

CD47 ANTIBODIES AND USES THEREOF FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of US Provisional Application No. 62/593,712, filed December 1, 2017, which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

[002] The present invention relates to the field of antibody-based cancer therapeutics. In particular, the present invention relates to novel humanized anti-CD47 antibodies and antigen-binding fragments or conjugates thereof, that may optionally be associated with a removable masking agent, and their use in the treatment of CD47-expressing cancers.

BACKGROUND

[003] Cluster of Differentiation 47 (CD47), also known as integrin associated protein (IAP), is a transmembrane receptor belonging to the immunoglobulin superfamily of proteins. CD47 is ubiquitously expressed on cells and serves as a marker for self-recognition, preventing phagocytosis by serving as a “don’t eat me” signal. CD47 mediates its effects through interactions with several other proteins, including thrombospondin (TSP) and signal regulatory protein-alpha (SIRP α). The interaction between SIRP α on phagocytic cells and CD47 on target cells helps ensure that target cells do not become engulfed.

[004] Certain cancers co-opt the CD47-based immune evasion mechanism of a cell by increasing expression of CD47 on the cell surface of the cancer cell, thus avoiding clearance by the immune system. However, therapies known in the art that target CD47-expressing cells in a subject target both cancerous and non-cancerous cells, which leads to toxicities in the subject, such as peripheral red blood cell and platelet depletion. Accordingly, there is a need for compositions and methods to selectively target CD47 in cancer cells without targeting non-cancerous cells.

SUMMARY

[005] The present disclosure is based on the discovery of novel humanized anti-CD47 antibodies and antigen-binding fragments thereof. In certain examples of the disclosure, humanized anti-CD47 antibodies or antigen-binding fragments thereof are provided that comprise a removable masking agent (e.g., a coiled coil masking agent) that prevents binding of the anti-CD47 antibodies or the antigen-binding fragments thereof to a CD47 protein. In certain examples, the masking agent

can be removed (e.g., cleaved) by one or more molecules (e.g., proteases) that are present in a cancer cell environment. Removal of the masking agent restores the ability of the anti-CD47 antibodies or the antigen-binding fragments thereof to bind CD47, thus enabling specific targeting of the anti-CD47 antibodies or the antigen-binding fragments thereof to the CD47 protein in the context of cancer cells.

[006] In one aspect, the present disclosure refers to a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, wherein the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 16, 19, 21, and 23; HCDR2 selected from SEQ ID NOs: 17, 20, 22, and 24; and HCDR3 of SEQ ID NO: 18; wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 31 and 34; LCDR2 selected from SEQ ID NOs: 32 and 35; and LCDR3 selected from SEQ ID NOs: 33 and 36; wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8; and wherein the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

[007] In one aspect, the present disclosure refers to a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 16, 19, 21, and 23; HCDR2 selected from SEQ ID NOs: 17, 20, 22, and 24; and HCDR3 of SEQ ID NO: 18; wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 31 and 34; LCDR2 selected from SEQ ID NOs: 32 and 35; and LCDR3 selected from SEQ ID NOs: 33 and 36; wherein the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or
- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering; and

wherein the light chain variable region comprises:

- a) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
- b) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.

[008] In some examples, the heavy chain variable region comprises HCDR1, HCDR2, and HCDR3 selected from: SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 19, 20, and 18; SEQ ID NOs: 21, 22, and 18; SEQ ID NOs: 16, 20, and 18; and SEQ ID NOs: 23, 24, and 18. In some examples, the light chain variable region comprises LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 31, 32, and 33; SEQ ID NOs: 31, 32, and 36; and SEQ ID NOs: 34, 35, and 33. In some examples, the antibody or antigen-binding fragment thereof comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 16, 17, 18, 31, 32, and 33; SEQ ID NOs: 16, 17, 18, 34, 35, and 33; SEQ ID NOs: 19, 20, 18, 31, 32, and 33; SEQ ID NOs: 19, 20, 18, 34, 35, and 33; SEQ ID NOs: 21, 22, 18, 31, 32, and 33; SEQ ID NOs: 21, 22, 18, 34, 35, and 33; SEQ ID NOs: 16, 20, 18, 31, 32, and 33; SEQ ID NOs: 16, 20, 18, 34, 35, and 33; SEQ ID NOs: 23, 24, 18, 31, 32, and 33; SEQ ID NOs: 23, 24, 18, 34, 35, and 33; SEQ ID NOs: 16, 17, 18, 31, 32, and 36; SEQ ID NOs: 19, 20, 18, 31, 32, and 36; SEQ ID NOs: 21, 22, 18, 31, 32, and 36; 16, 20, 18, 31, 32, and 36; and SEQ ID NOs: 23, 24, 18, 31, 32, and 36.

[009] In one aspect, the present disclosure refers to a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 25, 28, and 29; HCDR2 selected from SEQ ID NOs: 26 and 30; and HCDR3 of SEQ ID NO: 27; and wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 37 and 40; LCDR2 of SEQ ID NO: 38; and LCDR3 selected from SEQ ID NOs: 39 and 41; wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8; and wherein the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

[010] In one aspect, the present disclosure refers to a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable

region comprises HCDR1 selected from SEQ ID NOs: 25, 28, and 29; HCDR2 selected from SEQ ID NOs: 26 and 30; and HCDR3 of SEQ ID NO: 27; and wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 37 and 40; LCDR2 of SEQ ID NO: 38; and LCDR3 selected from SEQ ID NOs: 39 and 41; wherein the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or
- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering; and

wherein the light chain variable region comprises:

- a) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
- b) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.

[011] In some examples, the heavy chain variable region comprises HCDR1, HCDR2, and HCDR3 selected from: SEQ ID NOs: 25, 26, and 27; SEQ ID NOs: 28, 26, and 27; SEQ ID NOs: 29, 30, and 27; and SEQ ID NOs: 29, 26, and 27. In some examples, the light chain variable region comprises LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 37, 38, and 39; SEQ ID NOs: 40, 38, and 39; and SEQ ID NOs: 37, 38, and 41. In some examples, the antibody or antigen-binding fragment thereof comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 25, 26, 27, 37, 38, and 39; SEQ ID NOs: 25, 26, 27, 40, 38, and 39; SEQ ID NOs: 25, 26, 27, 37, 38, and 41; SEQ ID NOs: 28, 26, 27, 37, 38, and 39; SEQ ID NOs: 28, 26, 27, 40, 38, and 39; SEQ ID NOs: 28, 26, 27, 37, 38, and 41; SEQ ID NOs: 29, 30, 27, 37, 38, and 39; SEQ ID NOs: 29, 30, 27, 40, 38, and 39; SEQ ID NOs: 29, 30, 27, 37, 38, and 41; SEQ ID NOs: 29, 26, 27, 37, 38, and 39; SEQ ID NOs: 29, 26, 27, 40, 38, and 39; and SEQ ID NOs: 29, 26, 27, 37, 38, and 41.

[012] In some examples, the heavy chain variable region comprises an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8. In some examples, the light chain variable region comprises an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15. In some examples, the heavy chain variable region and light chain variable region comprise SEQ ID NOs: 2 and 10; SEQ ID NOs: 3 and 11; SEQ ID NOs: 3 and 12; SEQ ID NOs: 3 and 13; SEQ ID NOs: 3 and 14; SEQ ID NOs: 4 and 11; SEQ ID NOs: 4 and 12; SEQ ID NOs: 4 and 13; SEQ ID NOs: 4 and 14; SEQ ID NOs: 5 and 11; SEQ ID NOs: 5 and 12; SEQ ID NOs: 5 and 13; SEQ ID NOs: 5 and 14; SEQ ID NOs: 6 and 11; SEQ ID NOs: 6 and 12; SEQ ID NOs: 6 and 13; SEQ ID NOs: 6 and 14; SEQ ID NOs: 7 and 11; SEQ ID NOs: 7 and 12; SEQ ID NOs: 7 and 13; SEQ ID NOs: 7 and 14; SEQ ID NOs: 8 and 11; SEQ ID NOs: 8 and 12; SEQ ID NOs: 8 and 13; SEQ ID NOs: 8 and 14; SEQ ID NOs: 3 and 15.

