

[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 that specifically binds to human CD47, said antibody 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. 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 yet another aspect, the present disclosure refers to a pharmaceutical composition comprising an isolated 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. 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 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 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.

[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 that specifically binds to human CD47, said antibody comprising:
 - (i) 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
 - (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 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.

2. The isolated monoclonal antibody of claim 1, comprising two heavy chains each comprising the amino acid sequence of SEQ ID NO: 31; and two light chains each comprising the amino acid sequence of SEQ ID NO: 32.
3. The isolated monoclonal antibody of claim 2, wherein the isolated monoclonal antibody 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.
5. The isolated monoclonal antibody of claim 1, wherein the isolated monoclonal antibody prevents human CD47 from interacting with signal-regulatory-protein SIRP α .
6. The isolated monoclonal antibody of claim 1, wherein the isolated monoclonal antibody promotes macrophage-mediated phagocytosis of a CD47-expressing cell.
7. A pharmaceutical composition comprising the isolated monoclonal antibody of any one of claims 1-6, and a pharmaceutically acceptable carrier.
8. Use of an isolated monoclonal antibody of any one of claims 1-6 or a pharmaceutical composition of claim 7 in the manufacture of a medicament for treating a CD47-expressing cancer in a human subject in need thereof.
9. The use of claim 8, 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.

10. The use of claim 8, wherein the CD47-expressing cancer is a solid tumor.