

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

5 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:

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 ~~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~~ 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 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-T cell ~~(CAR-T cell) therapy~~, or an oncolytic virus.

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.~~

98. 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.

~~109.~~ The antibody or fragment thereof of claim 98, comprising a single domain (sd) heavy chain variable (VHH) domain sequence selected from the group consisting of:

QVKLEESGGGLVQAGGSLRLSCTASASISSFDIMGWYRQAPGKQRELVAAI
TTLDIANYRDSVKGRFTISRDNKNTVYLQMDSLKPEDTARYHCAAFQSDQN
YWGGGTQVTVSS (SEQ ID NO:119);

QVKLEESGGGLAQAGGSLRLSCAASGSISSINTIGWFRQAPGKQRELVAAS
DSGANRNYADSVKGRFTISRDNKNTVYLQMNNLKPEDTAIYYCRAWGTGT
ISTMYWGQGTQVTVSS (SEQ ID NO:123);

QVKLEESGGGLVQAGASLRLSCVASESIFGFNTMGWYRQAPGNERELVASI
SNSKRTMYADSVKGRFTISRDNKNTVNLQMNNLKPEDTAVYYCRAWGIITS
ATVYWGGGTQVTVSS (SEQ ID NO:124);

and

QVKLEESGGGLVQAGGSLRLSCAASGSSGMINTMGWYRQAPGKQRELVA
RRSTGGTTNYADSVKGRFTISRDDANNTVYLQMNSLKPEDTAVYYCAIIWRT
SDLTGRFNTWGQGTQVTVSS (SEQ ID NO:126).

~~11~~10. The antibody or fragment thereof of claim ~~98~~, which is a biparatopic antibody.

~~12~~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.

~~13~~12. The biparatropic antibody of claim ~~12~~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.

~~14~~13. An antibody drug conjugate (ADC) comprising the antibody of any one of claims 1 to ~~13~~12, conjugated to a drug moiety.

~~15~~14. The antibody drug conjugate of claim ~~14~~13, wherein the drug moiety is an anti-cancer drug.

~~16~~. 15. The antibody drug conjugate of claim ~~14~~13, wherein the drug moiety is DM1.

~~17~~16. A pharmaceutical composition comprising the ADC of any one of claims ~~14~~13 to ~~16~~15 and a pharmaceutically acceptable excipient.

~~18~~17. A fusion protein comprising the antibody according to claim 1 and a human IgG1 Fc.

~~19-18.~~ 18. A nucleic acid molecule encoding the antibody of any one of claims 1 to ~~109.~~

~~20-19.~~ 19. A vector comprising the nucleic acid molecule of claim ~~19~~18.

~~21-20.~~ 20. A host cell comprising the vector of claim ~~20~~19.

~~22-21.~~ 21. The host cell of claim ~~21~~20, which is a recombinant microbial host cell or a mammalian cell.

~~23-22.~~ 22. The host cell of claim ~~21~~20 or ~~22~~21, wherein said cell produces the antibody of any one of claims 1 to ~~109.~~

~~24.~~ 23. ~~A hybridoma or an organism that produces the antibody of any one of claims 1 to 10.~~

~~25-23.~~ 23. The antibody of any one of claims 1 to ~~13~~12, wherein the antibody is immobilized onto a surface.

~~26-24.~~ 24. The antibody of any one of claims 1 to ~~13~~12, wherein the antibody is linked to a cargo molecule.

~~27-25.~~ 25. The antibody of claim ~~26~~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.

~~28-26.~~ 26. The antibody of claim ~~27~~25, wherein the cargo molecule is from about 1 kDa to about 500 kDa in size.

~~29-27.~~ 27. The antibody of claim ~~27~~25, wherein the cargo molecule is loaded in one or more than one liposome or nanocarrier.

~~30-28.~~ 28. The antibody of claim 27, wherein the nanocarrier comprises one or more than one nanoparticle, nanowire, nanotube, or quantum dot.

~~31-29.~~ The antibody of claim ~~27~~25, wherein the viral vector is adenoviral, lentiviral or retroviral.

~~32-30.~~ The antibody of claim ~~27~~25, wherein the DNA-based molecule comprises an anti-sense oligonucleotide, microRNA, siRNA, or a plasmid.

~~33-31.~~ The antibody of any one of claims 24 to 27 wherein the cargo molecule is a cytotoxic agent.

~~34-32.~~ 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 ~~13~~12 linked to a detectable agent; and
- b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.

~~35-33.~~ The method of claim ~~34~~32, wherein method detects AXL in circulating cells and the sample is a serum sample.

~~36-34.~~ The method of claim ~~34~~32 or ~~35~~33, wherein the step of detecting is performed using optical imaging, immunohistochemistry, molecular diagnostic imaging, or ELISA.

~~37-35.~~ Use of one or more than one antibody of any one of claims 1 to ~~13~~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.

~~38-36.~~ The use of claim ~~37~~35, wherein the detecting is to be performed using PET, SPECT, or fluorescence imaging.

~~39-37.~~ Use of one or more than one antibody of any one of claims 1 to ~~13-12~~ in the manufacture of a medicament for treatment of cancer~~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~~a cytotoxic agent.

~~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.

~~45-38.~~ Use of an anti-cancer antibody drug conjugate comprising the antibody of any one of claims 1 to ~~13-12~~ in the manufacture of a medicament for treating cancer in a subject in need thereof.

~~46-39.~~ A chimeric antigen receptor-T cell or an oncolytic virus comprising Use of the antibody of any one of claims 1 to 13-12in the manufacture of a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus for treating cancer.

~~47-40.~~ Use of the antibody of any one of claims 1 to ~~13-12~~ for preparation of a medicament comprising an anti-cancer antibody drug conjugate, for treating cancer in a subject in need thereof.

~~48-41.~~ Use of the antibody of any one of claims 1 to ~~13-12~~ for preparation of a chimeric antigen receptor-T cell (~~CAR-T cell~~) therapy, or an oncolytic virus.