

CLAIMS

What is claimed is:

1. An anti-CD47 antibody comprising at least one antibody-antigen binding site, ~~wherein said antibody comprises:~~

~~(a) a heavy chain variable domain (V_H) comprising~~

~~i. a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 49, 51, 53, 55, 57, 59, 62-65, 86-87;—~~

~~ii. a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 145, 147, 149, 151, 153, 155, 158-161, 182-183; and~~

~~iii. a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 241, 243, 245, 247, 249, 251, 254-257, 278-279 and~~

~~(b) a light chain variable domain (V_L) comprising,—~~

~~i. a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, 54, 56, 58, 60, 76-79;—~~

~~ii. a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 146, 148, 150, 152, 154, 156, 172-175; and—~~

~~iii. a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 242, 244, 246, 248, 250, 252, 268-271,—~~

~~optionally,~~ wherein said antibody comprises any one of the following:

(1) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 65, 161 and 257, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 76, 172 and 268, respectively;

~~(1) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 49, 145 and 241, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 50, 146 and 242, respectively;—~~

(2) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 51, 147 and 243, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 52, 148 and 244, respectively;

(3) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 53, 149 and 245, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 54, 150 and 246, respectively;

(4) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 55, 151 and 247, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 56, 152 and 248, respectively;

(5) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 57, 153 and 249, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 58, 154 and 250, respectively;

(6) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 59, 155 and 251, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 60, 156 and 252, respectively;

(7) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 62, 158 and 254, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 76, 172 and 268, respectively;

(8) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 63, 159 and 255, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 77, 173 and 269, respectively;

(9) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 64, 160 and 256, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 78, 174 and 270, respectively;

(10) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 65, 161 and 257, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 79, 175 and 271, respectively;

(11) –a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 49, 145 and 241, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 50, 146 and 242, respectively.;

(11) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 65, 161 and 257, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 76, 172 and 268, respectively;

(12) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 86, 182 and 278, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 56, 152 and 248, respectively; and

(13) a V_H comprising the heavy chain CDR1, CDR2 and CDR3 sequences having the amino acid sequences of SEQ ID NOs: 87, 183 and 279, respectively, and a V_L comprising the light chain CDR1, CDR2 and CDR3 having the amino acid sequences of SEQ ID NOs: 56, 152 and 248, respectively.

2. The antibody of claim 1, wherein said antibody reduce the level of cell agglutination or does not cause hemagglutination of human red blood cells,

optionally, wherein the level of cell agglutination in the presence of said antibody is reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 99% compared to the level of agglutination of cells in the presence of CD47 antibody B6H12,—

optionally, wherein the antibody does not cause a significant level of cell agglutination at an antibody amount of between about 0.3 $\mu\text{g/ml}$ to about 200 $\mu\text{g/ml}$, optionally, about 100 $\mu\text{g/ml}$ to about 200 $\mu\text{g/ml}$.

3. The antibody of claim 1 or claim 2, wherein said anti-CD47 antibody blocks the interaction between human CD47 and human signal regulatory protein α (SIRP α),—

optionally, wherein the antibody blocks at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, or at least 99% of the interaction between CD47 and SIRP α as compared to the level of interaction between CD47 and SIRP α in the absence of the anti-CD47 antibody.

4. The antibody of any one of the preceding claims, wherein said antibody has potent anti-tumor activity,—

optionally, wherein said potent anti-tumor activity is measured by the ability of macrophages to phagocytose tumor cells being increased by at least 5%, at least 10%,

at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 99% in the presence of said antibody, compared to the ability of macrophages to phagocytose tumor cells in the presence of anti-CD47 antibody B6H12.

5. The antibody of anyone of the preceding claims, wherein said antibody does not promote clumping of CD47 positive cell lines.

