

antibodies bind to cell surface-expressed human AXL. Antibody-drug conjugates and fusions are described.

[0014] The present disclosure provides an isolated or purified antibody that specifically binds to an epitope in an immunoglobulin-like (IgL) domain of human AXL ectodomain (ECD).

[0014a] In one aspect, the present disclosure provides an isolated or purified antibody that specifically binds to an epitope in the immunoglobulin-like (IgL2) domain of human AXL ectodomain (ECD), wherein the antibody is capable of internalization into AXL expressing tumor cells and wherein the antibody comprises:

10 variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 20, 21, and 22, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 23, 24, and 25;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 86, 87, and 88, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 83, 84, and 85;

15 variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 32, 33, and 34, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 35, 36, and 37;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 80, 81, and 82, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 77, 78, and 79;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 9, 10, and 11, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 12, 13, and 14;

20 variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 98, 99, and 100, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 95, 96, and 97;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 15, 16, and 11, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 17, 18, and 19;

25 variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 92, 93, and 94, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 89, 90, and 91;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 104, 105, and 107, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 101, 102, and 103; or

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 116, 117, and 118, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 113, 114, and 115.

[0014b] In another aspect, the present disclosure provides an antibody or a fragment thereof comprising single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences selected from the group consisting of:

SEQ ID NO:127, 128, and 129;
SEQ ID NO:139, 140, and 141;
SEQ ID NO:142, 143, and 144; and
SEQ ID NO:148, 149, and 150.

[0014c] In yet another aspect, the present disclosure provides a biparatopic antibody comprising:

single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 127, 128, and 129 and single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 139, 140, and 141; or

single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 127, 128, and 129 and single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 142, 143, and 144.

[0014d] In yet another aspect, the present disclosure provides an antibody drug conjugate (ADC) comprising the antibody as described herein, conjugated to a drug moiety. In yet another aspect, the present disclosure provides a pharmaceutical composition comprising the ADC as described herein and a pharmaceutically acceptable excipient. In yet another aspect, the present disclosure provides a fusion protein comprising the antibody as described herein and a human IgG1 Fc. In yet another aspect, the present disclosure provides a nucleic acid molecule encoding the antibody as described herein. In yet another aspect, the present disclosure provides a vector comprising the nucleic acid molecule as described herein. In yet another aspect, the present disclosure provides a host cell comprising the vector as described herein. In yet another aspect, the present disclosure provides a hybridoma or an organism that produces the antibody as described herein.

[0014e] In a further aspect, the present disclosure provides an *in vitro* method of detecting AXL, comprising:

- a) contacting a sample with one or more than one isolated or purified antibody as described herein linked to a detectable agent; and
- 5 b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.

[0014f] In a further aspect, the present disclosure provides a use of one or more than one antibody as described herein in the manufacture of a composition for *in vivo* detection of AXL expression in a subject, wherein:

the antibody is linked to a detectable agent,

the composition is to be administered to the subject to allow the antibody to bind AXL, and

the detectable agent linked to the antibody bound to AXL is to be detected.

15 **[0014g]** In a further aspect, the present disclosure provides a use of one or more than one antibody as described herein in the manufacture of a medicament for transporting a molecule of interest into a subject's cells expressing AXL, wherein the antibody is linked to the molecule of interest, and wherein the antibody delivers the molecule of interest to the subject's cells expressing AXL.

20 **[0014h]** In a further aspect, the present disclosure provides a use of an anti-cancer antibody drug conjugate comprising the antibody as described herein in the manufacture of a medicament for treating cancer in a subject in need thereof. In a further aspect, the present disclosure provides a use of the antibody as described herein in the manufacture of a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus for treating cancer. In a further aspect, the present disclosure provides a use of the antibody as described herein for preparation of a medicament comprising an anti-cancer antibody drug conjugate, for treating cancer in a subject in need thereof. In a further aspect, the present disclosure provides a use of the antibody as described herein for preparation of a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus.

[0015] There is also provided an antibody or fragment thereof comprising variable light chain (VL) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: SEQ ID NO:98-100; SEQ ID NO:92-94; SEQ ID NO:110-112; SEQ ID NO:86-88; SEQ ID NO:80-82; SEQ ID NO:104-106; SEQ ID NO:116-118; and sequences substantially identical thereto.