[013] In one aspect, the present disclosure refers to a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 of SEQ ID NO: 16, HCDR2 of SEQ ID NO: 17, and HCDR3 of SEQ ID NO: 18; and wherein the light chain variable region comprises LCDR1 of SEQ ID NO: 31, LCDR2 of SEQ ID NO: 32, and LCDR3 of SEQ ID NO: 33; and wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 3 and the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13; and wherein the antibody has reduced hemagglutination of red blood cells compared to Ab47. In some examples, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 3 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 13.

[014] In some examples of the humanized antibody or antigen-binding fragment thereof provided herein, the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or

- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering.

In some such examples, H29 is F, H44 is R or G, H49 is A, H82 is M or I, H89 is I or V, H91 is F or Y, and H94 is R, according to Kabat numbering.

[015] In some examples of the humanized antibody or antigen-binding fragment thereof provided herein, the light chain variable region comprises:

- a) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
- b) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.

In some such examples, L4 is M, L21 is L, L49 is K, L69 is T or S, and L85 is V or T, according to Kabat numbering.

[016] In some examples, the antibody or antigen-binding fragment thereof is of an IgG1 isotype. In some examples, the antibody or antigen-binding fragment thereof has enhanced antibody dependent cellular cytotoxicity (ADCC) compared to its parental antibody. In some examples, the antibody or antigen-binding fragment thereof has enhanced antibody dependent cellular phagocytosis (ADCP) compared to its parental antibody. In some examples, the antibody or antigen-binding fragment thereof has enhanced complement-dependent cytotoxicity (CDC) compared to its parental antibody. In some examples, the antibody or antigen-binding fragment thereof is a Fab, a Fab', a F(ab')₂, a Fv fragment, a diabody, a single-chain antibody, an scFv fragment or an scFv-Fc. In some examples, the antibody or antigen-binding fragment thereof induces apoptosis of CD47-expressing cells *in vitro* and/or *in vivo*. In some examples, the antibody or antigen-binding fragment thereof has reduced core fucosylation compared to its parental antibody. In some examples, the antibody or antigen-binding fragment thereof is afucosylated. In some examples, the antibody or antigen-binding fragment thereof blocks an interaction between CD47 and SIRPα. In some examples, the antibody or antigen-binding fragment thereof has reduced hemagglutination of red blood cells compared to Ab47.

[017] In one aspect, the present disclosure refers to a nucleic acid sequence encoding an antibody or antigen-binding fragment provided herein. In one aspect, the present disclosure refers to an expression vector comprising the nucleic acid sequence. In one aspect, the present disclosure refers to a host cell comprising the nucleic acid or the expression vector. In one aspect, the present disclosure refers to a host cell that expresses an antibody or antigen-binding fragment provided herein. In one aspect, the present disclosure refers to a method of producing the antibody or antigen-binding fragment provided herein

comprising culturing the host cell. In some examples, the method further comprises isolating the antibody or antigen-binding fragment thereof.

[018] In some examples, methods of treating a CD47-expressing cancer in a subject are provided, comprising administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof provided herein. In one aspect, the present disclosure refers to use of the anti-CD47 antibody or antigen-binding fragment provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject.

[019] In some examples, methods of treating a CD47-expressing cancer in a subject are provided, comprising:

- a) identifying a subject as having a CD47-expressing cancer; and
- b) administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof provided herein.

In one aspect, the present disclosure refers to use of the anti-CD47 antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having a CD47-expressing cancer.

In some such examples, step a) or the use comprises:

- i) isolating cancer tissue; and
- ii) detecting CD47 in the isolated cancer tissue.

[020] In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising:

- a) identifying a subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue; and
- b) administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof provided herein.

In one aspect, the present disclosure refers to use of the anti-CD47 antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

In some such examples, step a) or the use comprises:

- i) isolating cancer tissue and surrounding non-cancer tissue from the subject;
- ii) detecting macrophages in the isolated cancer tissue and in non-cancer tissue; and
- iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue. In some examples, the macrophage staining is performed with an anti-CD163 antibody.

[021] In some examples, methods of treating cancer comprise identifying a subject as having a CD47-expressing cancer and elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

[022] In some examples, a method of inducing apoptosis of a CD47-expressing cell is provided, comprising contacting the cell with an antibody or antigen-binding fragment thereof provided herein. In some examples, the cell is *in vitro*. In some examples, the cell is *in vivo*. In one aspect, the present disclosure refers to an *in vitro* method of inducing apoptosis of a CD47-expressing cell comprising contacting the cell with an antibody or antigen-binding fragment thereof provided herein. In one aspect, the present disclosure refers to use of an antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for inducing apoptosis of a CD47-expressing cell *in vivo*.

[023] In one aspect, the present disclosure refers to a masked antibody comprising an antibody or antigen-binding fragment thereof that specifically binds to the human CD47 protein and at least one masking domain, wherein at least one masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44-55, 75-86, 94, and 95. In one aspect, the present disclosure refers to a masked antibody

comprising an antibody or antigen-binding fragment thereof provided herein and at least one masking domain. In some examples, at least one masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44-55, 75-86, 94, and 95.

[024] In some examples, the at least one masking domain reduces binding affinity of the antibody or antigen-binding fragment to human CD47 protein compared to the antibody or antigen-binding fragment thereof without the at least one masking domain. In some examples, the binding affinity is reduced at least about 100-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain. In some examples, the binding affinity is reduced between about 200-fold and about 1500-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

[025] In some examples, the antibody or antigen binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain is linked to a first masking domain; or wherein the light chain is linked to a second masking domain; or wherein the heavy chain is linked to a first masking domain and the light chain is linked to a second masking domain. In some examples, the first masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, 52, 54, 75, 77, 79, 81, 83, 85, and 94; and the second masking domain comprises an amino acid sequence selected from SEQ ID NOs: 45, 47, 49, 51, 53, 55, 76, 78, 80, 82, 84, 86, and 95. In some examples, the first masking domain and the second masking domain are a pair of masking domains selected from: SEQ ID NOs: 44 and 45; SEQ ID NOs: 46 and 47; SEQ ID NOs: 48 and 49; SEQ ID NOs: 50 and 51; SEQ ID NOs: 52 and 53; SEQ ID NOs: 54 and 55; SEQ ID NOs: 75 and 76; SEQ ID NOs: 77 and 78; SEQ ID NOs: 79 and 80; SEQ ID NOs: 81 and 82; SEQ ID NOs: 83 and 84; SEQ ID NOs: 85 and 86; and SEQ ID NOs: 94 and 95. In some examples, the first masking domain is linked to the N-terminus of the heavy chain and the second masking domain is linked to the N-terminus of the light chain.

[026] In some examples, each masking domain comprises a protease-cleavable linker and is linked to the heavy chain or light chain via the protease-cleavable linker. In some examples, the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site. In some examples, the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site. In some examples, following cleavage by an MMP, the heavy chain and/or light chain of the antibody or antigen-binding fragment thereof comprises a stub amino acid remnant of the MMP cleavage site. In some examples, the stub amino acid remnant comprises the sequence LRSG, SG, or VR at the N terminus of the antibody.

[027] In some examples, the antibody or antigen-binding fragment thereof is an antibody or antigen-binding fragment thereof provided herein. In some examples, the masked antibody

comprises a heavy chain linked to a first masking domain and having the amino acid sequence of SEQ ID NO: 42 and a light chain linked to a second masking domain and having the amino acid sequence of SEQ ID NO: 43.

