

ANTI OLIGOSACCHARIDE ANTIBODY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore application No.
5 10201702572P, filed 29 March 2017, the contents of it being hereby incorporated by
reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention is in the field of antibodies, in particular antibodies specific for
10 oligosaccharides of the Lewis X type.

BACKGROUND OF THE INVENTION

[0003] Hematological malignancies encompass a wide spectrum of cancers from
hematopoietic and lymphoid tissues. Under this grouping, the various forms of leukemia,
15 lymphoma and myeloma have been classified into more than 60 distinct disease types, each
having particular clinical features and disease outcomes. Among these diseases, curative
treatments with high long-term survival rate have been developed for some, e.g. Chronic
Myeloid Leukemia, while such effective regimens are still unavailable for many others, e.g.
Multiple Myeloma (MM) and Acute Myeloid Leukemia (AML).

20 [0004] Accordingly, there is a need for more effective treatment regimens for leukemia,
lymphoma and myeloma.

SUMMARY

[0005] According to one aspect, there is provided an antigen-binding protein, or an
antigen-binding fragment thereof, comprising (i) a heavy chain variable domain comprising a
25 VHCDR1 having the amino acid sequence GYTFTSYWIH (SEQ ID NO:1); a VHCDR2
having the amino acid sequence EINPSNGRTNFNEKFKN (SEQ ID NO:2) and a VHCDR3
having the amino acid sequence VDYDEAY (SEQ ID NO:3); and (ii) a light chain variable
domain comprising a VLCDR1 having the amino acid sequence RSSKSLHNSGITYLY
(SEQ ID NO:4), a VLCDR2 having the amino acid sequence QMSNLAS (SEQ ID NO:5),
30 and a VLCDR3 having the amino acid sequence AQNLELWT (SEQ ID NO:6).

[0006] According to another aspect, there is provided a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the antigen-binding protein, or an antigen-binding fragment thereof, as described herein.

[0007] According to another aspect, there is provided a use of an antigen-binding protein, or an antigen-binding fragment thereof, as described herein or composition as described herein, in the manufacture of a medicament for treating or preventing cancer.

[0008] According to another aspect, there is provided a method for detecting cancer in a subject, the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as described herein *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine the level of binding in the sample, wherein an increase in the level of binding in the sample relative to the control sample is indicative of cancer.

[0009] According to another aspect, there is provided a method for identifying a subject susceptible to cancer the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as described herein *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine the level of binding in the sample, wherein an increase in the level of binding in the sample relative to the control sample indicates that the subject is susceptible to cancer.

[0010] According to another aspect, there is provided a kit when used in the method as described herein, comprising an antigen-binding protein, or antigen-binding fragment thereof as described herein, together with instructions for use.

DEFINITIONS

[0011] The term “antigen binding protein” as used herein refers to antibodies, antibody fragments and other protein constructs, such as domains, which are capable of binding to SLAMF7.

[0012] The term “antibody” is used herein in the broadest sense to refer to molecules with an immunoglobulin-like domain and includes monoclonal, recombinant, polyclonal, chimeric, humanised, bispecific and heteroconjugate antibodies; a single variable domain, a domain antibody, antigen binding fragments, immunologically effective fragments, single

chain Fv, diabodies, Tandabs™, etc (for a summary of alternative “antibody” formats see Holliger and Hudson, Nature Biotechnology, 2005, Vol 23, No. 9, 1126-1136).

[0013] The phrase “single variable domain” refers to an antigen binding protein variable domain (for example, V_H , V_{HH} , V_L) that specifically binds an antigen or epitope independently of a different variable region or domain.

[0014] A “domain antibody” or “dAb” may be considered the same as a “single variable domain” which is capable of binding to an antigen. A single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and *Camelid* V_{HH} dAbs. Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanised according to standard techniques available in the art, and such domains are considered to be “domain antibodies”. As used herein V_H includes camelid V_{HH} domains.

[0015] As used herein the term “domain” refers to a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. A “single variable domain” is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain. A domain can bind an antigen or epitope independently of a different variable region or domain.

[0016] An antigen binding fragment may be provided by means of arrangement of one or more CDRs on non-antibody protein scaffolds such as a domain. The domain may be a domain antibody or may be a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4, lipocalin, SpA, an Affibody, an avimer, GroEl, transferrin, GroES and fibronectin/adnectin, which has been subjected to protein engineering in order to obtain binding to an antigen other than the natural ligand.

[0017] Neutralisation may be determined or measured using one or more assays known to the skilled person or as described herein. For example, antigen binding protein binding to SLAMF7 can be assessed in a sandwich ELISA, by BIAcoreTM, FMAT, FORTEbio, or similar *in vitro* assays.

- 5 [0018] An antigen binding fragment or an immunologically effective fragment may comprise partial heavy or light chain variable sequences. Fragments are at least 5, 6, 8 or 10 amino acids in length. Alternatively the fragments are at least 15, at least 20, at least 50, at least 75, or at least 100 amino acids in length.

[0019] The term “specifically binds” as used throughout the present specification in
10 relation to antigen binding proteins means that the antigen binding protein binds to an oligosaccharide of the Lewis X type, with no or insignificant binding to other (for example, unrelated) oligosaccharides. The antibody described herein does not bind to sialylated Lewis X.

