CLAIMS

1. An antibody-drug conjugate (ADC) comprising:
   (a) Brentuximab, wherein Brentuximab comprises at the C-terminus of the light chains, the heavy chains or all of the heavy and light chains of the Brentuximab a recognition sequence for tubulin tyrosine ligase and a non-natural amino acid; and
   (b) at least one drug moiety;

   wherein a drug moiety is coupled to each of the non-natural amino acids via a linker.

2. The ADC of claim 1,
   wherein the heavy chains of Brentuximab have an amino acid sequence that comprises or consists of SEQ ID NO: 1 or have a sequence identity of at least 95% to SEQ ID NO: 1; and/or
   wherein the light chains of Brentuximab have an amino acid sequence that comprises or consists of SEQ ID NO: 2 or have a sequence identity of at least 95% to SEQ ID NO: 2.

3. The ADC of claim 2, wherein Brentuximab consists of heavy chains consisting of the amino acid sequence of SEQ ID NO: 1 and light chains consisting of the amino acid sequence of SEQ ID NO: 2.

4. The ADC of any one of the preceding claims, wherein the drug moiety is selected from the group consisting of camptothecins, maytansinoids, calicheamycins, duocarmycins, tubulysins, amatoxins, dolastatins and auristatins such as monomethyl auristatin E (MMAE), pyrrolobenzodiazepine dimers, indolino-benzodiazepine dimers, radioisotopes, therapeutic proteins and peptides (or fragments thereof), nucleic acids, PROTACs, kinase inhibitors, MEK inhibitors, KSP inhibitors, and analogues or prodrugs thereof.

5. The ADC of claim 4, wherein the drug moiety is MMAE.

6. The ADC of any one of the preceding claims, wherein the recognition sequence for tubulin tyrosine ligase has at least the amino acid sequence $X_1X_2X_3X_4$ (SEQ ID NO: 3), wherein X_1 and X_2 is any amino acid, X_3 is E, D or C and X_4 is E.

7. The ADC of claim 6, wherein X_2 is G, S, A, V, or F and/or wherein X_1 is E, D, A, K, or P.

8. The ADC of claim 7, wherein the recognition sequence is EGEE (SEQ ID No. 4).

9. The ADC of claim 7, wherein the recognition sequence is VDSVEGEGEEEGEE (SEQ ID No. 5), SVEGEGEEEGEE (SEQ ID No. 6), SADGEDEGEE (SEQ ID No. 7), SVEAEAEEGEE (SEQ ID No. 8), SYEDEDEGEE (SEQ ID No. 9), or SFEEENEGEE (SEQ ID No. 10).

10. The ADC of any one of the preceding claims, wherein the unnatural amino acid is a 2-substituted, 3-substituted or 4-substituted tyrosine or a tyrosine derivative substituted at the benzylic position.

11. The ADC of claim 10, wherein said 3- or 4-substituted tyrosine derivative is 3-nitrotyrosine, 3-aminotyrosine, 3-azidotyrosine, 3-formyltyrosine, 3- acetyltyrosine, or 4-aminophenylalanine.

12. The ADC of claim 11, wherein said 3- or 4-substituted tyrosine derivative is 3-formyltyrosine.

13. The ADC of any one of the preceding claims, wherein the linker is cleavable.

14. The ADC of claim 13, wherein the linker is cleavable by a protease.

15. The ADC of claim 14, wherein the protease is a cathepsin such as cathepsin B.

16. The ADC of any one of the preceding claims wherein the linker comprises a valine-citrulline moiety.

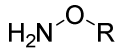
17. The ADC of any one of the preceding claims, wherein the linker comprises a hydroxylamine group and the unnatural amino acid comprises a formyl group ortho of a hydroxyl group in an aromatic ring, and wherein the hydroxylamine group of the linker forms an oxime with the formyl group of the unnatural amino acid after conjugation.

18. The ADC of any one of the preceding claims, wherein Brentuximab is conjugated to two, four, six, or eight drug moieties.

19. The ADC of claim 18, wherein Brentuximab is conjugated to two or four drug moieties.

20. The ADC of claim 19, wherein Brentuximab is conjugated to two drug moieties.

21. The ADC of any one of the preceding claims, wherein the linker has a structure as depicted in structure 1 before being coupled to the unnatural amino acid:



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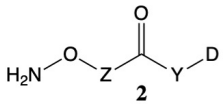
wherein R is one or more drug moieties, which are optionally coupled to the hydroxylamine of structure 1 by one or more cleavage sites.

