

over-expressed in cancers of the hepato-biliary system, pancreas and gastrointestinal tract, while its natural form, di-sialyl-Lewis^a which has an extra sialic acid sugar, is found in nonmalignant epithelial cells. Expression of sialyl-Lewis^a was found to increase metastatic potential in pancreatic adenocarcinoma (16, 27) and colon cancer (14, 15). In pancreatic and colon cancer, sialyl-Lewis^a is also used as a tumour marker to monitor responses to therapy (13,17,18). Sialyl-di-Lewis^a (this has the single sialic acid found in cancers but also has the Lewis' duplicated and is only found on proteins), is expressed by a wide range of pancreatic tumours but has a very restricted normal tissue expression. More recently, human sialyl-Lewis^a mAbs were produced using a patient vaccination strategy that showed specific binding to sialyl-Lewis^a and exhibited ADCC, CDC and anti-tumour activity in a xenograft model (20). One of these mabs, 5B1, is a human IgG1 which predominantly binds Sialyl Lewis' whether the neuraminic acid is endogenously produced (N-acetyl-neuraminic acid) or exogenously derived (N-glycolyl-neuraminic acid) and whether it is on a long or short spacer. Binding to Sialyl-di-lewis^a or Sialyl lewis^{a-x} is weak and insignificant. The second mab 7E3 is a human IgM which binds equally to Sialyl lewis^a whether the neuraminic acid is endogenously produced (N acetyl neuraminic acid) or exogenously derived (N-glycolyl-neuraminic acid) and whether it is on a long or short spacer, and to Sialyl-di-lewis^a or Sialyl lewis^{a-x}. Such anti-Sialyl Lewis^a mabs would have an unacceptable normal distribution, which is supported by the observation that GivaRex (a mouse monoclonal antibody) and its patent (W00191792) has been abandoned in preclinical studies.

An aim of the present invention is to provide an improved binding member for sialyl-di-Lewis^a

In one aspect of the invention, there is provided an isolated antibody or antibody fragment, wherein the antibody or antibody fragment binds specifically to sialyl-di-Lewis^a and sialyl-Lewis^{a-x} and mono-sialyl-Lewis^a, wherein the mono-sialyl-Lewis^a is bound to a glycoprotein, and does not bind to mono-sialyl-Lewis^a bound to a glycolipid, and wherein the antibody or antibody fragment comprises the following six CDRs:

- a) GFTFNTYA (CDRH1), IRSKSNNYAT (CDRH2) and VGYGSGGNY (CDRH3); and
- b) QSLNSGNQKNY (CDRL1), WAS (CDRL2) and QNDYSSPFT (CDRL3).

In another aspect of the invention, there is provided an antibody or antibody fragment as described herein for use as a medicament.

In another aspect of the invention, there is provided an antibody or antibody fragment as described herein for use in the prevention, treatment or diagnosis of cancer, wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.

In another aspect of the invention, there is provided a use of an antibody or antibody fragment as described herein in the manufacture of a medicament for treating or preventing cancer, wherein the medicament is to be administered to a subject in need of thereof, wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.

In another aspect of the invention, there is provided a nucleic acid comprising a sequence encoding an antibody or antibody fragment as described herein.

In another aspect of the invention, there is provided a recombinant host cell which comprises the nucleic acid as described herein.

In another aspect of the invention, there is provided a method for diagnosis of cancer comprising using an antibody or antibody fragment as describe herein to detect the glycans sialyl-di-Lewis^a and sialyl-Lewis^{a-x} and/or mono-sialyl-Lewis^a only attached to a glycoprotein in a sample obtained from an individual.

In another aspect of the invention, there is provided a pharmaceutical composition comprising the antibody or antibody fragment as described herein, and a pharmaceutically acceptable carrier.

In another aspect of the invention, there is provided a pharmaceutical composition as described herein, for use in the treatment of cancer wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.

According to a first aspect of the invention, there is provided an isolated specific binding member capable of binding sialyl-di-Lewis^a.

