGTTTCG
GCCAAGGGACCAAA

SEQ ID NO: 94: Light Chain Variable Domain Nucleic Acid Sequence of 4G4

GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTATCAACAAAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATAATGCGTCCAGGAGGGCCACTGGCATCCCAGACA
5 GGTTCAGTGGCAGTGGGTCTGGGGCAGACTTCACTCTCACCATCAGCAGACTGGATCCTGAAG
ATTTTGAGTGTATTTCTGTCAGCAGTATGGTAGCTCACCTCAGACGTTTCGGCCAAGGGACCA
AG

SEQ ID NO: 96: Light Chain Variable Domain Nucleic Acid Sequence of 1E1

GTAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
10 CCCTCTCCTGCAGGGCCAGTGAGAGTGTAGCAGCAGGTACTTAGCCTGGTACCAGCAGAGAC
CTGGCCAGGCTCCCAGGCTCCTCATTTACGGTGCATCCAGGCGGGCCACTGGCTCCCCAGAGA
GGTTCAGTGGCGGTGGGTCTGGGACAGACTTCACTCTCACCATCAGTAGACTGGAGCCCCGAAG
ATTTGCGAGTCTATTACTGTCAACATTATGGTAACTCACCGGGGACGTTTCGGCCAAGGGACCA
AG

15 **Example 8. Use as Anti-GPC-3 Bi-Specific T Cell-Engaging Antibodies**

The anti-GPC-3 antibodies were used to construct anti-GPC-3 bi-specific T cell-engaging antibodies. In these constructs, the Fab region of one arm from each of the 5 anti-GPC-3 antibody clones (1C4, 1D2, 2B5, 4H1, 5C4) was replaced by the anti-CD3 scFv fragment, as shown below.

Clone	Heavy Chain (V _H) Amino Acid Sequence	Light Chain (V _L) Amino Acid Sequence
Anti-CD3	SEQ ID NO: 97	SEQ ID NO: 98

20 Table D3. Anti-CD3 Heavy Chain and Light Chain Amino Acid Sequence ID Numbers (sequences set out below)

ANTI-CD3 HEAVY CHAIN VARIABLE DOMAIN AMINO ACID SEQUENCE (V_H)

SEQ ID NO: 97: Heavy Chain Variable Domain Amino Acid Sequence of Anti-CD3

25 DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYT
NYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTITVTVSS

ANTI-CD3 LIGHT CHAIN VARIABLE DOMAIN AMINO ACID SEQUENCE (VL)*SEQ ID NO: 98: Light Chain Variable Domain Amino Acid Sequence of Anti-CD3*

DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVP
ARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Clone	Heavy Chain (V _H) Nucleic Acid Sequence	Light Chain (V _L) Nucleic Acid Sequence
Anti-CD3	SEQ ID NO: 99	SEQ ID NO: 100

5 Table D4. Anti-CD3 Heavy Chain and Light Chain Nucleic Acid Sequence ID
Numbers (sequences set out below)

ANTI-CD3 HEAVY CHAIN VARIABLE DOMAIN NUCLEIC ACID SEQUENCE (V_H)*SEQ ID NO: 99: Heavy Chain Variable Domain Nucleic Acid Sequence of Anti-CD3*

gatgtgcagctggtgcagagcggggcagaggtgaaaaagcctggggcaagcgtcaaag
10 tcagttgtaaagcctccggctacacattcactaggtatactatgcactgggtgcgccaggccc
ctggccaggggctggagtggatcggctacattaaccaagcagaggggtacacaaattatgctg
actccgtgaaaggcaggtttactatcaccaccgataagtccacctctacagcatacatggagc
tgagcagcctgcgaagcgaagacactgcaacctactattgcgcccgggtactatgacgatcatt
actgtctggattattggggacagggcactaccgtgacagtctctagt

15 ANTI-CD3 LIGHT CHAIN VARIABLE DOMAIN NUCLEIC ACID SEQUENCE (V_L)*SEQ ID NO: 100: Heavy Chain Variable Domain Nucleic Acid Sequence of Anti-CD3*

