In one aspect, the present disclosure provides a conjugate as disclosed herein for use in therapy.

In one aspect, the present disclosure provides a method of making a light chain of an antibody or antigen-binding fragment, the method comprising:

expressing in a host cell the nucleic acid as disclosed herein.

In one aspect, the present disclosure provides a method of making a conjugate, the method comprising:

conjugating an agent to the antibody or antigen-binding fragment as disclosed herein, wherein the agent is conjugated to the cysteine residue of the C-terminal amino acid extension.

In one aspect, the present disclosure provides an antibody, or an antigen-binding fragment thereof, comprising a light chain polypeptide comprising a C-terminal amino acid extension comprising a cysteine residue, wherein the C-terminal amino acid extension is from 1 to 75 amino acids in length, and wherein:

(a) the antigen-binding fragment is a Fab, $F(ab')_2$ or a Fab', or

(b) the C-terminal amino acid extension comprises an endogenous human amino acid sequence or a modified human amino acid sequence.

In one aspect, the present disclosure provides a conjugate, comprising:

the antibody or antigen-binding fragment as described herein; and

an agent conjugated to the antibody or antigen-binding fragment via the cysteine residue of the C-terminal amino acid extension.

In one aspect, the present disclosure provides a nucleic acid encoding a light chain polypeptide of the antibody or antigen-binding fragment as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic illustration of an antibody that includes a C-terminal light chain extension according to one embodiment of the present disclosure.

FIG. 2 is a schematic illustration of a conjugate according to one embodiment of the present disclosure.

FIG. 3 provides cancer cell viability data for two example antibody conjugates according to embodiments of the present disclosure.

FIG. 4, Panels A and B show antibody binding data for unconjugated antibodies according to certain embodiments of the present disclosure.

FIG. 5, Panels A and B show antibody binding data for antibody-drug conjugates according to certain embodiments of the present disclosure.

FIG. 6 shows differential scanning calorimetry (DSC) data for an antibody having a C-terminal light chain extension according to one embodiment of the present disclosure. FIG. 7 is a gel image showing Alexa488 conjugation to an antibody according to an embodiment of the present disclosure.

C is a cysteine residue: and

a, b, c, d, e, f, g, h and i are integers independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20, where the sum of b, e and h is at least 1, and the sum of c, f and i is at least 1. X, X', and X'' may be the same or different. Where c is greater than 1 , then each X of $(X_aCb)_c$ may be the same or different amino acid sequence within each repeat unit of $(X_aC_b)_c$. Where d or f is greater than 1, then each X' of $(X'_dC_e)_f$ may the same or different amino acid sequence within each repeat unit of $(X'dC_e)_f$. Where g or i is greater than 1 , then each X" of $(X"_gC_h)i$ may the same or different amino acid sequence within each repeat unit of $(X''_gC_h)_i$.

A C-terminal extension of the present disclosure may include (e.g., consist of) an amino acid sequence selected from: EPKSCDKTHTC (SEQ ID NO:92) (also referred to herein as extension 1); EPKSCDKTHTCPPC (SEQ ID NO:93) (also referred to herein as extension 2); EPKSC (SEQ ID NO:94) (also referred to herein as extension 3); ESKYGPPC (SEQ ID NO:95) (also referred to herein as extension 4); ERKCCVECPPC (SEQ ID NO: 96) (also referred to herein as extension 5); ERKC (SEQ ID NO:97) (also referred to herein as extension 6);
DVITMDPKDNC (SEQ ID NO:98) (also referred to herein as extension 7);
DHVKPKETENTKQPSKSCHKPK (SEQ ID NO:99) (also referred to herein as extension 8);
ESSC (SEQ ID NO: 100) (also referred to herein as extension 9); ESSCDVKLV (SEQ ID NO: 101) (also referred to herein as extension 10); DHVKPKETENTKQPSKSC (SEQ ID NO: 102) (also referred to herein as extension 11); DVITMDPKDNCSKDAN (SEQ ID NO: 103) (also referred to herein as extension 12); CAA, CCAA, AACAA (SEQ ID NO: 129), and GGGGSCAA (SEQ ID NO:130).

The present disclosure also provides nucleic acids encoding any of the C-terminal extensions described herein (e.g., C-terminal extension of Formula II, etc.), as well as nucleic acids encoding any of the light chain polypeptides described herein (e.g., light chain polypeptides comprising a C-terminal extension of Formula II, etc.).