The binding member may be specific for sialyl-di-Lewis^a. In one embodiment, the binding member may be specific for sialyl-di-Lewis^a and sialyl-Lewis^{a-x}. The binding member may be specific for sialyl-di-Lewis^a. In one embodiment, the binding member may be specific for sialyl-di-Lewis^a and sialyl-Lewis^{a-x} present in tumour tissue. The binding member may not bind, or may not significantly bind, mono-sialyl-Lewis^a bound to a glycolipid. Additionally or alternatively, the binding member may not bind, or may not significantly bind, di-sialyl-

CLAIMS

1. An isolated antibody or antibody fragment, wherein the antibody or antibody fragment binds specifically to sialyl-di-Lewis^a and sialyl-Lewis^{a-x} and mono-sialyl-Lewis^a,
 5 wherein the mono-sialyl-Lewis^a is bound to a glycoprotein, and does not bind to mono-sialyl-Lewis^a bound to a glycolipid, and wherein the antibody or antibody fragment comprises the following six CDRs:

- a) GFTFNTYA (CDRH1), IRSKSNNYAT (CDRH2) and VYGSGGNY (CDRH3); and
 10 b) QSLNLSGNQKNY (CDRL1), WAS (CDRL2) and QNDYSSPFT (CDRL3).

2. The antibody or antibody fragment according to claim 1, wherein the mono-sialyl-Lewis^a is linked to the protein by a glycan chain comprising at least 4 glycan monomer units.

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3. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment has (i) a heavy chain amino acid sequence of:

MLLGLKWVFFVVFYQGVHCEVQLVESGGGLVQPKGSLKLSCAASGFTFNT
 YAMNWRQAPGKGLEWVARIRSKSNNYATYYADSVKDRFTISRDDSQSML
 20 YLQMNNLKKEDTAMYYCVGYGSGGNYWGQGTSTVTVSSAKTTPPSVYPLAP
 GSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLES DLYTLS
 SSVTVPPSSRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFI
 FPPKPKDVLITLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREE
 QFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
 25 VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIM
 NTNGSYFVYSKLVNPKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

and a light chain amino acid sequence of:

MESQTQVLMSLLFWVSTCGDIVMTQSPSSLTVTAGEKVTMSCKSSQSLL
 NSGNQKNYLTWYQQKPGQPPKVLIIYWASTRESGVP DRFTGSGSGTDFTLT
 30 ISSVQAEDLAVYYCQNDYSSPFTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSG
 GASVVCFLNFPKDIKWKIDGSEKQNGVLNSWTDQDSKSTYSMSSTL
 TLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

or (ii) the antibody has a heavy chain amino acid sequence of:

MLLGLKWVFFVVFYQGVHCEVQLVESGG GLVQPKGSLKLSCAASGFTF
 NTYAMNWVRQAPGKGLEWVARIRSKSNNYATYYADSVKDRFTISRDDSQS
 MLYLQMNNLKKEDTAMYYCVGYGSGGNYWGQGTSTVTVSSASTKGPSVFP
 5 LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
 10 QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
 TQKSLSLSPGK

and a light chain amino acid sequence of:

MESQTQVLMSLLFWVSTCGDIVMTQSPSSLTVTAGEKVTMSCKSSQSLL
 NSGNQKNYLTWYQQKPGQPPKVLIIYWASTRESGVP DRFTGSGSGTDFTLT
 15 ISSVQAEDLAVYYCQNDYSSPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSG
 TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYLSSTL
 TLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

or a fragment thereof.

20 4. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment is bispecific.

5. The antibody or antibody fragment according to claim 4, wherein the bispecific antibody or antibody fragment is additionally specific for CD3.

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6. The antibody or antibody fragment according to any preceding claim, wherein the antibody comprises a light chain variable sequence comprising CDRL1, CDRL2 and CDRL3 and a heavy chain variable sequence comprising CDRH1, CDRH2 and CDRH3.

30 7. The antibody or antibody fragment according to any preceding claim, wherein the CDRs are carried by a human antibody framework.

