

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 capable of binding sialyl-di-Lewis^a, wherein the binding member does not bind to mono-sialyl-Lewis^a bound to a glycolipid.

In another aspect of the invention, there is provided an antibody or antibody fragment which competes for binding to the same epitope as an antibody or antibody fragment as described herein, and optionally wherein the antibody or antibody fragment is in the same form as the binding member as described herein.

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.

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.

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-Lewie^{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.

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 capable of binding sialyl-di-Lewis^a, wherein the binding member does not bind to mono-sialyl-Lewis^a bound to a glycolipid.
2. The antibody or antibody fragment according to claim 1, wherein the antibody or antibody fragment is specific for sialyl-di-Lewis^a and sialyl-Lewis^{a-x}.
3. The antibody or antibody fragment according to claim 1 or claim 2, wherein the antibody or antibody fragment is specific for sialyl-di-Lewis^a, sialyl-Lewis^{a-x} and mono-sialyl-Lewis^a, wherein the mono-sialyl-Lewis^a is bound to a glycoprotein.
4. The antibody or antibody fragment according to claim 3, wherein the mono-sialyl-Lewis^a is linked to the protein by a glycan chain comprising at least 4 glycan monomer units.
5. 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
 YAMNWVRQAPGKGLEWVARIRSKSNNYATYYADSVKDRFTISRDDSQSML
 YLQMNNLKKEDTAMYYCVGYGSGGNYWGQGTSTVTSSAKTTPPSVYPLAP
 GSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLESPLYTLT
 SSVTVPSRPSETVTCNVAHPASSTKVDKIVPRDCGCKPCICTVPEVSSVFI
 FPPKPKDVLITLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREE
 QFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
 VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIM
 NTNGSYFVYSKLVNPKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

 and a light chain amino acid sequence of:

MESQTQVLMSLLFWVSTCGDIVMTQSPSSLTVTAGEKVTMSCKSSQSLL
 NSGNQKNYLTWYQQKPGQPPKVLIIWASTRESGVP DRFTGSGSGTDFTLT
 ISSVQAEDLAVYYCQNDYSSPFTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSG
 GASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKDYSTYSMSSTL

TLTKDEYERHNSYTCEATHKTSTSPIVKSFNRECE

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

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

and a light chain amino acid sequence of:

15 MESQTQVLMSLLFWVSTCGDIVMTQSPSSLT VTAGEKVTMSCKSSQSLL
 NSGNQKNYLTWYQQKPGQPPKVLIIWASTRESGVP DRFTGSGSGTDFTLT
 ISSVQAEDLAVYYCQNDYSSPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSG
 TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL
 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20 or a fragment thereof.

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

25 7. The antibody or antibody fragment according to claim 6, wherein the bispecific antibody or antibody fragment is additionally specific for CD3.

8. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment comprises the following six CDRs:

30 a) 26 to 33 (CDRH1), 50-59 (CDRH2) and 98-106 (CDRH3) of Figure 1a or 2a; and
 b) 27 to 38 (CDRL1), 56-58 (CDRL2) and 95-103 (CDRL3) of Figure 1b or 2b.

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

antibody comprises a light chain variable sequence comprising LCDR1, LCDR2 and LCDR3, wherein

LCDR1 comprises QSLNSGNQKNY,

LCDR2 comprises WAS, and

5 LCDR3 comprises QNDYSSPFT; and

a heavy chain variable sequence comprising HCDR1, HCDR2 and HCDR3, wherein

HCDR1 comprises GFTFNTYA

HCDR2 comprises IRSKSNNYAT, and

HCDR3 comprises VGYGSGGNY.

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10. The antibody or antibody fragment according to any preceding claim, wherein the binding domain(s) are carried by a human antibody framework.

11. The antibody or antibody fragment according to any preceding claim, wherein the
15 antibody or antibody fragment comprises a VH domain comprising residues 1 to 117 of the amino acid sequence of Figure 1a or 2a, and a VL domain comprising residues 1 to 114 of the amino acid sequence of Figure 1b or 2b.

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

13. 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.

25 14. 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.

30 15. The antibody or antibody fragment according to any of claims 1 to 13, 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, optionally further comprising either 5' or 3' purification tags.

16. 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).
- 5 17. The antibody or antibody fragment according to claim 16, 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.
18. The antibody or antibody fragment according to any preceding claim, wherein the
10 antibody or antibody fragment is monoclonal.
19. The antibody or antibody fragment according to any preceding claim, wherein the antibody or antibody fragment is a human, humanised, chimeric or veneered antibody.
- 15 20. 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).
21. An antibody or antibody fragment which competes for binding to the same epitope
20 as an antibody or antibody fragment according to any one of claims 1 to 20, and optionally wherein the antibody or antibody fragment is in the same form as the binding member according to any of claims 1 to 20.
22. An antibody or antibody fragment according to any preceding claim, for use as a
25 medicament.
23. An antibody or antibody fragment according to any of claims 1 to 21, for use in the prevention, treatment or diagnosis of cancer.
- 30 24. Use of an antibody or antibody fragment according to any of claims 1 to 21 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.

25. The antibody or antibody fragment for the use of claims 22 or 23, or the use of claim 24, wherein the cancer is colorectal cancer, gastric cancer, pancreatic cancer, lung cancer, ovarian cancer or breast cancer.
- 5 26. The antibody or antibody fragment for the use of claims 22, 23 or 25, or the use of claim 24 or claim 25, wherein the antibody or antibody fragment is to be administered, or arranged to be administered, alone or in combination with other treatments.
- 10 27. A nucleic acid comprising a sequence encoding an antibody or antibody fragment according to any of claims 1-21.
28. The nucleic acid according to claim 27, wherein the nucleic acid is a construct in the form of a plasmid, vector, transcription or expression cassette.
- 15 29. A recombinant host cell which comprises the nucleic acid according to claim 27 or claim 28.
30. A method for diagnosis of cancer comprising using an antibody or antibody fragment as claimed in any of claims 2 to 21 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.
- 20 31. The method according to claim 30, wherein the pattern of glycans detected by the antibody or antibody fragment is used to stratify therapy options for the individual.
- 25 32. A pharmaceutical composition comprising the antibody or antibody fragment according to any of claims 1 to 21, and a pharmaceutically acceptable carrier.
- 30 33. The pharmaceutical composition according to claim 32, further comprising at least one other pharmaceutical active.
34. The pharmaceutical composition according to claim 32 or claim 33, for use in the treatment of cancer.