[028] In one aspect, the present disclosure refers to a nucleic acid sequence encoding a masked antibody provided herein. In one aspect, the present disclosure refers to an expression vector comprising the nucleic acid. In one aspect, the present disclosure refers to a host cell comprising the nucleic acid or the expression vector. In one aspect, the present disclosure refers to a host cell that expresses the masked antibody provided herein. In one aspect, the present disclosure refers to a method of producing the masked antibody provided herein comprises culturing the host cell. In some examples, the method further comprises isolating the masked antibody.

[029] In some examples, methods of treating a CD47-expressing cancer in a subject are provided, comprising administering to the subject a therapeutically effective amount of a masked antibody provided herein. In one aspect, the present disclosure refers to use of the masked antibody provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject.

[030] In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising:

- a) identifying a subject as having elevated levels of MMP in the cancer relative to surrounding non-cancer tissue; and

- b) administering to the subject a therapeutically effective amount of a masked antibody provided herein, wherein each masking domain of the masked antibody comprises a protease-cleavable linker and wherein the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site. In one aspect, the present disclosure refers to use of the masked antibody provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, wherein each masking domain of the masked antibody comprises a protease-cleavable linker and wherein the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site. In some examples, the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site. In some examples, the MMP is selected from MMP2, MMP7, MMP9, and MMP13. In some examples, step a) or the use comprises:

- i) isolating cancer tissue and non-cancer tissue from the subject;
- ii) detecting MMPs in the isolated cancer tissue and the non-cancer tissue; and
- iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.

[031] In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising:

- a) identifying a subject as having a CD47-expressing cancer; and

- b) administering to the subject a therapeutically effective amount of a masked antibody provided herein.

In one aspect, the present disclosure refers to use of a masked antibody provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having a CD47-expressing cancer.

In some examples, step a) or the use comprises:

- i) isolating cancer tissue; and
- ii) detecting CD47 in the isolated cancer tissue.

[032] In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising:

- a) identifying a subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue; and
- b) administering to the subject a therapeutically effective amount of a masked antibody provided herein.

In one aspect, the present disclosure refers to use of the masked antibody provided herein in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

In some examples, step a) or the use comprises:

- i) isolating cancer tissue and surrounding non-cancer tissue from the subject;
- ii) detecting macrophages in the isolated cancer tissue and in non-cancer tissue; and
- iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue. In some examples, the macrophage staining is performed with an anti-CD163 antibody.

[033] In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising identifying a subject as having (a) elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, and (b) a CD47-expressing cancer. In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising identifying a subject as having (a) elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, and (b) elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue. In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising identifying a subject as having (a) a CD47-expressing cancer, and (b) elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue. In some examples, methods for treating a CD47-expressing cancer in a subject are provided, comprising identifying a subject as having (a) elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, (b) a CD47-expressing cancer, and (c) elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

[034] In some examples, the masked antibody comprises at least one masking domain comprising a protease-cleavable linker, and wherein the protease-cleavable linker is cleaved in the tumor microenvironment. In some examples, following cleavage of the protease-cleavable linker in the tumor microenvironment, the masking domain is released from the anti-CD47 antibody or antigen-binding fragment thereof. In some examples, the protease-cleavable linker comprises the amino acid sequence IPVSLRSG (SEQ ID NO: 73) or GPLGVR (SEQ ID NO: 57). In some examples, the protease-cleavable linker comprises a MMP cleavage site. In some examples, the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site. In some examples, following release of the anti-CD47 antibody or antigen-binding fragment thereof, the anti-CD47 antibody or antigen-binding fragment thereof has a stub amino acid remnant of the protease-

cleavable linker. In some examples, the stub amino acid remnant comprises the sequence of LRSG, SG, or VR at the N terminus of the antibody.

[035] In various examples, the CD47-expressing cancer is a hematological cancer or a solid cancer. In some examples, the CD47-expressing cancer is selected from non-Hodgkin lymphoma, B-lymphoblastic lymphoma; B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, Richter's syndrome, follicular lymphoma, multiple myeloma, myelofibrosis, polycythemia vera, cutaneous T-cell lymphoma, monoclonal gammopathy of unknown significance (MGUS), myelodysplastic syndrome (MDS), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, acute myeloid leukemia (AML), and anaplastic large cell lymphoma. In some examples, the CD47-expressing cancer is selected from lung cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicular cancer, kidney cancer, bladder cancer, spinal cancer, brain cancer, cervical cancer, endometrial cancer, colorectal cancer, anal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, gastrointestinal cancer, gastric cancer, carcinoma, head and neck cancer, skin cancer, melanoma, prostate cancer, pituitary cancer, stomach cancer, uterine cancer, vaginal cancer and thyroid cancer. In some examples, the CD47-expressing cancer is selected from lung cancer, sarcoma, colorectal cancer, head and neck cancer, ovarian cancer, pancreatic cancer, gastric cancer, melanoma, and breast cancer.

[036] In some examples, the anti-CD47 antibody or antigen-binding fragment thereof, or the masked antibody, is administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), PD-L2, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain containing 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1), CEACAM-5, V-domain Ig suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), CD160, 2B4 or TGFR. In some examples, the anti-CD47 antibody or antigen-binding fragment thereof, or the masked antibody, is administered in combination with an agonistic anti-CD40 antibody. In some examples, the agonistic anti-CD40 antibody has low fucosylation levels or is afucosylated. In some examples, the anti-CD47 antibody or antigen-binding fragment thereof, or the masked antibody, is administered in combination with an antibody drug conjugate (ADC), wherein the antibody of the ADC specifically binds to a protein that is expressed on the extracellular surface of a cancer cell and the antibody is conjugated to a drug-linker comprising a cytotoxic agent. In some examples, the cytotoxic agent is an auristatin. In some examples, the antibody of the ADC is conjugated to a drug-linker selected from vcMMAE and mcMMAF.

[037] In some examples, the anti-CD47 antibody or antigen-binding fragment thereof or the anti-CD47 antibody or antigen-binding fragment thereof of the masked antibody exhibits reduced hemagglutination *in vitro* compared to its parental anti-CD47 antibody. In some examples, the parental antibody is Ab47. In some examples, administration of the anti-CD47 antibody or masked antibody does not induce hemagglutination in the subject. In some examples, the anti-CD47 antibody or masked antibody induces apoptosis of CD47-expressing cells *in vitro* and/or *in vivo*. In some examples, the anti-CD47 antibody or masked antibody induces apoptosis of CD47-expressing cells *in vivo*. In some examples, the CD47-expressing cells are cancer cells.

[038] In one example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 16 (GYGMS), 17 (TITSGGTYTYYPDSVKG), and 18 (SLAGNAMDY), and a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88 [Figure 1A], wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering, is provided.

[039] In another example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 16 (GYGMS), 17 (TITSGGTYTYYPDSVKG), and 18 (SLAGNAMDY), and a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89 [Figure 1B], wherein framework position H49 is a donor residue, according to Kabat numbering, is provided.

[040] In yet another example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 16 (GYGMS), 17 (TITSGGTYTYYPDSVKG), and 18 (SLAGNAMDY), and a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90 [Figure 1C], wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering, is provided.

[041] In still another example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 16 (GYGMS), 17 (TITSGGTYTYYPDSVKG), and 18 (SLAGNAMDY), and a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91 [Figure

1D], wherein framework position H49 is a donor residue, according to Kabat numbering, is provided.

[042] In still another example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 31 (RASQTISDYLH), 32 (FASQSIG), and 33 (QNGHGFPRT); and a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92 [Figure 1G], wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering, is provided.