8. The antibody or antibody fragment according to any preceding claim, wherein the

antibody or antibody fragment comprises a VH domain comprising the amino acid sequence of:

a)

5 EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRS
KSNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKKEDTAMYYCVGYGSGG
NYWGQGTSVTVS; or

b)

EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRS
10 KSNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKKEDTAMYYCVGYGSGG
NYWGQGTSVTVS;

and a VL domain comprising the amino acid sequence:

15 c)

DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPPKVLII
YWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSSPFTFGSGTK
LEIKR; or

d)

20 DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPPKVLII
WASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSSPFTFGSGTKLEI
KR.

9. The antibody or antibody fragment according to any preceding claim, wherein the
25 antibody or antibody fragment comprises a human antibody constant region.

10. The antibody or antibody fragment according to any preceding claim, wherein the
antibody fragment is a Fab, (Fab')₂, scFv, Fv, dAb, Fd or a diabody.

30 11. The antibody or antibody fragment according to any preceding claim, wherein the
antibody fragment is an scFv comprising, in the following order, 1) leader sequence, 2)
heavy chain variable region, 3) 3x GGGGS spacer, 4) light chain variable region, and 5)
poly-Ala and a 6x His tag for purification.

12. The antibody or antibody fragment according to any of claims 1 to 10, wherein the antibody fragment is an scFv comprising, in the following order, 1) leader sequence, 2) light chain variable region, 3) 3x GGGGS spacer, and 4) heavy chain variable region,
5 optionally further comprising either 5' or 3' purification tags.

13. The antibody or antibody fragment according to any preceding claim, wherein the antibody fragment is provided in the form of a chimeric antigen receptor (CAR).

10 14. The antibody or antibody fragment according to claim 13, wherein the antibody fragment is an scFv provided in the form of a chimeric antigen receptor (CAR) either in the heavy chain-light chain orientation or the light chain-heavy chain orientation.

15 15. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment is monoclonal.

16. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment is a humanised, chimeric or veneered antibody.

20 17. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment is a drug conjugate, such as an antibody-drug conjugate (ADC).

25 18. The antibody or antibody fragment according to any preceding claim, for use as a medicament.

19. The antibody or antibody fragment according to any of claims 1 to 17, for use in the prevention, treatment or diagnosis of cancer, wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.

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20. Use of an antibody or antibody fragment according to any of claims 1 to 17 in the manufacture of a medicament for treating or preventing cancer, wherein the medicament is

to be administered to a subject in need of thereof, wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.

21. The antibody or antibody fragment for the use of claims 18-20, wherein the
5 antibody or antibody fragment is to be administered, or arranged to be administered, alone or in combination with other treatments.

22. A nucleic acid comprising a sequence encoding an antibody or antibody
fragment according to any of claims 1-17.

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23. The nucleic acid according to claim 22, wherein the nucleic acid is a construct in
the form of a plasmid, vector, transcription or expression cassette.

24. A recombinant host cell which comprises the nucleic acid according to claim 22
15 or claim 23.

25. A method for diagnosis of cancer comprising using an antibody or antibody
fragment as claimed in any of claims 1 to 17 to detect the glycans sialyl-di-Lewis^a and
sialyl-Lewis^{a-x} and/or mono-sialyl-Lewis^a only attached to a glycoprotein in a sample
20 obtained from an individual.

26. The method according to claim 25, wherein the pattern of glycans detected by the
antibody or antibody fragment is used to stratify therapy options for the individual.

25 27. A pharmaceutical composition comprising the antibody or antibody fragment
according to any of claims 1 to 17, and a pharmaceutically acceptable carrier.

28. The pharmaceutical composition according to claim 27, further comprising at least
one other active ingredient.

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29. The pharmaceutical composition according to claim 27 or claim 28, for use in the
treatment of cancer wherein the cancer is colorectal cancer, gastric cancer, pancreatic
cancer, lung cancer, ovarian cancer or breast cancer.