[0016] There is provided herein an antibody or fragment thereof comprising a variable light chain (VL) sequence selected from the group consisting of: SEQ ID NO:38; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:44; SEQ ID NO:46; SEQ ID NO:74; SEQ ID NO:76; and sequences substantially identical thereto.

[0017] Further, there is provided an antibody or fragment thereof comprising variable heavy chain (VH) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: SEQ ID NO:95-97; SEQ ID NO:89-91; SEQ ID NO:107-09; SEQ ID NO:83-85; SEQ ID NO:77-79; SEQ ID NO:101-103; SEQ ID NO:113-115; and sequences substantially identical thereto.

[0018] There is provided herein an antibody or fragment thereof comprising a variable heavy chain (VH) sequence selected from the group consisting of: SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; SEQ ID NO:45; SEQ ID NO:47; SEQ ID NO:73; SEQ ID NO:75; and sequences substantially identical thereto.

[0019] Further, there is provided an antibody or fragment thereof comprising a single domain (sd) heavy chain variable (VHH) domain sequence selected from the group consisting of: SEQ ID NOs:119-126; and sequences substantially identical thereto.

[0020] There is also provided an antibody or fragment thereof selected from the group consisting of: VH of SEQ ID NO:39, and VL of SEQ ID NO:38; VH of SEQ ID NO:41, and VL of SEQ ID NO:40; VH of SEQ ID NO:43, and VL of SEQ ID NO:42; VH of SEQ ID NO:45, and VL of SEQ ID NO:44; VH of SEQ ID NO:47, and VL of SEQ ID NO:46; VH of SEQ ID NO:73, and VL of SEQ ID NO:74; VH of SEQ ID NO:75, and VL of SEQ ID NO:76; VHH of SEQ ID NO:119; VHH of SEQ ID NO:120; VHH of SEQ ID NO:121; VHH of SEQ ID NO:122; VHH of SEQ ID NO:123; VHH of SEQ ID NO:124; VHH of SEQ ID NO:125; VHH of SEQ ID NO:126; and sequences having 85% or greater identity thereto.

[0021] Antibody drug conjugates (ADCs), pharmaceutical compositions, and fusion proteins are described, comprising the described antibodies. Nucleic acid molecules encoding the described antibodies, and vectors or host cells comprising same are also provided.

[0022] Methods of in vitro and in vivo detecting of AXL are described. Methods of transporting molecules of interest into cells expressing AXL are described. Methods and uses of the described antibodies for treating cancer, employing antibody drug conjugates are provided.

5 **[0023]** Other examples and features of the present disclosure will become apparent to those ordinarily skilled in the art upon review of the following description of specific examples in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **[0024]** Embodiments of the present disclosure will now be described, by way of example only, with reference to the attached Figures.

[0025] **Figure 1** (Prior Art) is a schematic depiction of AXL domain structure.

[0026] **Figure 2** shows results of the effect of anti-AXL mAbs on A549 cell survival.

15 **[0027]** **Figure 3** shows results of flow cytometry experiments determining the binding properties of anti-AXL mAb to cell surface-expressed AXL on H292 cells.

[0028] **Figure 4** shows results of the activity of anti-AXL-DM1 conjugates on cell viability in a H292 tumor cell line, a A549 cell line, human breast adenocarcinoma MDA-MB-231 tumor cell line, and immortalized HaCaT keratinocyte cells.

20 **[0029]** **Figure 5** is a schematic representation of the human AXL protein ectodomain (hAXL-ECD) and transmembrane domain. Domain binding is shown for ten peptide fragments of the hAXL-ECD.

[0030] **Figure 6** is a schematic representation of the strategy for isolation of anti-rhAXL ECD single domain antibodies (sdAbs).

25 **[0031]** **Figure 7** shows amino acid sequences of the mAb V_H/V_L domains with CDRs using IMGT definitions, shown in bold underlined font.

[0032] **Figure 8** shows amino acid sequences of the VHHs with CDRs, using IMGT definitions, represented in bold underlined font.