[043] In still another example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, the heavy chain variable comprising CDRs set forth as SEQ ID NOs: 31 (RASQTISDYLH), 32 (FASQSIG), and 33 (QNGHGFPRT); and a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93 [Figure 1H], wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering, is provided.

[044] In an example, the framework position L4 is occupied by M, L21 is occupied by L, L49 is occupied by K, L69 is occupied by T or S, and L85 is occupied by V or T, according to Kabat numbering.

[045] In an example, the antibody or antigen-binding fragment comprises a heavy chain variable region (HCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8, and a light chain variable region (LCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

[046] In an example, the antibody or antigen-binding fragment further comprises a G91A mutation in LCDR3, according to Kabat numbering.

[047] In an example, the antibody or antigen-binding fragment is of an IgG1 isotype.

[048] In an example, the antibody or antigen-binding fragment has enhanced antibody dependent cellular cytotoxicity (ADCC) compared to its parental antibody.

[049] In an example, the antibody or antigen-binding fragment has enhanced antibody dependent cellular phagocytosis (ADCP) compared to its parental antibody.

[050] In an example, the antibody or antigen-binding fragment has reduced core fucosylation compared to its parental antibody.

[051] In an example, the antibody or antigen-binding fragment blocks an interaction between CD47 and SIRP α .

[052] In an example, the antibody or antigen-binding fragment has reduced hemagglutination of red blood cells compared to its parental antibody.

[053] In one example, a nucleic acid sequence encoding a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, is provided.

[054] In an example, the antigen-binding fragment comprises a Fab, a Fab', a F(ab')₂, a Fv fragment, a diabody, a single-chain antibody, an scFv fragment or an scFv-Fc.

[055] In one example, a method for treating a CD47-expressing cancer in a subject, comprising administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof comprising a masking agent (also referred to as a “masking domain”), wherein the masking agent comprises one or more coiled coil peptides that reduce binding affinity of the antibody or antigen-binding fragment to human CD47 compared to the antibody or antigen-binding fragment thereof without the masking agent, is provided.

[056] In an example, a protease-cleavable linker attaches the masking agent to the antibody or antigen-binding fragment thereof.

[057] In an example, the protease-cleavable linker has an amino acid sequence comprising IPVSLRSG (SEQ ID NO: 73) or GPLGVR (SEQ ID NO: 57).

[058] In an example, the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site.

[059] In an example, the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.

[060] In an example, the masking agent is released from the anti-CD47 antibody or antigen-binding fragment thereof subsequent to cleavage of an MMP cleavage site in a tumor microenvironment by an MMP.

[061] In an example, the cleaved anti-CD47 antibody has a stub amino acid remnant of the MMP cleavage site.

[062] In an example, the stub amino acid remnant comprises the sequence of LRSG, SG, or VR at the N terminus of the antibody.

[063] In an example, one or more the coiled coil peptides comprise one or more sequences selected from SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, and 55. In an example, one or more the coiled coil peptides comprise one or more sequences selected from SEQ ID NOs: 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, and 86. In an example, one or more the coiled coil peptides comprise one or more sequences selected from SEQ ID NOs: 94 and 95.

[064] In an example, the antibody or antigen-binding fragment binding to CD47 is reduced at least about 100-fold compared to the antibody or antigen-binding fragment thereof without the masking agent.

[065] In an example, the antibody or antigen-binding fragment binding to CD47 is reduced between about 200-fold and about 1500-fold compared to the antibody or antigen-binding fragment thereof without the masking agent.

[066] In an example, the CD47-expressing cancer is a hematological cancer that causes a solid cancer.

[067] In an example, the hematological cancer is selected from non-Hodgkin lymphoma, B-lymphoblastic lymphoma; B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, Richter's syndrome, follicular lymphoma, multiple myeloma, myelofibrosis, polycythemia vera, cutaneous T-cell lymphoma, monoclonal gammopathy of unknown significance (MGUS), myelodysplastic syndrome (MDS), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, acute myeloid leukemia (AML), and anaplastic large cell lymphoma.

[068] In an example, the CD47-expressing cancer is a solid tumor.

[069] In an example, the solid tumor is selected from lung cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicular cancer, kidney cancer, bladder cancer, spinal cancer, brain cancer, cervical cancer, endometrial cancer, colorectal cancer, anal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, gastrointestinal cancer, gastric cancer, sarcoma, head and neck cancer, melanoma, skin cancer, prostate cancer, pituitary cancer, stomach cancer, uterine cancer, vaginal cancer and thyroid cancer.

[070] In an example, the solid tumor is selected from lung cancer, sarcoma, ovarian cancer, pancreatic cancer, gastric cancer, melanoma, colorectal cancer, head and neck cancer, and breast cancer.

[071] In an example, the subject is a human suffering from a solid cancer.

[072] In an example, the anti-CD47 antibody is administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), PD-L2, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain containing 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1), CEACAM-5, V-domain Ig suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), CD160, 2B4 or TGFR.

[073] In one example, an antibody or antigen-binding fragment thereof that specifically binds to the human CD47 protein comprising a masking agent, wherein the masking agent comprises one or more coiled coil peptides comprising the sequence of SEQ ID NO: 95 (QGASTTVAQLEEKVKTLRAENYELKSEVQRLEEQVAQLGS) and/or SEQ ID NO: 94 (QGASTSVDELQAEVDQLEDENYALKTKVAQLRKKVEKLGS), and wherein the one or

more coiled coil peptides reduce binding affinity of the antibody or antigen-binding fragment to human CD47 protein compared to the antibody or antigen-binding fragment thereof without the masking agent, is provided.

[074] In an example, the antibody or antigen-binding fragment comprises a heavy chain variable region (HCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8, and a light chain variable region (LCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

[075] In an example, the masking agent is attached to the antibody or antigen-binding fragment thereof via a protease-cleavable linker.

[076] In an example, the protease-cleavable linker has an amino acid sequence comprising IPVSLRSG (SEQ ID NO: 73) or GPLGVR (SEQ ID NO: 57).

[077] In an example, the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site.

[078] In an example, the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.

[079] In an example, the masking agent is removed from the anti-CD47 antibody after cleavage of an MMP cleavage site by an MMP.

[080] In an example, the anti-CD47 antibody has a stub amino acid remnant of the MMP cleavage site after cleavage of an MMP cleavage site by an MMP.

[081] In an example, the stub amino acid remnant comprises the sequence of LRSG, SG, or VR at the N terminus of the antibody.

[082] In an example, the binding is reduced at least about 100-fold compared to the antibody or antigen-binding fragment thereof without the masking agent.

[083] In an example, the binding is reduced between about 200-fold and about 1500-fold compared to the antibody or antigen-binding fragment thereof without the masking agent.

[084] In an example, the antibody or antigen-binding fragment comprises a heavy chain sequence of SEQ ID NO: 42 and a light chain sequence of SEQ ID NO: 43.

[085] In an example, the antibody or antigen-binding fragment comprises a variant Fc region which confers enhanced effector function selected from ADCC and/or CDC activity.

[086] In an example, the antibody or antigen-binding fragment is afucosylated.

[087] In one example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, wherein the antibody is an IgG1 isotype, is provided.

[088] In an example, the antibody comprises enhanced ADCC, enhanced ADCP, and/or enhanced CDC activity.

[089] In one example, a method for treating a CD47-expressing cancer in a subject, comprising the steps of:

[090] a) identifying the subject as having elevated levels of MMP in the cancer relative to surrounding non-cancer tissue; and

[091] b) administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof comprising a masking agent, wherein the masking agent comprises coiled coil peptides that reduce binding affinity of the antibody or antigen-binding fragment to human CD47 compared to the antibody or antigen-binding fragment thereof without the masking agent, if the subject has elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, is provided.

[092] In an example, the MMP is selected from the group consisting of: MMP2, MMP7, MMP9, and MMP13.