Gatattgtgctgaccagctctccagccacactgagctctgtcaccggcgaacgagcca
ccctgagctgccgggcccagccagctccgtctcttacatgaactggatcagcagaagcccggaa
aagcccctaagcgggtgatctacgacacaagcaaagtggcttccggcgtccccgcacgattca
20 gtggctcagggagcgggaactgactattctctgaccattaatagtctggaggctgaagatgccg
ctacctactattgtcagcagtggtcaagcaaccctctgacattcggggggggaactaaagtgg
aatcaag

ANTI-CD3 scFv AMINO ACID SEQUENCE*SEQ ID NO: 101: Amino Acid Sequence of Anti-CD3 scFv*

25 DVQLVQSGAEVKKPGASVKVSKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYT
NYADSVKGRFTITTDKSTSTAYMELSSLRSEDATATYYCARYYDDHYCLDYWGQGTITVTVSSGE

GTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWI
YDTSKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

ANTI-CD3 scFv NUCLEIC ACID SEQUENCE

SEQ ID NO: 102: Nucleic Acid Sequence of Anti-CD3 scFv

5 gatgtgcagctggtgcagagcggggcagaggtgaaaaagcctggggcaagcgtcaaag
tcagttgtaaagcctccggctacacattcactaggtatactatgcactgggtgcgccaggccc
ctggccaggggctggagtggtacggctacattaacccaagcagaggggtacacaaattatgctg
actccgtgaaaggcaggtttactatcaccaccgataagtccacctctacagcatacatggagc
tgagcagcctgcgaagcgaagacactgcaacctactattgcgcccgggtactatgacgatcatt
10 actgtctggattattggggacagggcactaccgtgacagtctctagtggggaggggaacatcca
ctgggtctggagggagtgagggtcaggaggagcagacgatattgtgctgacctcagctctccag
ccacactgagtctgtcaccggcgaacgagccaccctgagctgccggggccagccagtcctgtct
cttacatgaactggtatcagcagaagcccggaaaagcccctaagcgggtggatctacgacacaa
gcaaagtggcttccggcgtccccgcacgattcagtggctcagggagcggaaactgactattctc
15 tgaccattaatagtctggaggctgaagatgccgctacctactattgtcagcagtggtcaagca
accctctgacattcggggggggaactaaagtggaaatcaag

Knob-in-hole mutations were introduced to facilitate correct heavy-chain pairing and LALA mutations to remove Fcγ receptor binding (Figure 8A).

20 The ability of these anti-GPC-3 bi-specific T cell-engaging antibodies to specifically recognize and bind to both GPC-3 and human CD3 molecules on cell surface was measured using HepG2 (GPC-3^{Positive}), SK-Hep1 (GPC-3^{Negative}), and human T cells (CD3^{Positive}) cells. HepG2 cells and human T cells constitutively express high level of GPC-3 and CD3, respectively, while SK-Hep1 cells do not express GPC-3 or CD3.

25 Briefly, HepG2 (GPC-3^{Positive}), SK-Hep1 (GPC-3^{Negative}) and human T cells (CD3^{Positive}) were seeded in cell suspension at 100000 per well in 96-well round-bottom plate prior to incubation with different concentrations of anti-GPC-3 bi-specific T cell-engaging antibodies. Binding was measured by flow cytometry using a secondary labelled antibody. In this assay, all 5 anti-GPC-3 bi-specific T-cell engaging antibodies bound strongly to the GPC-3 expressing cell HepG2 (Figure 8B) as well as human T cells expressing CD3 (Figure 8D).
30 However, these antibodies did not bind to GPC-3 negative SK-Hep1 cells (Figure 8C). This

suggests that these anti-GPC-3 bi-specific T cell-engaging antibodies bind to surface of the cells in a highly specific manner dependent on GPC-3 and CD3 expression.

