

CLAIMS

1. A monoclonal anti-CD47 antibody which specifically binds to human CD47, wherein the anti-CD47 antibody comprises: (i) a heavy chain variable region (V_H) comprising complementarity determining regions (CDR) 1, CDR2, and CDR3 comprising amino acid sequences GFNIKDYLLH (SEQ ID NO: 14), WIDPDQGDTE (SEQ ID NO: 15), and NAAYGSSSYPM DY (SEQ ID NO: 16), respectively; (ii) a light chain variable region comprising CDR1, CDR2, and CDR3 comprising amino acid sequences KASQDIHRYLS (SEQ ID NO: 17), RANRLVS (SEQ ID NO: 18), and LQYDEFPYT (SEQ ID NO: 19), respectively, and (iii) wherein the V_H comprises the amino acid sequence:

$X_1QX_2QLVQSGAEVKKX_3GX_4SVKVSCKASGFNIKDYLLHWVRQAPGQX_5LEWMGWIDPDQ$
 $GDTEYAQKX_6QX_7RVTX_8TX_9DX_{10}SX_{11}STAYMELX_{12}SLRSX_{13}DTAX_{14}$
YYCNAAYGSSSYPM DYWGQGTTTVTV (SEQ ID NO: 20),

wherein X_1 is M or there is no amino acid at position X_1 , X_2 is an amino acid with hydrophobic side chains, X_3 is P, X_4 is S or A, X_5 is an amino acid having aliphatic side chains, X_6 is F or L, X_7 is G, X_8 is an amino acid with hydrophobic side chains, X_9 is R or T, X_{10} is R or T, X_{11} is T, X_{12} is R, X_{13} is a negatively charged amino acid, and X_{14} is V.

2. The anti-CD47 antibody of claim 1, wherein the X_2 is M or V.
3. The anti-CD47 antibody of any one of claims 1-2, wherein the X_5 is A or G.
4. The anti-CD47 antibody of any one of claims 1-3, wherein the X_8 is I or M.
5. The anti-CD47 antibody of any one of claims 1-4, wherein the X_{13} is E or D.
6. The anti-CD47 antibody of any one of claims 1-5, wherein the anti-CD47 antibody has an expression titer or yield when produced using a cell-free system that is higher than an expression titer or yield of a parental anti-CD47 antibody produced using the cell-free system, wherein the parental anti-CD47 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1 or a heavy chain comprising the amino acid sequence of SEQ ID NO: 2, 3 or 4.
7. The anti-CD47 antibody of claim 6, wherein the antibody expression titer or yield of the anti-CD47 antibody is higher by at least 1 fold, at least 2 fold, or at least 3 fold compared to the parental antibody.
8. The anti-CD47 antibody of any one of claims 1-7, which is a humanized antibody.

9. The anti-CD47 antibody of any one of claims 1-8, which is an IgG1 antibody.
10. The anti-CD47 antibody of any one of claims 1-8, which is an IgG4 antibody.
11. The anti-CD47 antibody of claim 10, which is an IgG4 antibody comprising a S228P amino acid substitution according to the EU numbering index.
12. The anti-CD47 antibody of claim 10, which is an IgG4 antibody comprising a S228P and L235E amino acid substitutions according to the EU numbering index.
13. The anti-CD47 antibody of claim 6 or 7, wherein the parental antibody comprises the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1.
14. The anti-CD47 antibody of claim 13, wherein the parental antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12.
15. The anti-CD47 antibody of claim 6 or 7, wherein the parental antibody comprises the heavy chain comprising the amino acid sequence of SEQ ID NO: 2, 3 or 4.
16. The anti-CD47 antibody of any one of claims 1-15, which comprises 13 amino acid modifications in the framework region of the heavy chain variable region of the amino acid sequence of SEQ ID NO:1.
17. The anti-CD47 antibody of any one of claims 1-15, which comprises 10 amino acid modifications in the framework region of the heavy chain variable region of the amino acid sequence of SEQ ID NO:1.
18. The anti-CD47 antibody of any one of claims 1-15, which comprises 14 amino acid modifications in the framework region of the heavy chain variable region of the amino acid sequence of SEQ ID NO:1.
19. The anti-CD47 antibody of any one of claims 1-15, wherein the V_H comprises the amino acid sequence of SEQ ID NO: 21.
20. The anti-CD47 antibody of any one of claims 1-15, wherein the V_H comprises the amino acid sequence of SEQ ID NO: 22.
21. The anti-CD47 antibody of any one of claims 1-15, wherein the V_H comprises a V_H amino acid sequence of the heavy chain amino acid sequence of SEQ ID NO: 7.

22. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 5.
23. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6.
24. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 7.
25. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 8.
26. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9.
27. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 10.
28. The anti-CD47 antibody of any one of claims 1-15, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 11.
29. The anti-CD47 antibody of any one of claims 1-28, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 13 or a light chain comprising the amino acid sequence of SEQ ID NO: 13 without amino acid M at the N-terminus.
30. The anti-CD47 antibody of any one of claims 1-29, which does not cause substantial red blood cell depletion, anemia, or both red blood cell depletion and anemia after administration.
31. The anti-CD47 antibody of claim 30, which does not cause substantial platelet depletion after administration.
32. The anti-CD47 antibody of claim 30, which does not cause substantial agglutination of cells after administration.
33. The anti-CD47 antibody of claim 30, which does not cause substantial hemagglutination of red blood cells after administration.
34. The anti-CD47 antibody of any one of claims 1-33, which inhibits CD47 from interacting with signal-regulatory-protein α (SIRP α).

35. The anti-CD47 antibody of any one of claims 1-34, which promotes macrophage-mediated phagocytosis of a CD47-expressing cell.
36. The anti-CD47 antibody of any one of claims 1-35, which does not cause a significant level of effector function.
37. The anti-CD47 antibody of any one of claims 1-36, which when expressed using a cell-free system, exhibits lower binding affinity, or does not bind, to an FcγR than when expressed using CHO cells.
38. The anti-CD47 antibody of claim 37, wherein the lower binding affinity is at least 1 log lower or at least 2 log lower.
39. The anti-CD47 antibody of claim 37 or 38, wherein the FcγR is FcγRI, FcγRIIA R131, FcγRIIA H131, FcγRIIB, or FcγRIIIA V158.
40. The anti-CD47 antibody of any one of claims 1-39, which is aglycosylated or has less glycosylation when expressed using a cell free system compared to when expressed in CHO cells.
41. The anti-CD47 antibody of any one of claims 1-40, which does not cause or promote a significant level of ADCC or CDC.
42. The anti-CD47 antibody of any one of claims 1-41, which is produced using a cell-free system, wherein the cell-free system comprises using S30 cell-free extract.
43. The anti-CD47 antibody of claim 42, wherein the S30 cell-free extract comprises prokaryotic disulfide bond isomerase DsbC.
44. The anti-CD47 antibody of any one of claims 1-43, which is a bispecific antibody.
45. The anti-CD47 antibody of any one of claims 1-44, which is conjugated to an agent.
46. The anti-CD47 antibody of claim 45, wherein the agent is a label or a toxin.
47. A pharmaceutical composition comprising a therapeutically effective amount of the anti-CD47 antibody according to any one of claims 1-46 or an antigen-binding fragment thereof.
48. The pharmaceutical composition of claim 47, further comprising a pharmaceutically acceptable carrier.

