

necessarily induce effective cell killing, an observation that may at least in part arise from differential endosomal/lysosomal processing pathways in different types of cancer cells.

Therapeutic antibodies may require substantial quantities of product over multiple dosages to be clinically effective (e.g. 200–350mg/m² per dose). A therapeutic dose of ADC may often be marginally less than that of naked therapeutic monoclonal antibodies (TMAs) (e.g. at 160 mg per dose). However, ADC construction involves additional technologies such as chemical conjugation steps that complicate production streams and can raise the cost of manufacture significantly. It is therefore desirable to provide therapeutic dose ADCs at lower cost where possible.

Despite the promise that antibodies offer as therapeutic agents, a need continues to exist for effective therapeutic antibodies to treat prostate, bladder, and/or pancreatic cancer.

Summary of the Invention

The present inventors have surprisingly identified that the BLCA-38 antibody referred to and used in the aforementioned prior art is a combination of two distinct monoclonal antibodies in a mixed population. It has been determined that only one of these antibody species is capable of binding strongly to the relevant target antigen present on prostate cancer cells, whilst the second species cannot. Moreover, it has been unexpectedly discovered that the antibody species is capable of binding to the relevant target antigen present on prostate cancer cells is internalised upon doing so, despite the aforementioned prior art indicating to the contrary. Moreover, the present inventors have demonstrated effective internalisation in prostate, bladder and pancreatic cancer cells providing a means of targeting and killing these cancer cell types.

Accordingly, in a first aspect the present invention provides a use of an anti-glypican-1 (anti-GPC1) antibody and/or an antigen-binding fragment thereof in the preparation of a medicament for treating prostate, bladder, and/or pancreatic cancer in a subject, wherein: the antibody and/or fragment is conjugated to at least one cytotoxic agent that is toxic to a prostate, bladder, and/or pancreatic cancer cell in the subject, and the antibody and/or fragment comprises: (a) a heavy chain variable region comprising: a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 50-54 of **SEQ ID NO: 3**; a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 69-85 of **SEQ ID NO: 3**; a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 118-126 of **SEQ ID NO: 3**; and (b) a light chain variable region comprising: a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 44-54 of **SEQ ID NO: 4**; a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 70-76 of **SEQ ID NO: 4**; a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by

positions 109-117 of **SEQ ID NO: 4**; and the medicament does not comprise an antibody with a light chain variable region comprising: a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 48-58 of **SEQ ID NO: 11**; a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 74-80 of **SEQ ID NO: 11**; a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 113-121 of **SEQ ID NO: 11**.. Also disclosed is a method for treating prostate, bladder, and/or pancreatic cancer in a subject, the method comprising administering to the subject a pharmaceutical preparation comprising an anti-glypican-1 (anti-GPC1) antibody and/or an antigen-binding fragment thereof, wherein the antibody and/or fragment is conjugated to at least one cytotoxic agent that is toxic to a prostate, bladder, and/or pancreatic cancer cell in the subject.

Also disclosed is a method for killing a prostate, bladder, and/or pancreatic cancer cell in a subject, the method comprising administering to the subject a pharmaceutical preparation comprising an anti-glypican-1 (anti-GPC1) antibody and/or an antigen-binding fragment thereof, wherein the antibody and/or fragment is

daunorubicin, dinaciclib, 3',5'-O-dioleoyl-FudR (FudR-d0), DM1, DM3, DM4, docetaxel, doxorubicin, doxorubicin glucuronide, duocarmycin, endostatin, entinostat, epidophyllotoxin, epirubicin glucuronide, erlotinib, estramustine, estrogen receptor binding agents, etoposide
5 glucuronide, etoposide phosphate, etoposide (VP16), exemestane, farnesyl-protein transferase inhibitors, fingolimod, flavopiridol, floxuridine (FudR), fludarabine, 5-fluorouracil, flutamide, fostamatinib, ganetespib, GDC-0834, gefitinib, gemcitabine, GS-1101, 10-hydroxycamptothecin, hydroxyurea, ibrutinib, idarubicin, idelalisib, ifosfamide, imatinib, irinotecan (CPT-11), lapatinib, lenolidamide, leucovorin, LFM-A13, lomustine,
10 mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mithramycin, mitomycin, mitotane, mitoxantrone, monomethylauristatin D (MMAD), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), navelbine, neratinib, nilotinib, nitrosurea, olaparib, paclitaxel, PCI-32765, pentostatin, plicomycin, a 2-PDox pro-drug (pro-2-PDox), procarbazine, PSI-341, 2-pyrrolinodoxorubicine (2-PDox), raloxifene,
15 semustine, SN-38, sorafenib, streptozocin, SU1 1248, sunitinib, tamoxifen, temazolomide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, transplatinum, uracil mustard, vatalanib, vinblastine, vinca alkaloids, vincristine, vinorelbine, and ZD 1839.

