Claims

1. An isolated antibody, or an antigen binding fragment thereof, which specifically binds to trophoblast cell-surface antigen-2 (Trop-2), wherein the antibody comprises

(a) a heavy chain variable (VH) region comprising (i) a VH complementary determining region one (CDR1) comprising the sequence SYGVH (SEQ ID NO: 30), GGSISSY (SEQ ID NO: 36), or GGSISSYGVH (SEQ ID NO: 37); (ii) a VH CDR2 comprising the sequence VIWTX$_1$GX$_2$TDYNSALMX$_3$, wherein X$_1$ is G or S; X$_2$ is S or V; X$_3$ is S or G (SEQ ID NO: 49), or WTX$_1$GX$_2$, wherein X$_1$ is G or S, X$_2$ is S or V (SEQ ID NO: 50); and iii) a VH CDR3 comprising the sequence DYDRYTX$_1$DY, wherein X$_1$ is E or M (SEQ ID NO: 82); and

(b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence RASKSVSTSX$_1$YSYMH, wherein X$_1$ is G, L, or N (SEQ ID NO: 63); (ii) a VL CDR2 comprising the sequence LASNLES (SEQ ID NO: 55); and (iii) a VL CDR3 comprising the sequence QHSRELPYT (SEQ ID NO: 56).

2. An isolated antibody, or an antigen binding fragment thereof, which specifically binds to Trop-2, wherein the antibody comprises:

a VH region comprising VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 4 or 5; and

a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 3, 6, or 7.

3. The antibody or the antigen binding fragment of claim 2, wherein the VH region comprises (i) a VH CDR1 comprising the sequence SYGVH (SEQ ID NO: 30), GGSISSY (SEQ ID NO: 36), or GGSISSYGVH (SEQ ID NO: 37); (ii) a VH CDR2 comprising the sequence VIWTSGVTDYNSALMG (SEQ ID NO: 38) or WTSGV (SEQ ID NO: 39); and (iii) a VH CDR3 comprising the sequence DGDYDRYTMDY (SEQ ID

NO: 35), and wherein the VL region comprises (i) a VL CDR1 comprising the sequence RASKSVSTSGYSYMH (SEQ ID NO: 54); (ii) a VL CDR2 comprising the sequence LASNLES (SEQ ID NO: 55); and (iii) a VL CDR3 comprising the sequence QHSRELPYT (SEQ ID NO: 56).

4. The antibody or the antigen binding fragment of claim 3, wherein the VH region comprises the sequence shown in SEQ ID NO: 5 or a variant with one or several conservative amino acid substitutions in residues that are not within a CDR and the VL region comprises the amino acid sequence shown in SEQ ID NO: 8 or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

5. The antibody or the antigen binding fragment of claim 4, wherein the antibody comprises a light chain comprising the sequence shown in SEQ ID NO: 66 and a heavy chain comprising the sequence shown in SEQ ID NO: 65.

6. The antibody or the antigen binding fragment of claim 3, wherein the antibody comprises a VH region produced by the expression vector with ATCC Accession No. PTA-12872.

7. The antibody or the antigen binding fragment of claim 3, wherein the antibody comprises a VL region produced by the expression vector with ATCC Accession No. PTA-12871.

8. The antibody, or the antigen binding fragment thereof of claim 1, which specifically binds to domain 3 and domain 4 of human Trop-2 (SEQ ID NO:27) with a monovalent antibody binding affinity (KD) of about 0.25 nM or less as measured by surface plasmon resonance.

9. The antibody of any one of claims 1-8, wherein the antibody comprises an acyl donor glutamine-containing tag engineered at a specific site.

10. The antibody of claim 9, wherein the tag comprises an amino acid sequence GGLLQGG (SEQ ID NO: 78), LLQGA (SEQ ID NO:79), or LLQ.

11. The antibody of claim 10, wherein the antibody further comprises an amino acid modification at position K222, K340, or K370.

12. The antibody of claim 11, wherein the amino acid modification is a substitution from lysine to arginine.

13. A conjugate of the antibody or the antigen binding fragment of any one of claims 1-12, wherein the antibody or the antigen binding fragment is conjugated to an agent, wherein the agent is selected from the group consisting of a cytotoxic agent, an immunomodulating agent, an imaging agent, a therapeutic protein, a biopolymer, and an oligonucleotide.

14. The conjugate of claim 13, wherein the agent is a cytotoxic agent.

15. The conjugate of claim 14, wherein the cytotoxic agent is MMAD (Monomethyl Auristatin D) or 0101 (2-methylalanyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide).

16. The conjugate of claim 15, wherein the conjugate comprises the formula: antibody-(acyl donor glutamine-containing tag)-(linker)-(cytotoxic agent).

