

CLAIMS:

1. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the one or more mutations include a mutation at light chain position 180 (positional numbering).
2. The antibody of claim 1 comprising two introduced site specific conjugation sites.
3. The antibody of any preceding claim wherein the wild type residue at position 180 is T; and/or wherein the mutated residue at position 180 is selected from cysteine (C), lysine (K), glutamine (Q), or a non-natural amino acid.
4. The antibody of any preceding claim further comprising one or more mutations at one or more positions selected from residues 206 (light chain), 160 (heavy chain), 190 (heavy chain), 443 (heavy chain), and 447 (heavy chain) (positional numbering); preferably wherein the mutated residues are independently selected from cysteine, lysine, glutamine, or a non-natural amino acid; and more preferably wherein the mutated residues are LC T180C in combination with HC S160C.
5. The antibody of any preceding claim further comprising one or more mutations selected from light chain (LC) S208, LC S171, LC S182, LC A184, LC V191, LC S202, LC S203, LC T206, heavy chain (HC) S160, HC T190, HC S443, HC S447, HC S139, HC S168, HC V170, HC V176, HC T200, HC S445 according to a positional numbering convention.
6. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody comprises the amino acid sequence of SEQ ID No 33; where X is selected from C, K, Q, or a non-natural amino acid.
7. The antibody of claim 6, further comprising one or more amino acid sequences selected from the group consisting of SEQ ID No 22 to 32 and 34 to 40; where each X is independently selected from C, K, Q, or a non-natural amino acid; and preferably wherein

the antibody comprises the amino acid sequences of SEQ ID No. 33 and SEQ ID No. 23, where X is C.

8. An isolated or engineered antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site by:

a) substituting a residue selected from the group consisting of light chain (LC) T180, LC S208, LC S171, LC S182, LC A184, LC V191, LC S202, LC S203, LC T206, heavy chain (HC) S160, HC T190, HC S443, HC S447, HC S139, HC S168, HC V170, HC V176, HC T200, HC S445 according to a positional numbering convention

with a cysteine residue, a lysine residue, a glutamine residue or a non-natural amino acid; or

b) introducing a cysteine substitution at a residue selected from the group consisting of LC T180C, LC S208C, LC S171C, LC S182C, LC A184C, LC V191C, LC S202C, LC S203C, LC T206C, HC S160C, HC T190C, HC S443C, HC S447C, HC S139C, HC S168C, HC V170C, HC V176C, HC T200C, HC S445C according to a positional numbering convention; or

c) comprising at least one amino acid sequence selected from the group consisting of: SEQ ID No 22 to 40; wherein X is a cysteine residue, a lysine residue, a glutamine residue or a non-natural amino acid; or

d) comprising at least one amino acid sequence selected from the group consisting of: SEQ ID No 22 to 40; wherein X is a cysteine residue.

9. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody includes a light chain constant region comprising the amino acid sequence of residues 109-214 of SEQ ID No 14.

10. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the one or more mutations include a mutation at light chain position 208 (positional numbering).

11. The antibody of claim 10 comprising two introduced site specific conjugation sites, and/or wherein the wild type residue at position 208 is S; and/or wherein the mutated residue at

position 208 is selected from cysteine, lysine, glutamine, or a non-natural amino acid and is preferably cysteine.

12. The antibody of any of claims 10 to 11, further comprising one or more mutations selected from light chain (LC) T180, LC S171, LC S182, LC A184, LC V191, LC S202, LC S203, LC T206, heavy chain (HC) S160, HC T190, HC S443, HC S447, HC S139, HC S168, HC V170, HC V176, HC T200, HC S445 according to a positional numbering convention; and preferably wherein the mutated residues are independently selected from cysteine, lysine, glutamine, or a non-natural amino acid, and is most preferably cysteine.

13. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody comprises the amino acid sequence of SEQ ID No 40; where X is selected from C, K, Q, or a non-natural amino acid.

14. The antibody of claim 13 further comprising one or more amino acid sequences selected from the group consisting of SEQ ID No 22-39; where each X is independently selected from C, K, Q, or a non-natural amino acid; and/or wherein X is C.

15. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody includes a light chain constant region comprising the amino acid sequence of residues 109-214 of SEQ ID No 21.

16. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody includes a light chain comprising the amino acid sequence of SEQ ID No 21; and preferably including a heavy chain comprising an amino acid sequence selected from SEQ ID No 2 to SEQ ID No 12.

17. The antibody of any preceding claim, wherein the antibody is selected from the group comprising IgG1, IgG2, IgG3, and IgG4.

18. The antibody of any preceding claim wherein the constant region comprises at least a portion of an IgG1 constant region; and preferably wherein the constant region comprises one or more, preferably all, of the C_κ, CH1 and CH3 domains of the IgG1 constant region.

19. The antibody of any preceding claim wherein the antibody is selected from the group consisting of Fabs, bi specific antibody fragments (tandem scFv-Fc, scFv-Fc knobs-into-holes, scFv-Fc-scFv, F(ab')₂, Fab-scFv, (Fab'scFv)₂, scDiabody-Fc, or scDiabody-C_H3), IgG-based bispecific antibodies (Hybrid hybridoma, Knobs-into-holes with common light chain, Two-in-one IgG, Dual V domain IgG, IgG-scFv, scFv-IgG, IgG-V, V-IgG), minibody, tribi-minibody, nanobodies, and di-diabody.

