

SUMMARY

[0008] Disclosed is an isolated heavy chain variable region polypeptide that specifically binds to the Ror2 protein. The polypeptide includes three complementarity determining regions H1, H2, and H3 sequences, wherein:

the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);

the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and

the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,

X₈ is T or D or E,

X₉ is A or M or T,

X₁₀ is R or H,

X₁₁ is G or E,

X₁₂ is L or F,

X₁₃ is S or G,

X₁₄ is G or D,

X₁₅ is N or E,

X₁₆ is D or L,

X₁₇ is Y or C or T,

X₁₈ is W or L,

X₂₉ is Y or E or R or T,

X₃₀ is K or N,

X₃₁ is R or G or H or W or Y,

X₃₂ is F or C or N or Q,

X₃₃ is E or S,

X₃₄ is G or E or F or H or M or Q or S,

X₃₅ is W or A or I or P or Q or T or V,

X₃₆ is Y or G or N or Q,

X₃₇ is G or S or T, and

X₃₈ is Y or I.

[0008a] In one aspect, the present disclosure refers to a conditionally active anti-Ror2 antibody or antibody fragment that specifically binds to Ror2 protein with a greater binding to the Ror protein at a value of a pH in a tumor microenvironment in comparison with a different value of pH that occurs in a non-tumor microenvironment, said antibody or antibody fragment comprising a heavy chain variable region and a light chain variable region, said heavy chain variable region comprising three complementarity determining regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);

(b) the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and

(c) the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,

X₈ is T or D or E,

X₉ is A or I or M or S or T,

X₁₀ is R or Q or H,

X₁₁ is G or E,

X₁₂ is L or F,

X₁₃ is S or G,

X₁₄ is G or D,

X₁₅ is N or E,

X₁₆ is D or L,

X₁₇ is Y or C or T,

X₁₈ is W or L,

X₂₉ is Y or E or R or T,
X₃₀ is K or S,
X₃₁ is R or G or H or W or Y,
X₃₂ is F or C or N or Q,
X₃₃ is E or S,
X₃₄ is G or E or F or H or M or Q or S,
X₃₅ is W or A or I or P or Q or T or V,
X₃₆ is Y or G or N or Q,
X₃₇ is G or S or T, and
X₃₈ is Y or I,

said light chain variable region comprising three light chain complementarity determining regions having L1, L2, and L3 sequences, wherein:

- (a) the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or
RASESVDRYGNSX₃₉IH (SEQ ID NO:10);
- (b) the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11); and
- (c) the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or
QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,
X₂₀ is S or D,
X₂₁ is Y or C or D,
X₂₂ is H or G or L,
X₂₃ is G or C or H or P,
X₂₄ is Q or E,
X₂₅ is R or H,
X₂₆ is S or D or G or I or Q or V,
X₂₇ is T or D,
X₂₈ is F or D or E,
X₃₉ is F or S or T,
X₄₀ is R or C or D or E or W,
X₄₁ is N or D,
X₄₂ is T or D or I or P,
X₄₃ is E or V,

X₄₄ is W or T, and

X₄₅ is F or S, and

wherein each of the heavy/light chain of the antibody or antibody fragment has only one single point mutation in comparison with the heavy chain variable region and/or the light chain variable region of the parent antibody in which X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅, X₁₆, X₁₇, X₁₈, X₂₉, X₃₀, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₁₉, X₂₀, X₂₁, X₂₂, X₂₃, X₂₄, X₂₅, X₂₆, X₂₇, X₂₈, X₃₉, X₄₀, X₄₁, X₄₂, X₄₃, X₄₄, and X₄₅, are F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, Y, K, R, F, E, G, W, Y, G, Y, V, S, Y, H, G, Q, R, S, T, F, F, R, N, T, E, W, and F, respectively.

[0009] In one example, this 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 SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or RASESVDRYGNSX₃₉IH (SEQ ID NO:10);

the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11); and

the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,

X₂₀ is S or D,

X₂₁ is Y or C or D,

X₂₂ is H or G or L,

X₂₃ is G or C or H or P,

X₂₄ is Q or E,

X₂₅ is R or H,

X₂₆ is S or D or G or I or Q or V,

X₂₇ is T or D,

X₂₈ is F or D or E,

X₃₉ is F or S or T,

X₄₀ is R or C or D or E or W,

X₄₁ is N or D,

X₄₂ is T or I or P,

X₄₃ is E or V,

X₄₄ is W or T, and

X₄₅ is F or T.

[0009a] In another example, there is provided an isolated polypeptide that specifically binds to Ror2 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 SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or
RASESVDRYGNSX₃₉IH (SEQ ID NO:10);
- (b) the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11); and
- (c) the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or
QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,

X₂₀ is S or D,

X₂₁ is Y or C or D,

X₂₂ is H or G or L,

X₂₃ is G or C or H or P,

X₂₄ is Q or E,

X₂₅ is R or H,

X₂₆ is S or D or G or I or Q or V,

X₂₇ is T or D,

X₂₈ is F or D or E,

X₃₉ is F or S or T,

X₄₀ is R or C or D or E or W,

X₄₁ is N or D,

X₄₂ is T or I or P,

X₄₃ is E or V,

X₄₄ is W or T, and

X₄₅ is F or T.