[0020] The term “an oligosaccharide of the Lewis X type” is intended to encompass
15 subsets of the Lewis X oligosaccharide including bioactive conformations of different forms of the Lewis X oligosaccharide epitope. The term “bioactive conformation” is intended to encompass the relative orientation of the sugar rings and their side chain orientations with respect to the dihedral angles ϕ and ψ around the glycosidic linkages in order to define a particular conformation of an oligosaccharide. It is an advantage of the antigen binding
20 protein as described herein that it can bind to a bioactive conformation of the Lewis X oligosaccharide epitope different to that of commercially available (for example, anti-SSEA1 antibody) anti-Lewis X antibodies.

[0021] “CDRs” are defined as the complementarity determining region amino acid
sequences of an antigen binding protein. These are the hypervariable regions of
25 immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, “CDRs” as used herein refers to all three heavy chain CDRs, all three light chain CDRs, all heavy and light chain CDRs, or at least two CDRs.

[0022] Throughout this specification, amino acid residues in variable domain sequences
30 and full length antibody sequences are numbered according to the Kabat numbering convention. Similarly, the terms “CDR”, “CDRL1”, “CDRL2”, “CDRL3”, “CDRH1”,

“CDRH2”, “CDRH3” used in the Examples follow the Kabat numbering convention. For further information, see Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987).

[0023] It will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full length antibody sequences. There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia et al. (1989) Nature 342: 877-883. The structure and protein folding of the antibody may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

[0024] Other numbering conventions for CDR sequences available to a skilled person include “AbM” (University of Bath) and “contact” (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the “minimum binding unit”. The minimum binding unit may be a sub-portion of a CDR.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0026] **Fig. 1** shows the schematic workflow of mouse immunization, hybridoma fusion and screening.

[0027] **Fig. 2** shows the binding of SLAM01 (1E10) and commercial anti-CD319 on MM cell lines: KMS28/BM and KMS11. SLAM01 binds only to KMS28/BM but not KMS11, despite the expression of CD319 (SLAMF7) on both cell lines.

[0028] **Fig. 3** shows an immunoblot of SLAM01 and anti-CD319 on ectodomain of CD319. SLAM01 only recognized a high molecular weight form (>140 kDa) of CD319 ectodomain under non-reducing condition (N), but not in reducing (R) condition. SLAM01 does not recognise the predicted size of SLAMF7 between 40 to 50 kDa (as probed by anti-CD319).

[0029] **Fig. 4** shows an immunoblot of SLAM01 and anti-CD319 on KMS28/BM lysate. SLAM01 recognized the high molecular weight form and a 40 kDa form of CD319 on

KMS28/BM lysate and not the expected size of the smear of full length CD319, which is between 40 to 65 kDa.

[0030] **Fig. 5** shows an immunoblot of SLAM01-immunoprecipitated (IP) product by anti-CD319. KMS28/BM lysate was immunoprecipitated with SLAM01 and the eluate was immunoblotted with anti-CD319. The high molecular weight band was observed on lane 1 (IP eluate) and lane 5 (KMS28/BM lysate).

[0031] **Fig. 6** summarizes the binding profile of SLAM01 and anti-CD319 on various hematological cell lines. SLAM01 and Anti-CD319 was screened against 7 MM lines, 1 Burkitt's Lymphoma line and 14 AML lines. SLAM01 preferentially binds to AML lines but not MM or B cell lines.

[0032] **Fig. 7** summarizes the binding profile of SLAM01 on solid tumour cell lines. SLAM01 is positive for non-small cell lung cancer cell line PC9; ovarian cancer cell lines OVCAR3 and SKOV3.

[0033] **Fig. 8** summarizes the binding profile of SLAM01 on normal cell lines. SLAM01 does not bind to various normal cell lines tested.

[0034] **Fig. 9** summarizes the immunohistochemistry binding profile of SLAM01 on in-house formalin-fixed paraffin-embedded (FFPE) cell line array. SLAM01 was negative for the normal cell lines. Besides being positive (2+ and 3+) for the AML line (F3), SLAM01 is also positive for some non-small cell lung cancer (NSCLC), ovarian, breast, gastric and colorectal cancer lines.

[0035] **Fig. 10** shows the immunohistochemistry staining of SLAM01 on Mono-Mac-1 (AML) and PC9 (NSCLC), both cores from in house FFPE cell line array. SLAM01 binds strongly to Mono-Mac-1 (3+) and PC9 (3+).

[0036] **Fig. 11** shows the isotype of SLAM01. SLAM01 was determined to be an IgM with κ light chain.

[0037] **Fig. 12** shows an immunoblot of SLAM01 on pronase-treated AML3 lysate. AML3 lysate was treated with pronase to digest the proteins in it. Control lysate was set up without the pronase enzyme. After digestion, the lysates were dotted onto membrane and allowed to dry before immunoblot with either anti-CD319 mAb, anti-SSEA1 mAb, anti- β -actin mAb or SLAM01. Binding of SLAM01 on pronase-treated lysate was similar to that of control, indicating that the binding of SLAM01 was not affected with the loss of protein and structure. This demonstrated that SLAM01 is binding to a glycan epitope.