17. The conjugate of claim 16, wherein the acyl donor glutamine-containing tag comprises an amino acid sequence GGLLQGG (SEQ ID NO: 78), LLQGA (SEQ NO: 79), LLQ, or $LLQX_1X_2X_3X_4X_5$, wherein X_1 is G or P, wherein X_2 is A, G, P, or absent, wherein X_3 is A, G, K, P, or absent, wherein X_4 is G, K, or absent, and wherein X_5 is K or absent (SEQ ID NO: 88), and wherein the linker comprises acetyl-lysine-valine-citrulline-*p*-aminobenzyloxycarbonyl or amino-PEG6-propionyl.

18. The conjugate of claim 17, wherein the conjugate is selected from the group consisting of 1) antibody-LLQGA (SEQ ID NO: 79)- (acetyl-lysine-valine-citrulline-*p*-aminobenzyloxycarbonyl (AcLys-VC-PABC))-0101; 2) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-MMAD; 3) antibody-$LLQX_1X_2X_3X_4X_5$ (SEQ ID NO: 88)-(AcLys-

VC-PABC)-0101; 4) antibody-LLQX$_1$X$_2$X$_3$X$_4$X$_5$ (SEQ ID NO: 88)-(AcLys-VC-PABC)-MMAD; 5) antibody –GGLLQGG (SEQ ID NO: 78)- (AcLys-VC-PABC)-0101; and 6) antibody–GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-MMAD.

19. The conjugate of claim 18, wherein the conjugate 1) antibody –GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-0101; 2) antibody–GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-MMAD; 3) antibody-LLQX$_1$X$_2$X$_3$X$_4$X$_5$ (SEQ ID NO: 88)-(AcLys-VC-PABC)-0101; 4) antibody-LLQX$_1$X$_2$X$_3$X$_4$X$_5$ (SEQ ID NO: 88)-(AcLys-VC-PABC)-MMAD; 5) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101; or 6) antibody-LLQGA (SEQ ID NO: 79)- AcLys-VC-PABC-MMAD comprises an amino acid substitution from lysine to arginine at position 222 of the antibody.

20. The conjugate of claim 18, wherein the conjugate is 1) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101; 2) antibody-LLQGA (SEQ ID NO: 79)-AcLys-VC-PABC-MMAD; 3) antibody-LLQX$_1$X$_2$X$_3$X$_4$X$_5$ (SEQ ID NO: 88)-(AcLys-VC-PABC)-0101; or 4) antibody-LLQX$_1$X$_2$X$_3$X$_4$X$_5$ (SEQ ID NO: 88)-(AcLys-VC-PABC)-MMAD, wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.

21. The conjugate of claim 15, wherein the conjugate comprises amino acid substitutions at position N297Q and K222R of the antibody, a linker comprising amino-PEG6-propionyl, and a cytotoxic agent comprising MMAD.

22. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of any one of claims 1-12 or the conjugate of any one of claims 22-30 and a pharmaceutically acceptable carrier.

23. An isolated polynucleotide comprising a nucleotide sequence encoding the antibody of any one of claims 1-8.

24. A vector comprising the polynucleotide of claim 23.

25. An isolated host cell that recombinantly produces the antibody of any one of claims 1-8.

26. A method of producing an antibody, comprising culturing the host cell of claim 25 under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture.

27. The pharmaceutical composition of claim 22 for use in the treatment of a condition associated with Trop-2 expression in a subject in need thereof.

28. The composition of claim 27, wherein the condition is a cancer.

29. The composition of claim 28, wherein the cancer is selected from the group consisting of bladder, breast, cervical, choriocarcinoma, colon, esophageal, gastric, glioblastoma, head and neck, kidney, lung, oral, ovarian, pancreatic, prostate, and skin cancer.

30. The pharmaceutical composition of claim 22 for use in the treatment of inhibiting tumor growth or progression in a subject who has a Trop-2 expressing tumor.

31. The pharmaceutical composition of claim 22 for use in the treatment of inhibiting metastasis of Trop-2 expressing cancer cells in a subject need thereof.

32. The pharmaceutical composition of claim 22 for use in the treatment of inducing regression in a subject who has a Trop-2 expressing tumor.

33. Use of the pharmaceutical composition of claim 22 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a condition associated with Trop-2 expression in a subject in need thereof, wherein the condition is selected from the group consisting of bladder, breast, cervical, choriocarcinoma, colon, esophageal, gastric, glioblastoma, head and neck, kidney, lung, oral, ovarian, pancreatic, prostate, and skin cancer.

34. Use of the pharmaceutical composition of claim 22 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inhibiting tumor growth or progression in a subject who has a Trop-2 expressing tumor.

35. Use of the pharmaceutical composition of claim 22 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inhibiting metastasis of Trop-2 expressing cancer cells in a subject need thereof.