[0010] Disclosed is an anti-Ror2 antibody or antibody fragment that includes the isolated heavy chain variable region polypeptide of the disclosure.

[0010a] In another example, there is provided an anti-Ror2 antibody or antibody fragment comprising the isolated heavy chain variable region polypeptide as described herein.

[0011] Disclosed is an immunoconjugate that includes the antibody or antibody fragment of the disclosure, optionally conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0011a] In another aspect, the present disclosure refers to an immunoconjugate comprising the antibody or antibody fragment as described herein.

[0012] Disclosed is a pharmaceutical composition that includes the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the disclosure, together with a pharmaceutically acceptable carrier.

[0012a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising: the antibody or antibody fragment as described herein, or the immunoconjugate as described herein; and a pharmaceutically acceptable carrier.

[0013] Disclosed is a kit for diagnosis or treatment including the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the present disclosure.

[0013a] In another aspect, the present disclosure refers to a kit for diagnosis or treatment, said kit comprising the antibody or antibody fragment as described herein, or the immunoconjugate as described herein, or the pharmaceutical composition as described herein and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 is a schematic diagram of the structure of human Ror2 protein. The protein contains an Ig-like domain (Ig), a frizzled or cysteine-rich (CRD) domain, and a kringle (Kr) domain in the extracellular domain. The extracellular and intracellular domains are separated by a transmembrane (TM) domain. The intracellular domain contains a tyrosine kinase (TK) domain and a proline-rich domain (PR) flanked by serine/threonine (ST) rich domains.

[0015] FIGS. 2A-2B show sequence alignments of exemplary heavy chain variable regions of anti-Ror2 antibodies of the present invention.

[0016] FIGS. 3A-3B show sequence alignments of exemplary light chain variable regions of anti-Ror2 antibodies of the present invention.

[0017] FIG. 4 shows a size exclusion chromatograph indicating that the anti-Ror2 antibodies of the invention do not aggregate, as described in Example 1.

[0018] FIG. 5 shows pH-dependent binding profiles of anti-Ror2 antibodies of the present invention for binding to Ror2, as described in Example 1.

[0019] FIGS. 6A-6B show on and off rates of conditionally active antibodies of the invention as measured by surface plasmon resonance (SPR) assay, as described in Example 1.

[0020] FIGS. 7A-7B show effects on tumor volume of treatment of xenografted mice with a paclitaxel-conjugated anti-Ror2 antibody of the present invention, as described in Example 2.

[0021] FIG. 8 shows the pH dependent binding affinity of an exemplary conditionally active antibody BAP048 measured by pH titration.

[0022] FIG. 9 shows the binding affinity of the conditionally active antibody BAP048 to Ror2 proteins of human, cynomolgus, and mouse.

WHAT IS CLAIMED IS:

1. A conditionally active anti-Ror2 antibody or antibody fragment that specifically binds to Ror2 protein with a greater binding to the Ror protein at a value of a pH in a tumor microenvironment in comparison with a different value of pH that occurs in a non-tumor microenvironment, said antibody or antibody fragment comprising a heavy chain variable region and a light chain variable region, said heavy chain variable region comprising three complementarity determining regions having H1, H2, and H3 sequences, wherein:

- (a) the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);
- (b) the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and
- (c) the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,

X₈ is T or D or E,

X₉ is A or I or M or S or T,

X₁₀ is R or Q or H,

X₁₁ is G or E,

X₁₂ is L or F,

X₁₃ is S or G,

X₁₄ is G or D,

X₁₅ is N or E,

X₁₆ is D or L,

X₁₇ is Y or C or T,

X₁₈ is W or L,

X₂₉ is Y or E or R or T,

X₃₀ is K or S,

X_{31} is R or G or H or W or Y,
 X_{32} is F or C or N or Q,
 X_{33} is E or S,
 X_{34} is G or E or F or H or M or Q or S,
 X_{35} is W or A or I or P or Q or T or V,
 X_{36} is Y or G or N or Q,
 X_{37} is G or S or T, and
 X_{38} is Y or I,

said light chain variable region comprising three light chain complementarity determining regions having L1, L2, and L3 sequences, wherein:

