

X₁₀ is S or D or M or N or Q,
 X₁₁ is Y or C or E or P,
 X₁₂ is F or E or N or S or T or V,
 X₁₃ is A or D or G or L or Y,
 X₁₄ is M or E or F,
 X₁₅ is W or A or D or H or L or N or P or R or T, and
 X₁₆ is G or H.

[0009a] In one aspect, the present disclosure refers to an antibody or antibody fragment that specifically binds to Axl protein, said antibody or antibody fragment comprising a heavy chain variable region including three complementarity determining regions and a light chain variable region including three complementarity determining regions, said heavy chain complementarity determining regions having H1, H2, and H3 sequences, wherein:

- (a) the H1 sequence is X₁GX₂X₃MX₄ (SEQ ID NO: 1);
- (b) the H2 sequence is LIKX₅SNGGTX₆YNQKFKG (SEQ ID NO: 2); and
- (c) the H3 sequence is GX₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄DYX₁₅X₁₆ (SEQ ID NO: 3),

wherein

X₁ is T or A or W,
 X₂ is H or A,
 X₃ is T or I,
 X₄ is N or I,
 X₅ is P or N,
 X₆ is S or I or T,
 X₇ is H or D or E or P or R or W,
 X₈ is Y or N,
 X₉ is E or A or D or F or G or H or I or L or M or N or R or V or Y,
 X₁₀ is S or D or M or N or Q,
 X₁₁ is Y or C or E or P,
 X₁₂ is F or E or N or S or T or V,
 X₁₃ is A or D or G or L or Y,
 X₁₄ is M or E or F,
 X₁₅ is W or A or D or H or L or N or P or R or T, and
 X₁₆ is G or H; and

said light chain complementarity determining regions having L1, L2, and L3 sequences, wherein:

- (a) the L1 sequence is KASQDX₁₇X₁₈SX₁₉VX₂₀ (SEQ ID NO: 4);
- (b) the L2 sequence is X₂₁X₂₂X₂₃TRX₂₄T (SEQ ID NO: 5); and
- (c) the L3 sequence is QEX₂₅X₂₆SX₂₇X₂₈X₂₉X₃₀ (SEQ ID NO: 6),

wherein

X₁₇ is V or D or G or N or W,
 X₁₈ is S or V,
 X₁₉ is A or L or M,
 X₂₀ is A or D or N or Q,
 X₂₁ is W or F,
 X₂₂ is A or I or N or P or Q,
 X₂₃ is S or D,
 X₂₄ is H or D,
 X₂₅ is H or C or F or I or L or Q or S or T or V or Y,
 X₂₆ is F or C or D or E or G or N or S,
 X₂₇ is T or C or P,

X₂₈ is P or A or C or D or E or H or K or S or T or V or W,
X₂₉ is L or G or R, and
X₃₀ is T or I or R, with the proviso that X₁ to X₃₀ cannot be T, H, T, N, P, S, H, Y, E, S, Y, F, A, M, W, G, V, S, A, A, W, A, S, H, H, F, T, P, L and T, respectively, at the same time.

[0010] Disclosed is that, the isolated heavy chain variable region polypeptide is combined with an isolated light chain variable region that includes three complementarity determining regions L1, L2, and L3 sequences, wherein:

the L1 sequence is KASQDX₁₇X₁₈SX₁₉VX₂₀ (SEQ ID NO: 4);

the L2 sequence is X₂₁X₂₂X₂₃TRX₂₄T (SEQ ID NO: 5); and

the L3 sequence is QEX₂₅X₂₆SX₂₇X₂₈X₂₉X₃₀ (SEQ ID NO: 6),

wherein

X₁₇ is V or D or G or N or W,

X₁₈ is S or V,

X₁₉ is A or L or M,

X₂₀ is A or D or N or Q,

X₂₁ is W or F,

X₂₂ is A or I or N or P or Q,

X₂₃ is S or D,

X₂₄ is H or D,

X₂₅ is H or C or F or I or L or Q or S or T or V or Y,

X₂₆ is F or C or D or E or G or N or S,

X₂₇ is T or C or P,

X₂₈ is P or A or C or D or E or H or K or S or T or V or W,

X₂₉ is L or G or R, and

X₃₀ is T or I or R.

