

[5] In keeping with the role of CD47 to inhibit phagocytosis of normal cells, there is evidence that it is transiently up-regulated on hematopoietic stem cells (HSCs) and progenitors just prior to and during their migratory phase, and that the level of CD47 on these cells determines the probability that they are engulfed *in vivo*.

[6] CD47 is also constitutively up-regulated on a number of cancers, including myeloid leukemias. Overexpression of CD47 on a myeloid leukemia line increases its pathogenicity by allowing it to evade phagocytosis. It has been concluded that CD47 up-regulation is an important mechanism for providing protection to normal HSCs during inflammation-mediated mobilization, and that leukemic progenitors co-opt this ability in order to evade macrophage killing.

[7] Certain CD47 antibodies have been shown to restore phagocytosis and prevent atherosclerosis. See, e.g., Kojima et al., Nature, Vol. 36, 86-90 (Aug. 4, 2016). The present invention provides novel CD47 antibodies or immunologically active fragments thereof that have low immunogenicity in humans and cause low or no level of red blood cell depletion. As well known to a person skilled in the art, such antibodies may be interchangeably called “anti-CD47 antibodies.”

SUMMARY

[7a] In one aspect, the present disclosure refers to an isolated monoclonal antibody ~~or immunologically active fragment thereof~~ that specifically binds to human CD47, ~~wherein the isolated monoclonal said antibody comprises~~comprising: a heavy chain comprising a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 31; and a light chain comprising a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 32; and wherein the VH CDR1, the VH CDR2, the VH CDR3, the VL CDR1, the VL CDR2, and the VL CDR3 are according to Kabat numbering scheme~~a variable heavy (VH) chain sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NO: 3, and SEQ ID NO: 31; and a variable light (VL) chain sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NO: 4, and SEQ ID NO: 32.~~ In one example, the present disclosure refers to an isolated monoclonal antibody or immunologically active fragment thereof that binds to human CD47, comprising: a variable heavy (VH) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63,

SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, and SEQ ID NO: 77; and a variable light (VL) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36,

SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, and SEQ ID NO: 78. ~~In another aspect, the present disclosure refers to an isolated bispecific monoclonal antibody comprising a first arm and a second arm, wherein the first arm comprises a first monoclonal antibody or immunologically active fragment thereof as disclosed herein which binds human CD47, and the second arm comprise a second monoclonal antibody that does not bind human CD47.~~ In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising an isolated monoclonal antibody ~~or immunologically active fragment thereof as disclosed herein or a bispecific monoclonal antibody~~ as disclosed herein, and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to use of an isolated monoclonal antibody or immunologically active fragment thereof as disclosed herein or a bispecific monoclonal antibody as disclosed herein, or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a CD47-expressing cancer in a human subject in need thereof ~~with enhanced expression of CD47, a fibrotic disease, a disease directly related to inhibition of phagocytosis, or a disease directly related to platelet aggregation.~~ In yet another example, the present disclosure provides the use of an isolated monoclonal antibody or immunologically active fragment thereof as disclosed herein or a bispecific monoclonal antibody as disclosed herein, or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer, a fibrotic disease, a disease related to inhibition of phagocytosis, or a disease related to platelet aggregation. ~~In yet another aspect, the present disclosure refers to a fusion protein comprising a first amino acid sequence and a second amino acid sequence, wherein the first amino acid sequence is SEQ ID NO: 3, or SEQ ID NO: 31, and the second amino acid sequence is SEQ ID NO: 4, or SEQ ID NO: 32.~~ In yet another example, the present disclosure refers to a fusion protein comprising a first amino acid sequence and a second amino acid sequence, wherein the first amino acid sequence is SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53,

SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, or SEQ ID NO: 77, or an amino acid sequence that is at least 95% identical thereto; and the second amino acid sequence is SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, or SEQ ID NO: 78, or an amino acid sequence that is at least 95% identical thereto. ~~In yet another aspect, the present disclosure refers to an immunodominant epitope comprising an amino acid sequence of a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47 when numbered in accordance with SEQ ID NO: 107. In yet another~~