22. The ADC of claim 21, wherein the hydroxylamine of structure 1 is conjugated to the unnatural amino acid.

23. The ADC of any one of the preceding claims, wherein the linker has a structure as depicted in structure 2 or 3 before being coupled to the unnatural amino acid:



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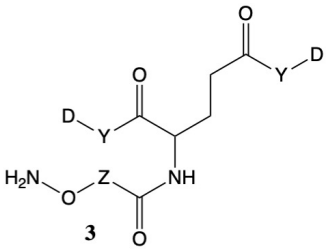

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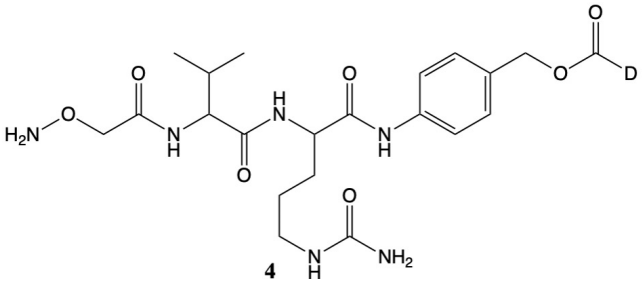
wherein Z is is selected from the group consisting of substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heteroarylalkyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl and substituted or unsubstituted heteroalkynyl;

wherein D is one or more drug moieties; and

wherein Y is a cleavage site such as a cleavage site for a cathepsin such as cathepsin B.

24. The ADC of claim 23, wherein the hydroxylamine of structure 2 or 3 is conjugated to the unnatural amino acid.

25. The ADC of any one of the preceding claims, wherein the linker has a structure as depicted in structure 4 or 5 before being coupled to the unnatural amino acid, wherein D is a drug moiety:



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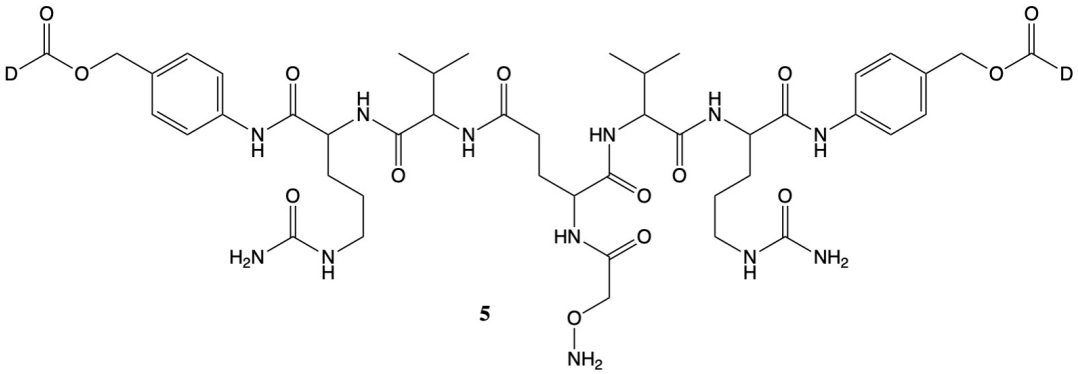

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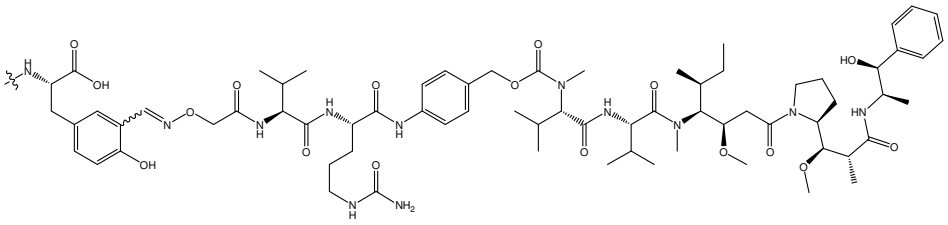
26. The ADC of claim 25, wherein the unnatural amino acid is 3-formyltyrosine and the hydroxylamine group of the linker forms an oxime with the 3-formyl group of the unnatural amino acid.

27. The ADC of claim 26, wherein the drug moiety is MMAE.

28. An antibody-drug conjugate (ADC) comprising:

(a) Brentuximab, wherein Brentuximab comprises at the C-terminus of each light chain a recognition sequence for tubulin-tyrosine ligase;
each light chain, including the recognition sequence, has SEQ ID NO: 11; and
each heavy chain of Brentuximab has SEQ ID NO: 1; and

(b) the C-terminus of the recognition sequence of each light chain is bound via an amide bond to a group having the following structure:



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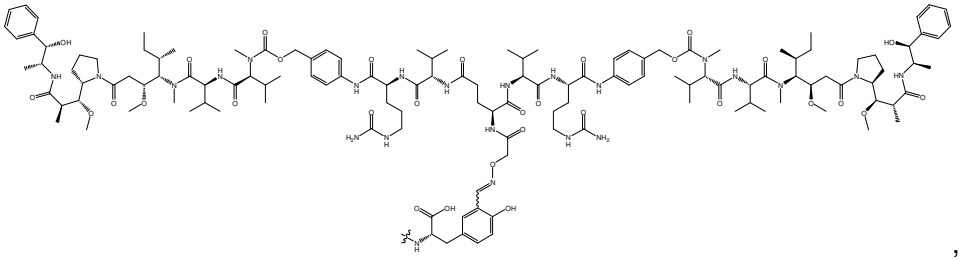
wherein the wavy line indicates attachment to the C-terminus of the recognition sequence of each light chain.