In still another example, the cytotoxic agent is a radioisotope selected from the group consisting of: ^{90}Y , ^{188}Re , ^{166}Ho , ^{165}Dy , ^{109}Pd , ^{111}Ag , ^{186}Re , ^{198}Au , ^{153}Sm , ^{64}Cu , ^{177}Lu , ^{131}I , ^{125}I ,
20 ^{67}Cu , ^{175}Yb , ^{166}Dy , ^{169}Er , ^{212}Bi , ^{213}Bi , ^{225}Ac , ^{212}Pb , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{86}Y , $^{94\text{m}}\text{Tc}$, and ^{89}Zr .

In still another example, the antibody or antigen-binding fragment thereof is conjugated with a chelating agent selected from the group consisting of: DOTA, DTPA, NOTA, NODAGA, MeCOSAR, TETA, TRAP, TE2A, CBTE2A.

In still another example, the cytotoxic agent and the antibody or antigen-binding
25 fragment thereof are conjugated with a chelating agent selected from the group consisting of: DOTA, DTPA, NOTA, NODAGA, MeCOSAR, TETA, TRAP, TE2A, CBTE2A.

In a further example, the subject is a mammalian subject or a human subject.

In a second aspect, the present invention provides an isolated antibody population comprising:

30 first anti-glypican-1 (anti-GPC1) antibodies and/or antigen binding fragments thereof, that are conjugated to at least one cytotoxic agent which is toxic to a prostate, bladder, and/or pancreatic cancer cell, wherein the first anti-glypican-1 (anti-GPC1) antibodies comprise:

CLAIMS

1. Use of an anti-glypican-1 (anti-GPC1) antibody and/or an antigen-binding fragment thereof in the preparation of a medicament for treating prostate, bladder, and/or pancreatic cancer in a subject, wherein:

the antibody and/or fragment is conjugated to at least one cytotoxic agent that is toxic to a prostate, bladder, and/or pancreatic cancer cell in the subject, and

the antibody and/or fragment comprises:

(a) a heavy chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 50-54 of **SEQ ID NO: 3**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 69-85 of **SEQ ID NO: 3**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 118-126 of **SEQ ID NO: 3**; and

(b) a light chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 44-54 of **SEQ ID NO: 4**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 70-76 of **SEQ ID NO: 4**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 109-117 of **SEQ ID NO: 4**; and

the medicament does not comprise an antibody with a light chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 48-58 of **SEQ ID NO: 11**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 74-80 of **SEQ ID NO: 11**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 113-121 of **SEQ ID NO: 11**.

2. The use according to claim 1, wherein the anti-glypican-1 (anti-GPC1) antibody is produced by a hybridoma cell as deposited at Cellbank Australia under accession number CBA20140026, or progeny thereof.

3. The use according to claim 1 or claim 2, wherein the anti-glypican-1 (anti-GPC1) antibody is:

- (i) a humanised anti-GPC1 antibody,
- (ii) a chimeric anti-GPC1 antibody,
- (i) a human anti-GPC1 antibody,
- (ii) a monoclonal anti-GPC1 antibody,
- (iii) a multimeric anti-GPC1 antibody,
- (iv) a synthetic anti-GPC1 antibody, or
- (v) a chimeric antibody comprising a heavy chain constant region comprising or consisting of an amino acid sequence as defined in residues 138-467 of **SEQ ID NO: 7**; and a light chain constant region comprising or consisting of an amino acid sequence as defined in residues of 128-234 **SEQ ID NO: 8**.

4. The use according to any one of claims 1 to 3, wherein the antigen-binding fragment thereof is: a single chain variable fragment (scFv), a single chain variable fragment (scFv) comprising a sequence as defined in **SEQ ID NO: 9**, a variable domain (Fv) fragment, a fragment antigen binding (Fab) fragment, a F(ab)₂ fragment, a peptide, or a proteolytic fragment containing an epitope binding region.