[093] In an example, step a) comprises:

[094] i) isolating cancer tissue and non-cancer tissue from the subject;

[095] ii) detecting MMPs in the isolated cancer tissue and the non-cancer tissue; and

[096] iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.

[097] In one example, a method for treating a CD47-expressing cancer in a subject, comprising the steps of:

[098] a) identifying the subject as having elevated levels of CD47 in the cancer relative to surrounding non-cancer tissue; and

[099] b) administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof comprising a masking agent, wherein the masking agent comprises coiled coil peptides that reduce binding affinity of the antibody or antigen-binding fragment to human CD47 compared to the antibody or antigen-binding fragment thereof without the masking agent, if the subject has elevated levels of CD47 in the cancer relative to surrounding non-cancer tissue, is provided.

[0100] In an example, step a) comprises:

[0101] i) isolating cancer tissue and surrounding non-cancer tissue from the subject;

[0102] ii) detecting CD47 in the isolated cancer tissue and surrounding non-cancer tissue; and

[0103] iii) comparing the amount of CD47 staining in the cancer tissue relative to CD47 staining the non-cancer tissue.

[0104] In one example, a method for treating a CD47-expressing cancer in a subject, comprising the steps of:

[0105] a) identifying the subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue; and

[0106] b) administering to the subject a therapeutically effective amount of an anti-CD47 antibody or antigen-binding fragment thereof comprising a masking agent, wherein the masking agent comprises one or more coiled coil peptides that reduce binding affinity of the antibody or antigen-binding fragment to human CD47 compared to the antibody or antigen-binding fragment thereof without the masking agent, if the subject has elevated levels of macrophage infiltration in the cancer relative to the non-cancer tissue, is provided.

[0107] In an example, step a) comprises:

[0108] i) isolating cancer tissue and surrounding non-cancer tissue from the subject;

[0109] ii) detecting macrophages in the isolated cancer tissue and in non-cancer tissue; and

[0110] iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.

[0111] In an example, the macrophage staining is performed with an anti-CD163 antibody.

[0112] In one example, a humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, comprising a heavy chain variable region (HCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8, and a light chain variable region (LCVR) having at least 90% sequence identity to any one of SEQ ID NOs: 10, 11, 12, 13, 14 and 15, wherein the antibody further comprises the sequence LRSG, SG, or VR at the N terminus of the HCVR and/or the LCVR, is provided.

[0113] In one example, a method of treating cancer by administering a combination of the masked CD47 antibody of the disclosure with an agonistic CD40 antibody.

[0114] In an example, the agonistic CD40 antibody has low fucosylation levels, e.g., SEA-CD40 antibody.

[0115] In one example, a method of treating cancer by administering a combination of the masked CD47 antibody of claim 37 with an antibody drug conjugate (ADC), wherein the antibody of the ADC specifically binds to a protein that is expressed on the extracellular surface of a cancer cell and the antibody is conjugated to a drug-linker comprising a cytotoxic agent, is provided.

[0116] In an example, the cytotoxic agent is an auristatin.

[0117] In an example, the antibody of the ADC is conjugated to a drug linker selected from vcMMAE and mcMMAF.

[0118] The summary of the disclosure described above is non-limiting, and other features and advantages of the disclosed antibodies and methods of making and using them will be apparent from the following drawings, the detailed description, the examples and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

CLAIMS

What is claimed:

1. A humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 16, 19, 21, and 23; HCDR2 selected from SEQ ID NOs: 17, 20, 22, and 24; and HCDR3 of SEQ ID NO: 18; wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 31 and 34; LCDR2 selected from SEQ ID NOs: 32 and 35; and LCDR3 selected from SEQ ID NOs: 33 and 36; wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8; and wherein the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

2. A humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 16, 19, 21, and 23; HCDR2 selected from SEQ ID NOs: 17, 20, 22, and 24; and HCDR3 of SEQ ID NO: 18; wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 31 and 34; LCDR2 selected from SEQ ID NOs: 32 and 35; and LCDR3 selected from SEQ ID NOs: 33 and 36; wherein the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or
- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering; and

wherein the light chain variable region comprises:

- c) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
 - d) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.
3. The humanized antibody or antigen-binding fragment thereof of claim 1 or claim 2, wherein the heavy chain variable region comprises HCDR1, HCDR2, and HCDR3 selected from: SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 19, 20, and 18; SEQ ID NOs: 21, 22, and 18; SEQ ID NOs: 16, 20, and 18; and SEQ ID NOs: 23, 24, and 18.
4. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the light chain variable region comprises LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 31, 32, and 33; SEQ ID NOs: 31, 32, and 36; and SEQ ID NOs: 34, 35, and 33.
5. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 16, 17, 18, 31, 32, and 33; SEQ ID NOs: 16, 17, 18, 34, 35, and 33; SEQ ID NOs: 19, 20, 18, 31, 32, and 33; SEQ ID NOs: 19, 20, 18, 34, 35, and 33; SEQ ID NOs: 21, 22, 18, 31, 32, and 33; SEQ ID NOs: 21, 22, 18, 34, 35, and 33; SEQ ID NOs: 16, 20, 18, 31, 32, and 33; SEQ ID NOs: 16, 20, 18, 34, 35, and 33; SEQ ID NOs: 23, 24, 18, 31, 32, and 33; SEQ ID NOs: 23, 24, 18, 34, 35, and 33; SEQ ID NOs: 16, 17, 18, 31, 32, and 36; SEQ ID NOs: 19, 20, 18, 31, 32, and 36; SEQ ID NOs: 21, 22, 18, 31, 32, and 36; 16, 20, 18, 31, 32, and 36; and SEQ ID NOs: 23, 24, 18, 31, 32, and 36.
6. A humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 25, 28, and 29; HCDR2 selected from SEQ ID NOs: 26 and 30; and HCDR3 of SEQ ID NO: 27; and wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 37 and 40; LCDR2 of SEQ ID NO: 38; and LCDR3 selected from SEQ ID NOs: 39 and 41; wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8; and wherein the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

7. A humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 selected from SEQ ID NOs: 25, 28, and 29; HCDR2 selected from SEQ ID NOs: 26 and 30; and HCDR3 of SEQ ID NO: 27; and wherein the light chain variable region comprises LCDR1 selected from SEQ ID NOs: 37 and 40; LCDR2 of SEQ ID NO: 38; and LCDR3 selected from SEQ ID NOs: 39 and 41; wherein the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or
- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering; and

wherein the light chain variable region comprises:

- c) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
- d) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.

8. The humanized antibody or antigen-binding fragment thereof of claim 6 or claim 7, wherein the heavy chain variable region comprises HCDR1, HCDR2, and HCDR3 selected from: SEQ ID NOs: 25, 26, and 27; SEQ ID NOs: 28, 26, and 27; SEQ ID NOs: 29, 30, and 27; and SEQ ID NOs: 29, 26, and 27.

9. The humanized antibody or antigen-binding fragment thereof of any one of claims 6-8, wherein the light chain variable region comprises LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 37, 38, and 39; SEQ ID NOs: 40, 38, and 39; and SEQ ID NOs: 37, 38, and 41.

10. The humanized antibody or antigen-binding fragment thereof of any one of claims 6-9, wherein the antibody or antigen-binding fragment thereof comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 selected from SEQ ID NOs: 25, 26, 27, 37, 38, and 39;

SEQ ID NOs: 25, 26, 27, 40, 38, and 39; SEQ ID NOs: 25, 26, 27, 37, 38, and 41; SEQ ID NOs: 28, 26, 27, 37, 38, and 39; SEQ ID NOs: 28, 26, 27, 40, 38, and 39; SEQ ID NOs: 28, 26, 27, 37, 38, and 41; SEQ ID NOs: 29, 30, 27, 37, 38, and 39; SEQ ID NOs: 29, 30, 27, 40, 38, and 39; SEQ ID NOs: 29, 30, 27, 37, 38, and 41; SEQ ID NOs: 29, 26, 27, 37, 38, and 39; SEQ ID NOs: 29, 26, 27, 40, 38, and 39; and SEQ ID NOs: 29, 26, 27, 37, 38, and 41.

11. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-10, wherein the heavy chain variable region comprises an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 6, 7 and 8.

12. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-11, wherein the light chain variable region comprises an amino acid sequence selected from SEQ ID NOs: 10, 11, 12, 13, 14 and 15.

13. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-12, wherein the heavy chain variable region and light chain variable region comprise SEQ ID NOs: 2 and 10; SEQ ID NOs: 3 and 11; SEQ ID NOs: 3 and 12; SEQ ID NOs: 3 and 13; SEQ ID NOs: 3 and 14; SEQ ID NOs: 4 and 11; SEQ ID NOs: 4 and 12; SEQ ID NOs: 4 and 13; SEQ ID NOs: 4 and 14; SEQ ID NOs: 5 and 11; SEQ ID NOs: 5 and 12; SEQ ID NOs: 5 and 13; SEQ ID NOs: 5 and 14; SEQ ID NOs: 6 and 11; SEQ ID NOs: 6 and 12; SEQ ID NOs: 6 and 13; SEQ ID NOs: 6 and 14; SEQ ID NOs: 7 and 11; SEQ ID NOs: 7 and 12; SEQ ID NOs: 7 and 13; SEQ ID NOs: 7 and 14; SEQ ID NOs: 8 and 11; SEQ ID NOs: 8 and 12; SEQ ID NOs: 8 and 13; SEQ ID NOs: 8 and 14; SEQ ID NOs: 3 and 15.

14. A humanized antibody or antigen-binding fragment thereof that specifically binds human CD47, the antibody or antigen-binding fragment comprising a light chain variable region and a heavy chain variable region, wherein the heavy chain variable region comprises HCDR1 of SEQ ID NO: 16, HCDR2 of SEQ ID NO: 17, and HCDR3 of SEQ ID NO: 18; and wherein the light chain variable region comprises LCDR1 of SEQ ID NO: 31, LCDR2 of SEQ ID NO: 32, and LCDR3 of SEQ ID NO: 33; and wherein the heavy chain variable region comprises an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 3 and the light chain variable region comprises an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13; and wherein the antibody has reduced hemagglutination of red blood cells compared to Ab47.

15. The humanized antibody or antigen-binding fragment thereof of claim 14, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 3 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 13.

16. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-15, wherein the heavy chain variable region comprises:

- a) a human IGHV3-23/HJ4 framework set forth in SEQ ID NO: 88, wherein framework positions H44, H49, H82, H89, H91, and H94 are donor residues, according to Kabat numbering; or
- b) a human IGHV3-48/HJ4 framework set forth in SEQ ID NO: 89, wherein framework position H49 is a donor residue, according to Kabat numbering; or
- c) a human IGHV3-66/HJ4 framework set forth in SEQ ID NO: 90, wherein framework position H29, H49, and H82 is a donor residue, according to Kabat numbering; or
- d) a human IGHV3-74/HJ4 framework set forth in SEQ ID NO: 91, wherein framework position H49 is a donor residue, according to Kabat numbering.

17. The humanized antibody or antigen-binding fragment thereof of claim 16, wherein H29 is F, H44 is R or G, H49 is A, H82 is M or I, H89 is I or V, H91 is F or Y, and H94 is R, according to Kabat numbering.

18. The humanized antibody or antigen-binding fragment thereof of any one of claims 1-17, wherein the light chain variable region comprises:

- a) a human IGKV6-21/KJ2 framework set forth in SEQ ID NO: 92, wherein framework positions L4, L21, L69, and L85 are donor residues, according to Kabat numbering; or
- b) a human IGKV1-27/KJ2 framework set forth in SEQ ID NO: 93, wherein framework positions L21, L49, and L69 are donor residues, according to Kabat numbering.

19. The humanized antibody or antigen binding fragment thereof of claim 18, wherein L4 is M, L21 is L, L49 is K, L69 is T or S, and L85 is V or T, according to Kabat numbering.

20. The antibody or antigen-binding fragment thereof of any one of claims 1-19, wherein the antibody or antigen-binding fragment thereof is of an IgG1 isotype.

21. The antibody or antigen-binding fragment thereof of any one of claims 1-20, wherein the antibody or antigen-binding fragment thereof has enhanced antibody dependent cellular cytotoxicity (ADCC) compared to its parental antibody.

22. The antibody or antigen-binding fragment thereof of any one of claims 1-21, wherein the antibody or antigen-binding fragment thereof has enhanced antibody dependent cellular phagocytosis (ADCP) compared to its parental antibody.

23. The antibody or antigen-binding fragment thereof of any one of claims 1-22, wherein the antibody or antigen-binding fragment thereof has enhanced complement-dependent cytotoxicity (CDC) compared to its parental antibody.

24. The antibody or antigen-binding fragment thereof of any one of claims 1-19, wherein the antibody or antigen-binding fragment thereof is a Fab, a Fab', a F(ab')₂, a Fv fragment, a diabody, a single-chain antibody, an scFv fragment or an scFv-Fc.

25. The antibody or antigen-binding fragment thereof of any one of claims 1-24, wherein the antibody or antigen-binding fragment thereof induces apoptosis of CD47-expressing cells *in vitro* and/or *in vivo*.

26. The antibody or antigen-binding fragment thereof of any one of claims 1-25, having reduced core fucosylation compared to its parental antibody.

27. The antibody or antigen-binding fragment thereof of any one of claims 1-26, which is afucosylated.

28. The antibody or antigen-binding fragment of any one of claims 1-27, wherein the antibody or antigen-binding fragment blocks an interaction between CD47 and SIRPα.

29. The antibody or antigen-binding fragment of any one of claims 1-28, wherein the antibody or antigen-binding fragment has reduced hemagglutination of red blood cells compared to Ab47.

30. A nucleic acid sequence encoding the antibody or antigen-binding fragment of any one of claims 1-29.

31. An expression vector comprising the nucleic acid of claim 29.

32. A host cell comprising the nucleic acid of claim 30 or the expression vector of claim 28.

33. A host cell that expresses the antibody or antigen-binding fragment of any one of claims 1-29.

34. A method of producing the antibody or antigen-binding fragment of any one of claims 1-23 comprising culturing the host cell of claim 32 or claim 33.

35. The method of claim 34, further comprising isolating the antibody or antigen-binding fragment thereof.

36. Use of the anti-CD47 antibody or antigen-binding fragment of any one of claims 1-29 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject.

37. Use of the anti-CD47 antibody or antigen-binding fragment thereof of any one of claims 1-29 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having a CD47-expressing cancer.

38. The use of claim 37, wherein the identifying comprises:

i) isolating cancer tissue; and

ii) detecting CD47 in the isolated cancer tissue.

39. Use of the anti-CD47 antibody or antigen-binding fragment thereof of any one of claims 1-26 in the manufacture of a medicament for treating a CD47-expressing cancer in

a subject, wherein the use comprises identifying the subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

40. The use of claim 39, wherein the identifying comprises:

- i) isolating cancer tissue and surrounding non-cancer tissue from the subject;
- ii) detecting macrophages in the isolated cancer tissue and in non-cancer tissue; and
- iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.