To assess the function of these anti-GPC-3 bi-specific T-cell engaging antibodies, T cells from healthy donors were isolated and incubated with either HepG2 (GPC-3^{Positive}) or SK-Hep1 (GPC-3^{Negative}) cells in the presence of anti-GPC-3 bi-specific T-cell engaging antibodies at 3 different concentrations of 2.5nM, 312.5pM and 39pM. 24 hours later, T cells were harvested and analysed for their activation status by assessing the surface expression of T cell activation markers CD25 and CD69.

The results showed that all five anti-GPC-3 bi-specific T-cell engaging antibodies could strongly activate human naïve T cells when co-cultured with GPC-3^{Positive} HepG2 cells (Figure 9A and Figure 9C) but not with GPC-3^{Negative} SK-Hep1 cells (Figure 9B and Figure 9D).

In addition, cell culture supernatants were collected and subjected to ELISA to assess the cytokine release of interferon- γ and IL-2 from anti-GPC-3 bi-specific T-cell engaging antibodies treated T cells.

When co-cultured with GPC-3^{Positive} HepG2 cells in the presence of anti-GPC-3 bi-specific T-cell engaging antibodies (clone 1C4, 1D2, 2B5, 4H1 and 5C4), activated human T cells secreted remarkably higher amount of interferon- γ and IL-2 (Figure 9E and Figure 9G). However, release of interferon- γ and IL-2 remained low when co-cultured with GPC-3^{Negative} SK-Hep1 cells (Figure 9F and Figure 9H), indicating the superior specificity of these anti-GPC-3 bi-specific T-cell engaging antibodies.

Efficacy of these anti-GPC-3 bi-specific T-cell engaging antibodies was further examined by subjecting them to the antibody dependent T cell mediated cytotoxicity assay.

Briefly, GPC-3^{Positive} HepG2 cells (T: Target cells) were seeded onto the xCelligence E-plate and cultured for overnight with cell index values measured. The next day, immediately after addition of anti-GPC-3 bi-specific T-cell engaging antibodies at various concentrations, primary T cells isolated from human PBMCs (E: Effector cells) were added to the target cells at Effector to Target ratio of 4 to 1 (E:T=4:1). The assay was continued for a further 72 hours to measure the target cell index values using xCelligence RTCA system.

Except for clone 2B5, which only induce profound cytolysis of target cell HepG2 (GPC-3^{Positive}) at high concentration of 10nM and 1nM, all the other anti-GPC-3 bi-specific T-cell engaging antibodies (clone 1C4, 1D2, 4H1 and 5C4) were able to kill 50-70% of HepG2 cells within 72 hours at a concentration as low as 0.1nM (Figure 10A and Figure 10B).

5 In summary, results from the above assays showcased the use of our anti-GPC-3 antibodies in the format of bi-specific T cell engaging antibody with high specificity and potency in inducing cell death of GPC-3 expressing cells.

Example 9. Use in a Chimeric Antigen Receptor (CAR) T Cell Construct

10 The *in vivo* activity of anti-GPC-3 CAR T cells was first assessed with a preventive model using immunocompromised NSG (NOD-*scid* IL2Rgamma^{null}) mice (Figure 11).

On day 0, two million Hep3B cells were subcutaneously injected into the right flanks of NSG mice (female only). Four days later when there is no measurable tumor observed, mice were randomly grouped and injected with ten million of either anti-GPC-3 CAR T cells (clone 5C4) or Mock T cells. All mice from both “Tumor only” group (no T cells injected) and “Mock T” group developed big tumors and were sacrificed on day 41, whereas all mice 15 from “CAR T” group remained tumor-free. Eighty-four days post initial tumor inoculation, all tumor-free mice from the CAR T group (4 mice) together with a group of naïve mice (5 mice, “Tumor only 2”) were challenged with one million of Hep3B cells subcutaneously injected into the right flanks and their tumor growth and body weights were continuously monitored. 20 While all five mice from “Tumor only 2” group developed into sizable tumors, two mice from CAR T group remained tumor free until day 165 (81 days post re-challenge) (Figure 11A).

