

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 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.

[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:

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

a first single domain (sd) heavy chain variable (VHH) domain comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 127, 128, and 129 and a second single domain (sd) heavy chain variable (VHH) domain comprising 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
- 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.

[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 treatment of cancer, wherein the antibody is linked to a cytotoxic agent.

[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 chimeric antigen receptor-T cell or an oncolytic virus comprising the antibody as described herein. 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-T cell, or an oncolytic virus.

WHAT IS CLAIMED IS:

1. An isolated or purified antibody that specifically binds to 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. 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.

9. The antibody or fragment thereof of claim 8, 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
ATVYWGGGTQVTVSS (SEQ ID NO:124);

and

QVKLEESGGGLVQAGGSLRLSCAASGSSGMINTMGWYRQAPGKQRELVA
RRSTGGTTNYADSVKGRFTISRDDANNTVYLQMNSLKPEDTAVYYCAIIWRT
SDLTGRFNTWGQGTQVTVSS (SEQ ID NO:126).

10. The antibody or fragment thereof of claim 8, which is a biparatopic antibody.

11. A biparatopic antibody comprising:

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

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

12. The biparatropic antibody of claim 11, 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.

13. An antibody drug conjugate (ADC) comprising the antibody of any one of claims 1 to 12, conjugated to a drug moiety.

14. The antibody drug conjugate of claim 13, wherein the drug moiety is an anti-cancer drug.

15. The antibody drug conjugate of claim 13, wherein the drug moiety is DM1.

16. A pharmaceutical composition comprising the ADC of any one of claims 13 to 15 and a pharmaceutically acceptable excipient.

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

18. A nucleic acid molecule encoding the antibody of any one of claims 1 to 9.

19. A vector comprising the nucleic acid molecule of claim 18.

20. A host cell comprising the vector of claim 19.

21. The host cell of claim 20, which is a recombinant microbial host cell or a mammalian cell.

22. The host cell of claim 20 or 21, wherein said cell produces the antibody of any one of claims 1 to 9.

23. The antibody of any one of claims 1 to 12, wherein the antibody is immobilized onto a surface.
24. The antibody of any one of claims 1 to 12, wherein the antibody is linked to a cargo molecule.
25. The antibody of claim 24, 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.
26. The antibody of claim 25, wherein the cargo molecule is from about 1 kDa to about 500 kDa in size.
27. The antibody of claim 25, wherein the cargo molecule is loaded in one or more than one liposome or nanocarrier.
28. The antibody of claim 27, wherein the nanocarrier comprises one or more than one nanoparticle, nanowire, nanotube, or quantum dot.
29. The antibody of claim 25, wherein the viral vector is adenoviral, lentiviral or retroviral.
30. The antibody of claim 25, wherein the DNA-based molecule comprises an anti-sense oligonucleotide, microRNA, siRNA, or a plasmid.
31. The antibody of any one of claims 24 to 27 wherein the cargo molecule is a cytotoxic agent.
32. 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 12 linked to a detectable agent; and
 - b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.

33. The method of claim 32, wherein method detects AXL in circulating cells and the sample is a serum sample.
34. The method of claim 32 or 33, wherein the step of detecting is performed using optical imaging, immunohistochemistry, molecular diagnostic imaging, or ELISA.
35. Use of one or more than one antibody of any one of claims 1 to 12 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.
36. The use of claim 35, wherein the detecting is to be performed using PET, SPECT, or fluorescence imaging.
37. Use of one or more than one antibody of any one of claims 1 to 12 in the manufacture of a medicament for treatment of cancer, wherein the antibody is linked to a cytotoxic agent.
38. Use of an anti-cancer antibody drug conjugate comprising the antibody of any one of claims 1 to 12 in the manufacture of a medicament for treating cancer in a subject in need thereof.
39. A chimeric antigen receptor-T cell or an oncolytic virus comprising the antibody of any one of claims 1 to 12.
40. Use of the antibody of any one of claims 1 to 12 for preparation of a medicament comprising an anti-cancer antibody drug conjugate, for treating cancer in a subject in need thereof.

41. Use of the antibody of any one of claims 1 to 12 for preparation of a chimeric antigen receptor-T cell, or an oncolytic virus.