41. The use of claim 40, wherein the macrophage staining is performed with an anti-CD163 antibody.

42. The use of any one of claims 36-41, wherein the CD47-expressing cancer is a hematological cancer or a solid cancer.

43. The use of any one of claim 36-42, wherein the CD47-expressing cancer is selected from non-Hodgkin lymphoma, B-lymphoblastic lymphoma; B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, Richter's syndrome, follicular lymphoma, multiple myeloma, myelofibrosis, polycythemia vera, cutaneous T-cell lymphoma, monoclonal gammopathy of unknown significance (MGUS), myelodysplastic syndrome (MDS), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, acute myeloid leukemia (AML), and anaplastic large cell lymphoma.

44. The use of any one of claims 36-42, wherein the CD47-expressing cancer is selected from lung cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicular cancer, kidney cancer, bladder cancer, spinal cancer, brain cancer, cervical cancer, endometrial cancer, colorectal cancer, anal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, gastrointestinal cancer, gastric cancer, carcinoma, head and neck cancer, skin cancer, melanoma, prostate cancer, pituitary cancer, stomach cancer, uterine cancer, vaginal cancer and thyroid cancer.

45. The use of any one of claims 36-42, wherein the CD47-expressing cancer is selected from lung cancer, sarcoma, colorectal cancer, head and neck cancer, ovarian cancer, pancreatic cancer, gastric cancer, melanoma, and breast cancer.

46. The use of any one of claims 36-45, wherein the anti-CD47 antibody or antigen-binding fragment thereof is to be administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), PD-L2, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain containing 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1), CEACAM-5, V-domain Ig suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), CD160, 2B4 or TGFR.

47. The use of any one of claims 36-46, wherein the anti-CD47 antibody or antigen-binding fragment thereof is to be administered in combination with an agonistic anti-CD40 antibody.

48. The use of claim 47, wherein the agonistic anti-CD40 antibody has low fucosylation levels or is afucosylated.

49. The use of any one of claims 36-48, wherein the anti-CD47 antibody or antigen-binding fragment thereof is to be administered in combination with an antibody drug conjugate (ADC), wherein the antibody of the ADC specifically binds to a protein that is expressed on the extracellular surface of a cancer cell and the antibody is conjugated to a drug-linker comprising a cytotoxic agent.

50. The use of claim 49, wherein the cytotoxic agent is an auristatin.

51. The use of claim 50, wherein the antibody of the ADC is conjugated to a drug-linker selected from the group consisting of vcMMAE and mcMMAF.

52. An *in vitro* method of inducing apoptosis of a CD47-expressing cell comprising contacting the cell with an antibody or antigen-binding fragment thereof of any one of claims 1-29.

53. Use of an antibody or antigen-binding fragment thereof of any one of claims 1-29 in the manufacture of a medicament for inducing apoptosis of a CD47-expressing cell *in vivo*.

54. A masked antibody comprising an antibody or antigen-binding fragment thereof that specifically binds to the human CD47 protein and at least one masking domain, wherein at least one masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44-55, 75-86, 94, and 95.

55. The masked antibody of claim 54, wherein the at least one masking domain reduces binding affinity of the antibody or antigen-binding fragment to human CD47 protein compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

56. The masked antibody of claim 55, wherein the binding affinity is reduced at least about 100-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

57. The masked antibody of claim 55, wherein the binding affinity is reduced between about 200-fold and about 1500-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

58. The masked antibody of any one of claims 54-57, wherein the antibody or antigen binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain is linked to a first masking domain; or wherein the light chain is linked to a second masking domain; or wherein the heavy chain is linked to a first masking domain and the light chain is linked to a second masking domain.

59. The masked antibody of claim 58, wherein the first masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, 52, 54, 75, 77, 79, 81, 83, 85, and 94; and the second masking domain comprises an amino acid sequence selected from SEQ ID NOs: 45, 47, 49, 51, 53, 55, 76, 78, 80, 82, 84, 86, and 95.

60. The masked antibody of claim 59, wherein the first masking domain and the second masking domain are a pair of masking domains selected from: SEQ ID NOs: 44 and 45; SEQ ID NOs: 46 and 47; SEQ ID NOs: 48 and 49; SEQ ID NOs: 50 and 51; SEQ ID NOs: 52 and 53; SEQ ID NOs: 54 and 55; SEQ ID NOs: 75 and 76; SEQ ID NOs: 77 and 78; SEQ ID NOs: 79 and 80; SEQ ID NOs: 81 and 82; SEQ ID NOs: 83 and 84; SEQ ID NOs: 85 and 86; and SEQ ID NOs: 94 and 95.

61. The masked antibody of any one of claims 58-60, wherein the first masking domain is linked to the N-terminus of the heavy chain and the second masking domain is linked to the N-terminus of the light chain.

62. The masked antibody of any one of claims 58-61, wherein each masking domain comprises a protease-cleavable linker and is linked to the heavy chain or light chain via the protease-cleavable linker.

63. The masked antibody of claim 62, wherein the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site.

64. The masked antibody of claim 63, wherein the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.

65. The masked antibody of claim 63 or claim 64, wherein following cleavage by an MMP, the heavy chain and/or light chain of the antibody or antigen-binding fragment thereof comprises a stub amino acid remnant of the MMP cleavage site.

66. The masked antibody of claim 65, wherein the stub amino acid remnant comprises the sequence LRSG, SG, or VR at the N terminus of the antibody.

67. The masked antibody of any one of claims 54-66, wherein the antibody or antigen-binding fragment thereof is an antibody or antigen-binding fragment thereof of any one of claims 1-29.

68. The masked antibody of any one of claims 54-67, comprising a heavy chain linked to a first masking domain and having the amino acid sequence of SEQ ID NO: 42 and a light chain linked to a second masking domain and having the amino acid sequence of SEQ ID NO: 43.

69. A masked antibody comprising an antibody or antigen-binding fragment thereof of any one of claims 1-29 and at least one masking domain.

70. The masked antibody of claim 69, wherein at least one masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44-55, 75-86, 94, and 95.

71. The masked antibody of claim 69, wherein each masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44-55, 75-86, 94, and 95.

72. The masked antibody of any one of claims 69-71, wherein the at least one masking domain reduces binding affinity of the antibody or antigen-binding fragment to human CD47 protein compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

73. The masked antibody of claim 72, wherein the binding affinity is reduced at least about 100-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

74. The masked antibody of claim 72, wherein the binding affinity is reduced between about 200-fold and about 1500-fold compared to the antibody or antigen-binding fragment thereof without the at least one masking domain.

75. The masked antibody of any one of claims 69-74, wherein the antibody or antigen binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain is linked to a first masking domain; or wherein the light chain is linked to a second masking domain; or wherein the heavy chain is linked to a first masking domain and the light chain is linked to a second masking domain.

76. The masked antibody of claim 75, wherein the first masking domain comprises an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, 52, 54, 75, 77, 79, 81, 83, 85, and 94; and the second masking domain comprises an amino acid sequence selected from SEQ ID NOs: 45, 47, 49, 51, 53, 55, 76, 78, 80, 82, 84, 86, and 95.

77. The masked antibody of claim 76, wherein the first masking domain and the second masking domain are a pair of masking domains selected from: SEQ ID NOs: 44 and 45; SEQ ID NOs: 46 and 47; SEQ ID NOs: 48 and 49; SEQ ID NOs: 50 and 51; SEQ ID NOs: 52 and 53; SEQ ID NOs: 54 and 55; SEQ ID NOs: 75 and 76; SEQ ID NOs: 77 and 78; SEQ ID NOs: 79 and 80; SEQ ID NOs: 81 and 82; SEQ ID NOs: 83 and 84; SEQ ID NOs: 85 and 86; and SEQ ID NOs: 94 and 95.

78. The masked antibody of any one of claims 75-77, wherein the first masking domain is linked to the N-terminus of the heavy chain and the second masking domain is linked to the N-terminus of the light chain.

79. The masked antibody of any one of claims 75-78, wherein each masking domain comprises a protease-cleavable linker and is linked to the heavy chain or light chain via the protease-cleavable linker.