29. An antibody-drug conjugate (ADC) comprising:

(a) Brentuximab, wherein Brentuximab comprises at the C-terminus of each light chain a recognition sequence for tubulin-tyrosine ligase;
each light chain, including the recognition sequence, has SEQ ID NO: 11; and
each heavy chain of Brentuximab has SEQ ID NO: 1; and

(b) the C-terminus of the recognition sequence of each light chain is bound via an amide bond to a group having the following structure:



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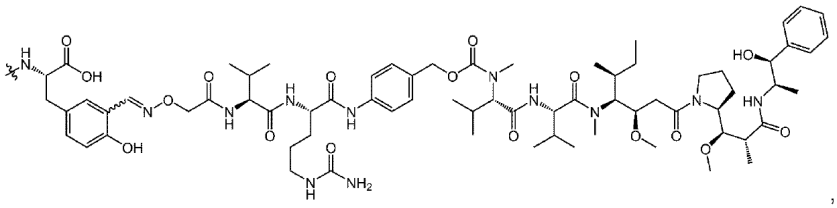
wherein the wavy line indicates attachment to the C-terminus of the recognition sequence of each light chain.

30. An antibody-drug conjugate (ADC) comprising:

(a) an anti-CD30 IgG antibody consisting of two light chains and two heavy chains, wherein the heavy chains each consist of the amino acid sequence as shown in SEQ ID NO: 1, wherein optionally the last residue K at position 447 of the heavy chains is omitted and wherein the light chains each consist of the amino acid sequence as shown in SEQ ID NO: 11; and

(b) the C-terminus of each light chain is bound via an amide bond to a group having the following structure:



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wherein the most left wavy line indicates attachment to the C-terminus of each light chain.

31~~0~~. A method of producing an ADC as defined in any one of claims 1-30~~29~~, comprising

(a) introducing or adding at the C-terminus of the light chain, the heavy chain or both the light chain and the heavy chain of Brentuximab a recognition sequence for tubulin tyrosine ligase;

(b) contacting the Brentuximab obtained in step (a) in the presence of tubulin tyrosine ligase and a non-natural amino acid under conditions suitable for the tubulin tyrosine ligase to ligate said Brentuximab with said non-natural amino acid; and

(c) conjugating an optionally cleavable linker comprising a drug moiety to said ligated Brentuximab obtained in step (b).

32~~1~~. An ADC obtainable or being obtained by the method of claim 31~~0~~.

33~~2~~. A pharmaceutical composition comprising the ADC of any one of claims 1 to 30~~29~~ or 32~~1~~.

~~33. The ADC of any one claims 1 to 29 or 31 for use in a method of treating a disease.~~

~~34. The ADC for use of claim 33, wherein the disease is associated with overexpression of CD30.~~

~~35. The ADC for use of claim 33 or 34, wherein the disease is selected from the group consisting of lymphoma, such as Hodgkin's lymphoma (HL), non-Hodgkin lymphoma (NHL), anaplastic large-cell lymphoma (ALCL), large B-cell lymphoma, paediatric lymphoma, T-cell lymphoma and enteropathy-associated T-cell lymphoma (EATL), leukaemia, such as acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and mast cell leukaemia, germ cell cancer, graft-versus-host disease (GvHD) and lupus, in particular systemic lupus erythematosus (SLE); or~~

~~wherein the disease is selected from the group consisting of peripheral T cell lymphoma – not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), enteropathy associated T cell lymphoma (EATL), adult T-cell leukemia/lymphoma (ATLL), extranodal natural killer/T-cell lymphoma (ENKTCL), hepatosplenic and intestinal γ/δ-T cell lymphoma, nodal peripheral T-cell lymphoma with TFH phenotype, and follicular T cell lymphoma.~~

~~36. The ADC for use of claim 35, wherein the disease is Hodgkin Lymphoma (HL) or anaplastic large cell lymphoma (ALCL); or~~

~~wherein the disease is peripheral T cell lymphoma (PTCL), including anaplastic large cell lymphoma (ALCL); or cutaneous T cell lymphoma (CTCL), including primary cutaneous anaplastic large cell lymphoma (pcALCL).~~