[0010a] In another example, there is provided an isolated polypeptide that specifically binds to Axl protein, said polypeptide comprising an light chain variable region including three complementarity determining regions having L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is KASQDX₁₇X₁₈SX₁₉VX₂₀ (SEQ ID NO: 4);

(b) the L2 sequence is X₂₁X₂₂X₂₃TRX₂₄T (SEQ ID NO: 5); and

(c) the L3 sequence is QEX₂₅X₂₆SX₂₇X₂₈X₂₉X₃₀ (SEQ ID NO: 6),

wherein

X₁₇ is V or D or G or N or W,

X₁₈ is S or V,

X₁₉ is A or L or M,

X₂₀ is A or D or N or Q,

X₂₁ is W or F,

X₂₂ is A or I or N or P or Q,

X₂₃ is S or D,

X₂₄ is H or D,

X₂₅ is H or C or F or I or L or Q or S or T or V or Y,

X₂₆ is F or C or D or E or G or N or S,

X₂₇ is T or C or P,

X₂₈ is P or A or C or D or E or H or K or S or T or V or W,

X₂₉ is L or G or R, and

X₃₀ is T or I or R.

[0011] Disclosed is an anti-Axl antibody or antibody fragment that includes the isolated heavy chain variable region polypeptide of the disclosure.

[0011a] In another example, there is provided an anti-Axl antibody or antibody fragment comprising the isolated heavy chain variable region polypeptide as described herein.

[0012] Disclosed is an immunoconjugate that includes the antibody or antibody fragment of the disclosure, optionally conjugated to an

[0112] In another aspect, the present invention identified the heavy chain variable regions as represented in FIG. 1A and the light chain variable regions as presented in FIG. 1B. Some heavy chain variable regions are encoded by DNA sequences with SEQ ID NOS: 11-13. The heavy chain variable regions in FIG. 1A have amino acid sequences with SEQ ID NOS: 14-67. Some light chain variable regions are encoded by DNA sequences with SEQ ID NOS: 7-10. The light chain variable regions in FIG. 1B have amino acid sequences with SEQ ID NOS: 68-116. These heavy and light chain variable regions can specifically bind to Axl. Antibodies comprising one of these heavy and light chain variable regions have been found to have a higher binding affinity to Axl at a pH found in the tumor microenvironment than at a pH in a non-tumor microenvironment.

[0113] The present invention also includes variants of the heavy and light chain variable regions presented in FIGS. 1A-1B and encoded by DNA sequences with SEQ ID NOS: 9-13 that can specifically bind to Axl. In order to derive these variants, it was determined that the complementarity determining regions (CDRs) of the heavy chain variable regions (H1-H3) and the CDRs of the light chain variable regions (L1-L3) should remain intact.

[0114] In deriving these variants, one is guided by the process as described herein. The variants of these heavy and light chain variable regions may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the heavy and light chain variable regions, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody or antibody fragment. Any combination of deletion(s), insertion(s), and substitution(s) can be made to arrive at the final construct, provided that the final construct possesses at least one of the desired characteristics, e.g., antigen-binding.

Substitution, Insertion, and Deletion Variants

[0115] In certain embodiments, antibody or antibody fragment variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and framework regions (FRs). Conservative substitutions are shown in Table 3 under the heading of “conservative substitutions.” More substantial changes are provided in Table 3 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody or antibody fragment of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, or decreased immunogenicity.

