

THERAPEUTIC CD47 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States Provisional Application Nos.
5 62/220,691 filed September 18, 2015; 62/263,544 filed December 4, 2015; 62/221,852 filed September 22, 2015; 62/220,725 filed September 18, 2015; 62/232,681 filed September 25, 2015; 62/252,171 filed November 6, 2015; and 62/354,592 filed June 24, 2016; the disclosures of which are hereby incorporated by reference as if written herein in their entireties.

FIELD OF THE DISCLOSURE

10 This disclosure is related generally to anti-CD47 monoclonal antibodies (anti-CD47 mAbs) with distinct functional profiles as described herein, methods to generate anti-CD47 mAbs, and to methods of using these anti-CD47 mAbs as therapeutics for the prevention and treatment of solid and hematological cancers, ischemia-reperfusion injury, cardiovascular diseases, autoimmune
15 diseases, or inflammatory diseases or as diagnostics for determining the level of CD47 in tissue samples.

BACKGROUND OF THE DISCLOSURE

CD47 is a cell surface receptor comprised of an extracellular IgV set domain, a 5
20 transmembrane domain, and a cytoplasmic tail that is alternatively spliced. Two ligands bind CD47: signal inhibitory receptor protein α (SIRP α) and thrombospondin-1 (TSP1). CD47 expression and/or activity have been implicated in a number of diseases and disorders. Accordingly, there exists a need for therapeutic compositions and methods for treating diseases and conditions associated with CD47 in humans and animals, including the prevention and
25 treatment of solid and hematological cancers, ischemia-reperfusion injury (IRI), cardiovascular diseases, or an autoimmune or inflammatory disease. There also exists a need for diagnostic compositions and methods for determining the level of CD47 expression in tumor samples.

SUMMARY OF THE DISCLOSURE

30 The present disclosure describes anti-CD47 mAbs with distinct functional profiles. These antibodies possess distinct combinations of properties selected from the following: 1) exhibit

cross-reactivity with one or more species homologs of CD47; 2) block the interaction between CD47 and its ligand SIRP α ; 3) increase phagocytosis of human tumor cells, 4) induce death of susceptible human tumor cells; 5) do not induce cell death of human tumor cells; 6) have reduced binding to human red blood cells (hRBCs); 7) have no detectable binding to hRBCs; 8) cause reduced agglutination of hRBCs; 9) cause no detectable agglutination of hRBCs; 10) reverse TSP1 inhibition of the nitric oxide (NO) pathway and/or 11) do not reverse TSP1 inhibition of the NO pathway. The antibodies of the disclosure are useful in various therapeutic methods for treating diseases and conditions associated with CD47 in humans and animals, including the prevention and treatment of solid and hematological cancers, autoimmune diseases, inflammatory diseases, IRI, and cardiovascular diseases. The antibodies of the disclosure are also useful as diagnostics to determine the level of CD47 expression in tissue samples. Embodiments of the disclosure include isolated antibodies and immunologically active binding fragments thereof; pharmaceutical compositions comprising one or more of the anti-CD47 monoclonal antibodies, preferably chimeric or humanized forms of said antibodies; methods of therapeutic use of such anti-CD47 monoclonal antibodies; and cell lines that produce these anti-CD47 monoclonal antibodies.

The embodiments of the disclosure include the mAbs, or antigen-binding fragments thereof, which are defined by reference to specific structural characteristics i.e. specified amino acid sequences of either the CDRs or entire heavy chain or light chain variable domains. All of these antibodies bind to CD47.

The monoclonal antibodies, or antigen binding fragments thereof may comprise at least one, usually at least three, CDR sequences as provided herein, usually in combination with framework sequences from a human variable region or as an isolated CDR peptide. In some embodiments, an antibody comprises at least one light chain comprising the three light chain CDR sequences provided herein situated in a variable region framework, which may be, without limitation, a murine or human variable region framework, and at least one heavy chain comprising the three heavy chain CDR sequences provided herein situated in a variable region framework, which may be, without limitation, a human or murine variable region framework.

Preferred embodiments are anti-CD47 mAbs, or antigen binding fragments thereof, comprising a heavy chain variable domain comprising a variable heavy chain CDR1, variable heavy chain CDR2, and a variable heavy chain CDR3, wherein said variable heavy chain CDR1 comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3; said variable heavy chain CDR2 comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6; and said variable heavy chain CDR3 comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10.

5 The heavy chain variable domain may comprise any one of the listed variable heavy chain CDR1 sequences (HCDR1) in combination with any one of the variable heavy chain CDR2 sequences (HCDR2) and any one of the variable heavy chain CDR3 sequences (HCDR3). However, certain embodiments of HCDR1 and HCDR2 and HCDR3 are particularly preferred, which derive from a single common V_H domain, examples of which are described herein.

10 The antibody or antigen binding fragment thereof may additionally comprise a light chain variable domain (V_L), which is paired with the V_H domain to form an antigen binding domain. Preferred light chain variable domains are those comprising a variable light chain CDR1, variable light chain CDR2, and a variable light chain CDR3, wherein said variable light chain CDR1 comprises an amino acid sequence selected from the group consisting of:

15 SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14; said variable light chain CDR2 optionally comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17; and said variable light chain CDR3 optionally comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20.

20 The light chain variable domain may comprise any one of the listed variable light chain CDR1 sequences (LCDR1) in combination with any one of the variable light chain CDR2 sequences (LCDR2) and any one of the variable light chain CDR3 sequences (LCDR3). However, certain embodiments of LCDR1 and LCDR2 and LCDR3 are particularly preferred, which derive from a single common V_L domain, examples of which are described herein.

25 Any given CD47 antibody or antigen binding fragment thereof comprising a V_H domain paired with a V_L domain will comprise a combination of 6 CDRs: variable heavy chain CDR1 (HCDR1), variable heavy chain CDR2 (HCDR2), variable heavy chain CDR3 (HCDR3), variable light chain CDR1 (LCDR1), variable light chain CDR2 (LCDR2), and variable light chain CDR1 (LCDR1). Although all combinations of 6 CDRs selected from the CDR sequence groups listed
30 above are permissible, and within the scope of the disclosure, certain combinations of 6 CDRs are particularly preferred.

Preferred combinations of 6 CDRs include, but are not limited to, the combinations of variable heavy chain CDR1 (HCDR1), variable heavy chain CDR2 (HCDR2), variable heavy chain CDR3 (HCDR3), variable light chain CDR1 (LCDR1), variable light chain CDR2 (LCDR2), and variable light chain CDR3 (LCDR3) selected from the group consisting of:

- (i) HCDR1 comprising SEQ ID NO:1, HCDR2 comprising SEQ ID NO:4, HCDR3 comprising SEQ ID NO:7, LCDR1 comprising SEQ ID NO:11, LCDR2 comprising SEQ ID NO:15, LCDR3 comprising SEQ ID NO:18;
- (ii) HCDR1 comprising SEQ ID NO:1, HCDR2 comprising SEQ ID NO:4, HCDR3 comprising SEQ ID NO:8, LCDR1 comprising SEQ ID NO:11, LCDR2 comprising SEQ ID NO:15, LCDR3 comprising SEQ ID NO:18;
- (iii) HCDR1 comprising SEQ ID NO:2, HCDR2 comprising SEQ ID NO:5, HCDR3 comprising SEQ ID NO:9, LCDR1 comprising SEQ ID NO:12, LCDR2 comprising SEQ ID NO:16, LCDR3 comprising SEQ ID NO:19;
- (iv) HCDR1 comprising SEQ ID NO:2, HCDR2 comprising SEQ ID NO:5, HCDR3 comprising SEQ ID NO:9, LCDR1 comprising SEQ ID NO:13, LCDR2 comprising SEQ ID NO:16, LCDR3 comprising SEQ ID NO:19; and
- (v) HCDR1 comprising SEQ ID NO:3, HCDR2 comprising SEQ ID NO:6, HCDR3 comprising SEQ ID NO:10, LCDR1 comprising SEQ ID NO:14, LCDR2 comprising SEQ ID NO:17, LCDR3 comprising SEQ ID NO:20.

Further preferred anti-CD47 antibodies include antibodies or antigen binding fragments thereof, comprising a heavy chain variable domain having an amino acid sequence selected from the group consisting of: the amino acid sequences of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO: 32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40 and amino acid sequences exhibiting at least 90%, 95%, 97%, 98%, or 99% sequence identity to one of the recited sequences. Alternatively or in addition, preferred anti-CD47 antibodies including antibodies or antigen binding fragments thereof may comprise a light chain variable domain having an amino acid sequence selected from the group consisting of: the amino acid sequences of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48,

SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, and SEQ ID NO:52 and amino acid sequences exhibiting at least 90%, 95%, 97%, 98%, or 99% sequence identity to one of the recited sequences.

Although all possible pairing of V_H domains and V_L domains selected from the V_H and V_L domain sequence groups listed above are permissible, and within the scope of the disclosure, certain combinations of V_H and V_L domains are particularly preferred. Accordingly, preferred CD47 antibodies, or antigen binding fragments thereof, are those comprising a combination of a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the combination is selected from the group consisting of:

- (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:41;
- (ii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:43;
- (iii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:34 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:49;
- (iv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
- (v) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
- (vi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:39 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
- (vii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:24 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:43;

- (viii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
- 5 (ix) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;
- (x) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:44;
- 10 (xi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:27 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:44; and
- (xii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51;
- 15 (xiii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:39 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51;
- (xiv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:40 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
- 20 (xv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51;
- 25 (xvi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:47;
- (xvii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:30 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:47;
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- (xviii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:31 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:47;
- 5 (xix) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:47;
- (xx) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:47;
- 10 (xxi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;
- (xxii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:30 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;
- 15 (xxiii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:31 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;
- (xxiv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;
- 20 (xxv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:43;
- 25 (xxvi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:27 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:43;
- (xxvii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:28 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:46;
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(xxviii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:35 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:50;

(xxix) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:29 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;

(xxx) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:30 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;

(xxxi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:31 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;

(xxxii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:48;

(xxxiii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51; and

(xxxiv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:40 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51.

Preferred anti-CD47 antibodies or antigen binding fragments thereof may also comprise a combination of a heavy chain variable domain and a light chain variable domain wherein the heavy chain variable domain comprises a VH sequence with at least 85% sequence identity, or at least 90% sequence identity, or at least 95% sequence identity, or at least 97%, 98% or 99% sequence identity, to the heavy chain amino acid sequences shown above in (i) to (xxxiv) and/or the light chain variable domain comprises a VL sequence with at least 85% sequence identity, or at least 90% sequence identity, or at least 95% sequence identity, or at least 97%, 98% or 99% sequence identity, to the light chain amino acid sequences shown above in (i) to (xxxiv). The specific VH and VL pairings or combinations in parts (i) through (xxxiv) may be preserved for anti-CD47

antibodies having VH and VL domain sequences with a particular percentage sequence identity to these reference sequences.

For all embodiments wherein the heavy chain and/or light chain variable domains of the antibodies or antigen binding fragments are defined by a particular percentage sequence identity to a reference sequence, the VH and/or VL domains may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

In another embodiment, the preferred CD47 antibodies, or antigen binding fragments thereof, are those comprising a combination of a heavy chain (HC) and a light chain (LC), wherein the combination is selected from the group consisting of:

- (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:76 and a light chain comprising the amino acid sequence SEQ ID NO:66 ;
- (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:77 and a light chain comprising the amino acid sequence SEQ ID NO:68 ;
- (iii) a heavy chain comprising the amino acid sequence of SEQ ID NO:78 and a light chain comprising the amino acid sequence SEQ ID NO:69 ;
- (iv) a heavy chain comprising the amino acid sequence of SEQ ID NO:79 and a light chain comprising the amino acid sequence SEQ ID NO:70 ;
- (v) a heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a light chain comprising the amino acid sequence SEQ ID NO:70 ;
- (vi) a heavy chain comprising the amino acid sequence of SEQ ID NO:81 and a light chain comprising the amino acid sequence SEQ ID NO:70 ;
- (vii) a heavy chain comprising the amino acid sequence of SEQ ID NO:82 and a light chain comprising the amino acid sequence SEQ ID NO:68 ;
- (viii) a heavy chain comprising the amino acid sequence of SEQ ID NO:83 and a light chain comprising the amino acid sequence SEQ ID NO:70;
- (ix) a heavy chain comprising the amino acid sequence of SEQ ID NO:84 and a light chain comprising the amino acid sequence SEQ ID NO:71;
- (x) a heavy chain comprising the amino acid sequence of SEQ ID NO:85 and a light chain comprising the amino acid sequence SEQ ID NO:72;

- (xi) a heavy chain comprising the amino acid sequence of SEQ ID NO:86 and a light chain comprising the amino acid sequence SEQ ID NO:72;
- (xii) a heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a light chain comprising the amino acid sequence SEQ ID NO:73;
- 5 (xiii) a heavy chain comprising the amino acid sequence of SEQ ID NO:81 and a light chain comprising the amino acid sequence SEQ ID NO:73;
- (xiv) a heavy chain comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino acid sequence SEQ ID NO:70;
- (xv) a heavy chain comprising the amino acid sequence of SEQ ID NO:79 and a light chain comprising the amino acid sequence SEQ ID NO:73;
- 10 (xvi) a heavy chain comprising the amino acid sequence of SEQ ID NO:88 and a light chain comprising the amino acid sequence SEQ ID NO:74;
- (xvii) a heavy chain comprising the amino acid sequence of SEQ ID NO:89 and a light chain comprising the amino acid sequence SEQ ID NO:74;
- 15 (xviii) a heavy chain comprising the amino acid sequence of SEQ ID NO:90 and a light chain comprising the amino acid sequence SEQ ID NO:74;
- (xix) a heavy chain comprising the amino acid sequence of SEQ ID NO:91 and a light chain comprising the amino acid sequence SEQ ID NO:74;
- (xx) a heavy chain comprising the amino acid sequence of SEQ ID NO:84 and a light chain comprising the amino acid sequence SEQ ID NO:74;
- 20 (xxi) a heavy chain comprising the amino acid sequence of SEQ ID NO:92 and a light chain comprising the amino acid sequence SEQ ID NO:71;
- (xxii) a heavy chain comprising the amino acid sequence of SEQ ID NO:89 and a light chain comprising the amino acid sequence SEQ ID NO:71;
- 25 (xxiii) a heavy chain comprising the amino acid sequence of SEQ ID NO:90 and a light chain comprising the amino acid sequence SEQ ID NO:31;
- (xxiv) a heavy chain comprising the amino acid sequence of SEQ ID NO:91 and a light chain comprising the amino acid sequence SEQ ID NO:71;
- (xxv) a heavy chain comprising the amino acid sequence of SEQ ID NO:85 and a light chain comprising the amino acid sequence SEQ ID NO:68;
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(xxvi) a heavy chain comprising the amino acid sequence of SEQ ID NO:86 and a light chain comprising the amino acid sequence SEQ ID NO:68;

(xxvii) a heavy chain comprising the amino acid sequence of SEQ ID NO:93 and a light chain comprising the amino acid sequence SEQ ID NO:100;

5 (xxviii) a heavy chain comprising the amino acid sequence of SEQ ID NO:94 and a light chain comprising the amino acid sequence SEQ ID NO:75;

(xxix) a heavy chain comprising the amino acid sequence of SEQ ID NO:95 and a light chain comprising the amino acid sequence SEQ ID NO:71;

10 (xxx) a heavy chain comprising the amino acid sequence of SEQ ID NO:96 and a light chain comprising the amino acid sequence SEQ ID NO:71;

(xxxi) a heavy chain comprising the amino acid sequence of SEQ ID NO:97 and a light chain comprising the amino acid sequence SEQ ID NO:71;

(xxxii) a heavy chain comprising the amino acid sequence of SEQ ID NO:98 and a light chain comprising the amino acid sequence SEQ ID NO:71;

15 (xxxiii) a heavy chain comprising the amino acid sequence of SEQ ID NO:83 and a light chain comprising the amino acid sequence SEQ ID NO:73;

(xxxiv) a heavy chain comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino acid sequence SEQ ID NO:73;

20 (xxxv) a heavy chain comprising the amino acid sequence of SEQ ID NO:102 and a light chain comprising the amino acid sequence SEQ ID NO:101;

(xxxvi) a heavy chain comprising the amino acid sequence of SEQ ID NO:104 and a light chain comprising the amino acid sequence SEQ ID NO:103;

25 wherein the VH amino acid sequence is at least 90%, 95%, 97%, 98% or 99% identical thereto and the a VL amino acid sequence is at least 90%, 95%, 97%, 98% or 99% identical thereto.

Preferred embodiments of the anti-CD47 antibodies described herein, are also characterized by combinations of properties which are not exhibited by prior art anti-CD47 antibodies proposed for human therapeutic use. Accordingly, the preferred anti-CD47 antibodies described herein are characterized by:

- 30 a. binds to human CD47,
b. blocks SIRP α binding to human CD47,

- c. increases phagocytosis of human tumor cells; and
- d. induces death of susceptible human tumor cells.

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- 5 a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- d. induces death of susceptible human tumor cells; and
- e. causes no agglutination of human red blood cells (hRBCs).

10 In yet another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- 15 d. induces death of susceptible human tumor cells; and
- e. causes reduced agglutination of human red blood cells (hRBCs).

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. specifically binds to human CD47,
- 20 b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells
- d. induces death of susceptible human tumor cells; and
- e. has reduced hRBC binding.

25 In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- d. causes no agglutination of human red blood cells (hRBCs); and
- 30 e. does not bind to hRBCs.

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. specifically binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- 5 c. increases phagocytosis of human tumor cells,
- d. causes no agglutination of human red blood cells (hRBCs); and
- e. has reduced hRBC binding.

In another preferred embodiment described herein, the monoclonal antibody, or antigen binding fragment thereof specifically also binds to non-human primate CD47, wherein non-human
10 primate may include, but is not limited to, cynomolgus monkey, green monkey, rhesus monkey and squirrel monkey.

In yet another preferred embodiment described herein, the monoclonal antibody, or antigen binding fragment thereof binds to human, non-human primate, mouse, rabbit, and rat CD47.

Various forms of the anti-CD47 mAbs disclosed are contemplated herein. For example,
15 the anti-CD47 mAbs can be full length humanized antibodies with human frameworks and constant regions of the isotypes, IgA, IgD, IgE, IgG, and IgM, more particularly, IgG1, IgG2, IgG3, IgG4, and in some cases with various mutations to alter Fc receptor function or prevent Fab arm exchange or an antibody fragment, e.g., a F(ab')₂ fragment, a F(ab) fragment, a single chain Fv fragment (scFv), etc., as disclosed herein.