These data indicated that 5C4 CAR T cells prevented GPC-3^{Positive} HCC tumor formation in xenograft models and also provided long-term protection to the NSG mice from developing GPC-3^{Positive} HCC tumors upon tumor re-challenge. In addition, the anti-GPC-3 25 CAR T cells (clone 5C4) did not induce severe toxicity to the NSG mice as there was no significant drop in mouse body weight upon anti-GPC-3 CAR T treatment (Figure 11B). The efficacy of the anti-GPC-3 CAR T cells was also reflected by the up-regulated blood serum level of T cell associated cytokines (Perforin, Granzyme B, sCD137, GM-CSF) from the “CAR T” group compared to the basal levels detected from the “Mock T” group after 6 days 30 of T cell injection (Figure 11C).

To test if the anti-GPC-3 CAR T cells are capable to suppress the growth of established tumors *in vivo*, we chose 3 different anti-GPC-3 antibody clones to generate anti-GPC-3 CAR constructs (clone 1D2, 4H1, and 5C4). Regardless of different clones, 20% of CAR expression was achieved by lentivirus transduction (Figure 12A).

5 These anti-GPC-3 CAR T cells exhibited strong and specific *in vitro* target cell cytotoxicity as they killed 70-80% of GPC-3^{Positive} Hep3B cells but not GPC-3^{Negative} SK-Hep1 cells in an xCelligence cell impedance assay (Figure 12B). Twenty-six days after subcutaneously inoculation of NSG mice (female only) with 2 million of GPC-3^{Positive} Hep3B cells, the mice were grouped according to the measurable tumor size for CAR T cell injection.
10 Ten million of different anti-GPC-3 CAR T cells (4 mice per group) or Mock T cells (3 mice) were intravenously injected into these mice via tail vein

On day 45 (19 days post CAR T cell injection), tumors in the mice treated with 1D2 CAR T cells at an average size of 1749mm³ started to shrink, to an average of 643mm³ on day 60. Tumors in the mice treated with 4H1 CAR T cells or with 5C4 CAR T cells grew much
15 slower than Mock T treated mice and a further decrease in tumor size was observed for 5C4 CAR T group from Day 53 onwards (Figure 12C).

Interestingly, while the body weights of Mock T group of mice dropped significantly after 45 days of tumor inoculation when average tumor size exceeded 2000mm³, the body weights of all CAR T injected mice remained stable (Figure 12D).

20 These data suggested that our anti-GPC-3 CAR T cells potently suppressed growth of GPC-3^{Positive} HCC xenograft *in vivo* without inducing severe toxicity.

Next, we aimed to test if our anti-GPC-3 CAR T cell therapy is effective with a combinatorial use of immune checkpoint blockade antibodies, such as anti-PD1 antibodies.

Similarly, two million Hep3B cells were subcutaneously injected into the right flank of
25 NSG mice (female only). Twenty-four days later, mice were grouped according to the measurable tumor size and injected intravenously with either anti-GPC-3 CAR T cells (clone 5C4) or Mock T cells via tail vein. Ten days after CAR T cell injection when both groups developed tumors exceeding an average size of 1000mm³, all mice were intraperitoneal injected with anti-PD1 IgG₄ antibodies for a total of 5 times with an interval of 3 to 5 days.

Mock T group of mice did not respond to anti-PD1 antibodies, and as a result tumors continued to grow. In contrast, within 5 days of the 1st anti-PD1 antibody injection, tumors from the CAR T group of mice exhibited a sharp drop in size and continued to shrink until the average tumor size reached less than 100mm³ and lasted for more than 20 days (Figure 13A).

5 The body weight from both Mock T and CAR T group of mice remained stable though a slight fluctuation was observed in CAR T group (Figure 13B).

As a result of the potent tumor suppression by combinatorial use of anti-GPC-3 CAR T and anti-PD1 antibodies, 75% of mice treated with the anti-GPC-3 CAR T cells followed by anti-PD1 antibodies survived for longer than 70 days, while the median survival durations of
10 the Mock T followed by anti-PD1 antibodies treated mice were only 45 days (Figure 13C).

