

Claims

1. An antibody which binds to AXL, wherein

- the antibody does not compete for AXL binding with the ligand Growth Arrest-Specific 6 (Gas6), and
- the antibody comprises at least one binding region, which comprises a variable heavy (VH) region and a variable light chain (VL) region selected from the group consisting of;
 - a) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 36, 37, and 38, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 39, GAS, and 40, respectively, **[107]**;
 - b) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 93, 94, and 95, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 96, GAS, and 97, respectively, **[613]**;
 - c) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 98, 99, and 100, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 101, DAS, and 102, respectively, **[613-08]**;
 - d) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 93, 94, and 95, respectively, and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 128, XAS, wherein X is D or G, and 129, respectively, **[613 / 613-08]**; and
 - e) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 93, 126, and 127, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 96, GAS, and 97, respectively **[613 / 608-01 / 610-01 / 620-06]**.

2. The antibody according to claim 1, wherein the antibody comprises at least one binding region, which comprises a variable heavy chain (VH) region and a variable light chain (VL) region, selected from the group consisting of;

- a) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 36, 37, and 38, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 39, GAS, and 40, respectively, **[107]**; and
- b) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 93, 94, and 95, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 96, GAS, and 97, respectively, **[613]**.

3. The antibody according to claim 2, wherein at least one binding region comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 36, 37, and 38, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 39, 40, and 41, respectively, [107].

4. The antibody according to claim 2, wherein at least one binding region comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 93, 94, and 95, respectively; and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos.: 96, 97, and 98, respectively, [613].

5. The antibody according to claim 1, wherein the antibody comprises a variable heavy chain (VH) region and a variable light chain (VL) region having at most 10 mutations or substitutions, across said VH region and said VL region, the VH region and the VL region being selected from the group consisting of;

- a) a VH region comprising SEQ ID No: 1 and a VL region comprising SEQ ID No: 2 [107];
- b) a VH region comprising SEQ ID No: 25 and a VL region comprising SEQ ID No: 26 [613];
- c) a VH region comprising SEQ ID No:21 and a VL region comprising SEQ ID No:22 [608-01];
- d) a VH region comprising SEQ ID No:23 and a VL region comprising SEQ ID No:24 [610-01];
- e) a VH region comprising SEQ ID No:27 and a VL region comprising SEQ ID No:28 [613-08]; and
- f) a VH region comprising SEQ ID No:29 and a VL region comprising SEQ ID No:30 [620-06].

6. The antibody according to claim 2, wherein the at least one binding region comprises a variable heavy chain (VH) region and a variable light chain (VL) region having at most 10 mutations or substitutions selected from conservative, physical or functional amino acids, across said variable heavy chain and variable light chain sequences selected from the group consisting of;

- a) a VH region comprising SEQ ID No: 1 and a VL region comprising SEQ ID No: 2 [107]; and
- b) a VH region comprising SEQ ID No: 25 and a VL region comprising SEQ ID No: 26 [613].

7. The antibody according to claim 1, wherein said at least one binding region comprises a VH region and a VL region selected from the group consisting of;

- a) a VH region comprising SEQ ID No: 1 and a VL region comprising SEQ ID No: 2 [107];
- b) a VH region comprising SEQ ID No: 25 and a VL region comprising SEQ ID No: 26 [613];

- c) a VH region comprising SEQ ID No:21 and a VL region comprising SEQ ID No:22 [608-01];
- d) a VH region comprising SEQ ID No:23 and a VL region comprising SEQ ID No:24 [610-01];
- e) a VH region comprising SEQ ID No:27 and a VL region comprising SEQ ID No:28 [613-08]; and
- f) a VH region comprising SEQ ID No:29 and a VL region comprising SEQ ID No:30 [620-06].

8. The antibody according to any one of the claims 2, 5 and 7, wherein the at least one binding region comprises a VH region comprising SEQ ID No: 1 and a VL region comprising SEQ ID No: 2 [107].

9. The antibody according to any one of claims 2, 6 and 7, wherein the at least one binding region comprises a VH region comprising SEQ ID No: 25 and a VL region comprising SEQ ID No: 26 [613].