36. Use of the pharmaceutical composition of claim 22 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inducing regression in a subject who has a Trop-2 expressing tumor.

37. The conjugate of claim 15, wherein the conjugate is antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101, wherein the conjugate comprises an amino acid substitution from lysine to arginine at position 222 of the antibody, and wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.

38. An antibody-drug conjugate which specifically binds to trophoblast cell-surface antigen-2 (Trop-2), wherein the conjugate is antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101, wherein the antibody comprises a heavy chain variable (VH) region comprising (i) a VH complementary determining region (CDR)1 comprising the sequence SYGVH (SEQ ID NO: 30), GGSISSY (SEQ ID NO: 36), or GGSISSYGVH (SEQ ID NO: 37); (ii) a VH CDR2 comprising the sequence VIWTSGVTDYNSALMG (SEQ ID NO: 38) or WTSGV (SEQ ID NO: 39); and (iii) a VH CDR3 comprising the sequence DGDYDRYTMDY (SEQ ID NO: 35), and a VL region comprising (i) a light chain variable (VL) CDR1 comprising the sequence RASKSVSTSGYSYMH (SEQ ID NO: 54); (ii) a VL CDR2 comprising the sequence LASNLES (SEQ ID NO: 55); and (iii) a VL CDR3 comprising the sequence QHSRELPYT (SEQ ID NO: 56), wherein the conjugate comprises an amino acid substitution from lysine to arginine at position 222 of the antibody, and wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.

Claims

1. An isolated antibody, or an antigen binding fragment thereof, which specifically binds to trophoblast cell-surface antigen-2 (Trop-2), wherein the antibody comprises

(a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence SYGVH (SEQ ID NO: 30), GGSISSY (SEQ ID NO: 36), or GGSISSYGVH (SEQ ID NO: 37); (ii) a VH CDR2 comprising the sequence VIWTSGVTDYNSALMG (SEQ ID NO: 38) or WTSGV (SEQ ID NO: 39); and iii) a VH CDR3 comprising the sequence DGDYDRYTMDY (SEQ ID NO: 35); and

(b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence RASKSVSTSGYSYMH (SEQ ID NO: 54); (ii) a VL CDR2 comprising the sequence LASNLES (SEQ ID NO: 55); and (iii) a VL CDR3 comprising the sequence QHSRELPYT (SEQ ID NO: 56).

2. The antibody or the antigen binding fragment of claim 1, wherein the VH region comprises the sequence shown in SEQ ID NO: 5 or a variant with one or several conservative amino acid substitutions in residues that are not within a CDR and the VL region comprises the amino acid sequence shown in SEQ ID NO: 3 or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

3. The antibody or the antigen binding fragment of claim 2, wherein the antibody comprises a light chain comprising the sequence shown in SEQ ID NO: 66 and a heavy chain comprising the sequence shown in SEQ ID NO: 65.

4. The antibody or the antigen binding fragment of claim 1, wherein the antibody comprises a VH region produced by the expression vector with ATCC Accession No. PTA-12872.

5. The antibody or the antigen binding fragment of claim 1, wherein the antibody comprises a VL region produced by the expression vector with ATCC Accession No. PTA-12871.

6. The antibody of any one of claims 1-5, wherein the antibody comprises an acyl donor glutamine-containing tag engineered at a specific site.

7. The antibody of claim 6, wherein the tag comprises an amino acid sequence GGLLQGG (SEQ ID NO: 78), or LLQGA (SEQ ID NO:79).

8. The antibody of claim 7, wherein the antibody further comprises an amino acid modification at position K222, K340, or K370.

9. The antibody of claim 8, wherein the amino acid modification is a substitution from lysine to arginine.

10. A conjugate of the antibody or the antigen binding fragment of any one of claims 1-9, wherein the antibody or the antigen binding fragment is conjugated to an agent, wherein the agent is selected from the group consisting of a cytotoxic agent, an immunomodulating agent, an imaging agent, a therapeutic protein, a biopolymer, and an oligonucleotide.

11. The conjugate of claim 10, wherein the agent is a cytotoxic agent.

12. The conjugate of claim 11, wherein the cytotoxic agent is MMAD (Monomethyl Auristatin D) or 0101 (2-methylalanyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide).

13. The conjugate of claim 12, wherein the conjugate comprises the formula: antibody-(acyl donor glutamine-containing tag)-(linker)-(cytotoxic agent).

14. The conjugate of claim 13, wherein the acyl donor glutamine-containing tag comprises an amino acid sequence GGLLQGG (SEQ ID NO: 78), or LLQGA (SE,Q

ID NO: 79) and wherein the linker comprises acetyl-lysine-valine-citrulline-*p*-aminobenzyloxycarbonyl or amino-PEG6-propionyl.