Interestingly, on the day after the second anti-PD1 antibody injection (Day40, 16 days after CAR T cell infusion), the total number of human T cells from mice of the CAR T group was significantly higher than that of mice treated with Mock T cells (Figure 13D). In addition, the persistence of peripheral CAR+CD3+ T cells, CD4+CD3+ T cells, and CD8+CD3+ T
15 cells was consistent with the total T cells (Figure 13E). Moreover, the efficacy of the combination therapy of anti-GPC-3 CAR T cells and anti-PD1 antibodies was also observed by the up-regulation in blood serum level of T cell associated cytokines (Perforin, Granzyme B, sCD137, GM-CSF) in the anti-GPC-3 CAR T cell treated group (Figure 13F).

Since most of the anti-GPC-3 antibodies clones can cross-react with the mouse
20 homolog of GPC-3 protein, we injected CAR T cells derived from 8 different mouse cross-reacting anti-GPC-3 clones (1C4, 1D2, 1F1, 1H1, 2B5, 4A5, 4H1, and 5C4) and 1 anti-GPC-3 clone, 1D8, which does not cross-react with mouse GPC-3, into NSG mice (6 mice per group, mixed genders). We tested the potential toxicity of these anti-GPC-3 CAR T cells against the normal mouse tissues in the absence of tumor cells expressing high level of GPC-3 proteins,
25 by monitoring the body weight as well as other abnormal physiological changes or behaviours of each mouse. The results clearly showed that similar to “No treatment” group (Figure 14A), Mock T group (Figure 14B), and 1D8 CAR T group (Figure 14E), the 8 different anti-GPC-3 CAR T cells which could bind to mouse GPC-3 expressing cells, did not induce noticeable toxicity *in vivo* up to the time point of 5 weeks (Figure 14C, Figure 14D, Figure 14F, Figure
30 14G, Figure 14H, Figure 14I, Figure 14J and Figure 14K), beyond which GvHD may happen anytime.

This indicated that either mouse GPC-3 did not express in normal mouse tissues, or the affinities between the mouse GPC-3 protein and the scFv fragment derived from the 8 anti-GPC-3 clones were at an optimal level that the low expression of mouse GPC-3 on normal mouse tissues would not induce strong activation of these anti-GPC-3 CAR T cells.

5 Finally, to examine if the use of CAR T cell therapy can be extended to the mouse system, we first stained parental MC38 cells and mouse GPC-3 stably transduced MC38 cells (named as “MC38-mGPC-3”) using a representative anti-GPC-3 IgG clone 5C4 followed by flow cytometry analysis (Figure 15A).

10 A mouse CAR T construct based on clone 5C4 was designed by replacing the human T cell activation domains to their counterpart of mouse homologs and cloned into a retrovirus-based vector. Anti-GPC-3 mouse CAR T cells were generated from mouse T cells isolated from mouse splenocytes by retroviral transduction process and the mouse CAR expression was detected by flow cytometry analysis (Figure 15B). The *in vitro* activity of these mouse CAR T cells was verified by more than 80% cytotoxicity of the MC38-mGPC-3 cells within
15 96 hours using xCelligence impedance assay (Figure 15C). To test the *in vivo* therapeutic efficacy of mouse anti-GPC-3 CAR T cells, 0.8 million MC38-mGPC-3 cells were subcutaneously injected into WT C57BL/6 mice. When the tumors grow to a measurable size (~100mm³), the mice were re-grouped and intravenously injected with anti-GPC-3 mouse CAR T cells (clone 5C4) or Mock T cells or left untreated (“Tumor only”).

20 By day 33 (21 days post CAR T cell infusion), all mice from both Mock T group and Tumor only group developed big sizes of tumors, while mice from anti-GPC-3 mouse CAR T cell injected group grew very small tumors with 1 mouse remained tumor-free (Figure 15D). Monitoring of mouse body weights revealed that these mouse CAR T cells did not induce noticeable toxicity both in the presence (Figure 15E) or in the absence of MC38-mGPC-3
25 tumor cells expressing mGPC-3 protein (Figure 15F).