[0038] **Fig. 13** shows an immunoblot of SLAM01 on OCI-AML3 lysate treated with PNGase. OCI-AML3 lysate was treated with PNGase F to remove N-linked glycan. Binding of SLAM01 to OCI-AML3 lysate was lost after PNGase F treatment; concluding that SLAM01 binding requires N-linked glycan on the antigen.

5 [0039] **Fig. 14** shows the competitive binding of SLAM01 on various AML cell lines. SLAM01 was pre-incubated with free Lewis X (Le^{X}), Lewis Y (Le^{Y}), Lewis A (Le^{A}) and Lewis B (Le^{B}) separately for 30 min. Each SLAM01-glycan mix was then tested for binding against five AML cell lines (AML2, AML3, KG1, MOLM14 and Mono-Mac-1). Only SLAM01 pre-incubated with Le^{X} displayed significantly reduced binding percentage as
10 compared to positive control (SLAM01 without glycan) across all 5 lines. This concludes that SLAM01 recognizes glycan with terminal Le^{X} structure.

[0040] **Fig. 15** shows the binding profile of SLAM01-pHrodo Red conjugate on AML2 and AML3. pHrodo Red dye fluoresces in acidic condition such as in endosome. AML2 and AML3 that were incubated with pHrodo Red conjugated SLAM01 for 60 min at room
15 temperature displayed increased fluorescence as compared to control. This indicates that SLAM01-pHrodo Red dye was internalised into endosome.

[0041] **Fig. 16** shows the relative viability of AML-2 and -3 after treating with SLAM01-Mab-ZAP complex for 3 days. Mab-ZAP is a secondary anti-mouse antibody conjugated with saporin. Saporin when internalised into the cytoplasm affects protein translation and results in
20 cell death. A reduced cell viability was observed only for cells treated with SLAM01-Mab-Zap complex (1st column from right). This concludes that SLAM01 can deliver a toxic payload into cells resulting in cell killing as an ADC.

[0042] **Fig. 17** highlights the positive correlation of SLAM01 binding percentage and relative cytotoxicity on AML cell lines. SLAM01 was screened against these AML cell lines.
25 Data on average binding percentage were collected via flow cytometry, while data on relative cytotoxicity were determined via Mab-Zap assay. The cell lines that were positive for SLAM01 binding (average binding percentage >20%) exhibit high cytotoxicity percentage when treated with SLAM01-Mab-Zap complex.

[0043] **Fig. 18A** shows the relative viability of AML-2 and -3 after treating with
30 SLAM01-Mab-ZAP complex for 3 days. Both AML cell lines were treated with various conditions as shown. The addition of Le^{X} to the SLAM01-Mab-Zap complex (1st column

from right) rescued the cells from the cytotoxic effect of SLAM01-Mab-Zap complex (2nd column from right).

[0044] **Fig. 18B** shows Binding histograms of SLAM01 on OCI-AML3, MONO-MAC-1 and PBMC. SLAM01 was incubated on OCI-AML3, MONO-MAC-1 and peripheral blood mononuclear cells (PBMCs). SLAM01 was positive for OCI-AML3 and MONO-MAC-1, while negative for PBMC.

[0045] **Fig. 19** shows the competitive inhibition flow cytometry of cSLAM01 and SLAM01 on AML3 and Mono-Mac-1. Competitive inhibition flow cytometry was set up between cSLAM01 and SLAM01. For the competing sample, SLAM01 was pre-incubated with the cells for 30 min at 4°C, before the addition of cSLAM01. The reduced binding histograms of cSLAM01 on the competing sample demonstrate that SLAM01 is blocking the binding of cSLAM01 to the epitope.

[0046] **Fig. 20** shows the binding histogram of cSLAM01 and SLAM01 on AML3, Mono-Mac-1, hME-1 and MRC5. Binding profile of cSLAM01 and SLAM01 on the cell lines was compared. cSLAM01 retained binding to SLAM01-positive cell lines, and not to SLAM01-negative cell lines.

[0047] **Fig. 21** shows the relative viability of AML-3 and Mono-Mac-1 after treating with various cSLAM01/Ig-toxin complex for 3 days. cSLAM01 was complexed with either Mab-ZAP, Ig-Monomethyl auristatin E (MMAE) or Ig-DM1 before treating AML3 and Mono-Mac-1. Controls were set up without cSLAM01. The reduced cell viability observed on cells treated with cSLAM01/Ig-toxin as compared to control, demonstrated that cSLAM01 retained the ADC potential.

[0048] **Fig. 22** shows the binding histogram of cSLAM01 and cSLAM01-MMAE on AML3 and Mono-Mac-1. Binding profile of cSLAM01-MMAE is similar to that of cSLAM01.

[0049] **Fig. 23** shows the dose response curve of AML-3 and Mono-Mac-1 after treating with cSLAM01-MMAE for 3 days. AML3 and Mono-Mac-1 were treated with increasing dose of cSLAM01-MMAE. cSLAM01-MMAE demonstrated its ADC effect on both AML3 and Mono-Mac-1.

[0050] **Fig. 24** shows a flow cytometry histogram of SLAM01 and anti-CD319 on PC9 and OVCAR3 cell lines. PC9 (non-small cell lung cancer cell line) and OVCAR3 (epithelial

ovarian cancer cell line) were incubated with either SLAM01 or anti-CD319 to test for expression of the respective antigen on cell surface.