20 The preferred embodiments of the disclosure provide pharmaceutical or veterinary compositions comprising one or more of the anti-CD47 mAbs or fragments disclosed herein, optionally chimeric or humanized forms, and a pharmaceutically acceptable carrier, diluent, or excipient.

Prior to the present disclosure, there was a need to identify anti-CD47 mAbs that possess
25 the functional profiles as described herein. The anti-CD47 mAbs of the present disclosure exhibit distinct combinations of properties, particularly combinations of properties that render the mAbs particularly advantageous or suitable for use in human therapy, particularly in the prevention and/or treatment of solid and hematological cancers, ischemia-reperfusion injury, autoimmune and/or inflammatory diseases.

30 Further scope of the applicability of the present disclosure will become apparent from the detailed description provided below. However, it should be understood that the detailed

description and specific examples, while indicating preferred embodiments of the disclosure, are given by way of illustration only since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

The above and other aspects, features, and advantages of the present disclosure will be better understood from the following detailed descriptions taken in conjunction with the accompanying drawing(s), all of which are given by way of illustration only, and are not limitative of the present disclosure.

FIG. 1A. Binding of VLX4 Humanized mAbs to Human OV10 Cells Expressing human CD47. Binding of VLX4 humanized mAbs (VLX4hum_01 IgG1, VLX4hum_02 IgG1, VLX4hum_01 IgG4 PE, and VLX4hum_02 IgG4 PE) to human CD47 was determined using a OV10 cell line expressing human CD47 (OV10 hCD47) cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 1B. Binding of VLX4 Humanized mAbs to Human OV10 Cells Expressing human CD47. Binding of VLX4 humanized mAbs (VLX4hum_06 IgG4 PE, VLX4hum_07 IgG4 PE, VLX4hum_12 IgG4 PE, and VLX4hum_13 IgG4 PE) to human CD47 was determined using an OV10 CD47 cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of VLX4 representative mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 2A. Binding of VLX4 Humanized mAbs to Human RBCs (hRBCs). Binding of VLX4 humanized mAbs (VLX4hum_01 IgG1, VLX4hum_02 IgG1, VLX4hum_01 IgG4 PE, and VLX4hum_02 IgG4PE) to human CD47 was determined using freshly isolated hRBCs. hRBCs were incubated for 60 minutes at 37°C with various concentrations of VLX4 mAbs, washed and incubated for 1hr with FITC-labeled donkey anti-human antibody. Cells were washed and antibody binding measured using flow cytometry.

FIG. 2B. Binding of VLX4 Humanized mAbs to Human RBCs. Binding of VLX4 humanized mAbs (VLX4hum_07 IgG4 PE, VLX4hum_12 IgG4 PE, and VLX4hum_13 IgG4 PE)

to human CD47 was determined using freshly isolated hRBCs. hRBCs were incubated for 60 minutes at 37°C with various concentrations of VLX4 mAbs, washed and incubated for 1hr with FITC-labeled donkey anti-human antibody. Cells were washed and antibody binding measured using flow cytometry.

FIG. 3A. Binding of VLX8 Humanized mAbs to Human OV10 hCD47 Cells. Binding of VLX8 IgG4PE chimera (xi) or humanized mAbs (VLX8hum_01 IgG4PE, VLX8hum_04 IgG4 PE, VLX8hum_07 IgG4 PE, and VLX8hum_09 IgG4 PE) to human CD47 was determined using an OV10 hCD47 cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of VLX8 representative mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 3B. Binding of VLX8 Humanized mAbs to Human OV10 hCD47 Cells. Binding of VLX8 chimera or humanized mAbs (VLX8hum_06 IgG2, VLX8hum_07 IgG2, VLX8hum_08 IgG2, and VLX8hum_09 IgG2) to human CD47 was determined using an OV10 hCD47 cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of VLX8 representative mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 4A. Binding of VLX8 Humanized mAbs to Human RBCs. Binding of VLX8 IgG4PE xi or humanized mAbs (VLX8hum_01 IgG4PE, VLX8hum_03 IgG4PE, VLX8hum_07 IgG4PE, and VLX8hum_10 IgG4PE) to human CD47 was determined using freshly isolated human RBCs. RBCs were incubated for 1 hr at 37°C with various concentrations of VLX8 mAbs, washed and incubated for 1hr with FITC-labeled donkey anti-human antibody. Cells were washed and antibody binding measured using flow cytometry.

FIG. 4B. Binding of VLX8 Humanized mAbs to Human RBCs. Binding of VLX8 IgG4PE xi or humanized mAbs (VLX8hum_06 IgG2, VLX8hum_07 IgG2, VLX8hum_08 IgG2 and VLX8hum_09 IgG2) to human CD47 was determined using freshly isolated human RBCs. RBCs were incubated for 1 hr at 37°C with various concentrations of VLX8 mAbs, washed and incubated for 1hr with FITC-labeled donkey anti-human antibody. Cells were washed and antibody binding measured using flow cytometry.

FIG. 5A. Binding of VLX9 Humanized mAbs to Human OV10 hCD47 Cells. Binding of VLX9 IgG2 xi or humanized mAbs (VLX9hum_01 IgG2, VLX9hum_02 IgG2, VLX9hum_03 IgG2, VLX9hum_04 IgG2 and VLX9hum_05 IgG2) to human CD47 was determined using an OV10 human CD47 cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 5B. Binding of VLX9 Humanized mAbs to Human OV10 hCD47 Cells. Binding of VLX9 IgG2 xi or humanized mAbs (VLX9hum_06 IgG2, VLX9hum_07 IgG2, VLX9hum_08 IgG2, VLX9hum_09 IgG2 and VLX9hum_10 IgG2) to human CD47 was determined using a OV10 hCD47 cell-based ELISA. OV10 hCD47 cells were plated into 96 well plates and were confluent at the time of assay. Various concentrations of mAbs were added to the cells for 1 hr. Cells were washed and then incubated with HRP-labelled secondary antibody for 1 hr followed by addition of peroxidase substrate.

FIG. 6. Binding of VLX9 Humanized mAbs to Human RBCs. Binding of VLX9 IgG2 xi or humanized mAbs to human CD47 was determined using freshly isolated human hRBCs. RBCs were incubated for 60 minutes at 37°C with various concentrations of VLX9 mAbs, washed and incubated for 1hr with FITC-labeled donkey anti-human antibody. Cells were washed and antibody binding measured using flow cytometry.

FIG. 7. VLX4, VLX8, and VLX9 Humanized mAbs Block SIRPα binding to CD47 on Jurkat Cells. 1.5×10^6 Jurkat cells were incubated with 5μg/ml of VLX4, VLX8 and VLX9 CD47 humanized mAbs (VLX4hum_01 IgG4 PE, VLX4hum_07 IgG4 PE, VLX8hum_10 IgG4 PE, VLX4hum_11 IgG4 PE, VLX9hum_03 IgG2, VLX9hum_06 IgG2, and VLX9hum_08 IgG2) or a control antibody in RPMI containing 10% media for 30 min at 37°C. An equal volume of fluorescently labeled SIRPα-Fc fusion protein was added and incubated for an additional 30 min at 37°C. Cells were washed and binding was assessed using flow cytometry.

FIG. 8. VLX4 CD47 Chimeric mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1×10^4 cells per well in a 96 well plate and allowed to adhere for 24 hrs. 5×10^4 CFSE (1μM) labeled human Jurkat T cells and 1 μg/ml of the VLX4 chimeric mAbs were added to the macrophage cultures and incubated at

37°C for 2 hrs. Non-phagocytosed Jurkat cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 9A. VLX4 Humanized mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1x10⁴ cells per well in a 96 well plate and allowed to adhere for 24 hrs. 5x10⁴ CFSE (1μM) labeled human Jurkat T cells and 1 μg/ml of antibody were added to the macrophage cultures and incubated at 37°C for 2 hrs. Non-phagocytosed Jurkat T cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 9B. VLX4 Humanized mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1x10⁴ cells per well in a 96 well plate and allowed to adhere for 24 hrs. 5x10⁴ CFSE (1μM) labeled human Jurkat T cells and 1 μg/ml of antibody were added to the macrophage cultures and incubated at 37°C for 2 hrs. Non-phagocytosed Jurkat T cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 10A. VLX8 CD47 Chimeric mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1x10⁴ cells per well in a 96 well plate and allowed to adhere for 24 hrs. 5x10⁴ CFSE (1μM) labeled human Jurkat T cells and 1 μg/ml of the VLX8 chimeric mAbs were added to the macrophage cultures and incubated at 37°C for 2 hrs. Non-phagocytosed Jurkat cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 10B. VLX8 Humanized mAbs Increase Phagocytosis of Jurkat Cells by Human Macrophages. Human macrophages were plated at a concentration of 1x10⁴ cells per well in a 96 well plate and allowed to adhere for 24 hrs. 5x10⁴ CFSE (1μM) labeled human Jurkat T cells and 1 μg/ml of antibody were added to the macrophage cultures and incubated at 37°C for 2 hrs. Non-phagocytosed Jurkat T cells were removed and macrophage cultures were washed extensively.

Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 11A VLX9 CD47 Chimeric mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1×10^4 cells per well in a 96 well plate and allowed to adhere for 24 hours. 5×10^4 CFSE (1 μ M) labeled human Jurkat T cells and 1 μ g/ml of the VLX9 chimeric mAbs were added to the macrophage cultures and incubated at 37°C for two hours. Non-phagocytosed Jurkat cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 11B. VLX9 Humanized mAbs Increase Phagocytosis of Jurkat T Cells by Human Macrophages. Human macrophages were plated at a concentration of 1×10^4 cells per well in a 96 well plate and allowed to adhere for 24 hours. 5×10^4 CFSE (1 μ M) labeled human Jurkat T cells and 1 μ g/ml of antibody were added to the macrophage cultures and incubated at 37°C for two hours. Non-phagocytosed Jurkat cells were removed and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14. Flow cytometry was used to determine the percentage of CD14⁺/CFSE⁺ cells in the total CD14⁺ population.

FIG. 12A. Induction of Cell Death in Human Jurkat T Cells by Soluble VLX4 Humanized mAbs. Jurkat T cells (1×10^4) were incubated with 1 μ g/ml VLX4 humanized mAbs in 1 ml of RPMI media for 24 hours at 37°C. Cells were then stained for annexin V and the signal was detected by flow cytometry.

FIG. 12B. Induction of Cell Death in Human Jurkat T Cells by Soluble VLX4 Humanized mAbs. Jurkat T cells (1×10^4) were incubated with 1 μ g/ml VLX4 humanized mAbs in 1 ml of RPMI media for 24 hours at 37°C. Cells were then stained for annexin V and the signal was detected by flow cytometry.

FIG. 13A. Induction of Cell Death in Human Jurkat Cells by Soluble VLX8 CD47 Chimeric mAbs. Jurkat T ALL cells (1×10^4) were incubated with 1 μ g/ml VLX8 humanized mAbs in 1 ml of RPMI media for 24 hrs at 37°C. Cells were then stained for annexin V and the signal was detected by flow cytometry.

FIG. 13B. Induction of Cell Death in Human Jurkat Cells by Soluble VLX8 Humanized mAbs. Jurkat T ALL cells (1×10^4) were incubated with 1 μ g/ml VLX8 humanized mAbs in 1 ml

of RPMI media for 24 hrs at 37°C. Cells were then stained for annexin V and the signal was detected by flow cytometry.

FIG. 14A. Induction of Cell Death of Human Jurkat Cells by Soluble VLX9 Murine/Human Chimeric mAbs. 1×10^4 Jurkat cells were incubated with 1 µg/ml of the VLX9 CD47 chimeric mAbs in 0.1 ml of RPMI media for 24 hours 37°C. Cells were then stained with annexin V and the signal analyzed by flow cytometry.

FIG. 14B. Induction of Cell Death in Human Jurkat Cells by Soluble VLX9 Humanized mAbs. Jurkat T ALL cells (1×10^4) were incubated with 1 µg/ml VLX9 humanized mAbs in 1 ml of RPMI media for 24 hours at 37°C. Cells were then stained for annexin V and the signal was detected by flow cytometry. VLX9 IgG2 (xi) is a murine/human chimera.

FIG. 15A. Agglutination of hRBCs by VLX4 Humanized mAbs. Hemagglutination was assessed following incubation of hRBCs with various concentrations of humanized VLX4 mAbs (25 µg/mL - 0.4 ng/mL). Blood was diluted (1:50) and washed 3 times with PBS/EDTA/BSA. hRBCs were added to U-bottomed 96 well plates with equal volumes of the antibodies (75 µl) and incubated for 3 hrs at 37°C and overnight at 4°C.

FIG. 15B. Agglutination of hRBCs by VLX8 Chimeric and Humanized mAbs. Hemagglutination was assessed following incubation of hRBCs with various concentrations of humanized VLX4 mAbs (25 µg/mL - 0.4 ng/mL). Blood was diluted (1:50) and washed 3 times with PBS/EDTA/BSA. hRBCs were added to U-bottomed 96 well plates with equal volumes of the antibodies (75 µl) and incubated for 3 hrs at 37°C and overnight at 4°C.

FIG. 16. Agglutination of Human RBCs by VLX9 Humanized mAbs. Hemagglutination was assessed following incubation of human RBCs with various concentrations of VLX9 IgG2 chimera (xi) and humanized VLX9 mAbs. Blood was diluted (1:50) and washed 3 times with PBS/EDTA/BSA. RBCs were added to U-bottomed 96 well plates with equal volumes of the antibodies (75 µl) and incubated for 3 hrs at 37°C and overnight at 4°C.

FIG. 17. VLX4 Humanized mAb Reduces Tumor Growth in Raji Xenograft Model. Female NSG mice were inoculated subcutaneously in the right flank with 0.1 mL of a 30% RPMI / 70% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of 5×10^6 Raji tumor cells. Five days following inoculation, tumor volumes were measured and mice with palpable tumor volumes of 31-74 mm³ were randomized into 8-10/group. VLX4hum_07 or PBS (control) administration was initiated at this time. Mice were treated with 5 mg/kg of antibody

5X/week for 4 weeks by intraperitoneal injection. Tumor volumes and body weights were recorded twice weekly.

FIG. 18. VLX8 Humanized mAb Reduces Tumor Growth in Raji Xenograft Model. Female NSG mice were inoculated subcutaneously in the right flank with 0.1 mL of a 30% RPMI / 70% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of 5×10^6 Raji tumor cells. Five days following inoculation, tumor volumes were measured and mice with palpable tumor volumes of 31-74 mm³ were randomized into 8-10/group. VLX8hum_10 or PBS (control) administration was initiated at this time. Mice were treated with 5 mg/kg of antibody 5X/week for 4 weeks by intraperitoneal injection. Tumor volumes and body weights were recorded twice weekly.

FIG. 19. VLX9 Humanized mAb Reduces Tumor Growth in Raji Xenograft Model. Female NSG mice were inoculated subcutaneously in the right flank with 0.1 mL of a 30% RPMI / 70% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of 5×10^6 Raji tumor cells. Five days following inoculation, tumor volumes were measured and mice with palpable tumor volumes of 31-74 mm³ were randomized into 8-10/group. VLX9hum_08 IgG2 or PBS (control) administration was initiated at this time. Mice were treated with 5 mg/kg of antibody 5X/week for 4 weeks by intraperitoneal injection. Tumor volumes and body weights were recorded twice weekly.

FIG. 20A. Hemoglobin Levels in Blood following Administration of a Humanized VLX9 mAb to Cynomolgus Monkeys by Intravenous Infusion. VLX9hum_08 IgG2 or vehicle were administered as a one hour intravenous infusion a dose of 5mg/kg on day 1 and a dose of 15mg/kg on day 18. Hemoglobin levels were monitored throughout the study and normalized to control values.

FIG. 20B. RBC Levels in Blood following Administration of Humanized VLX9 mAbs to Cynomolgus Monkeys by Intravenous Infusion. VLX9hum_08 IgG2 or vehicle was administered as a one hour intravenous infusion a dose of 5mg/kg on day 1 and a dose of 15mg/kg on day 18. RBC levels were monitored throughout the study and normalized to control values.

FIG. 21. Immunohistochemical Staining of CD47 in Human Tumor Tissue with Anti-Murine/Rabbit Chimeric mAb. CD47 was localized in human breast cancer tissue using VLX4 mouse/rabbit chimeric mAb. Paraffin-embedded tissue was sectioned, stained with 4 ug/ml of

purified antibody and localized with anti-rabbit HRP secondary antibody. Arrows denote positive areas of CD47 staining.

FIG. 22. Summary of Anti-CD47 Antibody Properties.

DETAILED DESCRIPTION OF THE EMBODIMENTS OF THE DISCLOSURE

Definitions

Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art.

As used herein, the term "CD47", "integrin-associated protein (IAP)", "ovarian cancer antigen OA3", "Rh-related antigen" and "MERG" are synonymous and may be used interchangeably.

The term "anti-CD47 antibody" refer to an antibody of the disclosure which is intended for use as a therapeutic or diagnostic agent, and therefore will typically possess the binding affinity required to be useful as a therapeutic and/or diagnostic agent.

As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By "specifically bind" or "immunoreacts" with or directed against is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at a much lower affinity ($K_d > 10^{-6}$). Antibodies include but are not limited to, polyclonal, monoclonal, chimeric, Fab fragments, Fab' fragments, F(ab')₂ fragments, single chain Fv fragments, and one-armed antibodies.

As used herein, the term "monoclonal antibody" (mAb) as applied to the present antibody compounds refers to an antibody that is derived from a single copy or clone including, for example, any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. mAbs of the present disclosure preferably exist in a homogeneous or substantially homogeneous population. Complete mAbs contain 2 heavy chains and 2 light chains.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab' -SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies
5 formed from antibody fragments.

As disclosed herein, "antibody compounds" refers to mAbs and antigen-binding fragments thereof. Additional antibody compounds exhibiting similar functional properties according to the present disclosure can be generated by conventional methods. For example, mice can be immunized with human CD47 or fragments thereof, the resulting antibodies can be recovered and
10 purified, and determination of whether they possess binding and functional properties similar to or the same as the antibody compounds disclosed herein can be assessed by the methods disclosed in Examples 3-11, below. Antigen-binding fragments can also be prepared by conventional methods. Methods for producing and purifying antibodies and antigen-binding fragments are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory*
15 *Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, chapters 5-8 and 15.

