amino acid extension and not to any cysteine residues outside the C-terminal amino acid extension.

In certain aspects, the C-terminal amino acid extension includes two or more cysteine residues, and two or more agents are conjugated to the cysteine residues of the C-terminal amino acid extension. The methods of making such antibody conjugates may further include reducing the sulfhydryl groups of the cysteines in the C-terminal amino acid extension prior to conjugating the agents to the antibody. In certain aspects, the conjugating includes crosslinking the agents to the reduced sulfhydryl groups using maleimide reaction chemistry, haloacetyl reaction chemistry, vinyl sulfone reaction chemistry or pyridyl disulfide reaction chemistry. According to certain aspects, the agents that are conjugated to the cysteine residues of the C- terminal amino acid extensions are therapeutic agents and/or labeling agents. In one
embodiment, the agents are conjugated to the cysteine residues of the C-terminal amino acid extension preferentially over cysteine residues outside the C-terminal amino acid extension. In one embodiment, the agents are conjugated to the cysteine residues of the C-terminal amino acid extension and not to any cysteine residues outside 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 5 to 50 amino acids in length,

wherein the C-terminal amino acid extension comprises an endogenous human amino acid sequence or a modified human amino acid sequence, and

wherein the endogenous human amino acid sequence comprises an amino acid sequence of a human antibody hinge region, or a portion thereof, or a human T cell receptor hinge region, or a portion thereof.

In another 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,

wherein the agent is a therapeutic agent or a labeling agent.

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

In another aspect, the present disclosure provides a vector comprising the nucleic acid as described herein.

In another aspect, the present disclosure provides a host cell comprising the nucleic acid or the vector as described herein.

In another aspect, the present disclosure provides a pharmaceutical composition comprising:

an antibody or an antigen-binding fragment as described herein, or a conjugate as described herein; and

a pharmaceutically acceptable excipient.

In another aspect, the present disclosure provides an antibody or an antigen-binding fragment as described herein for use in therapy.

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

In another 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 described herein.

In another 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 described herein, wherein the agent is conjugated to the cysteine residue of the C-terminal amino acid extension, and wherein the agent is a therapeutic agent or a labeling agent.

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.

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 5 to 50 amino acids in length,

wherein the C-terminal amino acid extension comprises an endogenous human amino acid sequence or a modified human amino acid sequence, and

wherein the endogenous human amino acid sequence comprises an amino acid sequence of a human antibody hinge region, or a portion thereof, or a human T cell receptor hinge region, or a portion thereof.

2. 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.

3. The antibody or antigen-binding fragment of Claim 1 or 2, wherein the C-terminal amino acid extension comprises an amino acid spacer that does not comprise a cysteine residue.

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

5. The antibody or antigen-binding fragment of Claim 3, wherein the spacer comprises from 4 to 17 amino acids.

6. The antibody or antigen-binding fragment of any one of Claims 3 to 5, wherein the spacer comprises one or more glycine (G) residues, and one or more serine (S) residues.

7. The antibody or antigen-binding fragment of any one of Claims 3 to 6, wherein the spacer comprises the sequence GGGS (SEQ ID NO:1).

8. The antibody or antigen-binding fragment of any one of Claims 1 to 7, wherein the endogenous human amino acid sequence comprises at least 2, 3, 4, 5 or 6 contiguous amino acids of a human antibody hinge region.

9. The antibody or antigen-binding fragment of any one of Claims 1 to 8, wherein the C-terminal amino acid extension comprises an endogenous human amino acid sequence into which one or more cysteine residues have been introduced by insertion or substitution.

10. The antibody or antigen-binding fragment of any one of Claims 1 to 9, wherein the antibody or antigen-binding fragment is selected from the group consisting of: an IgG, Fab, F(ab')2, Fab', Fv and scFv.

11. The antibody or antigen-binding fragment of any one of Claims 1 to 10, 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.

12. The antibody or antigen-binding fragment of any one of Claims 1 to 10, 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, or a portion thereof.

13. The antibody or antigen-binding fragment of any one of Claims 1 to 12, wherein the cysteine residue comprises a reduced sulfhydryl group.

14. The antibody or antigen-binding fragment of any one of Claims 1 to 13, comprising two light chain polypeptides each comprising a C-terminal amino acid extension comprising a cysteine residue.

15. The antibody or antigen-binding fragment of any one of Claims 1 to 13, 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 or both of the first and second light chain polypeptides comprises a C-terminal amino acid extension comprising a cysteine residue.

16. The antibody or antigen-binding fragment of any one of Claims 1 to 15, which is a bispecific antibody.

17. The antibody or antigen-binding fragment of any one of Claims 1 to 16, 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.

18. The antibody or antigen-binding fragment of Claim 17, wherein the agent is conjugated to the cysteine residue via a linker.

19. The antibody or antigen-binding fragment of Claim 17 or 18, 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.

20. A conjugate, comprising:

the antibody or antigen-binding fragment of any one of Claims 1 to 16; and

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.

21. The conjugate of Claim 20, wherein the agent is conjugated to the cysteine residue via a linker.

22. The conjugate of Claim 20 or 21, wherein the agent is a cytotoxic agent.

23. The conjugate of Claim 22, wherein the cytotoxic agent is an auristatin or analog thereof, or a hemiasterlin or an analog thereof.

24. The conjugate of Claim 20 or 21, wherein the agent is an *in vivo* imaging agent.

25. The conjugate of any one of Claims 20 to 24, 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.

26. The conjugate of any one of Claims 20 to 25, 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.

27. A nucleic acid encoding a light chain polypeptide of the antibody or antigen-binding fragment of any one of Claims 1 to 16.

28. A vector comprising the nucleic acid of Claim 27.

29. A host cell comprising the nucleic acid of Claim 27, or the vector of Claim 28.

30. A pharmaceutical composition comprising:

an antibody or an antigen-binding fragment of any one of Claims 1 to 19, or a conjugate of any one of Claims 20 to 26; and

a pharmaceutically acceptable excipient.

31. An antibody or an antigen-binding fragment of any one of Claims 1 to 19 for use in therapy.

32. A conjugate of any one of Claims 20 to 26 for use in therapy.

33. 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 27.

34. The method of Claim 33, wherein the method further comprises reducing the sulfhydryl group of the cysteine residue in the C-terminal amino acid extension.

35. 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 16, wherein the agent is conjugated to the cysteine residue of the C-terminal amino acid extension, and wherein the agent is a therapeutic agent or a labeling agent.

36. The method of Claim 35, 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.

37. The method of Claim 36, wherein the reducing comprises:

(a) 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, or

(b) 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.

38. The method of Claim 36 or 37, 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.