~~37. The pharmaceutical composition of claim 32 for use in a method of treating a disease.~~

~~38. The pharmaceutical composition for use of claim 37, wherein the disease is associated with overexpression of CD30.~~

~~39. The pharmaceutical composition for use of claim 37 or 38, wherein the disease is selected from the group consisting of lymphoma, such as Hodgkin's lymphoma (HL), non-Hodgkin lymphoma (NHL), anaplastic large-cell lymphoma (ALCL), large B-cell lymphoma, paediatric lymphoma, T-cell lymphoma and enteropathy-associated T-cell lymphoma (EATL), leukaemia, such as acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and mast cell leukaemia, germ cell cancer, graft-versus-host disease (GvHD) and lupus, in particular systemic lupus erythematosus (SLE); or~~

~~wherein the disease is selected from the group consisting of peripheral T cell lymphoma – not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), enteropathy associated T cell lymphoma (EATL), adult T-cell leukemia/lymphoma (ATLL), extranodal natural killer/T-cell lymphoma (ENKTCL), hepatosplenic and intestinal γ/δ-T cell lymphoma, nodal peripheral T-cell lymphoma with TFH phenotype, and follicular T cell lymphoma.~~

~~40. The pharmaceutical composition for use of claim 39, wherein the disease is Hodgkin Lymphoma (HL) or anaplastic large cell lymphoma (ALCL); or~~

~~wherein the disease is peripheral T cell lymphoma (PTCL), including anaplastic large cell lymphoma (ALCL); or cutaneous T cell lymphoma (CTCL), including primary cutaneous anaplastic large cell lymphoma (pcALCL).~~

34~~41~~. Use of an antibody drug conjugate (ADC) of any one of claims 1 to 30~~29~~ or 32~~1~~ for the manufacture of a medicament for treatment of a disease associated with overexpression of CD30.

35~~42~~. The use of claim 34~~41~~, wherein the disease is selected from the group consisting of lymphoma, such as Hodgkin's lymphoma (HL), non-Hodgkin lymphoma (NHL), anaplastic large-cell lymphoma (ALCL), large B-cell lymphoma, paediatric lymphoma, T-cell lymphoma and enteropathy-associated T-cell lymphoma (EATL), leukaemia, such as acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and mast cell leukaemia, germ cell cancer, graft-versus-host disease (GvHD) and lupus, in particular systemic lupus erythematosus (SLE); or

wherein the disease is selected from the group consisting of peripheral T cell lymphoma – not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), enteropathy associated T cell lymphoma (EATL), adult T-cell leukemia/lymphoma (ATLL), extranodal natural killer/T-cell lymphoma (ENKTCL), hepatosplenic and intestinal γ/δ-T cell lymphoma, nodal peripheral T-cell lymphoma with TFH phenotype, and follicular T cell lymphoma.

36~~43~~. The use of claim 35~~42~~, wherein the disease is Hodgkin Lymphoma (HL) or anaplastic large cell lymphoma (ALCL); or

wherein the disease is peripheral T cell lymphoma (PTCL), including anaplastic large cell lymphoma (ALCL); or cutaneous T cell lymphoma (CTCL), including primary cutaneous anaplastic large cell lymphoma (pcALCL).

37~~44~~. Use of the pharmaceutical composition of claim 33~~2~~ for the manufacture of a medicament for treatment of a disease associated with overexpression of CD30.

38~~45~~. The use of claim 37~~44~~, wherein the disease is selected from the group consisting of lymphoma, such as Hodgkin's lymphoma (HL), non-Hodgkin lymphoma (NHL), anaplastic large-cell lymphoma (ALCL), large B-cell lymphoma, paediatric lymphoma, T-cell lymphoma and enteropathy-associated T-cell lymphoma (EATL), leukaemia, such as acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and mast cell leukaemia, germ cell cancer, graft-versus-host disease (GvHD) and lupus, in particular systemic lupus erythematosus (SLE); or

wherein the disease is selected from the group consisting of peripheral T cell lymphoma – not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), enteropathy associated T cell lymphoma (EATL), adult T-cell leukemia/lymphoma (ATLL), extranodal natural killer/T-cell lymphoma (ENKTCL), hepatosplenic and intestinal γ/δ-T cell lymphoma, nodal peripheral T-cell lymphoma with TFH phenotype, and follicular T cell lymphoma.

39~~46~~. The use of claim 38~~45~~, wherein the disease is Hodgkin Lymphoma (HL) or anaplastic large cell lymphoma (ALCL); or

wherein the disease is peripheral T cell lymphoma (PTCL), including anaplastic large cell lymphoma (ALCL); or cutaneous T cell lymphoma (CTCL), including primary cutaneous anaplastic large cell lymphoma (pcALCL).