WHAT IS CLAIMED IS:

1. An antibody or antibody fragment that specifically binds to Axl protein, said antibody or antibody fragment comprising a heavy chain variable region including three complementarity determining regions and a light chain variable region including three complementarity determining regions, said heavy chain complementarity determining regions having H1, H2, and H3 sequences, wherein:

- (a) the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1);
- (b) the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2); and
- (c) the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3),

wherein

- X_1 is T or A or W,
- X_2 is H or A,
- X_3 is T or I,
- X_4 is N or I,
- X_5 is P or N,
- X_6 is S or I or T,
- X_7 is H or D or E or P or R or W,
- X_8 is Y or N,
- X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,
- X_{10} is S or D or M or N or Q,
- X_{11} is Y or C or E or P,
- X_{12} is F or E or N or S or T or V,
- X_{13} is A or D or G or L or Y,
- X_{14} is M or E or F,
- X_{15} is W or A or D or H or L or N or P or R or T, and
- X_{16} is G or H; and

said light chain complementarity determining regions having L1, L2, and L3 sequences, wherein:

- (a) the L1 sequence is $KASQDX_{17}X_{18}SX_{19}VX_{20}$ (SEQ ID NO: 4);
- (b) the L2 sequence is $X_{21}X_{22}X_{23}TRX_{24}T$ (SEQ ID NO: 5); and
- (c) the L3 sequence is $QEX_{25}X_{26}SX_{27}X_{28}X_{29}X_{30}$ (SEQ ID NO: 6),

wherein

- X_{17} is V or D or G or N or W,
- X_{18} is S or V,

X_{19} is A or L or M,
 X_{20} is A or D or N or Q,
 X_{21} is W or F,
 X_{22} is A or I or N or P or Q,
 X_{23} is S or D,
 X_{24} is H or D,
 X_{25} is H or C or F or I or L or Q or S or T or V or Y,
 X_{26} is F or C or D or E or G or N or S,
 X_{27} is T or C or P,
 X_{28} is P or A or C or D or E or H or K or S or T or V or W,
 X_{29} is L or G or R, and
 X_{30} is T or I or R, with the proviso that X_1 to X_{30} cannot be T, H, T, N, P, S, H, Y, E, S, Y, F, A, M, W, G, V, S, A, A, W, A, S, H, H, F, T, P, L and T, respectively, at the same time.

2. An anti-Axl antibody or antibody fragment of claim 1 in which up to three of X_1 to X_{16} can be other than T, H, T, N, P, S, H, Y, E, S, Y, F, A, M, W, G, respectively, at the same time, and up to five of X_{17} to X_{30} can be other than V, S, A, A, W, A, S, H, H, F, T, P, L and T, respectively, at the same time.

3. An anti-Axl antibody or antibody fragment of claim 2, in which only one of X_1 to X_{16} can be other than T, H, T, N, P, S, H, Y, E, S, Y, F, A, M, W, G, respectively, and only one of X_{17} to X_{30} can be other than V, S, A, A, W, A, S, H, H, F, T, P, L and T, respectively.

4. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by a DNA sequence selected from sequences of SEQ ID NOS: 11-13.

5. The antibody or antibody fragment of claim 1 or claim 2, wherein the light chain variable region is encoded by a DNA sequence selected from SEQ ID NOS: 7-10.

6. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 12 and the light chain variable region is encoded by SEQ ID NO: 9.

7. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 13 and the light chain variable region is encoded by SEQ ID NO: 10.
8. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 11 and the light chain variable region is encoded by SEQ ID NO: 7.
9. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 11 and the light chain variable region is encoded by SEQ ID NO: 8.
10. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 13 and the light chain variable region is encoded by SEQ ID NO: 7.
11. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region is encoded by SEQ ID NO: 12 and the light chain variable region is encoded by SEQ ID NO: 8.
12. The antibody or antibody fragment of any one of claims 1-11, wherein the antibody or antibody fragment has a higher binding affinity to Axl protein at a value of a condition in a tumor microenvironment in comparison with a different value of the same condition that occurs in a non-tumor microenvironment.
13. The antibody or antibody fragment of claim 12, wherein the condition is pH.
14. The antibody or antibody fragment of claim 13, wherein the pH in the tumor microenvironment is in a range of from 5.8 to 7.0 and the pH in the non-tumor microenvironment is in a range of 7.0-7.6.