The monoclonal antibodies encompass antibodies in which a portion of the heavy and/or light chain is identical with, or homologous to, corresponding sequences in murine antibodies, in particular the murine CDRs, while the remainder of the chain(s) is (are) identical with, or
20 homologous to, corresponding sequences in human antibodies. Other embodiments of the disclosure include antigen-binding fragments of these monoclonal antibodies that exhibit binding and biological properties similar or identical to the monoclonal antibodies. The antibodies of the present disclosure can comprise kappa or lambda light chain constant regions, and heavy chain IgA, IgD, IgE, IgG, or IgM constant regions, including those of IgG subclasses IgG1, IgG2, IgG3, and IgG4 and in some cases with various mutations to alter Fc receptor function.
25

The monoclonal antibodies containing the presently disclosed murine CDRs can be prepared by any of the various methods known to those skilled in the art, including recombinant DNA methods.

Reviews of current methods for antibody engineering and improvement can be found, for
30 example, in P. Chames, Ed., (2012) *Antibody Engineering: Methods and Protocols, Second Edition (Methods in Molecular Biology, Book 907)*, Humana Press, ISBN-10: 1617799734; C. R.

Wood, Ed., (2011) *Antibody Drug Discovery (Molecular Medicine and Medicinal Chemistry, Book 4)*, Imperial College Press; R. Kontermann and S. Dubel, Eds., (2010) *Antibody Engineering Volumes 1 and 2* (Springer Protocols), Second Edition; and W. Strohl and L. Strohl (2012) *Therapeutic antibody engineering: Current and future advances driving the strongest growth area in the pharmaceutical industry*, Woodhead Publishing.

Methods for producing and purifying antibodies and antigen-binding fragments are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, chapters 5-8 and 15.

A full-length antibody as it exists naturally is a “Y” shaped immunoglobulin (Ig) molecule comprising four polypeptide chains: two identical heavy (H) chains and two identical light (L) chains, interconnected by disulfide bonds. The amino terminal portion of each chain, termed the fragment antigen binding region (FAB), includes a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition via the complementarity determining regions (CDRs) contained therein. The carboxy-terminal portion of each chain defines a constant region (the “Fc” region) primarily responsible for effector function.

The CDRs are interspersed with regions that are more conserved, termed frameworks (“FRs”). Amino acid sequences of many FRs are well known in the art. Each light chain variable region (LCVR) and heavy chain variable region (HCVR) is composed of 3 CDRs and 4 FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The 3 CDRs of the light chain are referred to as “LCDR1, LCDR2, and LCDR3” and the 3 CDRs of the heavy chain are referred to as “HCDR1, HCDR2, and HCDR3.” The CDRs contain most of the residues which form specific interactions with the antigen. The numbering and positioning of CDR amino acid residues within the LCVR and HCVR regions are in accordance with the well-known Kabat numbering convention Kabat et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition. NIH Publication No. 91-3242.

As described herein, the “antigen-binding site” can also be defined as the “Hypervariable regions”, “HVRs”, or “HVs”, and refer to the structurally hypervariable regions of antibody variable domains as defined by Chothia and Lesk (Chothia and Lesk, Mol. Biol. 196:901-917, 1987). There are six HVRs, three in VH (H1, H2, H3) and three in VL (L1, L2, L3). We used herein CDRs as defined by Kabat except in H-CDR1, which is extended to include H1.

There are five types of mammalian immunoglobulin (Ig) heavy chains, denoted by the Greek letters α (alpha), δ (delta), ϵ (epsilon), γ (gamma), and μ (mu), which define the class or isotype of an antibody as IgA, IgD, IgE, IgG, or IgM, respectively. IgG antibodies can be further divided into subclasses, for example, IgG1, IgG2, IgG3, and IgG4.

Each heavy chain type is characterized by a particular constant region with a sequence well known in the art. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ , α , and δ have a constant region composed of three tandem immunoglobulin (Ig) domains, and a hinge region for added flexibility. Heavy chains μ and ϵ have a constant region composed of four Ig domains.

The hinge region is a flexible amino acid stretch that links the Fc and Fab portions of an antibody. This regions contains cysteine residues that can form disulfide bonds, connecting two heavy chains together.

The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

In mammals, light chains are classified as kappa (κ) or lambda (λ), and are characterized by a particular constant region as known in the art. A light chain has two successive domains: one variable domain at the amino-terminal end, and one constant domain at the carboxy-terminal end. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ , is present per antibody in mammals.

The Fc region, composed of two heavy chains that contribute three or four constant domains depending on the class of the antibody, plays a role in modulating immune cell activity. By binding to specific proteins, the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins. By doing this, it mediates different physiological effects, including opsonization, cell lysis, and degranulation of mast cells, basophils and eosinophils.

As used herein, the term "epitope" refers to a specific arrangement of amino acids located on a peptide or protein to which an antibody or antibody fragment binds. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains, and have specific three dimensional structural characteristics as well as specific charge characteristics.

Epitopes can be linear, i.e., involving binding to a single sequence of amino acids, or conformational, i.e., involving binding to two or more sequences of amino acids in various regions of the antigen that may not necessarily be contiguous in the linear sequence.

As used herein, the terms “specifically binds”, “bind specifically”, “specific binding”, and the like as applied to the present antibody compounds refer to the ability of a specific binding agent (such as an antibody) to bind to a target molecular species in preference to binding to other molecular species with which the specific binding agent and target molecular species are admixed. A specific binding agent is said specifically to recognize a target molecular species when it can bind specifically to that target.

As used herein, the term “binding affinity” refers to the strength of binding of one molecule to another at a site on the molecule. If a particular molecule will bind to or specifically associate with another particular molecule, these two molecules are said to exhibit binding affinity for each other. Binding affinity is related to the association constant and dissociation constant for a pair of molecules, but it is not critical to the methods herein that these constants be measured or determined. Rather, affinities as used herein to describe interactions between molecules of the described methods are generally apparent affinities (unless otherwise specified) observed in empirical studies, which can be used to compare the relative strength with which one molecule (e.g., an antibody or other specific binding partner) will bind two other molecules (e.g., two versions or variants of a peptide). The concepts of binding affinity, association constant, and dissociation constant are well known.

As used herein, the term “sequence identity” means the percentage of identical nucleotide or amino acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Identity can be readily calculated by known methods, including but not limited to those described in: *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the

sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs.

Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith & Waterman, by the homology alignment algorithms, by the search for similarity method or, by computerized implementations of these algorithms (GAP, BESTFIT, PASTA, and TFASTA in the GCG Wisconsin Package, available from Accelrys, Inc., San Diego, California, United States of America), or by visual inspection. See generally, Altschul, S. F. et al., *J. Mol. Biol.* 215: 403-410 (1990) and Altschul et al. *Nucl. Acids Res.* 25: 3389-3402 (1997).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in (Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; and Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold.

These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always, 0) and N (penalty score for mismatching residues; always, 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, a cutoff of 100, M = 5, N = -4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is in one embodiment less than about 0.1, in another embodiment less than about 0.01, and in still another embodiment less than about 0.001.

As used herein, the terms “humanized”, “humanization”, and the like, refer to grafting of the murine monoclonal antibody CDRs disclosed herein to human FRs and constant regions. Also encompassed by these terms are possible further modifications to the murine CDRs, and human FRs, by the methods disclosed in, for example, Kashmiri et al. (2005) *Methods* 36(1):25-34 and Hou et al. (2008) *J. Biochem.* 144(1):115-120, respectively, to improve various antibody properties, as discussed below.

As used herein, the term "humanized antibodies" refers to mAbs and antigen binding fragments thereof, including the antibody compounds disclosed herein, that have binding and functional properties according to the disclosure similar to those disclosed herein, and that have FRs and constant regions that are substantially human or fully human surrounding CDRs derived from a non-human antibody.

As used herein, the term "FR" or "framework sequence" refers to any one of FRs 1 to 4. Humanized antibodies and antigen binding fragments encompassed by the present disclosure include molecules wherein any one or more of FRs 1 to 4 is substantially or fully human, i.e., wherein any of the possible combinations of individual substantially or fully human FRs 1 to 4, is present. For example, this includes molecules in which FR1 and FR2, FR1 and FR3, FR1, FR2, and FR3, etc., are substantially or fully human. Substantially human frameworks are those that have at least 80% sequence identity to a known human germline framework sequence. Preferably, the substantially human frameworks have at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity, to a framework sequence disclosed herein, or to a known human germline framework sequence.

Fully human frameworks are those that are identical to a known human germline framework sequence. Human FR germline sequences can be obtained from the international ImMunoGeneTics (IMGT) database and from *The Immunoglobulin FactsBook* by Marie-Paule Lefranc and Gerard Lefranc, Academic Press, 2001, the contents of which are herein incorporated
5 by reference in their entirety.

The Immunoglobulin Facts Book is a compendium of the human germline immunoglobulin genes that are used to create the human antibody repertoire, and includes entries for 203 genes and 459 alleles, with a total of 837 displayed sequences. The individual entries comprise all the human immunoglobulin constant genes, and germline variable, diversity, and joining genes that have at
10 least one functional or open reading frame allele, and which are localized in the three major loci. For example, germline light chain FRs can be selected from the group consisting of: IGKV3D-20, IGKV2-30, IGKV2-29, IGKV2-28, IGKV1-27, IGKV3-20, IGKV1-17, IGKV1-16, 1-6, IGKV1-5, IGKV1-12, IGKV1D-16, IGKV2D-28, IGKV2D-29, IGKV3-11, IGKV1-9, IGKV1-39, IGKV1D-39 and IGKV1D-33 and IGKJ1-5 and germline heavy chain FRs can be selected from
15 the group consisting of: IGHV1-2, IGHV1-18, IGHV1-46, IGHV1-69, IGHV2-5, IGHV2-26, IGHV2-70, IGHV1-3, IGHV1-8, IGHV3-9, IGHV3-11, IGHV3-15, IGHV3-20, IGHV3-66, IGHV3-72, IGHV3-74, IGHV4-31, IGHV3-21, IGHV3-23, IGHV3-30, IGHV3-48, IGHV4-39, IGHV4-59 and IGHV5-51 and IGHJ1-6.

Substantially human FRs are those that have at least 80% sequence identity to a known
20 human germline FR sequence. Preferably, the substantially human frameworks have at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity, to a framework sequences disclosed herein, or to a known human germline framework sequence.

CDRs encompassed by the present disclosure include not only those specifically disclosed
25 herein, but also CDR sequences having sequence identities of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a CDR sequence disclosed herein. Alternatively, CDRs encompassed by the present disclosure include not only those specifically disclosed herein, but also CDR sequences having 1, 2, 3, 4, or 5 amino acid changes at corresponding positions compared to CDR sequences disclosed herein.
30 Such sequence identical, or amino acid modified, CDRs preferably bind to the antigen recognized by the intact antibody.

Humanized antibodies in addition to those disclosed herein exhibiting similar functional properties according to the present disclosure can be generated using several different methods Almagro et al. *Frontiers in Biosciences*. Humanization of antibodies. (2008) Jan 1; 13:1619-33.

In one approach, the parent antibody compound CDRs are grafted into a human framework that has a high sequence identity with the parent antibody compound framework. The sequence identity of the new framework will generally be at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identical to the sequence of the corresponding framework in the parent antibody compound. In the case of frameworks having fewer than 100 amino acid residues, one, two, three, four, five, six, seven, eight, nine, or ten amino acid residues can be changed. This grafting may result in a reduction in binding affinity compared to that of the parent antibody. If this is the case, the framework can be back-mutated to the parent framework at certain positions based on specific criteria disclosed by Queen et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:2869. Additional references describing methods useful to generate humanized variants based on homology and back mutations include as described in Olimpieri et al. *Bioinformatics*. 2015 Feb 1;31(3):434-435 and U.S. Patents 4,816,397, 5,225,539, and 5,693,761; and the method of Winter and co-workers (Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; and Verhoeven et al. (1988) *Science* 239:1534-1536.

Humanization began with chimerization, a method developed during the first half of the 1980's (Morrison, S. L., M. J. Johnson, L. A. Herzenberg & V. T. Oi: Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. USA*, 81, 6851-5 (1984)), consisting of combining the variable (V) domains of murine antibodies with human constant (C) domains to generate molecules with ~70% of human content.

Several different methods can be used to generate humanized antibodies, which are described herein. In one approach, the parent antibody compound CDRs are grafted into a human FR that has a high sequence identity with the parent antibody compound framework. The sequence identity of the new FR will generally be at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of the corresponding FR in the parent antibody compound. In the case of FRs having fewer than 100 amino acid residues, one, two, three, four, five, or more amino acid residues can be changed. This grafting

may result in a reduction in binding affinity compared to that of the parent antibody. If this is the case, the FR can be back-mutated to the parent framework at certain positions based on specific criteria disclosed by Queen et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:2869. Additional references describing methods useful to generate humanized variants based on homology and back mutations include as described in Olimpieri et al. *Bioinformatics*. 2015 Feb 1;31(3):434-435 and U.S. Patents 4,816,397, 5,225,539, and 5,693,761; and the method of Winter and co-workers (Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; and Verhoeven et al. (1988) *Science* 239:1534-1536.

The identification of residues to consider for back-mutation can be carried out as described below. When an amino acid falls under the following category, the framework amino acid of the human germ-line sequence that is being used (the "acceptor FR") is replaced by a framework amino acid from a framework of the parent antibody compound (the "donor FR"):

(a) the amino acid in the human FR of the acceptor framework is unusual for human frameworks at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human frameworks at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model.

When each of the amino acids in the human FR of the acceptor framework and a corresponding amino acid in the donor framework is generally unusual for human frameworks at that position, such amino acid can be replaced by an amino acid typical for human frameworks at that position. This back-mutation criterion enables one to recover the activity of the parent antibody compound.

Another approach to generating humanized antibodies exhibiting similar functional properties to the antibody compounds disclosed herein involves randomly mutating amino acids within the grafted CDRs without changing the framework, and screening the resultant molecules for binding affinity and other functional properties that are as good as, or better than, those of the parent antibody compounds. Single mutations can also be introduced at each amino acid position within each CDR, followed by assessing the effects of such mutations on binding affinity and other functional properties. Single mutations producing improved properties can be combined to assess their effects in combination with one another.

Further, a combination of both of the foregoing approaches is possible. After CDR grafting, one can back-mutate specific FRs in addition to introducing amino acid changes in the CDRs. This methodology is described in Wu et al. (1999) *J. Mol. Biol.* 294: 151-162.

Applying the teachings of the present disclosure, a person skilled in the art can use common techniques, e.g., site-directed mutagenesis, to substitute amino acids within the presently disclosed CDR and FR sequences and thereby generate further variable region amino acid sequences derived from the present sequences. Up to all naturally occurring amino acids can be introduced at a specific substitution site. The methods disclosed herein can then be used to screen these additional variable region amino acid sequences to identify sequences having the indicated *in vivo* functions. In this way, further sequences suitable for preparing humanized antibodies and antigen-binding portions thereof in accordance with the present disclosure can be identified. Preferably, amino acid substitution within the frameworks is restricted to one, two, three, four, or five positions within any one or more of the four light chain and/or heavy chain FRs disclosed herein. Preferably, amino acid substitution within the CDRs is restricted to one, two, three, four, or five positions within any one or more of the three light chain and/or heavy chain CDRs. Combinations of the various changes within these FRs and CDRs described above are also possible.

That the functional properties of the antibody compounds generated by introducing the amino acid modifications discussed above conform to those exhibited by the specific molecules disclosed herein can be confirmed by the methods in Examples disclosed herein.

As described above, to circumvent the problem of eliciting human anti-murine antibody (HAMA) response in patients, murine antibodies have been genetically manipulated to progressively replace their murine content with the amino acid residues present in their human counterparts by grafting their complementarity determining regions (CDRs) onto the variable light (V_L) and variable heavy (V_H) frameworks of human immunoglobulin molecules, while retaining those murine framework residues deemed essential for the integrity of the antigen-combining site. However, the xenogeneic CDRs of the humanized antibodies may evoke anti-idiotypic (anti-Id) response in patients.

To minimize the anti-Id response, a procedure to humanize xenogeneic antibodies by grafting onto the human frameworks only the CDR residues most crucial in the antibody-ligand interaction, called “SDR grafting”, has been developed, wherein only the crucial specificity determining residues (SDRs) of CDRs are grafted onto the human frameworks. This procedure,

described in Kashmiri et al. (2005) *Methods* 36(1):25-34, involves identification of SDRs through the help of a database of the three-dimensional structures of the antigen-antibody complexes of known structures, or by mutational analysis of the antibody-combining site. An alternative approach to humanization involving retention of more CDR residues is based on grafting of the
5 'abbreviated' CDRs, the stretches of CDR residues that include all the SDRs. Kashmiri et al. also discloses a procedure to assess the reactivity of humanized antibodies to sera from patients who had been administered the murine antibody.

Another strategy for constructing human antibody variants with improved immunogenic properties is disclosed in Hou et al. (2008) *J. Biochem.* 144(1):115-120. These authors developed
10 a humanized antibody from 4C8, a murine anti-human CD34 monoclonal antibody, by CDR grafting using a molecular model of 4C8 built by computer-assisted homology modelling. Using this molecular model, the authors identified FR residues of potential importance in antigen binding. A humanized version of 4C8 was generated by transferring these key murine FR residues onto a human antibody framework that was selected based on homology to the murine antibody FR,
15 together with the murine CDR residues. The resulting humanized antibody was shown to possess antigen-binding affinity and specificity similar to that of the original murine antibody, suggesting that it might be an alternative to murine anti-CD34 antibodies routinely used clinically.

Embodiments of the present disclosure encompass antibodies created to avoid recognition by the human immune system containing CDRs disclosed herein in any combinatorial form such
20 that contemplated mAbs can contain the set of CDRs from a single murine mAb disclosed herein, or light and heavy chains containing sets of CDRs comprising individual CDRs derived from two or three of the disclosed murine mAbs. Such mAbs can be created by standard techniques of molecular biology and screened for desired activities using assays described herein. In this way, the disclosure provides a "mix and match" approach to create novel mAbs comprising a mixture
25 of CDRs from the disclosed murine mAbs to achieve new, or improved, therapeutic activities.