[0051] **Fig. 25** shows western blots of SLAM01, anti-CD319 and anti-Lewis-X/SSEA1 on SLAM01-immunoprecipitated proteins. OCI-AML3 membrane protein extracts (MPXs) were immunoprecipitated with SLAM01. The immunoprecipitated proteins were resolved via SDS-PAGE and immunoblotted with SLAM01, anti-CD319 and anti-Lewis-X/SSEA1.

[0052] **Fig. 26** shows a 2D representation of glycan structures that were positive for SLAM01.

[0053] **Fig. 27** shows a 2D representation of glycan structures that were negative for SLAM01.

[0054] **Fig. 28** shows an immunohistochemical staining of SLAM01 and anti-Lewis-X/SSEA1 on FFPE cell line array.

[0055] **Fig. 29** shows binding histograms of SLAM01 and anti-Lewis-X/SSEA1 on A549, Colo205, H1299 and HCC827. SLAM01 and anti-Lewis-X/SSEA1 were incubated on A549 (lung cancer cell line), Colo205 (colorectal cancer cell line), H1299 (lung cancer cell lines) and HCC827 (lung cancer cell line) to determine their cell surface expression profiles.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0056] In a first aspect the present invention refers to an antigen-binding protein, or an antigen-binding fragment thereof, comprising (i) a heavy chain variable domain comprising a VHCDR1 having the amino acid sequence GYTFTSYWIH (SEQ ID NO:1); a VHCDR2 having the amino acid sequence EINPSNGRTNFNEKFKN (SEQ ID NO:2) and a VHCDR3 having the amino acid sequence VDYDEAY (SEQ ID NO:3); and (ii) a light chain variable domain comprising a VLCDR1 having the amino acid sequence RSSKSLLSNGITYLY (SEQ ID NO:4), a VLCDR2 having the amino acid sequence QMSNLAS (SEQ ID NO:5), and a VLCDR3 having the amino acid sequence AQNLELWT (SEQ ID NO:6).

[0057] The antigen-binding protein, or antigen-binding fragment thereof, may comprise heavy and light chain CDR regions that are about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% identical to the heavy and light chain CDR regions of (i) and (ii).

[0058] The heavy chain variable region may comprise the amino acid sequence QVKLQQSGAELAKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLEWIGEINPSNG

RTNFNEKFKNKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARVDYDEAYWGQGT
 VTVSS as set forth in SEQ ID NO:7.

[0059] In some embodiments, the heavy chain variable region comprises an amino acid sequence having about 80%, about 85%, about 90%, about 95%, about 96%, about 97%,
 5 about 98% or about 99% identity to the amino acid sequence set forth in SEQ ID NO:7.

[0060] In some embodiments, the light chain variable region may comprise the amino acid sequence

DIVMTQAAAFSNPVTLGTSASISCRSSKSLHNSNGITYLYWYLQKPGQSPQLLIYQMSN
 LASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELWTFGGGTKLEIK as set
 10 forth in SEQ ID NO:8.

[0061] The light chain variable region may comprise an amino acid sequence having about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% identity to the amino acid sequence set forth in SEQ ID NO:8.

[0062] In one embodiment, the antigen binding protein is selected from the group
 15 consisting of monoclonal, recombinant, polyclonal, chimeric, humanised, bispecific and heteroconjugate antibodies; a single variable domain, a domain antibody, antigen binding fragments, immunologically effective fragments, single chain Fv, a single chain antibody, a univalent antibody lacking a hinge region, a minibody, diabodies, and Tandabs™.

[0063] In some embodiments, the binding protein is a monoclonal antibody. In one
 20 embodiment the monoclonal antibody is SLAM01.

[0064] The monoclonal antibody described herein may be humanised. Alternatively, the monoclonal antibody is chimeric.

[0065] In one embodiment, the antigen-binding protein, or antigen-binding fragment thereof, as described herein, binds to an N-linked glycan. The N-linked glycan maybe
 25 located on an extracellular portion of SLAMF7. The N-linked glycan may be an oligosaccharide of the Lewis X type. In some embodiments, the antigen-binding protein, or antigen-binding fragment thereof, as described herein, does not bind to an oligosaccharide of the sialylated Lewis X type.

[0066] In one embodiment, the antigen-binding protein, or antigen-binding fragment
 30 thereof, as described herein may comprise a radioisotope or a cytotoxin conjugated thereto.

[0067] The antigen-binding protein, or antigen-binding fragment thereof, as described herein may be conjugated with a cytotoxin selected from the group consisting of monomethyl

auristatin E (MMAE-1), mertansine (DM-1), saporin, gemcitabine, irinotecan, etoposide, vinblastine, pemetrexed, docetaxel, paclitaxel, platinum agents (for example, cisplatin, oxaliplatin and carboplatin), vinorelbine, capecitabine, mitoxantrone, ixabepilone, eribulin, 5-fluorouracil, trifluridine and tipiracil.

5 [0068] In some embodiments, the antigen-binding protein, or an antigen-binding fragment thereof, may be internalized into a cell upon binding to said N-linked glycan on SLAMF7.

