

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 GFNIKDY Y_L H (SEQ ID NO: 14), WIDPDQGDTE (SEQ ID NO: 15), and NAA Y_G SSSY P_M 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 LQYDEF P_Y T (SEQ ID NO: 19), respectively, and (iii) wherein the V_H comprises the amino acid sequence:

X_1 Q X_2 QLVQSGAEVKK X_3 G X_4 SVKVSCKASGFNIKDY Y_L HWVRQAPGQ X_5 LEWMGW
IDPDQGDTEYAQK X_6 Q X_7 RV T_X X_8 T_X X_9 D X_{10} S X_{11} STAYMEL X_{12} SLRS X_{13} DTA X_{14}
YYCNAAYGSSSY P_M DYWGQGT T_V T_V (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 claim 1, wherein the X_5 is A or G.
4. The anti-CD47 antibody of claim 1, wherein the X_8 is I or M.
5. The anti-CD47 antibody of claim 1, 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.
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.
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.
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 Fc γ R 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 Fc γ R is Fc γ RI, Fc γ RIIA R131, Fc γ RIIA H131, Fc γ RIIB, or Fc γ RIIA 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 a V_H , a light chain variable region (V_L), or both a V_L and a 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. A polynucleotide comprising a nucleotide sequence encoding a heavy chain, a light chain, or both heavy chain and a light chain of the anti-CD47 antibody of any one of claims 1-46.
52. The polynucleotide of claim 50, wherein the nucleotide sequence of any one of SEQ ID NOs: 26-32 encodes the heavy chain.
53. The polynucleotide of claim 51 or 52, wherein the nucleotide sequence of SEQ ID NO: 33 encodes the light chain.
54. 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.
55. The population of polynucleotides of claim 54, wherein the first polynucleotide is operably linked to a first promoter, and wherein the second polynucleotide is operably linked to a second promoter.
56. A vector comprising the polynucleotide of any one of claims 49-53.

57. 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.
58. A composition for cell-free protein expression comprising a cell-free extract and one or more vectors of claim 56.
59. The composition of claim 58, further comprising S30 cell-free extract.
60. The composition of claim 58 or 59, further comprising prokaryotic disulfide bond isomerase DsbC.
61. 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.
62. 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.
63. The anti-CD47 antibody of claim 61 or 62, wherein the subject is to be further administered radiation or chemotherapy.
64. The anti-CD47 antibody of any one of claims 61-63, wherein the subject is to be further administered another anti-cancer agent.
65. The anti-CD47 antibody of any one of claims 61-64, wherein the cancer is a hematological cancer.
66. The anti-CD47 antibody of any one of claims 61-64, wherein the cancer is a solid cancer.
67. The anti-CD47 antibody of any one of claims 61-64, 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.

- 68. An isolated cell comprising the polynucleotide of any one of claims 49-53.
- 69. An isolated cell comprising the population of polynucleotides of claim 54 or 55.
- 70. An isolated cell comprising the vector of claim 56.
- 71. An isolated cell comprising the population of vectors of claim 57.
- 72. An isolated cell producing the anti-CD47 antibody or antigen-binding fragment of any one of claims 1-46.
- 73. 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.
- 74. 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 58-60.
- 75. A method of making an anti-CD47 antibody comprising expressing the anti-CD47 antibody using the cell of any one of claims 68-72.
- 76. The method of claim 73 or 74, further comprising purifying the anti-CD47 antibody.