15. The conjugate of claim 14, wherein the conjugate is selected from the group consisting of 1) antibody-LLQGA (SEQ ID NO: 79)- (acetyl-lysine-valine-citrulline-*p*-aminobenzyloxycarbonyl (AcLys-VC-PABC))-0101; 2) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-MMAD; 5) antibody –GGLLQGG (SEQ ID NO: 78)- (AcLys-VC-PABC)-0101; and 6) antibody–GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-MMAD.

16. The conjugate of claim 15, wherein the conjugate 1) antibody –GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-0101; 2) antibody–GGLLQGG (SEQ ID NO: 78)-(AcLys-VC-PABC)-MMAD; 3) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101; or 4) antibody-LLQGA (SEQ ID NO: 79)- AcLys-VC-PABC-MMAD comprises an amino acid substitution from lysine to arginine at position 222 of the antibody.

17. The conjugate of claim 15, wherein the conjugate is 1) antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101; or 2) antibody-LLQGA (SEQ ID NO: 79)-AcLys-VC-PABC-MMAD; wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.

18. The conjugate of claim 12, wherein the conjugate comprises amino acid substitutions at position N297Q and K222R of the antibody, a linker comprising amino-PEG6-propionyl, and a cytotoxic agent comprising MMAD.

19. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of any one of claims 1-9 or the conjugate of any one of claims 10-18 and a pharmaceutically acceptable carrier.

20. An isolated polynucleotide comprising a nucleotide sequence encoding the antibody of any one of claims 1-5.

21. A vector comprising the polynucleotide of claim 20.

22. An isolated host cell that recombinantly produces the antibody of any one of claims 1-5.

23. A method of producing an antibody, comprising culturing the host cell of claim 22 under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture.

24. The pharmaceutical composition of claim 19 for use in the treatment of a condition associated with Trop-2 expression in a subject in need thereof.

25. The composition of claim 24, wherein the condition is a cancer.

26. The composition of claim 25, wherein the cancer is selected from the group consisting of bladder, breast, cervical, choriocarcinoma, colon, esophageal, gastric, glioblastoma, head and neck, kidney, lung, oral, ovarian, pancreatic, prostate, and skin cancer.

27. The pharmaceutical composition of claim 19 for use in the treatment of inhibiting tumor growth or progression in a subject who has a Trop-2 expressing tumor.

28. The pharmaceutical composition of claim 19 for use in the treatment of inhibiting metastasis of Trop-2 expressing cancer cells in a subject need thereof.

29. The pharmaceutical composition of claim 19 for use in the treatment of inducing regression in a subject who has a Trop-2 expressing tumor.

30. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a condition associated with Trop-2 expression in a subject in need thereof, wherein the condition is selected from the group consisting of bladder, breast, cervical, choriocarcinoma, colon, esophageal, gastric, glioblastoma, head and neck, kidney, lung, oral, ovarian, pancreatic, prostate, and skin cancer.

31. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inhibiting tumor growth or progression in a subject who has a Trop-2 expressing tumor.

32. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inhibiting metastasis of Trop-2 expressing cancer cells in a subject need thereof.

33. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of inducing regression in a subject who has a Trop-2 expressing tumor.

34. The conjugate of claim 12, wherein the conjugate is antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101, wherein the conjugate comprises an amino acid substitution from lysine to arginine at position 222 of the antibody, and wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.

35. An antibody-drug conjugate which specifically binds to trophoblast cell-surface antigen-2 (Trop-2), wherein the conjugate is antibody-LLQGA (SEQ ID NO: 79)-(AcLys-VC-PABC)-0101, wherein the antibody comprises a heavy chain variable (VH) region comprising (i) a VH complementary determining region (CDR)1 comprising the sequence SYGVH (SEQ ID NO: 30), GGSISSY (SEQ ID NO: 36), or GGSISSYGVH (SEQ ID NO: 37); (ii) a VH CDR2 comprising the sequence VIWTSGVTDYNSALMG (SEQ ID NO: 38) or WTSGV (SEQ ID NO: 39); and (iii) a VH CDR3 comprising the sequence DGDYDRYTMDY (SEQ ID NO: 35), and a VL region comprising (i) a light chain variable (VL) CDR1 comprising the sequence RASKSVSTSGYSYMH (SEQ ID NO: 54); (ii) a VL CDR2 comprising the sequence LASNLES (SEQ ID NO: 55); and (iii) a VL CDR3 comprising the sequence QHSRELPYT (SEQ ID NO: 56), wherein the conjugate comprises an amino acid substitution from lysine to arginine at position 222 of the antibody, and wherein the amino acid lysine at the C-terminus of heavy chain of the antibody is deleted.