49. A polynucleotide comprising a nucleotide sequence encoding both a light chain variable region (V_L) and a heavy chain variable region (V_H), of the anti-CD47 antibody of any one of claims 1-46.
50. The polynucleotide of claim 49, wherein the V_H is encoded by the V_H region of any one of SEQ ID NOs: 26-32, and wherein the V_L is encoded by the V_L region of SEQ ID NO: 33.
51. The polynucleotide of claim 50, wherein the nucleotide sequence of any one of SEQ ID NOs: 26-32 encodes the heavy chain.
52. The polynucleotide of claim 51, wherein the nucleotide sequence of SEQ ID NO: 33 encodes the light chain.
53. A population of polynucleotides comprising (i) a first polynucleotide comprising nucleotide sequences encoding a V_H or a heavy chain of the anti-CD47 antibody of any one of claims 1-46, and (ii) a second polynucleotide comprising nucleotide sequences encoding a V_L or a light chain of the anti-CD47 antibody of any one of claims 1-46.
54. The population of polynucleotides of claim 53, wherein the first polynucleotide is operably linked to a first promoter, and wherein the second polynucleotide is operably linked to a second promoter.
55. A vector comprising the polynucleotide of any one of claims 49- 52.
56. A population of vectors comprising (i) a first vector comprising nucleotide sequences encoding a V_H or a heavy chain of the anti-CD47 antibody of any one of claims 1- 46, and (ii) a second vector comprising nucleotide sequences encoding a V_L or a light chain of the anti-CD47 antibody of any one of claims 1-46.
57. A composition for cell-free protein expression comprising a cell-free extract and one or more vectors of claim 55.
58. The composition of claim 57, further comprising S30 cell-free extract.
59. The composition of claim 57 or 58, further comprising prokaryotic disulfide bond isomerase DsbC.
60. The anti-CD47 antibody of any one of claims 1-46 for use in treating cancer in a subject in need thereof, wherein the anti-CD47 antibody is to be administered to the subject in an amount sufficient to treat the cancer in the subject.

61. The anti-CD47 antibody of any one of claims 1-46 for use in alleviating a symptom of a cancer in a subject in need thereof, wherein the anti-CD47 antibody is to be administered to the subject in an amount sufficient to alleviate one or more symptoms of the cancer in the subject.

62. The anti-CD47 antibody of claim 60 or 61, wherein the subject is to be further administered radiation or chemotherapy.

63. The anti-CD47 antibody of any one of claims 60 - 62, wherein the subject is to be further administered another anti-cancer agent.

64. The anti-CD47 antibody of any one of claims 60 - 63, wherein the cancer is a hematological cancer.

65. The anti-CD47 antibody of any one of claims 60 - 63, wherein the cancer is a solid cancer.

66. The anti-CD47 antibody of any one of claims 60 - 63, wherein the cancer is multiple myeloma, non-Hodgkin's lymphoma, acute myeloid leukemia (AML), breast cancer, bladder cancer, non-small cell lung cancer/carcinoma, hepatocellular carcinoma (HCC), sarcoma, or head and neck cancer.

67. An isolated cell comprising the polynucleotide of any one of claims 49- 52.

68. An isolated cell comprising the population of polynucleotides of claim 53 or 54.

69. An isolated cell comprising the vector of claim 55.

70. An isolated cell comprising the population of vectors of claim 56.

71. An isolated cell producing the anti-CD47 antibody or antigen-binding fragment of any one of claims 1-46.

72. A population of host cells comprising (i) a first host cell comprising a polynucleotide comprising nucleotide sequences encoding a V_H or a heavy chain of the anti-CD47 antibody of any one of claims 1-46, and (ii) a second host cell comprising a polynucleotide comprising nucleotide sequences encoding a V_L or a light chain of the anti-CD47 antibody of any one of claims 1-46.

73. A method of making an anti-CD47 antibody comprising expressing the anti-CD47 antibody using the composition for cell-free protein expression of any one of claims 57 -59.

74. A method of making an anti-CD47 antibody comprising expressing the anti-CD47 antibody using the cell of any one of claims 67- 71.

75. The method of claim 73, further comprising purifying the anti-CD47 antibody.