[0069] In some embodiments, the antigen-binding protein, or an antigen-binding fragment may selectively binds to a non-small cell lung cancer cell, an ovarian cancer cell, a breast cancer cell, an acute myeloid leukaemia cell and a colorectal cancer cell.

10 [0070] In another aspect the present disclosure provides a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the antigen-binding protein, or an antigen-binding fragment thereof, as described herein.

[0071] In some embodiments, the composition as described herein may comprise one or more further therapeutic compounds.

15 [0072] In another aspect, the present disclosure provides the use of an antigen-binding protein, or an antigen-binding fragment thereof, as described herein or composition as described herein, in the manufacture of a medicament for treating or preventing cancer.

[0073] The cancer may be selected from the group consisting of non-small cell lung cancer, ovarian cancer, breast cancer, acute myeloid leukaemia and colorectal cancer.

20 [0074] In one embodiment, the medicament may be administered with one or more further active pharmaceutical ingredients.

[0075] In another embodiment, the medicament may be administered with chemotherapy.

[0076] The one or more further pharmaceutical agents or chemotherapy may be administered separately, simultaneously or sequentially with said medicament.

25 [0077] In another aspect, the present disclosure provides a method for detecting cancer in a subject, the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as described herein *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine
30 the level of binding in the sample, wherein an increase in the level of binding in the sample relative to the control sample is indicative of cancer.

[0078] In another aspect, the present disclosure provides a method for identifying a subject susceptible to cancer the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as described herein *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding
5 fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine the level of binding in the sample, wherein an increase in the level of binding in the sample relative to the control sample indicates that the subject is susceptible to cancer.

[0079] In some embodiments, the control sample may be from the same subject.
10 Alternatively, the control sample may be from a different subject.

[0080] The antigen-binding protein, or antigen-binding fragment thereof, may comprise a detectable label. In some embodiments, the detectable label may be selected from the group consisting of a fluorescent label, a chemiluminescent label, an enzymatic label and a radionuclide label. The detectable label may be selected from the group consisting of biotin,
15 alkaline phosphatase, horseradish peroxidase, FITC, PE and Cy Dyes.

[0081] In some embodiments, the detectable label may be detected in an assay selected from flow cytometry, tissue section, immunofluorescence, immunocytochemistry or immunohistochemistry.

[0082] In one embodiment, the cancer may be selected from the group consisting of non-
20 small cell lung cancer, ovarian cancer, breast cancer, acute myeloid leukaemia and colorectal cancer.

[0083] In another aspect, the present disclosure provides a kit when used in the method as described herein, comprising an antigen-binding protein, or antigen-binding fragment thereof as described herein, together with instructions for use.

[0084] The invention illustratively described herein may suitably be practiced in the
25 absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention
30 in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the

present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

5 [0085] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

10 [0086] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

15

EXPERIMENTAL SECTION

[0087] Non-limiting examples of the invention and comparative examples will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

[0088] **Example 1. Immunization and generation of mAbs**

20 [0089] In this study, a panel of monoclonal antibodies (mAbs) was raised against SLAMF7 (CD319), a cell surface marker that is associated to MM. To generate these mAbs, 4 female BALB/c mice were immunized with recombinant human SLAMF7 (CD319) purchased from Sino Biological Inc. (Catalogue No. 11691-H08H) (Fig. 1). The DNA sequence that encodes the recombinant human SLAM7 (CD319), consists of an extracellular domain (Met1 to Met226) fused with a polyhistidine tag at the C-terminus, that was
25 expressed via human host cells.

[0090] Mice immunization started on 06 Feb 2014, and this was followed by an immunisation regime consisting of 5 weekly immunisations and 1 booster immunisation. Upon completion of the immunisation regime, plasma B cells were harvested and fused with
30 SP2/0 mouse myeloma lines on 13 March 2014 (Fig. 1). Hybridoma fusion was performed using STEMCELL Technologies ClonaCell™-HY kit and generated a panel of hybridomas that produces mAbs present in the culture supernatant. The mAbs were screened by flow

cytometry for binding with AML, MM, other cancer and normal cell lines, that subsequently identified a novel anti-SLAMF7 antibody, SLAM01.

[0001] **Example 2. Binding profile of SLAM01 mAb**

[0002] Flow cytometry analysis of 1E10 mAb (SLAM01) on MM cell lines expressing
5 CD319 (SLAMF7) indicates binding of SLAM01 to KMS28/BM but not KMS11 (Fig. 2). The binding between SLAM01 and high molecular weight form (>140 kDa) of CD319 ectodomain was observed by immunoblotting under non-reducing condition only (Fig. 3). Based on the immunoblot probed by anti-CD319 Ab, SLAM01 also does not recognize the predicted size of SLAMF7 between 40 to 50 kDa. The recognition of SLAM01 to the full
10 length form of CD319 was assessed by immunoblotting SLAM01 with KMS28/BM lysate under both non-reducing and reducing conditions (Fig. 4). The immunoblot shows that SLAM01 recognized both the high molecular weight and 40 kDa forms of CD319. However, SLAM01 does not recognize the entirety of the full length form of CD319 that appears as a smear (40 to 65 kDa) on the immunoblot with anti-CD319. To further confirm the
15 recognition of SLAM01 to CD319, KMS28/BM lysate was immunoprecipitated (IP) with SLAM01 and the eluate was immunoblotted with anti-CD319 Ab (Fig. 5). The immunoblot indicated binding of anti-CD319 Ab to the high molecular weight form of CD319 in both IP eluate (lane 1) and KMS28/BM lysate (lane 5). Thus, SLAM01 recognized the high molecular weight form of CD319.