The present disclosure provides antibodies having at least one light chain polypeptide having a C-terminal extension of the present disclosure. In some embodiments, the antibody has two light chain polypeptides having a C-terminal extension of the present disclosure. In some embodiments, such antibodies can be conjugated to an agent/payload (e.g., a drug) through covalent attachment to at least one cysteine in the C-terminal extension present in at least one of

CLAIMS

1. An antibody, or an antigen-binding fragment thereof, comprising a light chain polypeptide comprising a C-terminal amino acid extension comprising a cysteine residue, wherein the C-terminal amino acid extension is from 1 to 75 amino acids in length, and wherein:

(a) the antigen-binding fragment is a Fab, $F(ab')_2$ or a Fab', or

(b) the C-terminal amino acid extension comprises an endogenous human amino acid sequence or a modified human amino acid sequence.

2. The antibody or antigen-binding fragment of Claim 1, wherein the C-terminal amino acid extension is from 1 to 50 amino acids in length.

3. The antibody or antigen-binding fragment of Claim 1, wherein the C-terminal amino acid extension is from 5 to 50 amino acids in length.

4. The antibody or antigen-binding fragment of Claim 1, wherein the C-terminal amino acid extension is from 5 to 25 amino acids in length.

5. The antibody or antigen-binding fragment of Claim 1(a), wherein the C-terminal amino acid extension comprises an amino acid spacer that does not comprise a cysteine residue.

6. The antibody or antigen-binding fragment of Claim 5, wherein the spacer comprises from 3 to 20 amino acids.

7. The antibody or antigen-binding fragment of Claim 5, wherein the spacer comprises from 4 to 17 amino acids.

8. The antibody or antigen-binding fragment of any one of Claims 5 to 7, wherein the spacer comprises one or more glycine (G) residues, and one or more serine (S) residues.

9. The antibody or antigen-binding fragment of any one of Claims 5 to 8, wherein the spacer comprises the sequence GGGS (SEQ ID NO:1).

10. The antibody or antigen-binding fragment of any one of Claims 5 to 9, wherein the C-terminal amino acid extension comprises from 2 to 10 spacers, and wherein (a) the spacers have the same amino acid sequence, or (b) at least two of the spacers have different amino acid sequences.

11. The antibody or antigen-binding fragment of Claim 10, wherein a cysteine residue is present between each of the spacers.

12. The antibody or antigen-binding fragment of Claim 10, wherein at least two of the spacers are contiguous.

13. The antibody or antigen-binding fragment of Claim 1(a), wherein the C-terminal amino acid extension comprises a sequence as set forth in any one of SEQ ID NO: 60, 63 or 64.

14. The antibody or antigen-binding fragment of Claim 1(b), wherein the antibody or antigen-binding fragment is selected from the group consisting of: an IgG, Fab, F(ab')2, Fab', Fv and scFv.

15. The antibody or antigen-binding fragment of Claim 1(b) or 14, wherein the human amino acid sequence comprises an amino acid sequence of a human antibody hinge region, or a portion thereof, or a T cell receptor hinge region, or a portion thereof.

16. The antibody or antigen-binding fragment of Claim 1(b) or 14, wherein the C-terminal amino acid extension comprises a sequence as set forth in any one of SEQ ID NO: 32, 33, 34, 35, 36, 37, 38, 39 or 40, or a portion thereof.

17. The antibody or antigen-binding fragment of Claim 1(b) or 14, wherein the C-terminal amino acid extension comprises a sequence as set forth in any one of SEQ ID NO: 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 or 103.

18. The antibody or antigen-binding fragment of any one of Claims 1 to 17, wherein the cysteine residue comprises a reduced sulfhydryl group.

19. The antibody or antigen-binding fragment of any one of Claims 1 to 18, comprising two light chain polypeptides each comprising a C-terminal amino acid extension comprising a cysteine residue.

20. The antibody or antigen-binding fragment of any one of Claims 1 to 18, which is an antibody comprising a first monoepitopic antigen-binding domain comprising a first heavy chain polypeptide and a first light chain polypeptide, and a second monoepitopic antigen-binding domain comprising a second heavy chain polypeptide and a second light chain polypeptide, wherein one of the first and second light chain polypeptides comprises a C-terminal amino acid extension comprising a cysteine residue.