WHAT IS CLAIMED IS:

1. An isolated or purified antibody that specifically binds to an epitope in the immunoglobulin-like (IgL2) domain of human AXL ectodomain (ECD), wherein the antibody is capable of internalization into AXL expressing tumor cells and wherein the antibody comprises:

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 20, 21, and 22, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 23, 24, and 25;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 86, 87, and 88, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 83, 84, and 85;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 32, 33, and 34, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 35, 36, and 37;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 80, 81, and 82, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 77, 78, and 79;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 9, 10, and 11, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 12, 13, and 14;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 98, 99, and 100, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 95, 96, and 97;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 15, 16, and 11, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 17, 18, and 19;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 92, 93, and 94, and variable heavy chain CDR1, CDR2, and CDR3 sequences of 89, 90, and 91;

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 104, 105, and 107, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 101, 102, and 103; or

variable light chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 116, 117, and 118, and variable heavy chain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 113, 114, and 115.

2. The antibody of claim 1, wherein the antibody comprises:

a light chain sequence of SEQ ID NO: 44 and a heavy chain sequence of SEQ ID NO: 45;

a light chain sequence of SEQ ID NO: 46 and a heavy chain sequence of SEQ ID NO: 47;

a light chain sequence of SEQ ID NO: 38 and a heavy chain sequence of SEQ ID NO: 39;

a light chain sequence of SEQ ID NO: 40 and a heavy chain sequence of SEQ ID NO: 41;

a light chain sequence of SEQ ID NO: 74 and a heavy chain sequence of SEQ ID NO: 73; or

a light chain sequence of SEQ ID NO: 76 and a heavy chain sequence of SEQ ID NO: 75.

3. The antibody of claim 1, which is a monoclonal antibody, a humanized antibody, a chimeric antibody, a biparatopic antibody, a bispecific antibody, or an AXL-binding antibody fragment thereof.

4. The antibody of claim 3, wherein the chimeric antibody comprises framework regions from human IgG1.

5. The antibody of claim 4, wherein the chimeric antibody comprises framework regions from human kappa light chain and human IgG heavy chain.

6. The antibody of claim 1, comprising framework regions from IgA, IgD, IgE, IgG, or IgM.
7. The antibody of claim 1, comprising a full-length IgG, an Fv, an scFv, a Fab, or a F(ab')₂.
8. The antibody of any one of claims 1 to 7, wherein the binding to the epitope in the IgL2 domain of AXL ECD does not compete with the binding of Growth arrest-specific 6 (Gas6) ligand to AXL.
9. An antibody or a fragment thereof comprising single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences selected from the group consisting of:

SEQ ID NO:127, 128, and 129;
SEQ ID NO:139, 140, and 141;
SEQ ID NO:142, 143, and 144; and

SEQ ID NO:148, 149, and 150.10. The antibody or fragment thereof of claim 9, comprising a single domain (sd) heavy chain variable (VHH) domain sequence selected from the group consisting of:

QVKLEESGGGLVQAGGSLRLSCTASASISSFDIMGWYRQAPGKQRELVAAI
TTLDIANYRDSVKGRFTISRDNANTVYLQMDSLKPEDTARYHCAAFQSDQN
YWGGGTQVTVSS (SEQ ID NO:119);

QVKLEESGGGLAQAGGSLRLSCAASGSISSINTIGWFRQAPGKQRELVAAS
DSGANRNYADSVKGRFTISRDNANTVYLQMNNLKPEDTAIYYCRAWGTGT
ISTMYWGQGTQVTVSS (SEQ ID NO:123);

QVKLEESGGGLVQAGASLRLSCVASESIFGFNTMGWYRQAPGNERELVASI
SNSKRTMYADSVKGRFTISRDNANTVNLQMNNLKPEDTAVYYCRAWGIITS
ATVYWGQGTQVTVSS (SEQ ID NO:124);

and

QVKLEESGGGLVQAGGSLRLSCAASGSSGMINTMGWYRQAPGKQRELVA
RRSTGGTTNYADSVKGRFTISRDDANNTVYLQMNSLKPEDTAVYYCAIIWRT
SDLTGRFNTWGQGTQVTVSS (SEQ ID NO:126).

11. The antibody or fragment thereof of claim 9, which is a biparatopic antibody.

12. A biparatopic antibody comprising:

single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 127, 128, and 129 and single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 139, 140, and 141; or

single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 127, 128, and 129 and single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 142, 143, and 144.

13. The biparatopic antibody of claim 12, comprising single domain (sd) heavy chain variable (VHH) domain sequences of SEQ ID NO: 119 and 123 or single domain (sd) heavy chain variable (VHH) domain sequences of SEQ ID NO: 119 and 124.