~~example, the present disclosure refers to an immunodominant epitope encoded by the CD47 gene comprising a recombinant protein containing conformationally a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47. In yet another aspect, the present disclosure refers to an isolated antibody or antigen-binding portion that specifically binds to a conformational epitope having an amino acid sequence comprising a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47, wherein the isolated antibody or the antigen-binding portion specifically binds to CD47. In yet another example, the present disclosure provides a biological molecule that specifically binds to a conformational epitope having an amino acid sequence comprising a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47, wherein the antibody can specifically bind to CD47. In yet another aspect, the present disclosure refers to an isolated monoclonal antibody or immunologically active fragment thereof, the isolated monoclonal antibody comprising a VH CDR1, VH CDR2 and VH CDR3 of the VH sequence shown in SEQ ID NO: 3 or SEQ ID NO: 31, and a VL CDR1, VL CDR2 and VL CDR3 of the VL sequence shown in SEQ ID NO: 4 or SEQ ID NO: 32; wherein the VH CDR1 has the amino acid sequence of RAWMN, the VH CDR2 has the amino acid sequence of RIKRKTGGETTDYAAPVKG, the VH CDR3 has the amino acid sequence of SNRAFDI, the VL CDR1 has the amino acid sequence of KSSQSVLYAGNNRNLYLA, the VL CDR2 has the amino acid sequence of QASTRAS, and the VL CDR3 has the amino acid sequence of QQYYTPPLA.~~

[8] In one example, the present disclosure provides isolated monoclonal antibodies and their immunologically active fragments that bind to human CD47. For brevity, these CD47-binding isolated monoclonal antibodies and their immunologically active fragments are referred to hereinafter as “CD47 antibodies”. The CD47 antibodies of this disclosure are capable of modulating, e.g., blocking, inhibiting, reducing, antagonizing, neutralizing or otherwise interfering with, CD47 expression, activity and/or signaling, or the interaction between CD47 and SIRP α . Very significantly, the CD47 antibodies of this disclosure do not generally cause a significant level of depletion or hemagglutination of human red blood cells, and surprisingly in many cases do not cause any depletion or hemagglutination of human red blood cells at all. Additionally, the CD47 antibodies of this disclosure have exhibited potent anti-tumor activities.

[9] In some examples, the CD47 antibodies of this disclosure each include (a) a variable heavy (VH) chain sequence that is at least 90% (e.g., at least 95%) identical to an amino acid

What Is Claimed Is:

1. An isolated monoclonal antibody ~~or immunologically active fragment thereof~~ that specifically binds to human CD47, wherein the isolated monoclonal antibody comprises comprising:
 - (i) ~~a variable-heavy (VH)-chain sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NO: 3, and SEQ ID NO: 31~~ comprising a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 31; and
 - (ii) a light chain comprising a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 32;~~_____ ; and~~
a variable light (VL) chain and wherein the VH CDR1, the VH CDR2, the VH CDR3, the VL CDR1, the VL CDR2, and the VL CDR3 are according to Kabat numbering scheme~~sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NO: 4, and SEQ ID NO: 32.~~
2. The isolated monoclonal antibody ~~or immunologically active fragment thereof~~ of claim 1-, comprising two heavy chains each a VH/VL pair, wherein the VH/VL pair comprises comprising the amino acid sequence of VH and VL chain sequences selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4, and SEQ ID NO: 31; and two light chains each comprising the amino acid sequence of SEQ ID NO: 32.
3. The isolated monoclonal antibody ~~or immunologically active fragment thereof~~ of claim 21, wherein the isolated monoclonal antibody ~~or immunologically active fragment thereof is chimeric or humanized~~ comprises human IgG4 heavy chains and human kappa light chains.
4. The isolated monoclonal antibody of claim 1, wherein the isolated monoclonal antibody does not cause hemagglutination or depletion of red blood cells.
- 4.5. The isolated monoclonal antibody or immunologically active fragment thereof of claim 1, wherein the isolated monoclonal antibody or immunologically active fragment thereof prevents human CD47 from interacting with signal-regulatory-protein α -(SIRP α).