5. The use according to any one of claims 1 to 4, wherein the cytotoxic agent is selected from the group consisting of:

- (a) an adrenocortical suppressant, an alkylating agent, an alkyl sulfonate, an anthracycline, an anti-angiogenic agent, an antibiotic, an antimetabolite, an antimitotic, an auristatin, a calicheamycin, a camptothecin, a COX-2 inhibitor, an enzyme inhibitor, an epipodophyllotoxin, an ethylenimine derivative, a folic acid analog, an HDAC inhibitor, a heat shock protein (HSP90) inhibitor, a hormone antagonist, a maytansinoid, a methyl hydrazine derivative, an mTOR inhibitor, a nitrogen mustard, a nitrosourea, a platinum coordination complex, a pro-apoptotic agent, a proteasome inhibitor, a purine analog, a pyrimidine analog, a radioisotope, a substituted urea, a taxane, triazene, a tubulin inhibitor, a tyrosine kinase inhibitor, and a vinca alkaloid;

(b) afatinib, aplidin, anastrozole, anthracyclines, AVL-101, AVL-291, axitinib, azaribine, bendamustine, bleomycin, bortezomib, bosutinib, bryostatin-1, busulfan, camptothecans, carboplatin, calicheamycin, camptothecin, carboplatin, carmustine, celecoxib, chlorambucil, cisplatin, cladribine, COX-2 inhibitors, crizotinib, cyano-morpholino doxorubicin, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, dasatinib, daunorubicin, dinaciclib, 3',5'-O-dioleoyl-FudR (FUDR-d0), DM1, DM3, DM4, docetaxel, doxorubicin, doxorubicin glucuronide, duocarmycin, endostatin, entinostat, epidophyllotoxin, epirubicin glucuronide, erlotinib, estramustine, estrogen receptor binding agents, etoposide glucuronide, etoposide phosphate, etoposide (VP16), exemestane, farnesyl-protein transferase inhibitors, fingolimod, flavopiridol, floxuridine (FUDR), fludarabine, 5-fluorouracil, flutamide, fostamatinib, ganetespib, GDC-0834, gefitinib, gemcitabine, GS-1101, 10-hydroxycamptothecin, hydroxyurea, ibrutinib, idarubicin, idelalisib, ifosfamide, imatinib, irinotecan (CPT-11), lapatinib, lenolidamide, leucovorin, LFM-A13, lomustine, mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mithramycin, mitomycin, mitotane, mitoxantrone, monomethylauristatin D (MMAD), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), navelbine, neratinib, nilotinib, nitrosurea, olaparib, paclitaxel, PCI-32765, pentostatin, plicomycin, a 2-PDox pro-drug (pro-2-PDox), procarbazine, PSI-341, 2-pyrrolinodoxorubicine (2-PDox), raloxifene, semustine, SN-38, sorafenib, streptozocin, SU1 1248, sunitinib, tamoxifen, temazolomide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, transplatinum, uracil mustard, vatalanib, vinblastine, vinca alkaloids, vincristine, vinorelbine, and ZD 1839.

6. The use according to any one of claims 1 to 5, wherein the cytotoxic agent is:

- (a) a radioisotope;
- (b) a radioisotope selected from the group consisting of: ^{90}Y , ^{188}Re , ^{166}Ho , ^{165}Dy , ^{109}Pd , ^{111}Ag , ^{186}Re , ^{198}Au , ^{153}Sm , ^{64}Cu , ^{177}Lu , ^{131}I , ^{125}I , ^{67}Cu , ^{175}Yb , ^{166}Dy , ^{169}Er , ^{212}Bi , ^{213}Bi , ^{225}Ac , ^{212}Pb , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{64}Cu , ^{86}Y , $^{94\text{m}}\text{Tc}$, and ^{89}Zr .

7. The use according to any one of claims 1 to 6, wherein the subject is a mammalian subject or a human subject.

8. An isolated antibody population comprising:

first anti-glypican-1 (anti-GPC1) antibodies and/or antigen binding fragments thereof, that are conjugated to at least one cytotoxic agent which is toxic to a prostate, bladder, and/or pancreatic cancer cell, wherein the first anti-glypican-1 (anti-GPC1) antibodies comprise:

- (a) a heavy chain variable region comprising:
 - a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 50-54 of **SEQ ID NO: 3**;
 - a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 69-85 of **SEQ ID NO: 3**;
 - a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 118-126 of **SEQ ID NO: 3**; and
 - (b) a light chain variable region comprising:
 - a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 44-54 of **SEQ ID NO: 4**;
 - a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 70-76 of **SEQ ID NO: 4**;
 - a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 109-117 of **SEQ ID NO: 4**;
- and wherein the antibody population does not contain second antibodies comprising a light chain variable region comprising:
- a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 48-58 of **SEQ ID NO: 11**;
 - a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 74-80 of **SEQ ID NO: 11**;
 - a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 113-121 of **SEQ ID NO: 11**.

9. The antibody population according to claim 8, wherein the first anti-glypican-1 (anti-GPC1) antibodies and/or antigen binding fragments thereof are any one or more of monoclonal antibodies, humanised antibodies, chimeric antibodies, multimeric antibodies, synthetic antibodies, and/or chimeric antibodies comprising:

- (a) a heavy chain constant region comprising or consisting of an amino acid sequence as defined in residues 138-467 of **SEQ ID NO: 7**; and
- (b) a light chain constant region comprising or consisting of an amino acid sequence as defined in residues of 128-234 **SEQ ID NO: 8**.