62. The antibody of ~~any one of the preceding claims~~claim 1, wherein said antibody comprises a variable heavy chain selected from SEQ ID NOs: 349, 351, 353, 355, 357, 359, 361-373, 380-383, 388-392 and a variable light chain selected from SEQ ID NOs: 350, 352, 354, 356, 358, 360, 374-379, 384-387 and 393-396;~~or~~

~~wherein said antibody comprises a variable heavy chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence set forth in one of SEQ ID NOs: 349, 351, 353, 355, 357, 359, 361-373, 380-383, 388-392, and a variable light chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence set forth in one of SEQ ID NOs: 350, 352, 354, 356, 358, 360, 374-379, 384-387 and 393-396.~~

3. The antibody of claim 1 or 2, wherein said antibody comprises:

- (1) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 365, and a VL comprising the amino acid sequences of SEQ ID NOs: 376;
- (2) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 351, and a VL comprising the amino acid sequences of SEQ ID NOs: 352;
- (3) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 353, and a VL comprising the amino acid sequences of SEQ ID NOs: 354;
- (4) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 355, and a VL comprising the amino acid sequences of SEQ ID NOs: 356;
- (5) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 357, and a VL comprising the amino acid sequences of SEQ ID NOs: 358;
- (6) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 359, and a VL comprising the amino acid sequences of SEQ ID NOs: 360;
- (7) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 362, and a VL comprising the amino acid sequences of SEQ ID NOs: 376;

- (8) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 363, and a VL comprising the amino acid sequences of SEQ ID NOs: 377;
- (9) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 364, and a VL comprising the amino acid sequences of SEQ ID NOs: 378;
- (10) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 365, and a VL comprising the amino acid sequences of SEQ ID NOs: 379;
- (11) a VH comprising the sequences having the amino acid sequences of SEQ ID NOs: 349, and a VL comprising the amino acid sequences of SEQ ID NOs: 350.

~~7. The antibody of any one of the preceding claims, wherein CD47 expression or activity in the presence of said antibody is decreased by at least 50%, 55%, 60%, 75%, 80%, 85% or 90% as compared to the level of CD47 expression or activity in the absence of said antibody, optionally, wherein CD47 expression or activity in the presence of said antibody is decreased by at least 95%, 96%, 97%, 98%, 99% or 100% as compared to the level of CD47 expression or activity in the absence of said antibody.~~

~~84.~~ The antibody of any one of the preceding claims, wherein said antibody comprises a constant region modified at amino acid Asn297, Leu235 and/or Leu234,

~~Optionally and/or,~~ wherein said antibody comprises a constant region with an amino acid modification of N297A,

~~optionally and/or,~~ wherein said constant region has an amino acid modification of Leu235Glu (L235E) or Leu235Ala (L235A), and/or an amino acid modification of Leu234Ala (L234A).

~~95.~~ The antibody of any one of the preceding claims, wherein said antibody comprises a human IgG₃ constant region that is modified at amino acid Arg435, ~~optionally and/or,~~ wherein said human IgG₃ constant region has an amino acid modification of Arg435His (R435H).

~~106.~~ The antibody of any one of the preceding claims, wherein said antibody comprises a human IgG₄ constant region that is modified within the hinge region to prevent or reduce strand exchange,

~~optionally and/or~~, wherein said antibody comprises a human IgG₄ constant region with an amino acid modification of Ser228Pro (S228P);

~~optionally and/or~~, wherein said human IgG₄ constant region is further modified at amino acid 235;

~~optionally and/or~~, wherein said human IgG₄ constant region has an amino acid modification of Leu235Glu (S228P/L235E).

~~117~~. The antibody of any one of the preceding claims, wherein said antibody comprises a human IgG constant region modified to enhance FcRn binding, wherein said human IgG constant region has one or more amino acid modifications of Met252Tyr, Ser254Thr, Thr256Glu, Met428Leu or Asn434Ser (M252Y, S254T, T256E M428L, or N434S).

