

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).
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2. The antibody of claim 1 comprising two introduced site specific conjugation sites.
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3. The antibody of any preceding claim wherein the wild type residue at position 180 is T.
4. The antibody of any preceding claim wherein the mutated residue at position 180 is selected from cysteine (C), lysine (K), glutamine (Q), or a non-natural amino acid.
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5. 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).
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6. The antibody of claim 5, wherein the mutated residues are independently selected from cysteine, lysine, glutamine, or a non-natural amino acid.
7. The antibody of claim 5, wherein the mutated residues are LC T180C in combination with HC S160C.
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8. 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.
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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
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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.

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10. The antibody of claim 9, 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.

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11. The antibody of claim 10 comprising the amino acid sequences of SEQ ID No. 33 and SEQ ID No. 23, where X is C.

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12. 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:

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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

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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

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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.

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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 includes a light chain constant region comprising the amino acid sequence of residues 109-214 of SEQ ID No 14.

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14. 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).

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15. The antibody of claim 14 comprising two introduced site specific conjugation sites.

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16. The antibody of claim 14 or 15 wherein the wild type residue at position 208 is S.

17. The antibody of any of claims 14 to 16 wherein the mutated residue at position 208 is selected from cysteine, lysine, glutamine, or a non-natural amino acid.

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18. The antibody of claim 17 wherein the mutated residue at position 208 is cysteine.

19. The antibody of any of claims 14 to 18, 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.

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20. The antibody of claim 19 wherein the mutated residues are independently selected from cysteine, lysine, glutamine, or a non-natural amino acid, and is most preferably cysteine.

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21. 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

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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.

5 22. The antibody of claim 21 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.

23. The antibody of claim 21 or 22 wherein X is C.

10 24. 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
15 residues 109-214 of SEQ ID No 21.

25. 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
20 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.

26. The antibody of claim 25 including a heavy chain comprising an amino acid sequence selected from SEQ ID No 2 to SEQ ID No 12.

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

30 28. The antibody of any preceding claim wherein the constant region comprises at least a portion of an IgG1 constant region.

29. The antibody of claim 28 wherein the constant region comprises one or more, preferably all, of the Ck, CH1 and CH3 domains of the IgG1 constant region.

30. 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.

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

32. 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.

33. 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.

34. The antibody of claim 33, wherein the antibody includes a heavy chain comprising an amino acid sequence selected from SEQ ID No 2 to SEQ ID No 12.

35. The antibody of any preceding claim which lacks one or more Fc effector functions.

36. The antibody of claim 35 which lacks ADCC activity.

37. The antibody of any of claims 1 to 34 which has increased ADCC activity.

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

39. An immunoconjugate comprising an antibody according to any of claims 1 to 38, a payload, and a linker joining the payload to the antibody.

40. The antibody of claim 38, or the immunoconjugate of claim 39, 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.

41. The immunoconjugate of claim 39 or 40, 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.

42. 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,
 5 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
 10 LC T180 in combination with HC S445.

43. The immunoconjugate of claim 41, 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
 15 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..

20 44. The immunoconjugate of claim 41, wherein the payload is a microtubule disrupting
 agent, or a DNA modifying agent.

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

46. A method for generating an immunoconjugate, the method comprising conjugating
 an antibody according to any of claims 1 to 38 or 42 to a payload.

30 47. The antibody of any of claims 1 to 38 or 42 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.

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48. The antibody of claim 47 wherein the antibody is expressed in a prokaryotic or a eukaryotic cell; preferably a mammalian or an insect cell.

49. The antibody of claim 47 or 48, the process further comprising:

- 5 (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.

10 50. An isolated or recombinant polynucleotide encoding the antibody of any of claims 1-38 or 42.

51. A vector comprising the polynucleotide of claim 50.

15 52. The vector of claim 51 further comprising an inducible promoter operably linked to the polynucleotide.

53. A host cell comprising the vector of claim 51 or claim 52.

54. A method of producing an antibody comprising:

- 20 (a) providing a culture medium comprising the host cell of claim 53; and
(b) placing the culture medium in conditions under which the antibody is expressed, and optionally (c) isolating the antibody.

25 55. The antibody of claim 42, wherein the two or more mutations include LC T180 in combination with HC S160.

56. The antibody of claim 55, wherein the two or more mutations include LC T180C in combination with HC S160C.

30 57. The antibody of any one of claim 4, 8, or 9, wherein the mutated residue(s) are mutated to cysteine.

CLAIMS:

1. An antibody or a fragment thereof, having a variable region which binds a target molecule, and a constant region, wherein the light chain is a kappa light chain, and wherein the constant region comprises one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, or fragment to a payload, wherein the one or more mutations include a mutation to cysteine at light chain position 208 (positional numbering) and a mutation at one or more of heavy chain positions 160, 190, 168, 170, 176 or 200.
2. The antibody of claim 1 wherein the wild type residue at position 208 is S.
3. The antibody of claim 1 or 2, wherein the wild type residue at heavy chain position 160 is S, the wild type residue at heavy chain position 190 is T, the wild type residue at heavy chain position 168 is S, the wild type residue at heavy chain position 170 is V, the wild type residue at heavy chain position 176 is V or the wild type residue at heavy chain position 200 is T.
4. The antibody of any of claims 1-3, wherein the mutated residues are cysteine.
5. The antibody of any preceding claim, wherein the antibody is selected from the group comprising IgG1, IgG2, IgG3, and IgG4.
6. The antibody of any preceding claim wherein the constant region comprises at least a portion of an IgG1 constant region.
7. The antibody of claim 6 wherein the constant region comprises one or more of the Ck, CH1 and CH3 domains of the IgG1 constant region.
8. 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-CH3), 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.
9. The antibody of any preceding claim wherein the antibody is human, humanised, or chimeric.

10. The antibody of any preceding claim comprising one or more mutations introducing a site specific conjugation site selected so as to permit conjugation of the antibody, wherein the antibody is selected from Abciximab; Rituximab; Basiliximab ;Daclizumab; Palivizumab; Infliximab; Trastuzumab; Alemtuzumab; Adalimumab; Efalizumab; Cetuximab; Ibritumomab; 5 Omalizumab; Bevacizumab; Ranibizumab; Golimumab; Canakinumab; Ustekinumab; Tocilizumab; Ofatumumab; Belimumab; Ipilimumab; Brentuximab; Pertuzumab; Raxibacumab; Vedolizumab; Ramucirumab; Obinutuzumab; Siltuximab; Secukinumab; Dinutuximab.

11. The antibody of any preceding claim which lacks one or more Fc effector 10 functions.

12. The antibody of claim 11 which lacks ADCC activity.

13. The antibody of any of claims 1 to 11 which has increased ADCC activity. 15

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

15. An immunoconjugate comprising an antibody according to any of claims 1 to 14, a payload, and a linker joining the payload to the antibody. 20

16. The antibody of claim 14, or the immunoconjugate of claim 15, 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 25 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 30 is connected to the phenyl ring via the o-hydroxy group.

17. The immunoconjugate of claim 15 or 16, 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 35 enzyme-substrate label, an enzyme, a peptide, a peptidomimetic, a nucleotide, an siRNA, a microRNA, an RNA mimetic, and an aptamer.

18. The immunoconjugate of claim 17 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),
5 centanamycin, irinotecan, and doxorubicin, alpha-amanitin, melatonin, membrane disrupting peptide, Pseudomonas exotoxin A, Diphtheria toxin, ricin, polyethylene glycol, hydroxyethyl starch, and a mannosyl residue.

19. The immunoconjugate of claim 15 or 16, wherein the payload is a microtubule
10 disrupting agent, or a DNA modifying agent.

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

21. A method for generating an immunoconjugate, the method comprising conjugating
15 an antibody according to any of claims 1 to 14 to a payload.

22. An isolated or recombinant polynucleotide encoding the antibody of any of claims
20 1-13.

23. A vector comprising the polynucleotide of claim 22.

24. The vector of claim 23 further comprising an inducible promoter operably linked
25 to the polynucleotide.

25. A host cell comprising the vector of claim 23 or claim 24.

26. A method of producing an antibody comprising:

- 30 (a) providing a culture medium comprising the host cell of claim 25; and
(b) placing the culture medium in conditions under which the antibody is expressed,
and optionally (c) isolating the antibody.