Monoclonal antibodies or antigen-binding fragments thereof encompassed by the present disclosure that "compete" with the molecules disclosed herein are those that bind human CD47 at site(s) that are identical to, or overlapping with, the site(s) at which the present molecules bind. Competing monoclonal antibodies or antigen-binding fragments thereof can be identified, for
30 example, via an antibody competition assay. For example, a sample of purified or partially purified human CD47 extracellular domain can be bound to a solid support. Then, an antibody compound,

or antigen binding fragment thereof, of the present disclosure and a monoclonal antibody or antigen-binding fragment thereof suspected of being able to compete with such disclosure antibody compound are added. One of the two molecules is labeled. If the labeled compound and the unlabeled compound bind to separate and discrete sites on CD47, the labeled compound will bind to the same level whether or not the suspected competing compound is present. However, if the sites of interaction are identical or overlapping, the unlabeled compound will compete, and the amount of labeled compound bound to the antigen will be lowered. If the unlabeled compound is present in excess, very little, if any, labeled compound will bind. For purposes of the present disclosure, competing monoclonal antibodies or antigen-binding fragments thereof are those that decrease the binding of the present antibody compounds to CD47 by about 50%, about 60%, about 70%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%. Details of procedures for carrying out such competition assays are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Such assays can be made quantitative by using purified antibodies. A standard curve is established by titrating one antibody against itself, i.e., the same antibody is used for both the label and the competitor. The capacity of an unlabeled competing monoclonal antibody or antigen-binding fragment thereof to inhibit the binding of the labeled molecule to the plate is titrated. The results are plotted, and the concentrations necessary to achieve the desired degree of binding inhibition are compared.

Whether mAbs or antigen-binding fragments thereof that compete with antibody compounds of the present disclosure in such competition assays possess the same or similar functional properties of the present antibody compounds can be determined via these methods in conjunction with the methods described in Examples 3-5, below. In various embodiments, competing antibodies for use in the therapeutic methods encompassed herein possess biological activities as described herein in the range of from about 50% to about 100% or about 125%, or more, compared to that of the antibody compounds disclosed herein. In some embodiments, competing antibodies possess about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical biological activity compared to that of the antibody compounds disclosed herein as determined by the methods disclosed in the Examples presented below.

The mAbs or antigen-binding fragments thereof, or competing antibodies useful in the compositions and methods can be any of the isotypes described herein. Furthermore, any of these isotypes can comprise further amino acid modifications as follows.

5 The monoclonal antibody or antigen-binding fragment thereof, or competing antibody described herein can be of the human IgG1 isotype.

The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to alter antibody half-life. Antibody half-life is regulated in large part by Fc-dependent interactions with the neonatal Fc receptor (Roopenian and Alikesh, 2007). The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody can be modified to increase half-life include, but are not limited to amino acid modifications N434A, T307A/E380A/N434A (Petkova et al., 2006, Yeung et al., 2009); M252Y/S254T/T256E (Dall'Acqua et al., 2006); T250Q/M428L (Hinton et al., 2006); and M428L/N434S (Zalevsky et al., 2010).

15 As opposed to increasing half-life, there are some circumstances where decreased half-life would be desired, such as to reduce the possibility of adverse events associated with high Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) antibodies (Presta 2008). The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to decrease half-life and/or decrease endogenous IgG include, but are not limited to amino acid modifications I253A (Petkova et al., 2006); P257I/N434H, D376V/N434H (Datta-Mannan et al., 2007); and M252Y/S254T/T256E/H433K/N434F (Vaccaro et al., 2005).

25 The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to increase or decrease antibody effector functions. These antibody effector functions include, but are not limited to, Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC), Antibody-Dependent Cellular Phagocytosis (ADCP), C1q binding, and altered binding to Fc receptors.

30 The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to increase antibody effector function include, but are not limited to amino acid modifications S298A/E333A/K334 (Shields et al., 2001); S239D/I332E and S239D/A330L/I332E (Lazar et al., 2006); F234L/R292P/Y300L,

F234L/R292P/Y300L/P393L, and F243L/R292P/Y300L/V305I/P396L (Stevenhagen et al., 2007); G236A, G236A/S239D/I332E, and G236A/S239D/A330L/I332E (Richards et al., 2008); K326A/E333A, K326A/E333S and K326W/E333S (Idusogie et al., 2001); S267E and S267E/L328F (Smith et al., 2012); H268F/S324T, S267E/H268F, S267E/S234T, and
5 S267E/H268F/S324T (Moore et al., 2010); S298G/T299A (Sazinsky et al., 2008); E382V/M428I (Jung et al., 2010).

The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to decrease antibody effector
10 function include, but are not limited to amino acid modifications N297A and N297Q (Bolt et al., 1993, Walker et al., 1989); L234A/L235A (Xu et al., 2000); K214T/E233P/L234V/L235A/G236-deleted/A327G/P331A/D356E/L358M (Ghevaert et al., 2008); C226S/C229S/E233P/L234V/L235A (McEarchern et al., 2007); S267E/L328F (Chu et al., 2008).

The human IgG1 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to decrease antibody effector
15 function include, but are not limited to amino acid modifications V234A/G237A (Cole et al., 1999); E233D, G237D, P238D, H268Q, H268D, P271G, V309L, A330S, A330R, P331S, H268Q/A330S/V309L/P331S, H268D/A330S/V309L/P331S, H268Q/A330R/V309L/P331S, H268D/A330R/V309L/P331S, E233D/A330R, E233D/A330S, E233D/P271G/A330R, E233D/P271G/A330S, G237D/H268D/P271G, G237D/H268Q/P271G, G237D/ P271G/A330R,
20 G237D/ P271G/A330S, E233D/H268D/P271G/A330R, E233D/H268Q/P271G/A330R, E233D/H268D/P271G/A330S, E233D/H268Q/P271G/A330S, G237D/H268D/P271G/A330R, G237D/H268Q/P271G/A330R, G237D/H268D/P271G/A330S, G237D/H268Q/P271G/A330S, E233D/G237D/H268D/P271G/A330R, E233D/G237D/H268Q/P271G/A330R, E233D/G237D/H268D/P271G/A330S, E233D/G237D/H268Q/P271G/A330S,
25 P238D/E233D/A330R, P238D/E233D/A330S, P238D/E233D/P271G/A330R, P238D/E233D/P271G/A330S, P238D/G237D/H268D/P271G, P238D/G237D/H268Q/P271G, P238D/G237D/ P271G/A330R, P238D/G237D/ P271G/A330S, P238D/E233D/H268D/P271G/A330R, P238D/E233D/H268Q/P271G/A330R, P238D/E233D/H268D/P271G/A330S, P238D/E233D/H268Q/P271G/A330S,
30 P238D/G237D/H268D/P271G/A330R, P238D/G237D/H268Q/P271G/A330R, P238D/G237D/H268D/P271G/A330S, P238D/G237D/H268Q/P271G/A330S,

P238D/E233D/G237D/H268D/P271G/A330R, P238D/E233D/G237D/H268Q/P271G/A330R,
P238D/E233D/G237D/H268D/P271G/A330S, P238D/E233D/G237D/H268Q/P271G/A330S
(An et al., 2009, Mimoto, 2013).

The monoclonal antibody or antigen-binding fragment thereof, or competing antibody
5 described herein can be of the human IgG2 isotype.

The human IgG2 constant region of the monoclonal antibody, antigen-binding fragment
thereof, or competing antibody described herein can be modified to increase or decrease antibody
effector functions. These antibody effector functions include, but are not limited to, Antibody-
Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC),
10 Antibody-Dependent Cellular Phagocytosis (ADCP), and C1q binding, and altered binding to Fc
receptors.

The human IgG2 constant region of the monoclonal antibody, antigen-binding fragment
thereof, or competing antibody described herein can be modified to increase antibody effector
function include, but are not limited to the amino acid modification K326A/E333S (Idusogie et
15 al., 2001).

The human IgG2 constant region of the monoclonal antibody, antigen-binding fragment
thereof, or competing antibody described herein can be modified to decrease antibody effector
function include, but are not limited to amino acid modifications V234A/G237A (Cole et al.,
1999); E233D, G237D, P238D, H268Q, H268D, P271G, V309L, A330S, A330R, P331S,
20 H268Q/A330S/V309L/P331S, H268D/A330S/V309L/P331S, H268Q/A330R/V309L/P331S,
H268D/A330R/V309L/P331S, E233D/A330R, E233D/A330S, E233D/P271G/A330R,
E233D/P271G/A330S, G237D/H268D/P271G, G237D/H268Q/P271G, G237D/ P271G/A330R,
G237D/ P271G/A330S, E233D/H268D/P271G/A330R, E233D/H268Q/P271G/A330R,
E233D/H268D/P271G/A330S, E233D/H268Q/P271G/A330S, G237D/H268D/P271G/A330R,
25 G237D/H268Q/P271G/A330R, G237D/H268D/P271G/A330S, G237D/H268Q/P271G/A330S,
E233D/G237D/H268D/P271G/A330R, E233D/G237D/H268Q/P271G/A330R,
E233D/G237D/H268D/P271G/A330S, E233D/G237D/H268Q/P271G/A330S,
P238D/E233D/A330R, P238D/E233D/A330S, P238D/E233D/P271G/A330R,
P238D/E233D/P271G/A330S, P238D/G237D/H268D/P271G, P238D/G237D/H268Q/P271G,
30 P238D/G237D/ P271G/A330R, P238D/G237D/ P271G/A330S,
P238D/E233D/H268D/P271G/A330R, P238D/E233D/H268Q/P271G/A330R,

P238D/E233D/H268D/P271G/A330S, P238D/E233D/H268Q/P271G/A330S,
P238D/G237D/H268D/P271G/A330R, P238D/G237D/H268Q/P271G/A330R,
P238D/G237D/H268D/P271G/A330S, P238D/G237D/H268Q/P271G/A330S,
P238D/E233D/G237D/H268D/P271G/A330R, P238D/E233D/G237D/H268Q/P271G/A330R,
5 P238D/E233D/G237D/H268D/P271G/A330S, P238D/E233D/G237D/H268Q/P271G/A330S
(An et al., 2009, Mimoto, 2013).

The Fc region of a human IgG2 of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to alter isoform and/or agonistic activity, include, but are not limited to amino acid modifications C127S (C_{H1} domain), C232S,
10 C233S, C232S/C233S, C236S, and C239S (White et al., 2015, Lightle et al., 2010).

The monoclonal antibody or antigen-binding fragment thereof, or competing antibody described herein can be of the human IgG3 isotype.

The human IgG3 constant region of the monoclonal antibody, or antigen binding fragment thereof, wherein said human IgG3 constant region of the monoclonal antibody, or antigen-binding
15 fragment thereof can be modified at one or more amino acid(s) to increase antibody half-life, Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC), or apoptosis activity.

The human IgG3 constant region of the monoclonal antibody, or antigen-binding fragment thereof, wherein said human IgG3 constant region of the monoclonal antibody, or antigen-binding
20 fragment thereof can be modified at amino acid R435H to increase antibody half-life.

The monoclonal antibody or antigen-binding fragment thereof, or competing antibody described herein can be of the human IgG4 isotype.

The human IgG4 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to decrease antibody effector
25 functions. These antibody effector functions include, but are not limited to, Antibody-Dependent Cellular Cytotoxicity (ADCC) and Antibody-Dependent Cellular Phagocytosis (ADCP).

The human IgG4 constant region of the monoclonal antibody, antigen-binding fragment thereof, or competing antibody described herein can be modified to prevent Fab arm exchange and/or decrease antibody effector function include, but are not limited to amino acid modifications
30 F234A/L235A (Alegre et al., 1994); S228P, L235E and S228P/L235E (Reddy et al., 2000).

As used herein, the term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The terms "cancer", "cancerous", and "tumor" are not mutually exclusive as used herein. The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by aberrant cell growth/proliferation. Examples of cancers include, but are not limited to, carcinoma, lymphoma (i.e., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

The term "susceptible cancer" as used herein refers to a cancer, cells of which express CD47, and are responsive to treatment with an antibody or antigen binding fragment thereof, or competing antibody or antigen binding fragment thereof, of the present disclosure.

The term "autoimmune disease" as used herein refers to when the body's immune system turns against itself and mistakenly attacks healthy cells.

The term "inflammatory disease" as used herein refers to a disease characterized by inflammation which is a fundamental pathologic process consisting of a dynamic complex of histologically apparent cytologic changes, cellular infiltration, and mediator release that occurs in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent, including the local reactions and resulting morphologic changes; the destruction or removal of the injurious material; and the responses that lead to repair and healing.

The term "autoinflammatory disease" as used herein refers to a disease that results when the innate immune system causes inflammation for unknown reasons.

As used herein, the term "ischemia" refers to a vascular phenomenon in which a decrease in the blood supply to a bodily organ, tissue, or part is caused, for instance, by constriction or obstruction of one or more blood vessels. Ischemia sometimes results from vasoconstriction or

thrombosis or embolism. Ischemia can lead to direct ischemic injury, tissue damage due to cell death caused by reduced oxygen supply. Ischemia can occur acutely, as during surgery, or from trauma to tissue incurred in accidents, injuries and war settings, or following harvest of organs intended for subsequent transplantation, for example. It can also occur sub-acutely, as found in
5 atherosclerotic peripheral vascular disease, where progressive narrowing of blood vessels leads to inadequate blood flow to tissues and organs. When a tissue is subjected to ischemia, a sequence of chemical events is initiated that may ultimately lead to cellular dysfunction and necrosis. If ischemia is ended by the restoration of blood flow, a second series of injurious events ensue, producing additional injury. Thus, whenever there is a transient decrease or interruption of blood
10 flow in a subject, the resultant injury involves two components--the direct injury occurring during the ischemic interval, and the indirect or reperfusion injury that follows.

“Ischemic stroke” can be caused by several different kinds of diseases. The most common problem is narrowing of the arteries in the neck or head. This is most often caused by atherosclerosis, or gradual cholesterol deposition. If the arteries become too narrow, blood cells
15 may collect in them and form blood clots (thrombi). These blood clots can block the artery where they are formed (thrombosis), or can dislodge and become trapped in arteries closer to the brain (embolism). Cerebral stroke can occur when atherosclerotic plaque separates away partially from the vessel wall and occludes the flow of blood through the blood vessel.

As used herein, the term “Reperfusion” refers to restoration of blood flow to tissue that is
20 ischemic, due to decrease in blood flow. Reperfusion is a procedure for treating infarction or other ischemia, by enabling viable ischemic tissue to recover, thus limiting further necrosis. However, reperfusion can itself further damage the ischemic tissue, causing reperfusion injury. In addition to the immediate injury that occurs during deprivation of blood flow, “ischemic/reperfusion injury” involves tissue injury that occurs after blood flow is restored. Current understanding is that much
25 of this injury is caused by chemical products, free radicals, and active biological agents released by the ischemic tissues.

“Nitric oxide (NO) donor, precursor, or nitric oxide generating topical agent” refers to a compound or agent that either delivers NO, or that can be converted to NO through enzymatic or non-enzymatic processes. Examples include, but are not limited to, NO gas, isosorbide dinitrite,
30 nitrite, nitroprusside, nitroglycerin, 3-Morpholiniosydnonimine (SIN-1), S-nitroso-N-acetylpenicillamine (SNAP), Diethylenetriamine/NO (DETA/NO), S-nitrosothiols, Bidil[®], and arginine.

“Soluble guanylyl cyclase (sGC)” is the receptor for nitric oxide in vascular smooth muscle. In the cardiovascular system, nitric oxide is endogenously generated by endothelial nitric oxide synthase from L-arginine, and activates soluble guanylyl cyclase in adjacent vascular smooth muscle cells to increase cGMP levels, inducing vascular relaxation. Nitric oxide binds to the normally reduced heme moiety of soluble guanylyl cyclase, and increases the formation of cGMP from GTP, leading to a decrease in intracellular calcium, vasodilation, and anti-inflammatory effects. Oxidation of the heme iron on sGC decreases responsiveness of the enzyme to nitric oxide, and promotes vasoconstriction. The nitric oxide-sGC-cGMP pathway therefore plays an important role in cardiovascular diseases. Nitrogen-containing compounds such as sodium azide, sodium nitrite, hydroxylamine, nitroglycerin, and sodium nitroprusside have been shown to stimulate sGC, causing an increase in cGMP, and vascular relaxation. In contrast to stimulators of sGC, which bind to reduced sGC, activators of sGC activate the oxidized or heme-deficient sGC enzyme that is not responsive to nitric oxide, i.e., they stimulate sGC independent of redox state. While stimulators of sGC can enhance the sensitivity of reduced sGC to nitric oxide, activators of sGC can increase sGC enzyme activity even when the enzyme is oxidized and is therefore less, or unresponsive, to nitric oxide. Thus, sGC activators are non-nitric oxide based. Note the reviews of Nossaman et al. (2012) *Critical Care Research and Practice*, Volume 2012, article 290805, and Derbyshire and Marletta (2012) *Ann. Rev. Biochem.* 81:533-559.

“An agent that activates soluble guanylyl cyclase” refers, for example, to organic nitrates (Artz et al. (2002) *J. Biol. Chem.* 277:18253-18256); protoporphyrin IX (Ignarro et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:2870-2873); YC-1 (Ko et al. (1994) *Blood* 84:4226-4233); BAY 41-2272 and BAY 41-8543 (Stasch et al. (2001) *Nature* 410 (6825): 212–5), CMF-1571, and A-350619 (reviewed in Evgenov et al. (2006) *Nat. Rev. Drug. Discov.* 5:755-768); BAY 58-2667 (Cinaciguat; Frey et al. (2008) *Journal of Clinical Pharmacology* 48 (12): 1400–10); BAY 63-2521 (Riociguat; Mittendorf et al. (2009) *Chemmedchem* 4 (5): 853–65). Additional soluble guanylyl cyclase activators are disclosed in Stasch et al. (2011) *Circulation* 123:2263-2273; Derbyshire and Marletta (2012) *Ann. Rev. Biochem.* 81:533-559, and Nossaman et al. (2012) *Critical Care Research and Practice*, Volume 2012, Article ID 290805, pages 1-12.

cGMP can also be increased by inhibiting degradation using phosphodiesterase inhibitors. Examples of “an agent that inhibits cyclic nucleotide phosphodiesterases” include, tadalafil, vardenafil, udenafil, and sildenafil avanafil.

As used herein, term "treating" or "treat" or "treatment" means slowing, interrupting, arresting, controlling, stopping, reducing, or reversing the progression or severity of a sign, symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related signs, symptoms, conditions, or disorders. The term "treating" and the like refer to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop.