- ~~5.6.~~ The isolated monoclonal antibody ~~or immunologically active fragment thereof~~ of claim 1, wherein the isolated monoclonal antibody ~~or immunologically active fragment thereof~~ promotes macrophage-mediated phagocytosis of a CD47-expressing cell.
- ~~6.~~ The isolated monoclonal antibody or immunologically active fragment thereof of claim 1, wherein the isolated monoclonal antibody or immunologically active fragment thereof does not cause a significant level of hemagglutination or depletion of red blood cells.
- ~~7.~~ The isolated monoclonal antibody or immunologically active fragment thereof of claim 1, wherein the isolated monoclonal antibody or immunologically active fragment thereof does not cause hemagglutination or depletion of red blood cells.
- ~~8.~~ An isolated bispecific monoclonal antibody comprising a first arm and a second arm, wherein the first arm comprises a first monoclonal antibody or immunologically active fragment thereof of any of claims 1-7 which binds human CD47, and the second arm comprise a second monoclonal antibody that does not bind human CD47.
- ~~9.~~ The bispecific monoclonal antibody of claim 8, wherein the second arm binds to a cancer cell.
- ~~10.~~ The bispecific monoclonal antibody of claim 8, wherein the bispecific monoclonal antibody inhibits interaction between human CD47 and human SIRP α .
- ~~11.7.~~ A pharmaceutical composition comprising ~~an the~~ isolated monoclonal antibody ~~or immunologically active fragment thereof~~ of any one of claims 1-~~67~~ or a bispecific monoclonal antibody of any of claims ~~8-10~~, and a pharmaceutically acceptable carrier.
- ~~12.8.~~ Use of an isolated monoclonal antibody ~~or immunologically active fragment thereof~~ of any one of claims 1-~~76~~ or a pharmaceutical composition of claim ~~7~~ or a bispecific monoclonal antibody of any of claims ~~8-10~~, or a pharmaceutical composition of claim ~~11~~ in the manufacture of a medicament for treating a CD47-expressing cancer ~~in a human subject in need thereof~~ with enhanced expression of CD47, a fibrotic disease, a disease directly related to inhibition of phagocytosis, or a disease directly related to platelet aggregation.
- ~~13.9.~~ The use of claim ~~128~~, wherein the CD47-expressing cancer is selected from the group consisting of: ovarian cancer, colon cancer, breast cancer, lung cancer, head and

neck cancer, bladder cancer, colorectal cancer, pancreatic cancer, non-Hodgkin's lymphoma, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myelogenous leukemia, hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, adult T-cell leukemia, multiple myeloma, melanoma, leiomyoma, leiomyosarcoma, glioma, glioblastoma, myelomas, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, cervical cancer, gastric cancer, liver cancer, and solid tumors; ~~the fibrotic disease is selected from the group consisting of: myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis, bronchitis, and asthma; the disease related to inhibition of phagocytosis is a cardiovascular disease; the disease related to platelet aggregation is Glanzmann Thrombasthenia, immune thrombocytopenia (ITP), von Willebrand disease (vWD).~~

- ~~14. The use of claim 13, wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, and venous thrombosis.~~
- ~~15. A fusion protein comprising a first amino acid sequence and a second amino acid sequence, wherein the first amino acid sequence is SEQ ID NO: 3, or SEQ ID NO: 31, and the second amino acid sequence is SEQ ID NO: 4, or SEQ ID NO: 32.~~
- ~~16. The fusion protein of claim 15, comprising a combination of the first amino acid sequence and the second amino acid sequence, and the combination is SEQ ID NO: 3 and SEQ ID NO: 4, or SEQ ID NO: 31 and SEQ ID NO: 32.~~
- ~~17. The fusion protein of claims 15 and 16, further comprising an additional protein in addition to the first and second amino acid sequences.~~
- ~~18. The fusion protein of claim 17, wherein the additional protein is an antibody or a cytokine.~~
- ~~19. The fusion protein of any of claims 15-18, wherein the fusion protein is conjugated with a small molecule therapeutic agent or a marker.~~

- ~~20. The fusion protein of claim 19 wherein the small molecule therapeutic agent is an anti-cancer or anti-inflammation agent; and the marker is a biomarker or fluorescent marker.~~
- ~~21. An immunodominant epitope comprising an amino acid sequence of a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47 when numbered in accordance with SEQ ID NO: 107.~~
- ~~22. An isolated antibody or antigen-binding portion that specifically binds to a conformational epitope having an amino acid sequence comprising a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47, wherein the isolated antibody or the antigen-binding portion specifically binds to CD47.~~
- ~~23. An isolated monoclonal antibody or immunologically active fragment thereof, the isolated monoclonal antibody comprising a VH CDR1, VH CDR2 and VH CDR3 of the VH sequence shown in SEQ ID NO: 3 or SEQ ID NO: 31, and a VL CDR1, VL CDR2 and VL CDR3 of the VL sequence shown in SEQ ID NO: 4 or SEQ ID NO: 32;~~
- ~~10. wherein the VH CDR1 has the amino acid sequence of RAWMN, the VH CDR2 has the amino acid sequence of RIKRKTDGETTDYAAPVKG, the VH CDR3 has the amino acid sequence of SNRAFDI, the VL CDR1 has the amino acid sequence of KSSQSVLYAGNNRNYLA, the VL CDR2 has the amino acid sequence of QASTRAS, and the VL CDR3 has the amino acid sequence of QQYYTPPLA. The use of claim 8, wherein the CD47-expressing cancer is a solid tumor.~~