20 [0003] Among the mAbs, SLAM01 was surprisingly found to preferentially bind to SLAMF7 expressed on AML cell lines rather than MM (Fig. 6). In addition, SLAM01 demonstrates reactivity to several ovarian and NSCLC cell lines by flow cytometry (Fig. 7). SLAM01 does not have non-specific binding to fibroblast, epithelial cell lines, adult MSC and PBMC when tested via flow cytometry (Fig. 8). To further confirm the reactivity of
25 SLAM01 on the cell lines, immunohistochemistry was performed on in-house FFPE cell line array, and results were scored by ImmunoMembrane. The human cell line array showed that SLAM01 had high selectivity to the tumor antigen target on many AML cell lines and other cancer cell lines, such as breast, ovarian, gastric, colorectal and NSCLC, while no staining was observed on normal cell lines (Fig. 9 and 10). Thus, both flow cytometry and
30 immunohistochemistry revealed the preferential binding of SLAM01 to AML and other cancer cell lines, and not to MM, B cell lines or normal cells.

[0004] **Example 3. Determination of SLAM01 isotype and epitope nature**

[0091] SLAM01 was isotyped and determined to be a mouse IgM monoclonal antibody of κ isotype (Fig. 11). The translated sequence of the variable heavy and light chains, with the complementarity-determining regions (CDRs) underlined, are as follows:

[0092] Variable heavy chain (SEQ ID NO:7)

5 QVKLQQSGAE LAKPGASVKL SCKASGYTFT SYWIHWVKQR PGQGLEWIGE
INPSNGRTNF NEKFKNKATL TVDKSSSTAYMQLNSLTSED SAVYYCARVD
YDEAYWGQGT TTVVSS

[0093] Variable light chain (SEQ ID NO:8)

10 DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ
 LLIYQMSNLA SGVPDRFSST GSGTDFTLRI SRVEAEDVGV YYCAQNLELW
TFGGGTKLEI K

[0094] Epitope characterization revealed that SLAM01 recognized a glycan epitope as
 15 binding was not affected after AML3 was treated with pronase, an enzyme that digests protein to amino acid (Fig. 12). In addition, SLAM01 required the presence of N-linked glycan for binding (Fig. 13). SLAM01 binding to the antigen was abolished with PNGase treatment (lane 1), indicating the requirement of N-glycan for binding. In addition, pre-incubation of SLAM01 with Le^X resulted in reduced binding of SLAM01 to all AML cell
 20 lines. (Fig. 14). Thus, SLAM01 is a mouse IgM monoclonal antibody that recognizes terminal Le^X structure on N-glycosylated SLAMF7 on human cancer cells, with a defined translated protein sequence encoding the variable heavy and light antibody chains.

[0095] To further characterize the binding of SLAM01 to SLAMF7 PC9 (non-small cell lung cancer cell line) and OVCAR3 (epithelial ovarian cancer cell line) were incubated with
 25 either SLAM01 or anti-CD319 to test for expression of the respective antigen on cell surface. The histograms in Fig. 24 showed that the 2 cell lines are positive for SLAM01 but negative for CD319. This suggests that the epitope that SLAM01 is binding to on PC9 and OVCAR3, is not on CD319.

[0096] Western blots of SLAM01, anti-CD319 and anti-Lewis-X/SSEA1 on SLAM01-
 30 immunoprecipitated proteins we prepared (Fig. 25). OCI-AML3 membrane protein extracts (MPXs) were immunoprecipitated with SLAM01 and the immunoprecipitated proteins were resolved via SDS-PAGE and immunoblotted with SLAM01, anti-CD319 and anti-Lewis-X/SSEA1. The blots showed that SLAM01 has successfully immunoprecipitated the proteins from the MPX (left immunoblot, Lane 1). However, the immunoprecipitated proteins are not

recognized by anti-CD319 (middle immunoblot, Lane 1) despite the expression of CD319 by OCI-AML3 (middle immunoblot, Lane 7). These SLAM01-immunoprecipitated proteins are modified with Lewis-X (right immunoblot, Lane 1). These data demonstrate that SLAM01 does not recognize CD319, but glycoproteins with Lewis-X.

[0097] 2D representations of glycan structures that were positive for SLAM01 can be seen in Fig. 26. SLAM01 was found to be positive for Lewis-X and also 4 glycan structures that contain Lewis-X structure at the terminal, non-reducing end of glycan structure.

[0098] 2D representations of glycan structures that were negative for SLAM01 can be seen in Fig. 27. SLAM01 was negative for these glycan structures that contain Lewis-X structure at the terminal, non-reducing end of glycan structure. From this data, it is hypothesized, albeit in no way limiting, that binding of SLAM01 requires Lewis-X to be presented on a specific glycan backbone or SLAM01 recognizes an epitope that consist of terminal Lewis-X structure and other glycans.