20. The antibody of any preceding claim wherein the antibody is human, humanised, or chimeric.

21. The antibody of any preceding claim wherein the antibody is selected from Abciximab; Rituximab; Basiliximab ;Daclizumab; Palivizumab; Infliximab; Trastuzumab; Alemtuzumab; Adalimumab; Efalizumab; Cetuximab; Ibritumomab; Omalizumab; Bevacizumab; Ranibizumab; Golimumab; Canakinumab; Ustekinumab; Tocilizumab; Ofatumumab; Belimumab; Ipilimumab; Brentuximab; Pertuzumab; Raxibacumab; Vedolizumab; Ramucirumab; Obinutuzumab; Siltuximab; Secukinumab; Dinutuximab.

22. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the antibody includes a light chain comprising the amino acid sequence of SEQ ID No 14, and preferably wherein the antibody includes a heavy chain comprising an amino acid sequence selected from SEQ ID No 2 to SEQ ID No 12.

23. The antibody of any preceding claim which lacks one or more Fc effector functions, and preferably which lacks ADCC activity.

24. The antibody of any of claims 1 to 22 which has increased ADCC activity.

25. The antibody of any preceding claim further comprising a linker.

26. An immunoconjugate comprising an antibody according to any of claims 1 to 25, a payload, and a linker joining the payload to the antibody.

27. The antibody of claim 25, or the immunoconjugate of claim 26, wherein the linker is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), N-Succinimidyl, (4-iodo-acetyl) aminobenzoate (SIAB),), SPDB, hydrazone, maleimidocaproyl and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB); or is a branched linker which comprises a peptide chain and is derived from o-hydroxy p-amino benzylic alcohol, wherein the peptide chain is connected to the phenyl ring via the p-amino group, the payload is connected to the phenyl ring via the benzylic alcohol moiety, and the antibody is connected to the phenyl ring via the o-hydroxy group.

28. The immunoconjugate of claim 26 or 27, wherein the payload is selected from the group consisting of a radionuclide, a chemotherapeutic agent, a cytotoxic agent, a microbial toxin, a plant toxin, a polymer, a carbohydrate, a cytokine, a fluorescent label, a luminescent label, an enzyme-substrate label, an enzyme, a peptide, a peptidomimetic, a nucleotide, an siRNA, a microRNA, an RNA mimetic, and an aptamer; and preferably wherein the payload is selected from the group consisting of ⁹⁰Y, ¹³¹I, ⁶⁷Cu, ¹⁷⁷Lu, ²¹³Bi, ²¹¹At, dolastatin, vedotin, monomethyl auristatin F(MMAF), monomethyl auristatin E (MMAE); maytansinoids including DM1 and DM4, duocarmycin, duocarmycin analogs, calicheamicin, pyrrolobenzodiazepines (PBD), centanamycin, irinotecan, and doxorubicin, alpha-amanitin, melatonin, membrane disrupting peptide, Pseudomonas exotoxin A, Diphtheria toxin, ricin, polyethylene glycol, hydroxyethyl starch, and a mannosyl residue; or wherein the payload is a microtubule disrupting agent, or a DNA modifying agent.

29. An antibody, or a fragment or derivative thereof, having a variable region which binds a target molecule, and a constant region, wherein the constant region comprises two or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, fragment, or derivative to a payload, wherein the two or more mutations include mutations selected from the group consisting of:

LC T180 in combination with LC S208,

LC T180 in combination with LC S171,

LC T180 in combination with LC S182,

LC T180 in combination with LC A184,

LC T180 in combination with LC V191,
 LC T180 in combination with LC S202,
 LC T180 in combination with LC S203,
 LC T180 in combination with LC T206,
 LC T180 in combination with HC S160,
 LC T180 in combination with HC T190
 LC T180 in combination with HC S443,
 LC T180 in combination with HC S447,
 LC T180 in combination with HC S139,
 LC T180 in combination with HC S168,
 LC T180 in combination with HC V170,
 LC T180 in combination with HC V176,
 LC T180 in combination with HC T200, and
 LC T180 in combination with HC S445.

30. A pharmaceutical composition comprising an antibody or an immunoconjugate according to any preceding claim, and a pharmaceutically acceptable diluent, carrier or excipient.

31. A method for generating an immunoconjugate, the method comprising conjugating an antibody according to any of claims 1 to 25 or 29 to a payload.

32. The antibody of any of claims 1 to 25 or 29 prepared by a process comprising:

- (i) mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues with a mutant residue to encode the antibody;
- (ii) expressing the mutant antibody; and
- (iii) isolating the mutant antibody;

and preferably wherein the antibody is expressed in a prokaryotic or a eukaryotic cell; preferably a mammalian or an insect cell.

33. The antibody of claim 32, the process further comprising:

- (i) reacting the mutant antibody with a thiol-reactive affinity reagent to generate an affinity labelled, antibody; and
- (ii) measuring the binding of the affinity labelled antibody to a capture media.

34. An isolated or recombinant polynucleotide encoding the antibody of any of claims 1-25 or 29.

35. A vector comprising the polynucleotide of claim 34; preferably further comprising an inducible promoter operably linked to the polynucleotide.

36. A host cell comprising the vector of claim 35.

37. A method of producing an antibody comprising:

- (a) providing a culture medium comprising the host cell of claim 36; and
- (b) placing the culture medium in conditions under which the antibody is expressed, and optionally (c) isolating the antibody.

38. The antibody of claim 29, wherein the two or more mutations include LC T180 in combination with HC S160, preferably wherein the two or more mutations include LC T180C in combination with HC S160C.

39. The antibody of any one of claim 3, 5, or 6, wherein the mutated residue(s) are mutated to cysteine.