As used herein, term "effective amount" refers to the amount or dose of an antibody compound of the present disclosure which, upon single or multiple dose administration to a patient or organ, provides the desired treatment or prevention.

The precise effective amount for any particular subject will depend upon their size and health, the nature and extent of their condition, and the therapeutics or combination of therapeutics selected for administration. The effective amount for a given patient is determined by routine experimentation and is within the judgment of a clinician. Therapeutically effective amounts of the present antibody compounds can also comprise an amount in the range of from about 0.1 mg/kg to about 150 mg/kg, from about 0.1 mg/kg to about 100 mg/kg, from about 0.1 mg/kg to about 50 mg/kg, or from about 0.05 mg/kg to about 10 mg/kg per single dose administered to a harvested organ or to a patient. Known antibody-based pharmaceuticals provide guidance in this respect. For example, Herceptin™ is administered by intravenous infusion of a 21 mg/ml solution, with an initial loading dose of 4 mg/kg body weight and a weekly maintenance dose of 2 mg/kg body weight; Rituxan™ is administered weekly at 375 mg/m²; for example.

A therapeutically effective amount for any individual patient can be determined by the health care provider by monitoring the effect of the antibody compounds on tumor regression, circulating tumor cells, tumor stem cells or anti-tumor responses. Analysis of the data obtained by these methods permits modification of the treatment regimen during therapy so that optimal amounts of antibody compounds of the present disclosure, whether employed alone or in combination with one another, or in combination with another therapeutic agent, or both, are administered, and so that the duration of treatment can be determined as well. In this way, the dosing/treatment regimen can be modified over the course of therapy so that the lowest amounts of antibody compounds used alone or in combination that exhibit satisfactory efficacy are administered, and so that administration of such compounds is continued only so long as is necessary to successfully treat the patient. Known antibody-based pharmaceuticals provide

guidance relating to frequency of administration e.g., whether a pharmaceutical should be delivered daily, weekly, monthly, etc. Frequency and dosage may also depend on the severity of symptoms.

In some embodiments antibody compounds of the present disclosure can be used as medicaments in human and veterinary medicine, administered by a variety of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intraperitoneal, intrathecal, intraventricular, transdermal, transcutaneous, topical, subcutaneous, intratumoral, intranasal, enteral, sublingual, intravaginal, intravesicular or rectal routes. The compositions can also be administered directly into a lesion such as a tumor. Dosage treatment may be a single dose schedule or a multiple dose schedule. Hypo sprays may also be used to administer the pharmaceutical compositions. Typically, the therapeutic compositions can be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Veterinary applications include the treatment of companion/pet animals, such as cats and dogs; working animals, such as guide or service dogs, and horses; sport animals, such as horses and dogs; zoo animals, such as primates, cats such as lions and tigers, bears, etc.; and other valuable animals kept in captivity.

Such pharmaceutical compositions can be prepared by methods well known in the art. See, e.g., *Remington: The Science and Practice of Pharmacy*, 21st Edition (2005), Lippincott Williams & Wilkins, Philadelphia, PA, and comprise one or more antibody compounds disclosed herein, and a pharmaceutically or veterinarily acceptable, for example, physiologically acceptable, carrier, diluent, or excipient.

The present disclosure describes anti-CD47 mAbs with distinct functional profiles. These antibodies possess distinct combinations of properties selected from the following: 1) exhibit cross-reactivity with one or more species homologs of CD47; 2) block the interaction between CD47 and its ligand SIRP α ; 3) increase phagocytosis of human tumor cells, 4) induce death of susceptible human tumor cells; 5) do not induce cell death of human tumor cells; 6) have reduced binding to human red blood cells (hRBCs); 7) have no detectable binding to hRBCs; 8) cause reduced agglutination of hRBCs; 9) cause no detectable agglutination of hRBCs; 10) reverse TSP1 inhibition of the nitric oxide (NO) pathway and/or 11) do not reverse TSP1 inhibition of the NO pathway.

The anti-CD47 antibodies and antigen binding fragments thereof of the present disclosure

possess combinations of properties that are distinct from the anti-CD47 antibodies of the prior art. These properties and characteristics will now be described in further detail.

Binding to CD47 of Different Species

The anti-CD47 antibodies, and antigen binding fragments thereof, of the present disclosure
5 bind human CD47. In certain embodiments, the anti-CD47 antibodies exhibit cross-reactivity with one or more species homologs of CD47, for example CD47 homologs of non-human primate origin. In certain embodiments, the anti-CD47 antibodies and antigen binding fragments thereof of the present disclosure bind to human CD47 and to CD47 of non-human primate, mouse, rat, and/or rabbit origin. The cross-reactivity with other species homologs can be particularly
10 advantageous in the development and testing of therapeutic antibodies. For example, pre-clinical toxicology testing of therapeutic antibodies is frequently carried out in non-human primate species including, but not limited to, cynomolgus monkey, green monkey, rhesus monkey and squirrel monkey. Cross-reactivity with these species homologs can therefore be particularly advantageous for the development of antibodies as clinical candidates.

Blocking the Interaction Between CD47 and SIRP α and Promoting Phagocytosis

CD47, also known as integrin associated protein (IAP), is a 50 kDa cell surface receptor that is comprised of an extracellular N-terminal IgV domain, a five membrane spanning transmembrane domain, and a short C-terminal intracellular tail that is alternatively spliced.

Two ligands bind to CD47: Signal Regulatory Protein alpha (SIRP α) and
20 Thrombospondin-1 (TSP1). TSP1 is present in plasma and synthesized by many cells, including platelets. SIRP α is expressed on hematopoietic cells, which include macrophages and dendritic cells.

When SIRP α on a phagocyte engages CD47 on a target cell, this interaction prevents phagocytosis of the target cell. The interaction of CD47 and SIRP α effectively sends a “don’t eat
25 me” signal to the phagocyte (Oldenborg et al. *Science* 288: 2051-2054, 2000). Blocking the interaction of SIRP α and CD47 with an anti-CD47 mAb in a therapeutic context can provide an effective anti-cancer treatment by promoting the uptake and clearance of cancer cells by the host’s immune system. Thus, an important functional characteristic of some anti-CD47 mAbs is the ability to block the interaction of CD47 and SIRP α , resulting in phagocytosis of CD47 expressing
30 tumor cells by macrophages. Several anti-CD47 mAbs have been shown to block the interaction of CD47 and SIRP α , including B6H12 (Seiffert et al. *Blood* 94:3633-3643, 1999; Latour et al. *J.*

Immunol. 167: 2547-2554, 2001; Subramanian et al. *Blood* 107: 2548-2556, 2006; Liu et al. *J Biol. Chem.* 277: 10028-10036, 2002; Rebres et al. et al. *J. Cellular Physiol.* 205: 182-193, 2005), BRIC126 (Vernon-Wilson et al. *Eur J Immunol.* 30: 2130-2137, 2000; Subramanian et al. *Blood* 107: 2548-2556, 2006), CC2C6 (Seiffert et al. *Blood* 94:3633-3643,1999), and 1F7 (Rebres et al. 5 *J. Cellular Physiol.* 205: 182-193, 2005). B6H12 and BRIC126 have also been shown to cause phagocytosis of human tumor cells by human and mouse macrophages (Willingham et al. *Proc Natl Acad Sci USA* 109(17):6662-6667, 2012; Chao et al. *Cell* 142:699-713, 2012; EP 2 242 512 B1). Other existing anti-CD47 mAbs, such as 2D3, does not block the interaction of CD47 and SIRP α (Seiffert et al. *Blood* 94:3633-3643,1999; Latour et al. *J. Immunol.* 167: 2547-2554, 2001; 10 Rebres et al. *J. Cellular Physiol.* 205: 182-193, 2005), and does not cause phagocytosis of tumor cells (Willingham et al. *Proc Natl Acad Sci USA* 109(17):6662-6667, 2012; Chao et al. *Cell* 142:699-713, 2012; EP 2 242 512 B1).

As used herein, the term “blocks SIRP α binding to human CD47” refers to a greater than 50% reduction of SIRP α -Fc binding to CD47 on Jurkat cells by an anti-CD47 mAb.

15 The anti-CD47 mAbs of the disclosure described herein, block the interaction of CD47 and SIRP α and increase phagocytosis of human tumor cells.

“Phagocytosis” of cancer cells refers to the engulfment and digestion of such cells by macrophages, and the eventual digestion or degradation of these cancer cells and the release of digested or degraded cellular components extracellularly, or intracellularly to undergo further 20 processing. Anti-CD47 monoclonal antibodies that block SIRP α binding to CD47 increase macrophage phagocytosis of cancer cells. SIRP α binding to CD47 on cancer cells would otherwise allow these cells to escape macrophage phagocytosis. The cancer cell may be viable or living cancer cells.

Inducing Death of Tumor Cells

25 Some soluble anti-CD47 mAbs initiate a cell death program on binding to CD47 on tumor cells, resulting in collapse of mitochondrial membrane potential, loss of ATP generating capacity, increased cell surface expression of phosphatidylserine (detected by increased staining for annexin V) and cell death without the participation of caspases or fragmentation of DNA. Such soluble anti-CD47 mAbs have the potential to treat a variety of solid and hematological cancers. Several 30 soluble anti-CD47 mAbs which have been shown to induce tumor cell death, including MABL-1, MABL-2 and fragments thereof (US Patent 8,101,719; Uno et al. *Oncol Rep.* 17: 1189-94, 2007;

Kikuchi et al. *Biochem Biophys Res. Commun.* 315: 912-8, 2004), Ad22 (Pettersen et al. *J. Immuno.* 166: 4931-4942, 2001; Lamy et al. *J. Biol. Chem.* 278: 23915-23921, 2003), and 1F7 (Manna et al. *J. Immunol.* 170: 3544-3553, 2003; Manna et al. *Cancer Research*, 64: 1026-1036, 2004). Some of the anti-CD47 mAbs of the disclosure described herein induce cell death of human
5 tumor cells.

The terms “inducing cell death” or “kills” and the like, are used interchangeably herein to mean that addition of an antibody compound of the present disclosure to cultured cancer cells causes these cells to display quantifiable characteristics associated with cell death including any one, or more, of the following:

- 10 1. Increased binding of Annexin V (in the presence of calcium ion) to the tumor cells as detected by flow cytometry or confocal fluorescence microscopy;
2. Increased uptake of the fluorescent compound propidium iodide (as assayed by flow cytometry) or 7-aminoactinomycin D (7-AAD as assayed by flow cytometry) or trypan blue (scored with light microscopy) by the tumor cells
- 15 3. Loss of mitochondrial function and membrane potential by the tumor cells as assayed by one of several available measures (potentiometric fluorescent dyes such as DiO-C6 or JC1 or formazan-based assays such as MTT or WST-1).

Induction of cell death refers to the ability of certain of the soluble anti-CD47 antibodies, murine antibodies, chimeric antibodies, humanized antibodies, or antigen-binding fragments
20 thereof (and competing antibodies and antigen-binding fragments thereof) disclosed herein to kill cancer cells via a cell autonomous mechanism without participation of complement or other cells including, but not limited to, T cells, neutrophils, natural killer cells, macrophages, or dendritic cells. Quantifiably, induction of cell death includes, but is not limited to, a greater than 2-fold increase in annexin V staining of human tumor cells caused by soluble anti-CD47 mAb compared
25 to the background obtained with the negative control antibody (humanized, isotype-matched antibody).

Among the present humanized or chimeric mAbs, those that induce cell death of human tumor cells cause increased Annexin V binding similar to the findings reported for anti-CD47 mAbs Ad22 (Pettersen et al. *J. Immuno.* 166: 4931-4942, 2001; Lamy et al. *J. Biol. Chem.* 278:
30 23915-23921, 2003); 1F7 (Manna and Frazier *J. Immunol.* 170:3544-3553, 2003; Manna and

Frazier *Cancer Res.* 64:1026-1036, 2004); and MABL-1 and 2 (US Patent 7,531,643 B2; US Patent 7,696,325 B2; US Patent 8,101,719 B2).

Cell viability assays are described in NCI/NIH guidance manual that describes numerous types of cell based assays that can be used to assess induction of cell death caused by CD47 antibodies: “Cell Viability Assays”, Terry L Riss, PhD, Richard A Moravec, BS, Andrew L Niles, MS, Helene A Benink, PhD, Tracy J Worzella, MS, and Lisa Minor, PhD. Contributor Information, published May 1, 2013.

Binding to hRBCs

CD47 is expressed on human erythrocytes (hRBCs) (Brown. *J Cell Biol.* 111: 2785-2794, 1990; Avent. *Biochem J.*, (1988) 251: 499-505; Knapp. *Blood*, (1989) Vol. 74, No. 4, 1448-1450; Oliveira et al. *Biochimica et Biophysica Acta* 1818: 481–490, 2012; Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271). It has been shown that anti-CD47 mAbs bind to RBCs, including B6H12 (Brown et al. *J. Cell Biol.*, 1990, Oliveira et al. *Biochimica et Biophysica Acta* 1818: 481–490, 2012, Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271), BRIC125 (Avent. *Biochem J.*, (1988) 251: 499-505), BRIC126 (Avent. *Biochem J.*, (1988) 251: 499-505; Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271), 5F9 (Uger R. et al. *Cancer Res* 2014; 74(19 Suppl): Abstract nr 5011, Liu et al. *PLoS One.* 2015 Sep 21;10(9): e0137345; Sikic B. et al. *J Clin Oncol* 2016;34 (suppl; abstract 3019)), anti-CD47 antibodies disclosed in US Patent Publication 2014/0161799, WO Publication 2014/093678, US Patent Publication 2014/0363442, and CC2C6 (Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271, Uger R. et al. *Cancer Res* 2014; 74(19 Suppl): Abstract nr 5011). It has also been shown that a SIRP α -Fc fusion protein, which binds to human CD47, has reduced binding to human RBCs compared to other human cells (Uger R. et al. *Cancer Res* 2014; 74(19 Suppl): Abstract nr 5011). Binding to RBCs can be reduced by generation of bi-specific antibodies with only one CD47 binding arm (Masternak et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 2482).

Because some anti-CD47 mAbs have been shown to result in reduction of RBCs when administered to cynomolgus monkeys (Mounho-Zamora B. et al. *The Toxicologist, Supplement to Toxicological Sciences*, 2015; 144 (1): Abstract 596: 127, Liu et al. *PLoS One.* 2015 Sep 21;10(9): e0137345; Pietsch et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 2470), it is highly desirable to identify anti-CD47 mAbs that do not bind to CD47-expressing RBCs.

As used herein, the terms “red blood cell(s)” and “erythrocyte(s)” are synonymous and used interchangeably herein.

As used herein, the terms “reduced binding to hRBCs”, refers to the K_d of an anti-CD47 mAb binding to a hRBC which is 10-fold or greater than the K_d on a human tumor cell, wherein the tumor cell is an OV10hCD47 cell.

As used herein, the term “no binding” or “NB”, refers to no measurable binding to hRBCs at an anti-CD47 mAb concentration up to and including 100 $\mu\text{g/ml}$.

Prior to the disclosure described herein, no anti-CD47 mAbs have been reported that do not bind to human RBCs expressing CD47.

Some of the anti-CD47 mAbs, disclosed herein, have reduced or no detectable binding to human RBCs.

Agglutination of RBCs

Red blood cell (RBC) agglutination or hemagglutination is a homotypic interaction that occurs when RBCs aggregate or clump together following incubation with various agents, including antibodies to RBC antigens and cell surface proteins such as CD47. Many anti-CD47 antibodies have been reported to cause hemagglutination of isolated human RBCs in vitro, in a concentration dependent manner, including B6H12, BRIC126, MABL-1, MABL-2, CC2C6, and 5F9 (Uger R. et al. *Cancer Res* 2014; 74(19 Suppl): Abstract nr 5011, US Patent 9,045,541, Uno et al. *Oncol Rep.* 17: 1189-94, 2007; Kikuchi et al. *Biochem Biophys Res. Commun.* 315: 912-8, 2004; Sikic B. et al. *J Clin Oncol* 2016;34 (suppl; abstract 3019)). This functional effect requires binding to RBCs by an intact, bivalent antibody and can be reduced or eliminated by generating antibody fragments, either a F(ab') or svFv (Uno et al. *Oncol Rep.* 17: 1189-94, 2007; Kikuchi et al. *Biochem Biophys Res. Commun.* 315: 912-8, 2004) or bi-specific antibodies with only one CD47 binding arm (Masternak et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 2482). Other functional properties of these fragments, including cell killing, were shown to be either reduced or retained in these fragments (Uno et al. *Oncol Rep.* 17: 1189-94, 2007; Kikuchi et al. *Biochem Biophys Res. Commun.* 315: 912-8, 2004). The mouse antibody 2D3 is an example of an anti-CD47 antibody that binds to CD47 on red blood cells but does not cause hemagglutination (US Patent 9,045,541, Petrova et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271).

Hemagglutination has been shown to be reduced/eliminated by reducing the binding selectively to human RBCs, but not other cells, using a SIRP α -Fc fusion protein (Uger R. et al. *Blood* 2013;

122(21): 3935). In addition, mouse anti-CD47 mAb 2A1 and humanized versions of 2A1 have been reported to block CD47/SIRP α but do not exhibit hemagglutination activity (US Patent 9,045,541). A small number of a panel of mouse anti-human CD47 antibodies (3 of 23) were reported to not cause hemagglutination of human RBCs (Pietsch E et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 2470). Therefore, prior to the disclosure described herein, there was a need to identify CD47 mAbs that block SIRP α /CD47 binding, have no detectable or reduced binding to RBCs and/or cause no hemagglutination. The term “agglutination” refers to cellular clumping, while the term “hemagglutination” refers to clumping of a specific subset of cells, i.e., RBCs. Thus, hemagglutination is a type of agglutination.

As used herein, the term "reduced hemagglutination" refers to measurable agglutination activity of hRBCs at anti-CD47 mAb concentrations greater than 1.85 μ g/ml, and no measurable activity at concentrations less than or equal to 1.85 μ g/ml.

As used herein, the term “no detectable hemagglutination”, refers to no measurable agglutination activity of hRBCs at anti-CD47 mAb concentrations greater than or equal to 0.3 pg/mL to a concentration less than or equal to 50 μ g/mL.

Some of the anti-CD47 antibodies described herein, cause reduced or no detectable hemagglutination of human RBCs.