[0099] SLAM01 and anti-Lewis-X/SSEA1 were stained on an in-house FFPE cell line array (Fig.28). SLAM01 stained 5/48 of the cell line cores while anti-Lewis-X/SSEA1 stained 16/48 cores. All SLAM01-positive cores were positive for Lewis-X, supporting the hypothesis that SLAM01 is recognizing either Lewis-X on specific glycan backbone or SLAM01 epitope consist of terminal Lewis-X structure and other glycans.

[00100] SLAM01 and anti-Lewis-X/SSEA1 were incubated on A549 (lung cancer cell line), Colo205 (colorectal cancer cell line), H1299 (lung cancer cell lines) and HCC827 (lung cancer cell line) to determine their cell surface expression profiles (Fig. 29). SLAM01 had different expression profiles than that of anti-Lewis-X/SSEA1 across all 4 cell lines, suggesting that SLAM01 is recognizing either Lewis-X on a specific glycan backbone or SLAM01 epitope consists of a terminal Lewis-X structure and other glycans.

[00101] **Example 4. ADC potential of SLAM01**

[00102] SLAM01 can potentially be used as an ADC. Flow cytometry data show some degree of internalization of SLAM01 after binding, as observed by the increase in intracellular red fluorescence when SLAM01 was conjugated to pHrodo Red (Fig. 15).

SLAM01 exhibited cytotoxic activity as an ADC when indirectly conjugated to saporin via a secondary antibody on binding cancer lines *in vitro* (Fig. 16). Indirect conjugation of SLAM01 with the ribosome toxin, saporin (Mab-ZAP) as an ADC demonstrated killing

percentage ranging from 30%-90% on the various SLAM01 positive cell lines (Fig. 17). Killing percentage was observed to be positively correlated to the binding percentage. The binding of SLAM01 to cells can be competitively inhibited by soluble free Le^x (Fig. 18A). Similarly, the killing efficient of SLAM01 on target cells is reduced in the presence of Le^x.
5 In addition, flow cytometry screening and agglutination assays on blood tissue samples (n=3) demonstrated no binding on PBMC, making it safe for therapeutic applications (Fig 18B). Functionally, SLAM01, when complexed with saporin-conjugated secondary mAb, was able to internalize into and kill AML cell lines.

[00103] **Example 5. Validation of chimeric SLAM01**

- 10 [00104] The constant region of SLAM01 was converted to that of human IgG1 to create a chimeric SLAM01 (cSLAM01). Binding of cSLAM01 to SLAM-01 positive cell lines such as AML3 and Mono-Mac-1, was confirmed in a competitive inhibition study by flow cytometry (Fig. 19). Furthermore, cSLAM01 also retained the SLAM-01 target binding profile as binding to SLAM-01 positive cell lines but not SLAM01 negative cell lines such as
15 hME-1 and MRC5 was observed (Fig. 20). Functionally, treatment with cSLAM01 and Ig-toxin complex such as DM1, a highly potent maytansinoid, and monomethyl auristatin E (MMAE), an anti-mitotic toxin, reduced the cell viability of AML3 and Mono-Mac-1 (Fig. 21). Thus, cSLAM01 retained both the binding profile and ADC potential on SLAM-01 positive cells.
- 20 [00105] Following the formation and validation of cSLAM01, MMAE was directly conjugated with cSLAM01 to form cSLAM01-MMAE. Binding of cSLAM01-MMAE to both AML3 and Mono-Mac-1 was observed by flow cytometry (Fig. 22). As the binding profile of cSLAM01-MMAE is similar to cSLAM01, the ADC potential was next tested in a viability assay. Based on the dose response curve of cSLAM01-MMAE, ADC effect was
25 observed on both AML3 and Mono-Mac-1 with a stronger potency on the latter (Fig. 23). Overall, these data highlight the potential for SLAM01 to be developed into an ADC for AML.

CLAIMS

1. An antigen-binding protein, or an antigen-binding fragment thereof, comprising (i) a heavy chain variable domain comprising a VHCDR1 having the amino acid sequence
GYTFTSYWIH; a VHCDR2 having the amino acid sequence EINPSNGRTNFNEKFKN
5 and a VHCDR3 having the amino acid sequence VDYDEAY; and (ii) a light chain variable domain comprising a VLCDR1 having the amino acid sequence RSSKSLLHSNGITYLY, a VLCDR2 having the amino acid sequence QMSNLAS, and a VLCDR3 having the amino acid sequence AQNLELWT.
- 10
2. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 1, wherein the heavy chain variable region comprises the amino acid sequence
QVKLQQSGAELAKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLEWIGEINPS
NGRTNFNEKFKNKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARVDYDEAYWG
15 QGTTVTVSS as set forth in SEQ ID NO:1.
3. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 1, wherein the light chain variable region comprises the amino acid sequence
20 DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQM
SNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELWTFGGGKLEIK
as set forth in SEQ ID NO:2.
- 25
- 30

4. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in any one of claims 1 to 3, wherein the antigen binding protein is selected from the group consisting of monoclonal, recombinant, polyclonal, chimeric, humanised, bispecific and heteroconjugate antibodies; a single variable domain, a domain antibody, antigen binding fragments, immunologically effective fragments, single chain Fv, a single chain antibody, a univalent antibody lacking a hinge region, a minibody, diabodies, and tandem diabodies.
5. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 4, wherein the binding protein is a monoclonal antibody.
6. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 5, wherein the monoclonal antibody is humanised.
7. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 5, wherein the monoclonal antibody is chimeric.
8. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in any one of claims 1 to 7, wherein the antigen-binding protein, or antigen-binding fragment thereof binds to an N-linked glycan.
9. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 8, wherein the antigen-binding protein, or antigen-binding fragment thereof binds to an N-linked glycan on SLAMF7.

10. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 8 or 9, wherein the N-linked glycan is an oligosaccharide of the Lewis X type.
- 5 11. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in any one of claims 1 to 10, wherein the antigen-binding protein, or antigen-binding fragment thereof does not bind to an oligosaccharide of the sialylated Lewis X type.
12. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in any one of claims 1 to 11, comprising a radioisotope or a cytotoxin conjugated thereto.
- 10 13. The antigen-binding protein, or antigen-binding fragment thereof, as claimed in claim 12, wherein the antibody is conjugated with a cytotoxin selected from the group consisting of monomethyl auristatin E (MMAE-1), mertansine (DM-1), saporin, gemcitabine, irinotecan, etoposide, vinblastine, pemetrexed, docetaxel, paclitaxel, platinum agents (for example, 15 cisplatin, oxaliplatin and carboplatin), vinorelbine, capecitabine, mitoxantrone, ixabepilone, eribulin, 5-fluorouracil, trifluridine and tipiracil.
14. The antigen-binding protein, or antigen-binding fragment thereof as claimed in any one of the preceding claims, wherein the antigen-binding protein, or an antigen-binding fragment 20 is internalized into a cell upon binding to the N-linked glycan on SLAMF7.
15. The antigen-binding protein, or antigen-binding fragment thereof as claimed in any one of the preceding claims, wherein the antigen-binding protein, or an antigen-binding fragment selectively binds to a non-small cell lung cancer cell, an ovarian cancer cell, a breast cancer 25 cell, an acute myeloid leukaemia cell and a colorectal cancer cell.
16. A composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the antigen-binding protein, or an antigen-binding fragment thereof, as claimed in any one of claims 1 to 15.
- 30 17. The composition as claimed in claim 16, comprising one or more further therapeutic compounds.

18. Use of an antigen-binding protein, or an antigen-binding fragment thereof, as claimed in any one of claims 1 to 15 or composition as claimed in claims 16 or 17, in the manufacture of a medicament for treating or preventing cancer.
- 5 19. The use of claim 18, wherein the cancer is selected from the group consisting of non-small cell lung cancer, ovarian cancer, breast cancer, acute myeloid leukaemia and colorectal cancer.
- 10 20. The use of claims 18 or 19, wherein the medicament is to be administered with a further active pharmaceutical ingredient.
21. The use of claims 18 or 19, wherein the medicament is to be administered with chemotherapy.
- 15 22. The use of claims 20 or 21, wherein the further pharmaceutical agent or chemotherapy is to be administered separately, simultaneously or sequentially with said medicament.
- 20 23. A method for detecting cancer in a subject, the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as claimed in any one of claims 1 to 15 *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine the level of binding in the sample, wherein an increase in the level of binding in the sample relative to the control sample is indicative of cancer.
- 25 24. A method for identifying a subject susceptible to cancer the method comprising: contacting a sample obtained from the subject with an antigen-binding protein, or an antigen-binding fragment thereof as claimed in any one of claims 1 to 15 *in vitro*; detecting the binding of the antigen-binding protein, or an antigen-binding fragment thereof in the sample; correlating the binding with a level of binding in a control sample to determine the level of binding in the sample, wherein an increase in the level of
- 30

binding in the sample relative to the control sample indicates that the subject is susceptible to cancer.

- 5 25. The method according to claims 23 or 24, wherein the control sample is from the same subject.
26. The method according to claims 23 or 24, wherein the control sample is from a different subject.
- 10 27. The method according to any one of claims 23 to 26, wherein the antigen-binding protein, or antigen-binding fragment thereof, binds SLAMF7.
28. The method according to any one of claims 23 to 27, wherein the antigen-binding protein, or antigen-binding fragment thereof, comprises a detectable label.
- 15 29. The method according to claim 28, wherein the detectable label is selected from the group consisting of a fluorescent label, a chemiluminescent label, an enzymatic label and a radionuclide label.
- 20 30. The method according to claim 28 or claim 29, wherein the detectable label is selected from the group consisting of biotin, alkaline phosphatase, horseradish peroxidase, FITC, PE and Cy Dyes.
- 25 31. The method according to claims any one of claims 28 to 30, wherein the detectable label is detected in an assay selected from flow cytometry, tissue section, immunofluorescence, immunocytochemistry or immunohistochemistry.
- 30 32. The method as claimed in any one of claims 23 to 31, wherein the cancer is selected from the group consisting of non-small cell lung cancer, ovarian cancer, breast cancer, acute myeloid leukaemia and colorectal cancer.

33. A kit when used in the method of any one of claims 23 to 32, comprising an antigen-binding protein, or antigen-binding fragment thereof as claimed in any one of claims 1 to 15, together with instructions for use.