pharmacokinetic properties. Additionally, such heterogeneity can present challenges in manufacturing of the conjugated antibody.

SUMMARY

[0006] Disclosed herein are methods for generating a conjugated immunoglobulin, the methods comprising: decapping a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of an immunoglobulin derived from rabbit, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0007] Also provided are methods for generating an antigen-binding molecule, the methods comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein: the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("$Cys80^1$") wherein $Cys80^1$ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group; and the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("$Cys80^2$") wherein $Cys80^2$ is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group. In one aspect, the present invention refers to a method for generating a conjugated immunoglobulin, the method comprising: incubating a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof with a mild reducing buffer comprising cysteine, dithiothreitol (DTT), or tris (2-carboxyethyl) phosphine (TCEP) to decap a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of the immunoglobulin, the Cys80 based upon the Kabat or Chothia numbering system, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the decapped Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group. In another aspect, the present invention refers to a method for generating an antigen-binding molecule, the method comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein: the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys801") wherein the Cys801 is conjugated to a first thiol-reactive

compound comprising a first thiol-reactive group, and wherein the immunoglobulin is a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof and the Cys801 is based upon the Kabat or Chothia numbering system; and the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys802") wherein the Cys802 is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group, and wherein the immunoglobulin is a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof and the Cys802 is based upon the Kabat or Chothia numbering system.

[0008] Immunoglobulins comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83 are also provided herein, as are nucleic acid molecules encoding the immunoglobulins and host cells comprising the nucleic acid molecules.

[0009] Further provided are conjugated immunoglobulins comprising the disclosed immunoglobulins, wherein the cysteine at position 80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group.

[0010] Also disclosed herein are methods of treating cancer in a subject comprising administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises: any of the disclosed mesothelin immunoglobulins, and a thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent.

[0011] Provided are antigen-binding molecules comprising: a first conjugated immunoglobulin comprising a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("$Cys80^1$"), wherein $Cys80^1$ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and a second conjugated immunoglobulin comprising a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("$Cys80^2$") wherein $Cys80^2$ is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group. In yet another aspect, the present invention refers to an antigen-binding molecule produced according to the method disclosed herein.

[0012] Light chain variable regions for use in a conjugated immunoglobulin, the light chain variable region having a cysteine at amino acid position 80 ("Cys80") and an amino acid residue other than Phe, Lys, or Cys at amino acid position 83, wherein the Cys80 is unpaired are also disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions, there are shown in the drawings exemplary embodiments; however, the methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions are not limited to the specific embodiments disclosed. In the drawings:

[0014] **FIG. 1** represents an alignment of rabbit and human light chain sequences. **(A)** Alignment of the germline Vκ sequences from rabbit (IGKV1S2, X02336) and human (IGKV1-5, Z00001). The arrow indicates Cys80 (according to either Kabat or Chothia numbering) in the rabbit sequence. **(B)** Alignment of the germline Cκ sequences from rabbit (IGKC1, K01360) and human (IGKC, J00241). The arrow indicates Cys171 (EU numbering) in the rabbit sequence.

[0015] **FIG. 2** represents structural models of **(A)** rabbit mAb, showing the Cys80-Cys171 disulfide bond, **(B)** human mAb, and **(C)** rabbit-human chimeric mAb showing the unpaired Cys80.

[0016] **FIG. 3** illustrates an alignment of rabbit germline Vκ families. The residue at position 80 is indicated by the arrow.

What is Claimed:

1. A method for generating a conjugated immunoglobulin, the method comprising:

incubating a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof with a mild reducing buffer comprising cysteine, dithiothreitol (DTT), or tris (2-carboxyethyl) phosphine (TCEP) to decap a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of the immunoglobulin, the Cys80 based upon the Kabat or Chothia numbering system, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and

conjugating a thiol-reactive compound to the decapped Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

2. The method of claim 1, further comprising incubating the immunoglobulin or antigen-binding fragment thereof with a Tris-based, glutamine-based, arginine-based, or primary amine-based oxidizing buffer after the incubating with the mild reducing buffer .

3. The method of claim 2, further comprising immobilizing the immunoglobulin or antigen-binding fragment thereof on a matrix prior to the incubating with the mild reducing buffer and eluting the immunoglobulin from the matrix following the incubating with the oxidizing buffer.

4. The method of claim 1, wherein the thiol-reactive compound is attached to a functional agent.

5. The method of claim 4, wherein the functional agent comprises a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.

6. The method of claim 1, wherein the thiol-reactive compound is bound to a second thiol-reactive compound, the second thiol-reactive compound being bound to amino acid position 80 in a light chain variable region of a second chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof ("$Cys80^2$"), the $Cys80^2$ based upon the Kabat or Chothia numbering system.

7. The method of claim 1, wherein the Cys80 is unpaired with Cys171 of the light chain.

8. The method of claim 1, further comprising substituting an amino acid at position 83 with an amino acid residue other than Phe, Lys, or Cys, the position 83 based upon the Kabat or Chothia numbering system.

9. A method for generating an antigen-binding molecule, the method comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein:

the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("$Cys80^1$") wherein the $Cys80^1$ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and wherein the immunoglobulin is a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof and the $Cys80^1$ is based upon the Kabat or Chothia numbering system; and

the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("$Cys80^2$") wherein the $Cys80^2$ is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group, and wherein the immunoglobulin is a chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof and the $Cys80^2$ is based upon the Kabat or Chothia numbering system.

10. The method of claim 9, wherein the $Cys80^1$ is unpaired with $Cys171^1$ of the first light chain and/or the $Cys80^2$ is unpaired with $Cys171^2$ of the second light chain.

11. The method of claim 9, further comprising, prior to the incubating step,

incubating a first chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof with a mild reducing buffer comprising cysteine, dithiothreitol DTT), or tris (2-carboxyethyl) phosphdne (TCEP) to decap the $Cys80^1$ and/or incubating a second chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof with a mild reducing buffer comprising cysteine,

dithiothreitol (DTT), or tris (2-carboxyethyl) phosphine (TCEP) to decap the $Cys80^2$; and conjugating a first thiol-reactive compound to the decapped $Cys80^1$ and/or a second thiol-reactive compound to the decapped $Cys80^2$, wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

12. The method of claim 9, wherein the first thiol-reactive compound further comprises a first functional agent and/or the second thiol-reactive compound further comprises a second functional agent.

13. The method of claim 9, wherein the first chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof is a first Fab and/or the second chimeric or humanized rabbit immunoglobulin or antigen-binding fragment thereof is a second Fab.

14. The method of claim 9, further comprising substituting an amino acid at position 83 of the first light chain variable region with an amino acid residue other than Phe, Lys, or Cys, and/or substituting an amino acid at position 83 of the second light chain variable region with an amino acid residue other than Phe, Lys, or Cys, the position 83 based upon the Kabat or Chothia numbering system.

15. An antigen-binding molecule produced according to the method of claim 9.