Modulation of the NO Pathway

As noted above, TSP1 is also a ligand for CD47. The TSP1/CD47 pathway opposes the beneficial effects of the NO pathway in many cell types, including, but not limited to, vascular cells. The NO pathway consists of any of three enzymes (nitric oxide synthases, NOS I, NOS II and NOS III) that generate bioactive gas NO using arginine as a substrate. NO can act within the cell in which it is produced, or in neighboring cells, to activate the enzyme soluble guanylyl cyclase that produces the messenger molecule cyclic GMP (cGMP). The proper functioning of the NO/cGMP pathway is essential for protecting the cardiovascular system against stresses including, but not limited to, those resulting from wounding, inflammation, hypertension, metabolic syndrome, ischemia, and IRI. In the context of these cellular stresses the inhibition of the NO/cGMP pathway by the TSP1/CD47 system exacerbates the effects of stress. This is a particular problem in the cardiovascular system where both cGMP and cAMP play important protective roles. There are many cases in which ischemia and reperfusion injury cause or contribute to disease, trauma, and poor outcomes of surgical procedures.

As disclosed herein, one of more of the chimeric or humanized anti-CD47 antibodies will reverse TSP1 inhibition of cGMP production. Reversal will be complete (>80 %) or intermediate (20% -80%). This reversal of TSP1 inhibition of cGMP production will demonstrate that the anti-CD47 mAbs have the ability to increase NO signaling and suggest utility in protecting the cardiovascular system against stresses including, but not limited to, those resulting from wounding, inflammation, hypertension, metabolic syndrome, ischemia, and ischemia-reperfusion injury (IRI). Additional assay systems, for example smooth muscle cell contraction, will also be expected to show that some of the chimeric or humanized antibodies reverse the inhibitory actions of TSP1 on downstream effects resulting from the activation of NO signaling.

As disclosed herein, “complete reversal of NO pathway inhibition” refers to greater than 80% reversal of TSP1 inhibition of NO signaling by an anti-CD47 mAb compared to a negative control, humanized isotype-matched antibody.

As disclosed herein, “intermediate reversal of NO pathway inhibition” refers to 20-80% reversal of TSP1 inhibition of NO signaling by an anti-CD47 mAb compared to a negative control, humanized isotype-matched antibody.

As disclosed herein, “no reversal of NO pathway inhibition” refers to less than 20% reversal of TSP1 inhibition of NO signaling by an anti-CD47 mAb compared to a negative control, humanized isotype-matched antibody.

Preferred Combinations of Functional Properties

Anti-CD47 mAbs exist in the prior art with combinations of some, but not all, of the functional characteristics described herein. Previously, it has been shown that humanized anti-CD47 mAbs such as AB6.12 IgG1, AB6.12-IgG4P, and AB6.12-IgG4PE (US Patent 9,045,541, US Patent Publication 2014/0161799, WO Publication 2014/093678, US Patent Publication 2014/0363442) and 5F9 (Mounho-Zamora B. et al. *The Toxicologist, Supplement to Toxicological Sciences*, 2015; 144 (1): Abstract 596: 127, Liu et al. *PLoS One*. 2015 Sep 21; 10(9): e0137345) bind human CD47, block the interaction of CD47 and SIRP α and cause phagocytosis of human tumor cells. The humanized CD47 mAbs AB6.12 IgG1, AB6.12-IgG4P, and AB6.12-IgG4PE also do not cause hemagglutination of human RBCs (US Patent 9,045,541). The 5F9 humanized anti-CD47 mAb binds to and causes hemagglutination of human RBCs (Uger R. et al. *Cancer Res* 2014; 74(19 Suppl): Abstract nr 5011, Sikic B. et al. *J Clin Oncol* 2016;34 (suppl; abstract 3019). Murine anti-CD47 mAbs B6H12, BRIC126, and CC2C6 block the interaction of CD47 and

SIRP α , cause phagocytosis, and bind to and cause hemagglutination of human RBCs (Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271, Seiffert et al. *Blood* 94:3633-3643,1999; Vernon-Wilson et al. *Eur J Immunol.* 30: 2130-2137, 2000; Latour et al. *J. Immunol.* 167: 2547-2554, 2001; Subramanian et al. *Blood* 107: 2548-2556, 2006; Liu et al. *J Biol. Chem.* 277: 10028-10036, 2002). Murine anti-CD47 mAbs MABL-1 and MABL-2 bind to human CD47, induce tumor cell death and cause RBC hemagglutination (US Patent 8,101,719); murine mAb Ad22 binds to human CD47 and induces tumor cell death (Pettersen et al. *J. Immunol.* 166: 4931-4942, 2001; Lamy et al. *J Biol Chem.* 278: 23915-23921, 2003); and murine mAb 1F7 binds to human CD47, blocks the interaction of CD47 and SIRP α and induces tumor cell death (Rebres et al. *J. Cellular Physiol.* 205: 182-193, 2005; Manna et al. *J. Immunol.* 170: 3544-3553, 2003; Manna et al. *Cancer Research*, 64: 1026-1036, 2004).

Preferred embodiments of the anti-CD47 antibodies described herein, are also characterized by combinations of properties which are not exhibited by prior art anti-CD47 antibodies proposed for human therapeutic use. Accordingly, the preferred anti-CD47 antibodies described herein are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells; and
- d. induces death of susceptible human tumor cells.

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- d. induces death of susceptible human tumor cells; and
- e. causes no agglutination of human red blood cells (hRBCs).

In yet another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,

- d. induces death of susceptible human tumor cells; and
- e. causes reduced agglutination of human red blood cells (hRBCs).

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- 5 a. specifically binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells
- d. induces death of susceptible human tumor cells; and
- e. has reduced hRBC binding.

10 In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. binds to human CD47,
- b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- 15 d. causes no agglutination of human red blood cells (hRBCs); and
- e. does not bind to hRBCs.

In another preferred embodiment described herein, the anti-CD47 antibodies are characterized by:

- a. specifically binds to human CD47,
- 20 b. blocks SIRP α binding to human CD47,
- c. increases phagocytosis of human tumor cells,
- d. causes no agglutination of human red blood cells (hRBCs); and
- e. has reduced hRBC binding.

25 In another preferred embodiment described herein, the monoclonal antibody, or antigen binding fragment thereof also specifically binds to non-human primate CD47, wherein non-human primate may include, but is not limited to, cynomolgus monkey, green monkey, rhesus monkey and squirrel monkey.

In yet another preferred embodiment described herein, the monoclonal antibody, or antigen binding fragment thereof binds human, non-human primate, mouse, rabbit, and rat CD47.

30 Described herein, are anti-CD47 mAbs with distinct functional profiles. These antibodies possess distinct combinations of properties selected from the following: 1) exhibit cross-reactivity

with one or more species homologs of CD47; 2) block the interaction between CD47 and its ligand SIRP α ; 3) increase phagocytosis of human tumor cells, 4) induce death of susceptible human tumor cells; 5) do not induce cell death of human tumor cells; 6) have reduced binding to human red blood cells (hRBCs); 7) have no detectable binding to hRBCs; 8) cause reduced agglutination of hRBCs; 9) cause no detectable agglutination of hRBCs; 10) reverse TSP1 inhibition of the nitric oxide (NO) pathway and/or 11) do not reverse TSP1 inhibition of the NO pathway.

CD47 Antibodies

Many human cancers up-regulate cell surface expression of CD47 and those expressing the highest levels of CD47 appear to be the most aggressive and the most lethal for patients. Increased CD47 expression is thought to protect cancer cells from phagocytic clearance by sending a “don’t eat me” signal to macrophages via SIRP α , an inhibitory receptor that prevents phagocytosis of CD47-bearing cells (Oldenborg et al. *Science* 288: 2051-2054, 2000; Jaiswal et al. (2009) *Cell* 138(2):271-851; Chao et al. (2010) *Science Translational Medicine* 2(63):63ra94). Thus, the increase of CD47 expression by many cancers provides them with a cloak of “selfness” that slows their phagocytic clearance by macrophages and dendritic cells.

Antibodies that block CD47 and prevent its binding to SIRP α have shown efficacy in human tumor in murine (xenograft) tumor models. Such blocking anti-CD47 mAbs exhibiting this property increase the phagocytosis of cancer cells by macrophages, which can reduce tumor burden (Majeti et al. (2009) *Cell* 138 (2): 286-99; US 9,045,541; Willingham et al. (2012) *Proc Natl Acad. Sci. USA* 109(17):6662-6667; Xiao et al. (2015) *Cancer Letters* 360:302-309; Chao et al. (2012) *Cell* 142:699-713; Kim et al. (2012) *Leukemia* 26:2538-2545) and may ultimately lead to generation of an adaptive immune response to the tumor (Tseng et al. (2013) *PNAS* 110 (27):11103-11108; Soto-Pantoja et al. (2014) *Cancer Res.* 74 (23): 6771-6783; Liu et al. (2015) *Nat. Med.* 21 (10): 1209-1215).

However, there are mechanisms by which anti-CD47 mAbs can attack transformed cells that have not yet been exploited in the treatment of cancer. Multiple groups have shown that particular anti-human CD47 mAbs induce cell death of human tumor cells. Anti-CD47 mAb AD22 induces cell death of multiple human tumor cells lines (Pettersen et al. *J. Immuno.* 166: 4931-4942, 2001; Lamy et al. *J. Biol. Chem.* 278: 23915-23921, 2003). AD22 was shown to induce rapid mitochondrial dysfunction and rapid cell death with early phosphatidylserine exposure and a drop in mitochondrial membrane potential (Lamy et al. *J. Biol. Chem.* 278: 23915-23921, 2003). Anti-

CD47 mAb MABL-2 and fragments thereof induce cell death of human leukemia cell lines, but not normal cells *in vitro* and had an anti-tumor effect in *in vivo* xenograft models. (Uno et al. (2007) *Oncol. Rep.* 17 (5): 1189-94). Anti-human CD47 mAb 1F7 induces cell death of human T cell leukemias (Manna and Frazier (2003) *J. Immunol.* 170: 3544-53) and several breast cancers (Manna and Frazier (2004) *Cancer Research* 64 (3):1026-36). 1F7 kills CD47-bearing tumor cells without the action of complement or cell mediated killing by NK cells, T cells, or macrophages. Instead, anti-CD47 mAb 1F7 acts via a non-apoptotic mechanism that involves a direct CD47-dependent attack on mitochondria, discharging their membrane potential and destroying the ATP-generating capacity of the cell leading to rapid cell death. It is noteworthy that anti-CD47 mAb 1F7 does not kill resting leukocytes, which also express CD47, but only those cells that are “activated” by transformation. Thus, normal circulating cells, many of which express CD47, are spared while cancer cells are selectively killed by the tumor-toxic CD47 mAb (Manna and Frazier (2003) *J. Immunol.* 170: 3544-53). This mechanism can be thought of as a proactive, selective and direct attack on tumor cells in contrast to the passive mechanism of causing phagocytosis by simply blocking CD47/SIRP α binding. Importantly, mAb 1F7 also blocks binding of SIRP α to CD47 (Rebres et al et al. *J. Cellular Physiol.* 205: 182-193, 2005) and thus it can act via two mechanisms: (1) direct tumor toxicity, and (2) causing phagocytosis of cancer cells. A single mAb that can accomplish both functions may be superior to one that only blocks CD47/SIRP α binding.

Following periods of tissue ischemia, the initiation of blood flow causes damage referred to as "ischemia-reperfusion injury" or IRI. IRI contributes to poor outcomes in many surgical procedures where IRI occurs due to the necessity to stop blood flow for a period of time, in many forms/causes of trauma in which blood flow is interrupted and later restored by therapeutic intervention and in procedures required for organ transplantation, cardio/pulmonary bypass procedures, reattachment of severed body parts, reconstructive and cosmetic surgeries and other situations involving stopping and restarting blood flow. Ischemia itself causes many physiological changes that, by themselves would eventually lead to cell and tissue necrosis and death. Reperfusion poses its own set of damaging events including generation of reactive oxygen species, thrombosis, inflammation and cytokine mediated damage. The pathways that are limited by the TSP1-CD47 system are precisely those that would be of most benefit in combating the damage of IRI, including the NO pathway. Thus, blocking the TSP1-CD47 pathway, as with the antibodies disclosed herein, will provide more robust functioning of these endogenous protective pathways.

Anti-CD47 mAbs have been shown to reduce organ damage in rodent models of renal warm ischemia (Rogers et al. *J Am Soc Nephrol.* 23: 1538-1550, 2012), liver ischemia-reperfusion injury (Isenberg et al. *Surgery.* 144: 752-761, 2008), renal transplantation (Lin et al. *Transplantation.* 98: 394-401, 2014; Rogers et al. *Kidney International.* 90: 334-347, 2016)) and liver transplantation, including steatotic
 5 livers (Xiao et al. *Liver Transpl.* 21: 468-477, 2015; Xiao et al. *Transplantation.* 100: 1480-1489, 2016). In addition, anti-CD47 mAb caused significant reductions of right ventricular systolic pressure and right ventricular hypertrophy in the monocrotaline model of pulmonary arterial hypertension (Bauer et al. *Cardiovasc Res.* 93: 682-693, 2012). Studies in skin flap models have shown that modulation of CD47, including with anti-CD47 mAbs, inhibits TSP1-mediated CD47 signaling. This
 10 results in increased activity of the NO pathway, resulting in reduced IRI (Maxhimer et al. *Plast Reconstr Surg.* 124: 1880-1889, 2009; Isenberg et al. *Arterioscler Throm Vasc Biol.* 27: 2582-2588, 2007; Isenberg et al. *Curr Drug Targets.* 9: 833-841, 2008; Isenberg et al. *Ann Surg.* 247: 180-190, 2008)

Anti-CD47 mAbs have also been shown to be efficacious in models of other cardiovascular diseases. In the mouse transverse aortic constriction model of pressure overload left ventricular heart
 15 failure, anti-CD47 mAb mitigated cardiac myocyte hypertrophy, decreased left ventricular fibrosis, prevented an increase in left ventricular weight, decreased ventricular stiffness, and normalized changes in the pressure volume loop profile (Sharifi-Sanjani et al. *J Am Heart Assoc.*, 2014). An anti-CD47 mAb ameliorated atherosclerosis in multiple mouse models (Kojima et al. *Nature.*, 2016).

Cancer Indications

Presently disclosed are anti-CD47 mAbs and antigen binding fragments thereof effective
 20 as cancer therapeutics which can be administered to patients, preferably parenterally, with susceptible hematologic cancers and solid tumors including, but not limited to, leukemias, including systemic mastocytosis, acute lymphocytic (lymphoblastic) leukemia (ALL), T cell – ALL, acute myeloid leukemia (AML), myelogenous leukemia, chronic lymphocytic leukemia
 25 (CLL), chronic myeloid leukemia (CML), myeloproliferative disorder / neoplasm, monocytic cell leukemia, and plasma cell leukemia; multiple myeloma (MM); Waldenstrom's Macroglobulinemia; lymphomas, including histiocytic lymphoma and T cell lymphoma, B cell lymphomas, including Hodgkin's lymphoma and non-Hodgkin's lymphoma, such as low grade/follicular non-Hodgkin's lymphoma (NHL), cell lymphoma (FCC), mantle cell lymphoma
 30 (MCL), diffuse large cell lymphoma (DLCL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL; solid

tumors, including ovarian cancer, breast cancer, endometrial cancer, colon cancer (colorectal cancer), rectal cancer, bladder cancer, urothelial cancer, lung cancer (non-small cell lung cancer, adenocarcinoma of the lung, squamous cell carcinoma of the lung), bronchial cancer, bone cancer, prostate cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma (liver cancer, hepatoma), gall bladder cancer, bile duct cancer, esophageal cancer, renal cell carcinoma, thyroid cancer, squamous cell carcinoma of the head and neck (head and neck cancer), testicular cancer, cancer of the endocrine gland, cancer of the adrenal gland, cancer of the pituitary gland, cancer of the skin, cancer of soft tissues, cancer of blood vessels, cancer of brain, cancer of nerves, cancer of eyes, cancer of meninges, cancer of oropharynx, cancer of hypopharynx, cancer of cervix, and cancer of uterus, glioblastoma, medulloblastoma, astrocytoma, glioma, meningioma, gastrinoma, neuroblastoma, myelodysplastic syndrome, and sarcomas including, but not limited to, osteosarcoma, Ewing's sarcoma, leiomyosarcoma, synovial sarcoma, alveolar soft part sarcoma, angiosarcoma, liposarcoma, fibrosarcoma, rhabdomyosarcoma, and chondrosarcoma; and melanoma.

Treatment of Cancer

As is well known to those of ordinary skill in the art, combination therapies are often employed in cancer treatment as single-agent therapies or procedures may not be sufficient to treat or cure the disease or condition. Conventional cancer treatments often involve surgery, radiation treatment, the administration of a combination of cytotoxic drugs to achieve additive or synergistic effects, and combinations of any or all of these approaches. Especially useful chemotherapeutic and biologic therapy combinations employ drugs that work via different mechanisms of action, increasing cancer cell control or killing, increasing the ability of the immune system to control cancer cell growth, reducing the likelihood of drug resistance during therapy, and minimizing possible overlapping toxicities by permitting the use of reduced doses of individual drugs.

Classes of conventional anti-tumor/anti-neoplastic agents useful in the combination therapies encompassed by the present methods are disclosed, for example, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, Twelfth Edition (2010) L.L. Brunton, B.A. Chabner, and B. C. Knollmann Eds., Section VIII, "Chemotherapy of Neoplastic Diseases", Chapters 60-63, pp. 1665-1770, McGraw-Hill, NY, and include, for example, alkylating agents, antimetabolites, natural products, a variety of miscellaneous agents, hormones and antagonists, targeted drugs, monoclonal antibodies and other protein therapeutics.

In addition to the foregoing, the methods of the present disclosure are related to treatment of cancer indications and further comprises treating the patient via surgery, radiation, and/or administering to a patient in need thereof an effective amount of a chemical small molecule or biologic drug including, but not limited to, a peptide, polypeptide, protein, nucleic acid therapeutic, conventionally used or currently being developed, to treat tumorous conditions. This includes antibodies and antigen-binding fragments, other than those disclosed herein, cytokines, antisense oligonucleotides, siRNAs, and miRNAs.

The therapeutic methods disclosed and claimed herein include the use of the antibodies disclosed herein alone, and/or in combinations with one another, and/or with antigen-binding fragments thereof of the present disclosure that bind to CD47, and/or with competing antibodies exhibiting appropriate biological/therapeutic activity, as well, for example, all possible combinations of these antibody compounds to achieve the greatest treatment efficacy.

