

SUMMARY OF THE DISCLOSURE

[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, there is provided an isolated polypeptide that specifically binds to Ror2 protein, said polypeptide comprising a heavy chain variable region including three complementarity determining regions, said 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 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,

X_{29} is Y or E or R or T,
 X_{30} is K or N,
 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.

[0009] In one embodiment, 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

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 I or P,
 X_{43} is E or V,

X₄₄ is W or T, and

X₄₅ is F or T.

[0009a] In another aspect, 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 aspect, 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, there is provided 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, there is provided a pharmaceutical composition comprising: the polypeptide as described herein, 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, there is provided a kit for diagnosis or treatment, said kit comprising the polypeptide as described herein, 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.

18. The immunoconjugate of claim 17, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.
19. The immunoconjugate of claim 18, comprising at least two said agents.
20. The immunoconjugate of any one of claims 18-19, wherein the antibody or antibody fragment and the at least one agent are covalently bonded to a linker molecule.
21. The immunoconjugate of any one of claims 18-20, wherein the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.
22. A pharmaceutical composition comprising:
the polypeptide of any one of claims 1-9, the antibody or antibody fragment of any one of claims 10-16, or the immunoconjugate of any one of claims 14-18; and
a pharmaceutically acceptable carrier.
23. The pharmaceutical composition of claim 22, further comprising a tonicity agent.
24. A kit for diagnosis or treatment, said kit comprising the polypeptide of any one of claims 1-9, the antibody or antibody fragment of any one of claims 10-16, or the immunoconjugate of any one of claims 17-21, or the pharmaceutical composition of any one of claims 22-23 and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.