10. The antibody according to any one of the preceding claims, wherein maximal antibody binding to AXL in the presence of Gas6 is at least 90%, such as at least 95%, such as at least 97%, such as at least 99%, such as 100%, of binding in the absence of Gas6 as determined in an assay comprising the steps of

- i) incubating AXL-expressing cells with Gas6,
- ii) adding anti-AXL antibodies to be tested,
- iii) adding a fluorescently labelled secondary reagent detecting anti-AXL antibodies and
- iv) analyzing the cells by FACS.

11. The antibody according to any one of the preceding claims, wherein maximal antibody binding to AXL in the presence of Gas6 is at least 90%, such as at least 95%, such as at least 97%, such as at least 99%, such as 100%, of binding in the absence of Gas6 as determined by a competition assay, wherein competition between said antibody binding to AXL and said Gas6 is determined on A431 cells preincubated with Gas6 and without Gas6.

12. The antibody according to any one of the preceding claims, wherein said antibody has a binding affinity (K_D) in the range of 0.3×10^{-9} to 63×10^{-9} M to human AXL, and wherein said binding affinity is measured using a Bio-layer Interferometry using soluble AXL extracellular domain.

13. The antibody according to any one of the preceding claims, wherein said antibody has a dissociation rate of 9.7×10^{-5} to $4.4 \times 10^{-3} \text{ s}^{-1}$ to AXL, and wherein the dissociation rate is measured by Bio-layer Interferometry using soluble recombinant AXL extracellular domain.

14. The antibody according to any one of the preceding claims, wherein AXL is human AXL as specified in SEQ ID NO:130.
15. The antibody according to any one of claims 1 to 13, wherein AXL is cynomolgus monkey AXL as specified in SEQ ID NO:147.
16. The antibody according to any one of the preceding claims, wherein AXL is human AXL as specified in SEQ ID NO:130 and cynomolgus monkey AXL as specified in SEQ ID NO:147.
17. The antibody according to any of the preceding claims, wherein said antibody comprises a heavy chain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.
18. The antibody according to claim 17, wherein the isotype is IgG1, optionally allotype IgG1m(f).
19. The antibody of any one of the preceding claims, which is a full-length monoclonal antibody, such as a full-length monoclonal IgG1, κ antibody.
20. The antibody according to any one of claims 1 to 19, wherein said antibody is an effector-function-deficient antibody, a stabilized IgG4 antibody or a monovalent antibody.
21. The antibody according to claim 20, wherein said heavy chain has been modified such that the entire hinge region has been deleted.
22. The antibody according to any one of claims 20 and 21, wherein the sequence of said antibody has been modified so that it does not comprise any acceptor sites for N-linked glycosylation.
23. The antibody according to any one of claims 1 to 22, wherein said antibody is a single-chain antibody.
24. A bispecific antibody comprising a first binding region of an antibody according to any one of the preceding claims, and a second binding region which binds a different target or epitope than said first binding region.

25. The bispecific antibody according to claim 24, wherein said bispecific antibody comprises a first and a second heavy chain, each of said first and second heavy chain comprises at least a hinge region, a CH2 and CH3 region, wherein in said first heavy chain at least one of the amino acids in the positions corresponding to positions selected from the group consisting of K409, T366, L368, K370, D399, F405, and Y407 in a human IgG1 heavy chain has been substituted, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of F405, T366, L368, K370, D399, Y407, and K409 in a human IgG1 heavy chain has been substituted, and wherein said substitutions of said first and said second heavy chains are not in the same positions.

26. The bispecific antibody according to any one of claims 24 to 25, wherein the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said second heavy chain, or vice versa.

27. An immunoconjugate comprising the antibody according to any one of claims 1 to 23, or bispecific antibody according to any one of claims 24 to 26, and a therapeutic moiety, such as a cytotoxic agent, a chemotherapeutic drug, a cytokine, an immunosuppressant, antibiotic, or a radioisotope.

28. The immunoconjugate according to claim 27, wherein the therapeutic moiety is a cytotoxic agent.

29. The immunoconjugate according to any one of claims 27 to 28, wherein said cytotoxic agent is linked to said antibody with a cleavable linker, such as *N*-succinimydyl 4-(2-pyridyldithio)-pentanoate (SSP), maleimidocaproyl-valine-citrulline-*p*-aminobenzyloxycarbonyl (mc-vc-PAB) or AV-1 K-lock valine-citrulline.

30. The immunoconjugate according to any one of claims 27 to 28, wherein said cytotoxic agent is linked to said antibody, or fragment thereof, with a non-cleavable linker, such as succinimidyl-4(*N*-maleimidomethyl)cyclohexane-1-carboxylate (MCC) or maleimidocaproyl (MC).