10. The antibody population according to claim 8 or claim 9, wherein the antigen binding fragments are any one or more of single chain variable fragments (scFv), single chain variable fragments (scFv) comprising a sequence as defined in **SEQ ID NO: 9**, variable domain (Fv) fragments, fragment antigen binding (Fab) fragments, F(ab)₂ fragments, peptides, or proteolytic fragments containing an epitope binding region.

11. The antibody population according to any one of claims 8 to 10, wherein the first anti-glypican-1 (anti-GPC1) antibodies comprise or consist of a heavy chain sequence as defined by positions 20-461 of **SEQ ID NO: 3** and a light chain sequence as defined by positions 21-234 of **SEQ ID NO: 4**.

12. The antibody population according to any one of claims 8 to 11, wherein the cytotoxic agent is selected from the group consisting of:

(a) an adrenocortical suppressant, an alkylating agent, an alkyl sulfonate, an anthracycline, an anti-angiogenic agent, an antibiotic, an antimetabolite, an antimitotic, an auristatin, a calicheamycin, a camptothecin, a COX-2 inhibitor, an enzyme inhibitor, an epipodophyllotoxin, an ethylenimine derivative, a folic acid analog, an HDAC inhibitor, a heat shock protein (HSP90) inhibitor, a hormone antagonist, a maytansinoid, a methyl hydrazine derivative, an mTOR inhibitor, a nitrogen mustard, a nitrosourea, a platinum coordination complex, a pro-apoptotic agent, a proteasome inhibitor, a purine analog, a pyrimidine analog, a radioisotope, a substituted urea, a taxane, triazene, a tubulin inhibitor, a tyrosine kinase inhibitor, and a vinca alkaloid;

(b) afatinib, aplidin, anastrozole, anthracyclines, AVL-101, AVL-291, axitinib, azaribine, bendamustine, bleomycin, bortezomib, bosutinib, bryostatin-1, busulfan, camptothecans, carboplatin, calicheamycin, camptothecin, carboplatin, carmustine, celecoxib, chlorambucil, cisplatin, cladribine, COX-2 inhibitors, crizotinib, cyano-morpholino doxorubicin, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, dasatinib, daunorubicin, dinaciclib, 3',5'-O-dioleoyl-FudR (FUDR-d0), DM1, DM3, DM4, docetaxel, doxorubicin, doxorubicin glucuronide, duocarmycin, endostatin, entinostat, epipodophyllotoxin, epirubicin glucuronide, erlotinib, estramustine, estrogen receptor binding agents, etoposide glucuronide, etoposide phosphate, etoposide (VP16), exemestane, farnesyl-protein transferase inhibitors, fingolimod, flavopiridol, floxuridine (FUDR), fludarabine, 5-fluorouracil, flutamide, fostamatinib, ganetespib, GDC-0834, gefitinib, gemcitabine, GS-1101, 10-

hydroxycamptothecin, hydroxyurea, ibrutinib, idarubicin, idelalisib, ifosfamide, imatinib, irinotecan (CPT-11), lapatinib, lenolidamide, leucovorin, LFM-A13, lomustine, mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mithramycin, mitomycin, mitotane, mitoxantrone, monomethylauristatin D (MMAD), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), navelbine, neratinib, nilotinib, nitrosurea, olaparib, paclitaxel, PCI-32765, pentostatin, plicomycin, a 2-PDox pro-drug (pro-2-PDox), procarbazine, PSI-341, 2-pyrrolinodoxorubicine (2-PDox), raloxifene, semustine, SN-38, sorafenib, streptozocin, SU1 1248, sunitinib, tamoxifen, temazolomide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, transplatinum, uracil mustard, vatalanib, vinblastine, vinca alkaloids, vincristine, vinorelbine, and ZD 1839.

13. The antibody population according to any one of claims 8 to 12, wherein the cytotoxic agent is:

- (a) a radioisotope;
- (b) a radioisotope selected from the group consisting of: ^{90}Y , ^{188}Re , ^{166}Ho , ^{165}Dy , ^{109}Pd , ^{111}Ag , ^{186}Re , ^{198}Au , ^{153}Sm , ^{64}Cu , ^{177}Lu , ^{131}I , ^{125}I , ^{67}Cu , ^{175}Yb , ^{166}Dy , ^{169}Er , ^{212}Bi , ^{213}Bi , ^{225}Ac , ^{212}Pb , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{64}Cu , ^{86}Y , $^{94\text{m}}\text{Tc}$, and ^{89}Zr .