 These experiments not only demonstrated the efficacy of mouse anti-GPC-3 CAR T cells both *in vitro* and *in vivo*, but also shed light on the extended use of the anti-GPC-3 antibody sequences for future research of anti-GPC-3 CAR T therapy in syngeneic mouse models, as a format of mouse CAR T construct.

Example 10. Binding Affinity of Anti-GPC-3 IgG₁ Antibodies

Affinity of 24 anti-GPC-3 IgG₁ antibodies was measured via Surface Plasmon Resonance (SPR) analysis using Biacore™ T200 system and shown in Table E3 (below).

Clone	K _D (nM)	K _a (1/Ms)	K _d (1/s)
1A12	27.64	1.141 x 10 ⁵	3.155 x 10 ⁻³
1C4	14.08	9.49 x 10 ⁵	1.336 x 10 ⁻²
1D2	4.177	6.147 x 10 ⁵	2.567 x 10 ⁻³
1D3	17.21	1.704 x 10 ⁵	2.932 x 10 ⁻³
1D8	13.65	3.880 x 10 ⁵	5.295 x 10 ⁻³
1E1	16.16	1.445 x 10 ⁵	2.334 x 10 ⁻³
1F1	13.85	3.986 x 10 ⁵	5.520 x 10 ⁻³
1F4	7.201	1.793 x 10 ⁵	1.291 x 10 ⁻³
1H1	33.87	1.758 x 10 ⁵	5.955 x 10 ⁻³
1H10	19.67	7.480 x 10 ⁵	1.467 x 10 ⁻²
2B5	12.45	3.056 x 10 ⁵	3.804 x 10 ⁻³
2F1	14.69	1.235 x 10 ⁵	1.814 x 10 ⁻³
3A9	12.4	1.513 x 10 ⁵	1.876 x 10 ⁻³
3C6	8.176	5.774 x 10 ⁴	4.721 x 10 ⁻⁴
3C12	31.22	2.509 x 10 ⁵	7.835 x 10 ⁻³
3D12	32.4	1.081 x 10 ⁵	3.501 x 10 ⁻³
3G12	12.54	1.347 x 10 ⁵	1.688 x 10 ⁻³
4A5	12.5	7.569 x 10 ⁴	9.459 x 10 ⁻⁴
4A12	22.87	2.664 x 10 ⁵	6.094 x 10 ⁻³
4F9	10.49	1.644 x 10 ⁵	1.725 x 10 ⁻³
4G4	12.94	1.508 x 10 ⁵	1.952 x 10 ⁻³
4G11	15.5	1.474 x 10 ⁵	2.285 x 10 ⁻³
4H1	44.17	3.108 x 10 ⁵	1.373 x 10 ⁻²
5C4	9.015	1.788 x 10 ⁵	1.612 x 10 ⁻³

Table E3. Binding affinity (K_D), association rate (K_a) and dissociation rate (K_d) of 24 anti-GPC-3 IgG₁ antibodies to human GPC-3 protein measured by SPR analysis.

All 24 antibody clones exhibited binding affinity to the recombinant GPC-3 protein at the nanomolar scales ranging from the lowest affinity at 44.17nM (clone 4H1) to the highest affinity at 4.177nM (clone 1D2).

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In this document and in its claims, the verb “to comprise” and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one of the element is

present, unless the context clearly requires that there be one and only one of the elements. The indefinite article “a” or “an” thus usually means “at least one”.

Each of the applications and patents mentioned in this document, and each document cited or referenced in each of the above applications and patents, including during the
5 prosecution of each of the applications and patents (“application cited documents”) and any manufacturer’s instructions or catalogues for any products cited or mentioned in each of the applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer’s
10 instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific
15 preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

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,
5 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
10 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
15 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.
- 20 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
25 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
30 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.