~~128~~. The antibody of any one of the preceding claims, wherein said antibody comprises a human IgG constant region modified to alter antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), ~~optionally and/or~~,

wherein said antibody comprises a human IgG constant region modified to induce heterodimerization, wherein said antibody has an amino acid modification of T366W or T366S and/or an amino acid modification of L368A, or Y407V, S354C, or Y349C.

~~139~~. The antibody of any one of the preceding claims, wherein said antibody is a humanized antibody or a human antibody,—

~~optionally, said antibody is a humanized or human antibody~~ having a variable heavy chain region (V_H) and/or variable light (V_L) chain region that at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence selected from the group consisting of: SEQ ID NOs: 361-373, 375-379, 381-383, 385-387, 389-392, 394-396, 399-404;.

~~10~~. The antibody of claim 9, ~~optionally, wherein~~ said antibody is a humanized or human antibody having a variable heavy chain region (V_H) and/or variable light (V_L) chain region selected from the group consisting of: SEQ ID NOs: 361-373, 375-379, 381-383, 385-387, 389-392, 394-396, 399-404.

~~14~~11. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

~~15~~12. The isolated nucleic acid of claim ~~14~~11, wherein the nucleic acid comprises a sequence selected from the group consisting of: SEQ ID NOs: 337-348 and 413-421 or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence selected from the group consisting of: SEQ ID NOs: 337-348 and 413-421.

~~16~~13. A vector comprising the ~~isolated~~ nucleic acid of claim ~~14 or 15~~11 or 12, ~~or comprising a nucleic acid encoding a heavy chain region (V_H) of an antibody and/or variable light (V_L) chain region of an antibody that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence selected from the group consisting of: SEQ ID NOs: 337-348; 413-421.~~

~~17~~14. A cell comprising the nucleic acid of any one of claims ~~14-15~~11-12 or the vector of claim ~~16~~13, wherein said cell expresses the antibody of any one of claims 1-~~13~~10.

~~18~~15. A pharmaceutical composition comprising the antibody of any one of claims 1-~~13~~10 and a pharmaceutically acceptable excipient.

~~19. An antibody or pharmaceutical composition for use in treating, delaying the progression of, preventing relapse of, or alleviating a symptom of a cancer or other neoplastic condition in a human patient with cancer or other neoplastic condition, wherein the antibody comprises at least one antibody-antigen binding site, said antibody binds human CD47, inhibits, blocks, antagonizes, neutralizes or otherwise interferes with CD47 expression, activity and/or signaling, and does not cause significant agglutination of cells; and wherein the pharmaceutical composition comprises said antibody and a pharmaceutical excipient, wherein said antibody is the antibody according to any one of claims 1-13, and wherein said pharmaceutical composition is the pharmaceutical composition of claim 18.~~

~~20~~16. Use of an effective amount of the antibody of any one of claims 1-~~13~~10, or the pharmaceutical composition of claim ~~18-15~~15 in the manufacture of a medicament for treating a cancer or other neoplastic condition.

17. The use of claim 16, optionally, wherein said cancer or other neoplastic condition is selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), and multiple myeloma (MM), breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, colorectal cancer, pancreatic cancer, lung cancer, leiomyoma, leiomyosarcoma, glioma, glioblastoma, breast tumors, ovarian tumors, lung tumors, pancreatic tumors, prostate tumors, melanoma tumors, colorectal tumors, lung tumors, head and neck tumors, bladder tumors, esophageal tumors, liver tumors, and kidney tumors, Myeloproliferative disorder/neoplasm (MPDS), myelodysplasia syndrome, Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma(NHL), Burkitt's lymphoma, follicular lymphoma (small cell and large cell), multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, light chain or Bence-Jones myeloma.

178. The use of claim 16 or 17, wherein said cancer or other neoplastic condition is selected from the group consisting of non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), small cell lung cancer (SCLC), esophageal tumors, and Myelodysplastic syndromes (MDS).