- (a) the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or RASESVDRYGNSX₃₉IH (SEQ ID NO:10);
- (b) the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11); and
- (c) the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X_{19} is V or E,
 X_{20} is S or D,
 X_{21} is Y or C or D,
 X_{22} is H or G or L,
 X_{23} is G or C or H or P,
 X_{24} is Q or E,
 X_{25} is R or H,
 X_{26} is S or D or G or I or Q or V,
 X_{27} is T or D,
 X_{28} is F or D or E,
 X_{39} is F or S or T,
 X_{40} is R or C or D or E or W,
 X_{41} is N or D,
 X_{42} is T or D or I or P,
 X_{43} is E or V,
 X_{44} is W or T, and
 X_{45} is F or S, and

wherein each of the heavy/light chain of the antibody or antibody fragment has only one single point mutation in comparison with the heavy chain variable region and/or the light chain variable region of the parent antibody in which X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅, X₁₆, X₁₇, X₁₈, X₂₉, X₃₀, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₁₉, X₂₀, X₂₁, X₂₂, X₂₃, X₂₄, X₂₅, X₂₆, X₂₇, X₂₈, X₃₉, X₄₀, X₄₁, X₄₂, X₄₃, X₄₄, and X₄₅, are F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, Y, K, R, F, E, G, W, Y, G, Y, V, S, Y, H, G, Q, R, S, T, F, F, R, N, T, E, W, and F, respectively.

2. The antibody or antibody fragment of claim 1, wherein the heavy chain variable region has an amino acid sequence selected from sequences of SEQ ID NOS: 18-26.
3. The antibody or antibody fragment of any one of claims 1-2, wherein the light chain variable region has an amino acid sequence selected from SEQ ID NOS: 13-17 and 27.
4. The antibody or antibody fragment of any one of claims 1-3, wherein the X₂₉ is Y.
5. The antibody or antibody fragment of any one of claims 1-3, wherein the X₂₉ is E.
6. The antibody or antibody fragment of any one of claims 1-2 and 4-5, wherein the light chain complementarity determining regions have amino acid sequences of SEQ ID NOS: 10-12, respectively.
7. The anti-Ror2 antibody or antibody fragment of claim 1, wherein the heavy chain variable region has one single point mutation in comparison with the heavy chain variable region of the parent antibody in which X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅, X₁₆, X₁₇, X₁₈, X₂₉, X₃₀, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, and X₃₈, which are F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, Y, K, R, F, E, G, W, Y, G, and Y, respectively.
8. The antibody or antibody fragment of any one of claims 1 and 7, wherein the light chain variable region one single point mutation in comparison with the light chain variable region of the parent antibody in which X₁₉, X₂₀, X₂₁, X₂₂, X₂₃, X₂₄, X₂₅, X₂₆, X₂₇, X₂₈, X₃₉, X₄₀, X₄₁, X₄₂, X₄₃, X₄₄, and X₄₅, which are V, S, Y, H, G, Q, R, S, T, F, F, R, N, T, E, W, and F, respectively.

9. The antibody or antibody fragment of claim 1, wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅, X₁₆, X₁₇, X₁₈, X₁₉, X₂₀, X₂₁, X₂₂, X₂₃, X₂₄, X₂₅, X₂₆, X₂₇, X₂₈ are F, Y, T, M, G, I, N, T, A, H, G, L, S, G, N, D, Y, W, V, S, Y, H, G, Q, R, S, T and F, respectively.

10. The antibody or antibody fragment of any one of claims 1-9, wherein the pH in the tumor microenvironment is in a range of from 5.8 to 6.8 and the pH in the non-tumor microenvironment is in a range of 7.0 to 7.6.

11. The antibody or antibody fragment of any one of claims 1-10, wherein the antibody or antibody fragment has a ratio of binding affinity to the Ror2 protein at a value of a condition in a tumor microenvironment to a binding affinity to the Ror2 protein at a different value of the same condition in a non-tumor microenvironment of at least 2:1, at least 3:1, at least 4:1, at least 5:1, at least 6:1, at least 7:1, at least 8:1, at least 9:1, at least 10:1, at least 20:1, at least 30:1, at least 50:1, at least 70:1, or at least 100:1.

12. The antibody or antibody fragment of any one of claims 1-110, wherein the antibody or antibody fragment is a chimeric antibody, a multispecific antibody, or a humanized antibody.

13. An immunoconjugate comprising the antibody or antibody fragment of any one of claims 1-12.

14. The immunoconjugate of claim 13, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

15. The immunoconjugate of claim 14, comprising at least two said agents.

16. The immunoconjugate of any one of claims 14-15, wherein the antibody or antibody fragment and the at least one or two agents are covalently bonded to a linker molecule.

17. The immunoconjugate of any one of claims 14-16, wherein the at least one or two agents are selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolbenzodiazepines, and anthracyclines.

18. A pharmaceutical composition comprising:
- the antibody or antibody fragment of any one of claims 1-12, or the immunoconjugate of any one of claims 13-17; and
 - a pharmaceutically acceptable carrier.
19. The pharmaceutical composition of claim 18, further comprising a tonicity agent.
20. A kit for diagnosis or treatment, said kit comprising the antibody or antibody fragment of any one of claims 1-12, or the immunoconjugate of any one of claims 13-17, or the pharmaceutical composition of any one of claims 18-19 and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.