31. The immunoconjugate according to any one of claims 27 to 30, wherein said cytotoxic agent is selected from the group: DNA-targeting agents, e.g. DNA alkylators and cross-linkers, such as calicheamicin, duocarmycin, rachelmycin (CC-1065), pyrrolo[2,1-*c*][1,4] benzodiazepines (PBDs), and

indolinobenzodiazepine (IGN); microtubule-targeting agents, such as duostatin, such as duostatin-3, auristatin, such as monomethylauristatin E (MMAE) and monomethylauristatin F (MMAF), dolastatin, maytansine, *N*(2')-deacetyl-*N*(2')-(3-mercapto-1-oxopropyl)-maytansine (DM1), and tubulysin; and nucleoside analogs; or an analogs, derivatives, or prodrugs thereof.

32. The immunoconjugate according to any one of the claims 27 to 31, wherein said immunoconjugate comprises a combination of;

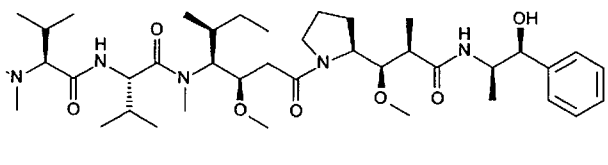
- i) said cytotoxic agent and said cleavable linker having bystander kill capacity;
- ii) said cytotoxic agent and said cleavable linker not having bystander kill capacity;
- iii) said cytotoxic agent and said non-cleavable linker having bystander kill capacity; or
- iv) said cytotoxic agent and said non-cleavable linker not having bystander kill capacity.

33. The immunoconjugate according to any one of claims 27 to 29, and 31 to 32, wherein said linker is mc-vc-PAB and the cytotoxic agent is MMAE; or the linker is SSP and the cytotoxic agent is DM1.

34. The immunoconjugate according to any one of claims 27 to 31, wherein said immunoconjugate comprises said linker mc-vc-PAB, said cytotoxic agent MMAE and said antibody wherein said at least one binding region comprises a VH region comprising SEQ ID No: 1 and a VL region comprising SEQ ID No: 2 [107].

35. The immunoconjugate according to any one of claims 27, and 29 to 30, wherein said linker is MMC and the cytotoxic agent is DM1; or the linker is MC and the cytotoxic agent is MMAF.

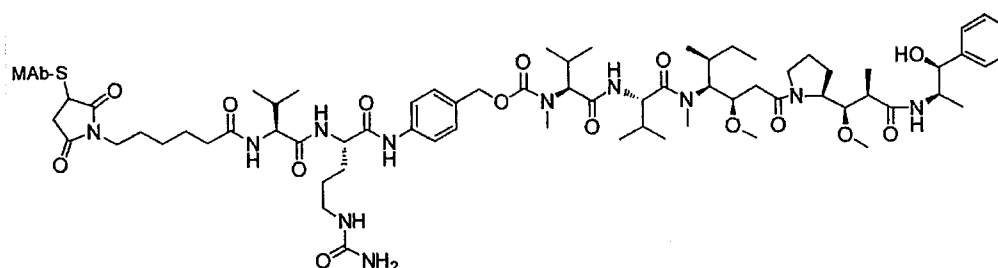
36. The immunoconjugate according to any one of claims 28 to 29 and 31 to 34, wherein the cytotoxic agent is monomethyl auristatin E (MMAE);



MMAE is linked to the antibody via a valine-citrulline (VC) linker at the nitrogen (N) on the left hand side of the structure of MMAE.

37. The immunoconjugate according to claim 36, wherein MMAE is linked to the antibody via a linker, which is valine-citrulline-*p*-aminobenzyloxycarbonyl (mc-vc-PAB).

38. The immunoconjugate according to claim 37, wherein the cytotoxic agent monomethyl auristatin E (MMAE) is linked to the antibody via a valine-citrulline (VC) linker and a maleimidocaproyl (MC) linker, wherein the combination of the cytotoxic agent and the linkers has the chemical structure;



wherein MAb is the antibody.

39. The immunoconjugate according to any one of claims 27 to 38, wherein the number of cytotoxic agents per antibody is from 1 to 8, such as 2 to 7, such as 2 to 6, such as 2 to 5, such as 2 to 4, and such as 2 to 3.