In addition, the present therapeutic methods also encompass the use of these antibodies, antigen-binding fragments thereof, competing antibodies and combinations thereof further in combination with: (1) any one or more anti-tumor therapeutic treatments selected from surgery, radiation, anti-tumor, anti-neoplastic agents, and combinations of any of these, or (2) any one or more of anti-tumor biological agents, or (3) equivalents of any of the foregoing of (1) or (2) as would be apparent to one of ordinary skill in the art, in appropriate combination(s) to achieve the desired therapeutic treatment effect for the particular indication.

Antibody and small molecule drugs that increase the immune response to cancer by modulating co-stimulatory or inhibitory interactions that influence the T cell response to tumor antigens, including inhibitors of immune checkpoints and modulators of co-stimulatory molecules, are also of particular interest in the context of the combination therapeutic methods encompassed herein and include, but are not limited to, other anti-CD47 antibodies. Administration of therapeutic agents that bind to the CD47 protein, for example, antibodies or small molecules that bind to CD47 and prevent interaction between CD47 and SIRP α , are administered to a patient, causing the clearance of cancer cells via phagocytosis. The therapeutic agent that binds to the CD47 protein is combined with a therapeutic agent such as an antibody, a chemical small molecule or biologic drug disclosed herein, directed against one or more additional cellular targets of CD70 (Cluster of Differentiation 70), CD200 (OX-2 membrane glycoprotein, Cluster of Differentiation 200), CD154 (Cluster of Differentiation 154, CD40L, CD40 ligand, Cluster of Differentiation 40

ligand), CD223 (Lymphocyte-activation gene 3, LAG3, Cluster of Differentiation 223), KIR (Killer-cell immunoglobulin-like receptors), GTR (TNFRSF18, glucocorticoid-induced TNFR-related protein, activation-inducible TNFR family receptor, AITR, Tumor necrosis factor receptor superfamily member 18), CD28 (Cluster of Differentiation 28), CD40 (Cluster of Differentiation 40, Bp50, CDW40, TNFRSF5, Tumor necrosis factor receptor superfamily member 5, p50), CD86 (B7-2, Cluster of Differentiation 86), CD160 (Cluster of Differentiation 160, BY55, NK1, NK28), CD258 (LIGHT, Cluster of Differentiation 258, Tumor necrosis factor ligand superfamily member 14, TNFSF14, HVEM, HVEM ligand, herpesvirus entry mediator ligand, LTg), CD270 (HVEM, Tumor necrosis factor receptor superfamily member 14, herpesvirus entry mediator, Cluster of Differentiation 270, LIGHTR, HVEA), CD275 (ICOSL, ICOS ligand, Inducible T-cell co-stimulator ligand, Cluster of Differentiation 275), CD276 (B7-H3, B7 homolog 3, Cluster of Differentiation 276), OX40L (OX40 Ligand), B7-H4 (B7 homolog 4, VTCN1, V-set domain-containing T-cell activation inhibitor 1), GITRL (Glucocorticoid-induced tumor necrosis factor receptor-ligand, glucocorticoid-induced TNFR-ligand), 4-1BBL (4-1BB ligand), CD3 (Cluster of Differentiation 3, T3D), CD25 (IL2R α , Cluster of Differentiation 25, Interleukin-2 Receptor α chain, IL-2 Receptor α chain), CD48 (Cluster of Differentiation 48, B-lymphocyte activation marker, BLAST-1, signaling lymphocytic activation molecule 2, SLAMF2), CD66a (Ceacam-1, Carcinoembryonic antigen-related cell adhesion molecule 1, biliary glycoprotein, BGP, BGP1, BGPI, Cluster of Differentiation 66a), CD80 (B7-1, Cluster of Differentiation 80), CD94 (Cluster of Differentiation 94), NKG2A (Natural killer group 2A, killer cell lectin-like receptor subfamily D member 1, KLRD1), CD96 (Cluster of Differentiation 96, TActILE, T cell activation increased late expression), CD112 (PVRL2, nectin, Poliovirus receptor-related 2, herpesvirus entry mediator B, HVEB, nectin-2, Cluster of Differentiation 112), CD115 (CSF1R, Colony stimulating factor 1 receptor, macrophage colony-stimulating factor receptor, M-CSFR, Cluster of Differentiation 115), CD205 (DEC-205, LY75, Lymphocyte antigen 75, Cluster of Differentiation 205), CD226 (DNAM1, Cluster of Differentiation 226, DNAX Accessory Molecule-1, PTA1, platelet and T cell activation antigen 1), CD244 (Cluster of Differentiation 244, Natural killer cell receptor 2B4), CD262 (DR5, TrailR2, TRAIL-R2, Tumor necrosis factor receptor superfamily member 10b, TNFRSF10B, Cluster of Differentiation 262, KILLER, TRICK2, TRICKB, ZTNFR9, TRICK2A, TRICK2B), CD284 (Toll-like Receptor-4, TLR4, Cluster of Differentiation 284), CD288 (Toll-like Receptor-8, TLR8, Cluster of Differentiation 288), TNFSF15 (Tumor necrosis factor

superfamily member 15, Vascular endothelial growth inhibitor, VEGI, TL1A), TDO2 (Tryptophan
 2,3-dioxygenase, TPH2, TRPO), IGF-1R (Type 1 Insulin-like Growth Factor), GD2
 (Disialoganglioside 2), TMIGD2 (Transmembrane and immunoglobulin domain-containing
 protein 2), RGMB (RGM domain family, member B), VISTA (V-domain immunoglobulin-
 5 containing suppressor of T-cell activation, B7-H5, B7 homolog 5), BTNL2 (Butyrophilin-like
 protein 2), Btn (Butyrophilin family), TIGIT (T cell Immunoreceptor with Ig and ITIM domains,
 Vstm3, WUCAM), Siglecs (Sialic acid binding Ig-like lectins), Neuropilin, VEGFR (Vascular
 endothelial growth factor receptor), ILT family (LIRs, immunoglobulin-like transcript family,
 leukocyte immunoglobulin-like receptors), NKG families (Natural killer group families, C-type
 10 lectin transmembrane receptors), MICA (MHC class I polypeptide-related sequence A), TGFβ
 (Transforming growth factor β), STING pathway (Stimulator of interferon gene pathway),
 Arginase (Arginine amidinase, canavanase, L-arginase, arginine transamidinase), EGFRvIII
 (Epidermal growth factor receptor variant III), and HHLA2 (B7-H7, B7y, HERV-H LTR-
 associating protein 2, B7 homolog 7), inhibitors of PD-1 (Programmed cell death protein 1, PD-
 15 1, CD279, Cluster of Differentiation 279), PD-L1 (B7-H1, B7 homolog 1, Programmed death-
 ligand 1, CD274, cluster of Differentiation 274), PD-L2 (B7-DC, Programmed cell death 1 ligand
 2, PDCD1LG2, CD273, Cluster of Differentiation 273), CTLA-4 (Cytotoxic T-lymphocyte-
 associated protein 4, CD152, Cluster of Differentiation 152), BTLA (B- and T-lymphocyte
 attenuator, CD272, Cluster of Differentiation 272), Indoleamine 2,3-dioxygenase (IDO, IDO1),
 20 TIM3 (HAVCR2, Hepatitis A virus cellular receptor 2, T cell immunoglobulin mucin-3, KIM-3,
 Kidney injury molecule 3, TIMD-3, T cell immunoglobulin mucin-domain 3), A2A adenosine
 receptor (ADO receptor), CD39 (ectonucleoside triphosphate diphosphohydrolase-1, Cluster of
 Differentiation 39, ENTPD1), and CD73 (Ecto-5'-nucleotidase, 5'-nucleotidase, 5'-NT, Cluster of
 Differentiation 73), CD27 (Cluster of Differentiation 27), ICOS (CD278, Cluster of Differentiation
 25 278, Inducible T-cell Co-stimulator), CD137 (4-1BB, Cluster of Differentiation 137, tumor
 necrosis factor receptor superfamily member 9, TNFRSF9), OX40 (CD134, Cluster of
 Differentiation 134), and TNFSF25 (Tumor necrosis factor receptor superfamily member 25),
 including antibodies, small molecules, and agonists, are also specifically contemplated herein.
 Additional agents include IL-10 (Interleukin-10, human cytokine synthesis inhibitory factor,
 30 CSIF) and Galectins.

YERVOY[®] (ipilimumab; Bristol-Meyers Squibb) is an example of an approved anti-CTLA-4 antibody.

KEYTRUDA[®] (pembrolizumab; Merck) and OPDIVO[®] (nivolumab; Bristol-Meyers Squibb Company) are examples of approved anti-PD-1 antibodies.

5 TECENTRIQ[™] (atezolizumab; Roche) is an example of an approved anti-PD-L1 antibody.

Ischemia-Reperfusion Injury (IRI)-Related, Autoimmune, Autoinflammatory, Inflammatory, Cardiovascular Conditions and Diseases

Administration of a CD47 mAb or antigen binding fragment thereof disclosed herein can be used to treat a number of diseases and conditions in which IRI is a contributing feature, and to
10 treat various autoimmune, autoinflammatory, inflammatory and cardiovascular diseases. These include: organ transplantation in which a mAb or antigen binding fragment thereof of the present invention is administered to the donor prior to organ harvest, to the harvested donor organ in the organ preservation solution, to the recipient patient, or to any combination thereof; skin grafting; surgical resections or tissue reconstruction in which such mAb or fragment is administered either
15 locally by injection to the affected tissue or parenterally to the patient; reattachment of body parts; treatment of traumatic injury; pulmonary hypertension; pulmonary arterial hypertension; sickle cell disease (crisis); myocardial infarction; cerebrovascular disease; stroke; surgically-induced ischemia; acute kidney disease/kidney failure; any other condition in which IRI occurs and contributes to the pathogenesis of disease; autoimmune and inflammatory diseases, including
20 arthritis, rheumatoid arthritis, multiple sclerosis, psoriasis, psoriatic arthritis, Crohn's disease, inflammatory bowel disease, ulcerative colitis, lupus, systemic lupus erythematosus, juvenile rheumatoid arthritis, juvenile idiopathic arthritis, Grave's disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, dermatomyositis, multiple sclerosis, myasthenia gravis, pernicious anemia, Sjogren syndrome, type I diabetes, vasculitis, uveitis and ankylosing
25 spondylitis; autoinflammatory diseases, including familial Mediterranean fever, neonatal onset multisystem inflammatory disease, tumor necrosis factor (TNF) receptor-associated periodic syndrome, deficiency of the interleukin-1 receptor antagonist, Behcet's disease; cardiovascular diseases, including coronary heart disease, coronary artery disease, atherosclerosis, myocardial infarction, heart failure, and left ventricular heart failure.

30 Anti-CD47 mAbs and antigen binding fragments thereof of the present invention can also be used to increase tissue perfusion in a subject in need of such treatment. Such subjects can be

identified by diagnostic procedures indicating a need for increased tissue perfusion. In addition, the need for increased tissue perfusion may arise because the subject has had, is having, or will have, a surgery selected from integument surgery, soft tissue surgery, composite tissue surgery, skin graft surgery, resection of a solid organ, organ transplant surgery, or reattachment or an appendage or other body part.

Treatment of Ischemia-Reperfusion Injury (IRI)-Related Indications

The methods of the present disclosure, for example those related to treatment of IRI-related indications, can further comprise administering to a patient in need thereof an effective amount of therapeutic agent that binds to the CD47 protein and a nitric oxide donor, precursor, or both; a nitric oxide generating topical agent; an agent that activates soluble guanylyl cyclase; an agent that inhibits cyclic nucleotide phosphodiesterases; or any combination of any of the foregoing.

In these methods, the nitric oxide donor or precursor can be selected from NO gas, isosorbide dinitrate, nitrite, nitroprusside, nitroglycerin, 3-Morpholiniosydnonimine (SIN-1), S-nitroso-N-acetylpenicillamine (SNAP), Diethylenetriamine/NO (DETA/NO), S-nitrosothiols, Bidil[®], and arginine.

The agent that activates soluble guanylyl cyclase can be a non-NO (nitric oxide)-based chemical activator of soluble guanylyl cyclase that increases cGMP levels in vascular cells. Such agents bind soluble guanylyl cyclase in a region other than the NO binding motif, and activate the enzyme regardless of local NO or reactive oxygen stress (ROS). Non-limiting examples of chemical activators of soluble guanylyl cyclase include organic nitrates (Artz et al. (2002) *J. Biol. Chem.* 277:18253-18256); protoporphyrin IX (Ignarro et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:2870-2873); YC-1 (Ko et al. (1994) *Blood* 84:4226-4233); BAY 41-2272 and BAY 41-8543 (Stasch et al. (2001 *Nature* 410 (6825): 212-5), CMF-1571, and A-350619 (reviewed in Evgenov et al. (2006) *Nat. Rev. Drug. Discov.* 5:755-768); BAY 58-2667 (Cinaciguat; Frey et al. (2008) *Journal of Clinical Pharmacology* 48 (12): 1400-10); BAY 63-2521 (Riociguat; Mittendorf et al. (2009) *Chemmedchem* 4 (5): 853-65). Additional soluble guanylyl cyclase activators are disclosed in Stasch et al. (2011) *Circulation* 123:2263-2273; Derbyshire and Marletta (2012) *Ann. Rev. Biochem.* 81:533-559, and Nossaman et al. (2012) *Critical Care Research and Practice*, Volume 2012, Article ID 290805, pages 1-12.

The agent that inhibits cyclic nucleotide phosphodiesterases can be selected from, tadalafil, vardenafil, udenafil, sildenafil and avanafil.

Treatment of Autoimmune, Autoinflammatory, Inflammatory Diseases and Cardiovascular Diseases

A therapeutic agent that binds to the CD47 protein for the treatment of an autoimmune, autoinflammatory, inflammatory disease and / or cardiovascular disease can be combined with one or more therapeutic agent(s) such as an antibody, a chemical small molecule, or biologic or a medical or surgical procedure which include, but are not limited to the following. For the treatment of autoimmune, autoinflammatory and inflammatory diseases, the combined therapeutic agents are: hydroxychloroquine, leflunomide, methotrexate, minocycline, sulfasalazine, abatacept, rituximab, tocilizumab, anti-TNF inhibitors or blockers (adalimumab, etanercept, infliximab, certolizumab pegol, golimumab), non-steroidal anti-inflammatory drugs, glucocorticoids, corticosteroids, intravenous immunoglobulin, anakinra, canakinumab, rilonacept, cyclophosphamide, mycophenolate mofetil, azathioprine, 6-mercaptopurine, belimumab, beta interferons, glatiramer acetate, dimethyl fumarate, fingolimod, teriflunomide, natalizumab, 5-aminosalicylic acid, mesalamine, cyclosporine, tacrolimus, pimecrolimus, vedolizumab, ustekinumab, secukinumab, ixekizumab, apremilast, budesonide and tofacitinib. For the treatment of atherosclerosis, the combined therapeutic agents or procedures are: medical procedures and/or surgery, including percutaneous coronary intervention (coronary angioplasty and stenting), coronary artery bypass grafting, and carotid endarterectomy; therapeutic agents, including angiotensin-converting enzyme (ACE) inhibitors (including ramipril, quinapril, captopril, and enalapril), calcium channel blockers (including amlodipine, nifedipine, verapamil, felodipine and diltiazem), angiotensin-receptor blockers (including eprosartan, olmesartan, azilsartan, valsartan, telmisartan, losartan, candesartan, and irbesartan), the combination of ezetimibe and simvastatin, PCSK9 inhibitors (including alirocumab and evolocumab), anacetrapib, and HMG-CoA inhibitors (including atorvastatin, pravastatin, simvastatin, rosuvastatin, pitavastatin, lovastatin and fluvastatin). For the treatment of heart failure, the combined therapeutic agents are: ACE inhibitors, angiotensin receptor blockers, angiotensin receptor neprilysin inhibitors (including the combination of sacubitril and valsartan), diuretics, digoxin, inotropes, beta blockers and aldosterone antagonists. For the treatment of pulmonary hypertension the combined therapeutic agents are: sildenafil, tadalafil, ambrisentan, bosentan, macitentan, riociguat, treprostinil, epoprostenol, iloprost, and selexipag.

As disclosed herein, the anti-CD47 mAb is administered before, at the same time or after the combined therapeutic agents or medical or surgical procedures.

Another useful class of compounds for the combination therapies contemplated herein includes modulators of SIRP α /CD47 binding such as antibodies to SIRP α , as well as soluble protein fragments of this ligand, or CD47 itself, inhibiting binding of, or interfering with binding of, SIRP α to CD47. It should be noted that the therapeutic methods encompassed herein include the use of the antibodies disclosed herein alone, in combination with one another, and/or with antigen-binding fragments thereof as well, for example, all possible combinations of these antibody compounds.

The examples illustrate various embodiments of the present disclosure, but should not be considered as limiting the disclosure to only these particularly disclosed embodiments.

Diagnostics for CD47 Expression

Diagnostics (including complementary and companion) have been an area of focus in the field of oncology. A number of diagnostic assays have been developed for targeted therapeutics such as Herceptin (Genentech), Tarceva (OSI Pharmaceuticals / Genentech), Iressa (Astra Zeneca), and Erbitux (Imclone / Bristol Myers Squibb). The anti-CD47 mAbs antibodies of the disclosure are particularly well-suited to use in diagnostic applications. Accordingly, the disclosure provides a method to measure CD47 expression in tumor and / or immune cells, using an anti-CD47 mAb of the disclosure.

The anti-CD47 mAbs of the disclosure may be used in a diagnostic assay and / or *in vitro* method to measure CD47 expression in tumor and / or immune cells present in a patient's tumor sample. In particular, the anti-CD47 mAbs of the disclosure may bind CD47 on approximately 1% or more of tumor and / or immune cells present in a patient's sample as compared to a reference level. In another embodiment, the anti-CD47 mAbs may bind CD47 on approximately 5% or more of tumor and / or immune cells in a patient's sample as compared to a reference level, for example, or binding at least 10%, or at least 20%, or at least 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or between 10-100% as compared to a reference level. In yet another embodiment, the anti-CD47 mAbs may bind CD47 on tumor and / or immune cells in a patient's sample to at least about a 2-fold increase as compared to a reference level, or at least about 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or between 2-fold and 10-fold or

greater as compared to a reference level. As described herein, the measurement of CD47 expression in a patient's sample provides biological and/or clinical information that enables decision making about the development and use of a potential drug therapy, notably the use of anti-CD47 antibodies for treating solid and hematological cancers, autoimmune disease, inflammatory disease, atherosclerosis, heart failure, in which the CD47 receptor plays a role.