21. The antibody or antigen-binding fragment of any one of Claims 1 to 18, which is an antibody comprising a first monoepitopic antigen-binding domain comprising a first heavy chain polypeptide and a first light chain polypeptide, and a second monoepitopic antigen-binding domain comprising a second heavy chain polypeptide and a second light chain polypeptide, wherein both the first and second light chain polypeptides comprise a C-terminal amino acid extension comprising a cysteine residue.

22. The antibody or antigen-binding fragment of any one of Claims 1 to 21, which is a bispecific antibody.

23. The antibody or antigen-binding fragment of any one of Claims 1 to 22, comprising an agent conjugated to the antibody or antigen-binding fragment via the cysteine residue of the C-terminal amino acid extension, wherein the agent is a therapeutic agent or a labeling agent.

24. The antibody or antigen-binding fragment of Claim 23, wherein the agent is conjugated to the cysteine residue via a linker.

25. The antibody or antigen-binding fragment of Claim 23 or 24, wherein the C-terminal amino acid extension comprises two or more cysteine residues, and wherein at least two of the two or more cysteine residues are conjugated to an agent independently selected from a therapeutic agent and a labeling agent.

26. A conjugate, comprising:

the antibody or antigen-binding fragment of any one of Claims 1 to 22; and

an agent conjugated to the antibody or antigen-binding fragment via the cysteine residue of the C-terminal amino acid extension.

27. The conjugate of Claim 26, wherein the agent is conjugated to the cysteine residue via a linker.

28. The conjugate of Claim 26 or 27, wherein the agent is a therapeutic agent.

29. The conjugate of Claim 28, wherein the therapeutic agent is a cytotoxic agent.

30. The conjugate of Claim 29, wherein the cytotoxic agent is an auristatin or analog thereof, or a hemiasterlin or an analog thereof.

31. The conjugate of Claim 26 or 27, wherein the agent is a labeling agent.

32. The conjugate of Claim 31, wherein the labeling agent is an *in vivo* imaging agent.

33. The conjugate of any one of Claims 26 to 32, wherein the antibody or antigen-binding fragment does not include an agent conjugated to a cysteine residue other than a cysteine residue of the C-terminal amino acid extension.

34. The conjugate of any one of Claims 26 to 33, wherein the C-terminal amino acid extension comprises two or more cysteine residues, and wherein at least two of the two or more cysteine residues are conjugated to an agent independently selected from a therapeutic agent and a labeling agent.

35. A nucleic acid encoding a light chain polypeptide of the antibody or antigen-binding fragment of any one of Claims 1 to 22.

36. A vector comprising the nucleic acid of Claim 35.

37. A host cell comprising the nucleic acid of Claim 35, or the vector of Claim 36.

38. The host cell of Claim 37, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

39. A pharmaceutical composition comprising:

an antibody or an antigen-binding fragment of any one of Claims 1 to 25, or a conjugate of any one of Claims 26 to 34; and

a pharmaceutically acceptable excipient.

40. An antibody or an antigen-binding fragment of any one of Claims 1 to 25 for use in therapy.

41. A conjugate of any one of Claims 26 to 34 for use in therapy.

42. A method of making a light chain of an antibody or antigen-binding fragment, the method comprising:

expressing in a host cell the nucleic acid of Claim 35.

43. The method of Claim 42, wherein the method further comprises reducing the sulfhydryl group of the cysteine residue in the C-terminal amino acid extension.

44. A method of making a conjugate, the method comprising:

conjugating an agent to the antibody or antigen-binding fragment of any one of Claims 1 to 22, wherein the agent is conjugated to the cysteine residue of the C-terminal amino acid extension.

45. The method of Claim 44, wherein the method further comprises reducing the sulfhydryl group of the cysteine residue in the C-terminal amino acid extension prior to conjugating the agent to the antibody or antigen-binding fragment.

46. The method of Claim 45, wherein the reducing comprises preferentially reducing one or more cysteine residues in the C-terminal amino acid extension over a cysteine residue not in the C-terminal amino acid extension.

47. The method of Claim 45, wherein the reducing comprises reducing one or more cysteine residues in the C-terminal amino acid extension and not reducing any cysteine residues not in the C-terminal amino acid extension.

48. The method of any one of Claims 45 to 47, wherein the conjugating comprises crosslinking the agent to the reduced sulfhydryl group using maleimide reaction chemistry, haloacetyl reaction chemistry, vinyl sulfone reaction chemistry, or pyridyl disulfide reaction chemistry.

49. The method of any one of Claims 44 to 48, wherein the agent is a therapeutic agent or a labeling agent.