40. A composition comprising the antibody according to any one of claims 1 to 23, a bispecific antibody according to any one of claims 24 to 26, or an immunoconjugate according to any one of claims 27 to 39.

41. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 23, a bispecific antibody according to any one of claims 24 to 26, or an immunoconjugate according to any one of claims 27 to 39, and a pharmaceutical acceptable carrier.

42. A nucleic acid construct encoding one or more corresponding amino acid sequences defined in any of claims 1 to 9.

43. A nucleic acid construct encoding an antibody according to any one of claims 1 to 23.

44. An expression vector comprising one or more nucleic acid constructs according to any one of claims 42 and 43.

45. A host cell comprising a vector according to claim 44.

46. The host cell according to claim 45, wherein said host cell is a recombinant host cell, such as a recombinant prokaryotic, recombinant eukaryotic, or recombinant microbial host cell.

47. The host cell according to any one of claims 45 to 46, wherein the host cell produces an antibody according to any one of claims 1 to 23, or a bispecific antibody according to any one of claims 24 to 26.

48. A hybridoma which produces an antibody according to any one of claims 1 to 23.

49. The antibody according to any one of claims 1 to 23, the bispecific antibody according to any one of claims 24 to 26, or the immunoconjugate according to any one of claims 27 to 39, for use as a medicament.

50. The use of an antibody according to any one of claims 1 to 23, the bispecific antibody according to any one of claims 24 to 26, or the immunoconjugate according to any one of claims 27 to 39, in the manufacture of a medicament for the treatment of cancer.

51. A composition according to claim 40, or a pharmaceutical composition according to claim 41, for use in the treatment of cancer.

52. An antibody according to any one of claims 1 to 23, a bispecific antibody according to any one of claims 24 to 26, an immunoconjugate according to any one of claims 27 to 39, a composition according to claim 40, or a pharmaceutical composition according to claim 41, for use in diagnosing a disease characterized by involvement or accumulation of AXL-expressing cells, optionally wherein the antibody is labeled with a detectable agent, and wherein the amount of AXL-expressing cells correlates with or is indicative of disease.

53. The use according to claim 50, wherein the cancer is a solid tumor expressing AXL or an AXL-expressing hematological cancer.

54. The use according to claim 50, wherein the hematological cancer is selected from the group consisting of leukemia, such as chronic lymphocytic leukemia, myeloid leukemia, acute myeloid

leukemia (AML) and chronic myeloid leukemia, lymphoma such as Non-Hodgkin lymphoma and multiple myeloma.

55. The use according to 53, wherein the solid tumor expressing AXL is lung cancer or epidermoid carcinoma.

56. The use according to claim 53, wherein the cancer is selected from the group consisting of colorectal cancer, such as colorectal carcinoma and colorectal adenocarcinoma, bladder cancer, bone cancer such as chondrosarcoma, breast cancer such as triple-negative breast cancer, cancers of the central nervous system such as glioblastoma, astrocytoma, neuroblastoma, cervical cancer, connective tissue cancer, endometrium cancer, fibroblast cancer, gastric cancer such as gastric carcinoma, head and neck cancer, kidney cancer, liver cancer such as hepatocellular carcinoma, lung cancer such as NSCLC and lung squamous cell carcinoma, muscle cancer, neural tissue cancer, ovarian cancer, pancreatic cancer such as pancreatic ductal carcinoma and pancreatic adenocarcinoma, skin cancer such as malignant melanoma and soft tissue sarcoma.

57. A method for producing an antibody of any one of claims 1 to 23, said method comprising the steps

- a) culturing a host cell according to any one of claims 45 to 47 or a hybridoma according to claim 48, and
- b) purifying the antibody from the culture media.

58. A diagnostic composition comprising an antibody according to any one of claims 1 to 23 or a bispecific antibody according to any one of claims 24 to 26.

59. A method for detecting the presence of AXL antibody, or a cell expressing AXL, in a sample comprising the steps of;

- a) contacting the sample with an antibody according to any one of claims 1 to 23, a bispecific antibody according to any one of claims 24 to 26, or an immunoconjugate according to any one of claims 27 to 39, under conditions that allow for formation of a complex between the antibody, bispecific antibody or immunoconjugate and AXL; and
- b) analyzing whether a complex has been formed.

60. An anti-idiotypic antibody which binds to an anti-AXL antibody of any one of claims 1 to 23.