14. An antibody drug conjugate (ADC) comprising the antibody of any one of claims 1 to 13, conjugated to a drug moiety.

15. The antibody drug conjugate of claim 14, wherein the drug moiety is an anti-cancer drug.

16. The antibody drug conjugate of claim 14, wherein the drug moiety is DM1.

17. A pharmaceutical composition comprising the ADC of any one of claims 14 to 16 and a pharmaceutically acceptable excipient.

18. A fusion protein comprising the antibody according to claim 1 and a human IgG1 Fc.

19. A nucleic acid molecule encoding the antibody of any one of claims 1 to 10.

20. A vector comprising the nucleic acid molecule of claim 19.
21. A host cell comprising the vector of claim 20.
22. The host cell of claim 21, which is a recombinant microbial host cell or a mammalian cell.
23. The host cell of claim 21 or 22, wherein said cell produces the antibody of any one of claims 1 to 10.
24. A hybridoma or an organism that produces the antibody of any one of claims 1 to 10.
25. The antibody of any one of claims 1 to 13, wherein the antibody is immobilized onto a surface.
26. The antibody of any one of claims 1 to 13, wherein the antibody is linked to a cargo molecule.
27. The antibody of claim 26, wherein the cargo molecule is a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector.
28. The antibody of claim 27, wherein the cargo molecule is from about 1 kDa to about 500 kDa in size.
29. The antibody of claim 27, wherein the cargo molecule is loaded in one or more than one liposome or nanocarrier.
30. The antibody of claim 27, wherein the nanocarrier comprises one or more than one nanoparticle, nanowire, nanotube, or quantum dot.
31. The antibody of claim 27, wherein the viral vector is adenoviral, lentiviral or retroviral.

32. The antibody of claim 27, wherein the DNA-based molecule comprises an anti-sense oligonucleotide, microRNA, siRNA, or a plasmid.
33. The antibody of any one of claims 24 to 27 wherein the cargo molecule is a cytotoxic agent.
34. An *in vitro* method of detecting AXL, comprising:
- a) contacting a sample with one or more than one isolated or purified antibody of any one of claims 1 to 13 linked to a detectable agent; and
 - b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.
35. The method of claim 34, wherein method detects AXL in circulating cells and the sample is a serum sample.
36. The method of claim 34 or 35, wherein the step of detecting is performed using optical imaging, immunohistochemistry, molecular diagnostic imaging, or ELISA.
37. Use of one or more than one antibody of any one of claims 1 to 13 in the manufacture of a composition for *in vivo* detection of AXL expression in a subject, wherein:
- the antibody is linked to a detectable agent,
 - the composition is to be administered to the subject to allow the antibody to bind AXL, and
 - the detectable agent linked to the antibody bound to AXL is to be detected.
38. The use of claim 37, wherein the detecting is to be performed using PET, SPECT, or fluorescence imaging.
39. Use of one or more than one antibody of any one of claims 1 to 13 in the manufacture of a medicament for transporting a molecule of interest into a subject's cells expressing AXL, wherein the antibody is linked to the molecule of interest, and

wherein the antibody delivers the molecule of interest to the subject's cells expressing AXL.

40. The use of claim 39, wherein the molecule of interest is a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector.

41. The use of claim 39, wherein the molecule of interest is loaded on one or more than one liposome or nanocarrier.

42. The use of claim 41, wherein the nanocarrier comprises one or more than one nanoparticle, nanowire, nanotube, or quantum dot.

43. The use of any one of claims 38 to 42, wherein the molecule of interest comprises a cytotoxic agent.

44. The use of claim 43, wherein the cytotoxic agent is for use in treating cancer.

45. Use of an anti-cancer antibody drug conjugate comprising the antibody of any one of claims 1 to 13 in the manufacture of a medicament for treating cancer in a subject in need thereof.

46. Use of the antibody of any one of claims 1 to 13 in the manufacture of a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus for treating cancer.

47. Use of the antibody of any one of claims 1 to 13 for preparation of a medicament comprising an anti-cancer antibody drug conjugate, for treating cancer in a subject in need thereof.

48. Use of the antibody of any one of claims 1 to 13 for preparation of a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus.