

CLAIMS:

1. An antibody, or a fragment 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 or fragment to a payload, wherein the one or more mutations include a mutation at light chain position 180 of the light chain wherein the mutated residue at light chain position 180 is cysteine and wherein the fragment retains the specific binding properties of the antibody and wherein said antibody or fragment thereof has a reduced aggregation propensity compared to an antibody or fragment thereof without the one or more mutation.
2. The antibody of claim 1 wherein the wild type residue at position 180 is T.
3. The antibody of any preceding claim further comprising a mutation at residue 206 of the light chain.
4. 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.
5. An antibody, or a fragment 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 to a payload, wherein the antibody comprises the amino acid sequence of SEQ ID No 33; where X is C, and wherein the fragment retains the specific binding properties of the antibody.
6. The antibody of claim 5, 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.
7. An isolated or engineered antibody or a fragment 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, and wherein the fragment retains the specific binding properties of the antibody.

8. The antibody of any preceding claim, wherein the antibody is selected from the group comprising IgG1, IgG2, IgG3, and IgG4, wherein preferably the antibody is human, humanised or chimeric.

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

10. 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.

11. 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.

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

13. An immunoconjugate comprising an antibody according to any of claims 1 to 12, a payload, and a linker joining the payload to the antibody.

14. The antibody of claim 12, or the immunoconjugate of claim 13, 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.

15. The immunoconjugate of claim 13 or 14, 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; wherein preferably the payload is selected from the group consisting of ^{90}Y , ^{131}I , ^{67}Cu , ^{177}Lu , ^{213}Bi , ^{211}At , 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.

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

17. A method for generating an immunoconjugate, the method comprising conjugating an antibody according to any of claims 1 to 12 to a payload.

18. The antibody of any of claims 1 to 12 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; preferably in a prokaryotic or a eukaryotic cell; preferably a mammalian or an insect cell; and

(iii) isolating the mutant antibody,

and wherein preferably the process further comprises:

(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.

19. An isolated or recombinant polynucleotide encoding the antibody of any of claims 1 to 12 or a

vector comprising the polynucleotide, wherein preferably the vector further comprises an inducible promoter operably linked to the polynucleotide or a host cell comprising the vector.

20. A method of producing an antibody comprising:

(a) providing a culture medium comprising the host cell of claim 19; and

(b) placing the culture medium in conditions under which the antibody is expressed, and optionally (c) isolating the antibody.