80. The masked antibody of claim 79, wherein the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site.

81. The masked antibody of claim 80, wherein the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.

82. The masked antibody of claim 80 or claim 81, wherein following cleavage by an MMP, the heavy chain and/or light chain of the antibody or antigen-binding fragment thereof comprises a stub amino acid remnant of the MMP cleavage site.

83. The masked antibody of claim 82, wherein the stub amino acid remnant comprises the sequence LRSG, SG, or VR at the N terminus of the antibody.
84. A nucleic acid sequence encoding the masked antibody of any one of claims 54-83.
85. An expression vector comprising the nucleic acid of claim 84.
86. A host cell comprising the nucleic acid of claim 85 or the expression vector of claim 77.
87. A host cell that expresses the masked antibody of any one of claims 54-83.
88. A method of producing the masked antibody of any one of claims 54-75 comprising culturing the host cell of claim 86 or claim 87.
89. The method of claim 88, further comprising isolating the masked antibody.
90. Use of the masked antibody of any one of claims 54-83 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject.
91. Use of the masked antibody of any one of claims 54-83 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having elevated levels of MMP in the cancer relative to surrounding non-cancer tissue, wherein each masking domain of the masked antibody comprises a protease-cleavable linker and wherein the protease-cleavable linker comprises a matrix metalloprotease (MMP) cleavage site.
92. The use of claim 91, wherein the MMP cleavage site is selected from an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.
93. The use of claim 91 or claim 92, wherein the MMP is selected from the group consisting of: MMP2, MMP7, MMP9, and MMP13.
94. The use of any one of claims 91-93, wherein the identifying comprises:
- i) isolating cancer tissue and non-cancer tissue from the subject;
 - ii) detecting MMPs in the isolated cancer tissue and the non-cancer tissue; and
 - iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.
95. Use of a masked antibody of any one of claims 54-83 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having a CD47-expressing cancer.
96. The use of claim 95, wherein the identifying comprises:
- i) isolating cancer tissue; and
 - ii) detecting CD47 in the isolated cancer tissue.
97. Use of the masked antibody of any one of claims 54-83 in the manufacture of a medicament for treating a CD47-expressing cancer in a subject, wherein the use comprises identifying the subject as having elevated levels of macrophage infiltration in cancer tissue relative to non-cancer tissue.

98. The use of claim 97, wherein the identifying comprises:

- i) isolating cancer tissue and surrounding non-cancer tissue from the subject;
- ii) detecting macrophages in the isolated cancer tissue and in non-cancer tissue; and
- iii) comparing the amount of staining in the cancer tissue relative to the non-cancer tissue.

99. The use of claim 98, wherein the macrophage staining is performed with an anti-CD163 antibody.

100. The use of any one of claims 90-99, wherein the CD47-expressing cancer is a hematological cancer or a solid cancer.

101. The use of any one of claims 90-100, wherein the CD47-expressing cancer is selected from non-Hodgkin lymphoma, B-lymphoblastic lymphoma; B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, Richter's syndrome, follicular lymphoma, multiple myeloma, myelofibrosis, polycythemia vera, cutaneous T-cell lymphoma, monoclonal gammopathy of unknown significance (MGUS), myelodysplastic syndrome (MDS), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, acute myeloid leukemia (AML), and anaplastic large cell lymphoma.

102. The use of any one of claims 90-100, wherein the CD47-expressing cancer is selected from lung cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicular cancer, kidney cancer, bladder cancer, spinal cancer, brain cancer, cervical cancer, endometrial cancer, colorectal cancer, anal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, gastrointestinal cancer, gastric cancer, sarcoma, head and neck cancer, melanoma, skin cancer, prostate cancer, pituitary cancer, stomach cancer, uterine cancer, vaginal cancer and thyroid cancer.

103. The use of any one of claims 90-100, wherein the CD47-expressing cancer is selected from lung cancer, sarcoma, colorectal cancer, head and neck cancer, ovarian cancer, pancreatic cancer, gastric cancer, melanoma, and breast cancer.

104. The use of any one of claims 90-103, wherein the anti-CD47 antibody is to be administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), PD-L2, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain containing 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1), CEACAM-5, V-domain Ig suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), CD160, 2B4 or TGFR.

105. The use of any one of claims 90-104, wherein the anti-CD47 antibody or antigen-binding fragment thereof is to be administered in combination with an agonistic anti-CD40 antibody.

106. The use of claim 105, wherein the agonistic anti-CD40 antibody has low fucosylation levels or is afucosylated.

107. The use of any one of claims 90-106, wherein the anti-CD47 antibody or antigen-binding fragment thereof is to be administered in combination with an antibody drug conjugate (ADC), wherein the antibody of the ADC specifically binds to a protein that is expressed on the extracellular surface of a cancer cell and the antibody is conjugated to a drug-linker comprising a cytotoxic agent.

108. The use of claim 107, wherein the cytotoxic agent is an auristatin.

109. The use of claim 108, wherein the antibody of the ADC is conjugated to a drug-linker selected from the group consisting of vcMMAE and mcMMAF.

110. The use of any one of claims 90-109, wherein the masked antibody comprises at least one masking domain comprising a protease-cleavable linker, and wherein the protease-cleavable linker is cleaved in the tumor microenvironment.

111. The use of claim 110, wherein following cleavage of the protease-cleavable linker in the tumor microenvironment, the masking domain is released from the anti-CD47 antibody or antigen-binding fragment thereof.

112. The use of claim 110 or claim 111, wherein the protease-cleavable linker comprises the amino acid sequence IPVSLRSG (SEQ ID NO: 73) or GPLGVR (SEQ ID NO: 57).

113. The use of any one of claims 110-112, wherein the protease-cleavable linker comprises a MMP cleavage site.

114. The use of claim 113, wherein the MMP cleavage site is selected from the group consisting of an MMP2 cleavage site, an MMP7 cleavage site, an MMP9 cleavage site and an MMP13 cleavage site.

115. The use of any one of claims 110-114, wherein following release of the anti-CD47 antibody or antigen-binding fragment thereof, the anti-CD47 antibody or antigen-binding fragment thereof has a stub amino acid remnant of the protease-cleavable linker.

116. The use of claim 115, wherein the stub amino acid remnant comprises the sequence of LRSG, SG, or VR at the N terminus of the antibody.

117. The use of any one of claims 110-116, wherein following cleavage in the tumor microenvironment, the released anti-CD47 antibody or antigen-binding fragment thereof binds to CD47 with an affinity at least about 100-fold stronger than the affinity of the masked antibody for CD47.

118. The use of any one of claims 110-116, wherein following cleavage in the tumor microenvironment, the released anti-CD47 antibody or antigen-binding fragment thereof binds to CD47 with an affinity from 200-fold to 1500-fold stronger than the affinity of the masked antibody for CD47.

119. The use of any one of claims 36-51 and 90-118, wherein the anti-CD47 antibody or antigen-binding fragment thereof or the anti-CD47 antibody or antigen-binding

fragment thereof of the masked antibody exhibits reduced hemagglutination *in vitro* compared to its parental anti-CD47 antibody.

120. The use of claim 119, wherein the parental antibody is Ab47.

121. The use of any one of claims 36-51 and 90-120, wherein administration of the anti-CD47 antibody or masked antibody does not induce hemagglutination in the subject.

122. The use of any one of claims 36-51 and 90-121, wherein the anti-CD47 antibody or masked antibody induces apoptosis of CD47-expressing cells *in vitro* and/or *in vivo*.

123. The use of claim 122, wherein the anti-CD47 antibody or masked antibody induces apoptosis of CD47-expressing cells *in vivo*.

124. The use of claim 123, wherein the CD47-expressing cells are cancer cells.