In one embodiment, the *in vitro* method comprises, obtaining a patient sample, contacting the patient sample with a monoclonal antibody, or antigen-binding fragment thereof, which specifically binds to an epitope within the sequence of SEQ ID NO:65, and assaying for binding of the antibody to the patient sample, wherein binding of the antibody to the patient sample is diagnostic of CD47 expression in a patient sample.

In another embodiment, the preferred CD47 antibodies, or antigen binding fragments thereof, for the *in vitro* method, are those comprising a combination of a heavy chain (HC) and a light chain (LC), listed from the combination of:

- (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:102 and a light chain comprising the amino acid sequence SEQ ID NO:101;
- (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:104 and a light chain comprising the amino acid sequence SEQ ID NO:103;

wherein the V_H amino acid sequence is at least 90%, 95%, 97%, 98% or 99% identical thereto and the a V_L amino acid sequence is at least 90%, 95%, 97%, 98% or 99% identical thereto.

Accordingly, a diagnostic assay in accordance with the disclosure may comprise contacting tumor and / or immune cells in a patient's sample with an anti-CD47 mAb, or an antigen binding fragment thereof, and assaying for binding of the anti-CD47 mAb to a patient's tumor sample, wherein binding of the anti-CD47 mAb to the patient sample is diagnostic of CD47 expression. Preferably, the patient's sample is a sample containing tumor cells. In this case, binding of the anti-CD47 mAb of the disclosure, or antigen binding fragment thereof, to the tumor cells may be assessed for CD47 expression. The levels of CD47 expression by tumor cells and/or immune cells of a patient's tumor sample may be predictive of clinical outcome in a patient.

Increased binding of anti-CD47 mAbs binding to cells in a patient's sample is associated with increased CD47 expression. In one embodiment, the anti-CD47 mAbs of the disclosure may bind to approximately 5% or more of tumor cells in a patient's sample and this may indicate that

the patient would benefit from rapid intervention to a solid and hematological cancer. A diagnostic assay of this sort may be used to determine suitable therapeutic regimes for solid and hematological cancers with relatively high binding of anti-CD47 mAbs of the disclosure, i.e., increased CD47 expression.

It will be appreciated that the diagnostic assay disclosed herein has a number of advantages. The most important of these advantages is that the diagnostic assay of the disclosure may allow the user a greater deal of confidence in the CD47 expression in tumor and / or immune cells. The increased sensitivity of the diagnostic assay of the disclosure allows detection of CD47 in a patient's sample at lower levels than has previously been the case.

The anti-CD47 mAbs of the disclosure may be used as a diagnostic assay in relation to many forms of cancer. Particular forms of cancer that may advantageously be investigated for CD47 expression include susceptible hematologic cancers and solid tumors including, but not limited to, leukemias, lymphomas, and solid tumors.

The diagnostic assays of the disclosure may utilize any suitable means for detecting binding of an anti-CD47 mAb to measure CD47 expression. Suitable methods may be selected with reference to the nature of any reporter moiety used to label the anti-CD47 mAbs of the disclosure. Suitable techniques include, but are by no means limited to, flow cytometry, and enzyme linked immunosorbent assays (ELISA) and assays utilizing nanoparticles. It is particularly preferred that a diagnostic assay of the invention be one involving immunohistochemistry in which a tumor sample is exposed to an anti-CD47 mAb of the disclosure, and the level of cell labelling is assessed by immunohistochemistry.

EXAMPLES

Example 1

Amino Acid Sequences

Light Chain CDRs

	LCDR1	LCDR2	LCDR3
	<i>Vx4-LCDR1</i>	<i>Vx4-LCDR2</i>	<i>Vx4-LCDR3</i>
	RSRQSIVHTNGNTYLG (SEQ ID NO:11)	KVSNRFS (SEQ ID NO:15)	FQGSHPYT (SEQ ID NO:18)
	<i>Vx8-LCDR1</i>	<i>Vx8-LCDR2</i>	<i>Vx8-LCDR3</i>
	RASQDISNYLN (SEQ ID NO:12)	YTSRLYS (SEQ ID NO:16)	QQGNTLPWT (SEQ ID NO:19)

	<i>Vx8-LCDR1</i>		
	RASQSIISNYLN (SEQ ID NO:13)		
	<i>Vx9-LCDR1</i>	<i>Vx9-LCDR2</i>	<i>Vx9-LCDR3</i>
	RSSQNIVQSNGNTYLE (SEQ ID NO:14)	KVFHRFS (SEQ ID NO:17)	FQGSHPVWT (SEQ ID NO:20)

Heavy Chain CDRs

	HCDR1	HCDR2	HCDR3
	<i>Vx4-HCDR1</i>	<i>Vx4-HCDR2</i>	<i>Vx4-HCDR3</i>
	GYTFTNYVIH (SEQ ID NO:1)	YIYPYNDGILYNEKFKG (SEQ ID NO:4)	GGYYVPDY (SEQ ID NO:7)
			<i>Vx4-HCDR3</i>
			GGYYVYDY (SEQ ID NO:8)
	<i>Vx8-HCDR1</i>	<i>Vx8-HCDR2</i>	<i>Vx8-HCDR3</i>
	GYSFTNYIIH (SEQ ID NO:2)	YIDPLNGDTTYNQKFKG (SEQ ID NO:5)	GGKRAMDY (SEQ ID NO:9)
	<i>Vx9-HCDR1</i>	<i>Vx9-HCDR2</i>	<i>Vx9-HCDR3</i>
	GYTFTNYWIIH (SEQ ID NO:3)	YTDPRTDYTEYNQKFKD (SEQ ID NO:6)	GGRVGLGY (SEQ ID NO:10)

Murine Light Chain Variable Domains

5 >Vx4murL01

DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVS NRF
SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGGGKLEIK (SEQ ID
NO:41).

>Vx4murL02

10 DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVS NRF
SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGQGTKVEIK (SEQ ID
NO:42).

>Vx8murL03

15 DIQMTQTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLYSGVPS
RFGSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIK (SEQ ID NO:46).

>Vx9murL04

DVFMTQTPLSLPVSLGDQASISCRSSQNIVQSNGNTYLEWYLQKPGQSPKLLIYKVFHRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPWTFGGGTKVEIK(SEQ ID
NO:50)

5 Murine Heavy Chain Variable Domains

>Vx4murH01

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYVIHWVKRRPGQGLEWIGYIYPYNDGIL
YNEKFKGKATVTSDKSSSTAYMDLSSLTSEDSAVYYCTRGGYYVPDYWGQGTTLVSS
(SEQ ID NO:21).

10 > Vx4mur-H02

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYVIHWVKRRPGQGLEWIGYIYPYNDGIL
YNEKFKGKATVTSDKSSSTAYMDLSSLTSEDSAVYYCTRGGYYVPDYWGQGTLLTVSS
(SEQ ID NO:22).

> Vx8murH03

15 EVQLQQSGPELMKPGASVKISCKASGYSTFTNYVIHWVNQSHGKSLEWIGYIDPLNGDTT
YNQKFKGKATLTVDKSSSTAYMRLSSLTSADSAVYYCARGGKRAMDYWGQGTSTVTVSS
(SEQ ID NO:28).

>Vx9murH04

20 QVQLQQFGAELAKPGASVQMSCKASGYTFTNYWIHWVKQRPQGLEWIGYTDPRTDY
TEYNQKFKDKATLAADRSSSTAYMRLSSLTSEDSAVYYCAGGGRVGLGYWGHGSSVTV
SS (SEQ ID NO:35)

Human Light Chain Variable Domains

>Vx4humL01

25 DIVMTQSPLSLPVTPGEPASISCRSRQSIHTNGNTYLGWYLQKPGQSPRLLIYKVSNRFS
GVPDRFSGSGSGTDFTLKISRVEADDVGIYYCFQGSHVPYTFGQGTKLEIK (SEQ ID
NO:43)

>Vx4humL02

DVVMTQSPSLSPVTLGQPASISCRSRQSIVHTNGNTYLGWFQQRPGQSPRRLIYKVSNRFS
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIK (SEQ ID
NO:44)

5 >Vx4humL03

DIVMTQSPDSLAVSLGERATINCRSRQSIVHTNGNTYLGWYQQKPGQPPKLLIYKVSNRFS
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCFQGSHVPYTFGQGTKLEIK (SEQ ID
NO:45)

> Vx8humL04

10 DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLYSGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQGNTLPWTFGQGTKVEIK (SEQ ID NO:47).

> Vx8humL05

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK (SEQ ID NO:48).

15 > Vx8humL06

DIVMTQSPSLSPVTPGEPASISCRASQDISNYLNWYLQKPGQSPRLLIYYTSRLYSGVPDR
FSGSGSGTDFTLKISRVEADDVGIYYCQQGNTLPWTFGQGTKLEIK (SEQ ID NO:49)

>Vx9humL07

20 DVVMTQSPSLSPVTLGQPASISCRSSQNIVQSNGNTYLEWFQQRPGQSPRRLIYKVFHRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIK (SEQ ID
NO:51).

>Vx9humL08

25 DIVMTQSPDSLAVSLGERATINCRSSQNIVQSNGNTYLEWYQQKPGQPPKLLIYKVFHRF
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCFQGSHVPYTFGQGTKLEIK (SEQ ID
NO:52).

Human Heavy Chain Variable Domains

>Vx4humH01

QVQLVQSGAEVKKPGASVQVSCKASGYTFTNYVIHWLRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGYYVPDYWGQATLTVS
S (SEQ ID NO:23).

5 >Vx4humH02

QVQLVQSGAEVKKPGASVQVSCKASGYTFTNYVIHWLRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGYYVYDYWGQATLTVS
S (SEQ ID NO:24).

>Vx4humH03

10 EVQLVQSGAEVKKPGATVKISCKVSGYTFTNYVIHWVQQAPGKGLEWMGYIYPYNDGI
LYNEKFKGRVTITADTSTDYMELESLRSEDYAVYYCATGGYYVPDYWGQGTTVTVSS
(SEQ ID NO:25)

>Vx4humH04

15 EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYVIHWVRQMPGKGLEWMGYIYPYNDGI
LYNEKFKGQVTISADKSISTAYLQWSSLKASDTAMYVCARGGYYVPDYWGQGTTVTVS
S (SEQ ID NO:26)

>Vx4humH05

20 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYVIHWVRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARGGYYVPDYWGQGTTVTV
SS (SEQ ID NO:27)

>Vx8humH06

QVQLVQSGAEVKKPGASVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTRDTSTSTVYMELESLRSEDYAVYYCARGGKRAMDYWGQGLTVT
VSS (SEQ ID NO:29).

25 >Vx8humH07

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGKRAMDYWGQGTLVTV
SS (SEQ ID NO:30).

>Vx8humH08

5 EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIHWVRQMPGKGLEWMGYIDPLNGDT
TYNQKFKGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGKRAMDYWGQGTLVTV
SS (SEQ ID NO:31).

>Vx8humH09

10 QVQLVQSGAEVKKPGSSVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGKRAMDYWGQGTLVTVS
S (SEQ ID NO:32).

>Vx8humH10

15 EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIHWVRQMPGKGLEWMGYIDPLNGDT
TYSFSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGKRAMDYWGRGTLVTVS
S (SEQ ID NO:33).

>Vx8humH11

20 QVQLVQSGAEVKKPGASVQVVSCKASGYTFTNYYIHWLRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGKRAMDYWGQATLVTV
SS (SEQ ID NO:34)

>Vx9humH12

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIHWVRQAPGQGLEWMGYTDPRTD
YTEYNQKFKDRVTMTRDTSTSTVYMELSSLRSEDVAVYYCARGGRVGLGYWGQGTLVT
VSS (SEQ ID NO:36).

>Vx9humH13

25 QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYWIHWVRQAPGQGLEWMGYTDPRTDY
TEYNQKFKDRVTITADESTSTAYMELSSLRSEDVAVYYCARGGRVGLGYWGQGTLVTVS
S (SEQ ID NO:37).

>Vx9humH14

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYWIHWVRQMPGKGLEWMGYTDPRTDY
TEYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYYCARGGRVGLGYWGQGTLVTV
SS (SEQ ID NO:38).

5 >Vx9humH15

QVQLVQSGAEVKKPGSSVKVSCASGYTFTNYWIHWVRQAPGQGLEWMGYTDPRTDY
TEYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGRVGLGYWGQGTLVTVS
S (SEQ ID NO:39).

>Vx9humH16

10 EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYWIHWVRQMPGKGLEWMGYTDPRTDY
TEYSPSFQGGVTISADKSISTAYLQWSSLKASDTAMYYCARGGRVGLGYWGQGTLVTVS
S (SEQ ID NO:40).

Human IgG-Fc

>Human Fc IgG1

15 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
20 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:53).

>Human Fc IgG1-N297Q

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
25 QSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:54).

>Human Fc-IgG2

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
5 FRVSVSLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:55).

>Human Fc-IgG3

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
10 GLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSC
DTPPCPRCPEPKSCDTPPCPRCPEPKSCDTPPCPRCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVSVSLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
15 EALHNRFTQKSLSLSPGK (SEQ ID NO:56)

>Human Fc-IgG4

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDPHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR
20 VVSVSLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:57).

>Human Fc-IgG4 S228P

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
25 LYSLSVVTVPSSSLGTQTYTCNVDPHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR
VVSVSLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLGLG (SEQ ID NO:58).

>Human Fc-IgG4 PE

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
5 GLYSLSSVVTVPSSSLGTQKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLGLG (SEQ ID NO:59)

10 >Human Fc-IgG4 PE'

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
15 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLGLG (SEQ ID NO:99)

>Human kappa LC

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:60).

20 >Rat Fc-IgG2c

ARTTAPSVYPLVPGCSGTSGSLVTLGCLVKGYFPEPVTVKWNSGALSSGVHTFPAVLQS
GLYTLSSSVTVPSSTWSSQTVTCVAHPATKSNLIKRIEPRRPKPRPPTDICSCDDNLGRPS
VFIFPPKPKDILMITLTPKVTCTVVDVSEEEPDVQFSWFVDNVRVFTAQTQPHEEQLNGT
FRVVSSTLHIQHQQDWMSGKEFKCKVNNKDLPSPIEKTISKPRGKARTPQVYTIPPPREQMS
25 KKNVSLTCMVTSFYPASISVEWERNGELEQDYKNTLPVLDSDESFLYSKLSVDTDSW
MRGDIYTCVVHEALHNHHTQKNLSRSPGK (SEQ ID NO:61).

>Rat kappa LC

RADAAPTVSIFPPSMEQLTSGGATVVCFVNNFYPRDISVKWKIDGSEQRDGVLDSDVTDQ
DSKDSTYSMSSTLSLTKVEYERHNLYTCEVVHKTSSSPVVKSFNRNEC (SEQ ID NO:62).

Rabbit IgG-Fc

>Rabbit IgG

5 GQPKAPSVFPLAPCCGDTSPSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSS
GLYSLSSVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPP
KPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVST
LPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSL
TCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSGSYFLYSKLSVPTSEWQRGDVFT
10 CSVMHEALHNHYTQKSISRSPGK (SEQ ID NO:63).

>Rabbit kappa LC

RDPVAPTVLIFFPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQN
SADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFNRGDC (SEQ ID NO:64).

>CD47

15 MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKW
KFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVT
ELTREGETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALL
VAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVI
QVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVE (SEQ ID NO:65).

20 Chimera and Human Light Chains

>Vx4murL01 Full length

DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVSNRF
SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
25 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:66).

>Vx4murL01 Full length

DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVSNRFS
SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSST LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:67).

5 >Vx4humL01 Full length LC

DIVMTQSPLSLPVTTPGEPASISCRSRQSIVHTNGNTYLGWYLGKPGQSPRLLIYKVSNRFS
GVPDRFSGSGSGTDFTLKISRVEADDVGIIYYCFQGSHVPYTFGQGTKLEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
ST LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:68).

10 >Vx8humL03 Full length LC

DIVMTQSPLSLPVTTPGEPASISCRASQDISNYLNWYLGKPGQSPRLLIYYTSRLYSGVPDR
FSGSGSGTDFTLKISRVEADDVGIIYYCQQGNTLPWTFGQGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:69).

15 >Vx9humL02 Full length LC

DIVMTQSPDSLAVSLGERATINCRSSQNIVQSNNGNTYLEWYQQKPGQPPKLLIYKVFHRF
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCFQGSHVPYTFGQGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSST LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:70).

20 >Vx8humL02 Full length LC

DIQMTQSPSSLSASVGDRVTITCRASQSSISNYLNWYQQKPGKAPKLLIYYTSRLYSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:71).

25 >Vx4humL02 Full length LC

DVVMTQSPLSLPVTLGQPASISCRSRQSIVHTNGNTYLGWFQQRPQGQSPRRLIYKVSNRFS
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIKRTVAAPSV

FIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:72).

>Vx9humL07 Full length LC

DVVMTQSPSLPVTLGQPASISCRSSQNIVQSNNGNTYLEWFQQRPGQSPRRLIYKVFHRF
5 SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
LSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:73).

>Vx8humL01 Full length LC

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLYSGVPS
10 RFSGSGSGTDFTFTISSLPEDIAITYYCQQGNTLPWTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:74).

>Vx8murL03 Full length LC

DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLYSGVPS
15 RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:100).

>Vx9mur_L04 Full length LC

DVFMTQTPLSLPVSLGDQASISCRSSQNIVQSNNGNTYLEWYLQKPGQSPKLLIYKVFHRF
20 SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPWTFGGGKLEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDY
SLST LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:75).

Chimera and Human Heavy Chains

>Vx4murH01 Full length HC

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYYVHWVKRRPGQGLEWIGYIYPYNDGIL
25 YNEKFKGKATVTSKSSSTAYMDLSSLTSEDSAVYYCTRGGYVPDYWGQGTTLTVSS
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS

GLYSLSSVVTVPSSSLGTKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
5 QEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID NO:76).

>Vx4humH01 Full length HC

QVQLVQSGAEVKKPGASVQVSCKASGYTFTNYYVIHWLRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGYVDPDYWGQATLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
10 SSGLYSLSSVVTVPSSSLGTKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID NO:77).

15 > Vx8humH11 Full length HC

QVQLVQSGAEVKKPGASVQVSCKASGYSFTNYYIHWLRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGKRAMDYWGQATLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGP
20 SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID NO:78).

>Vx9humH12 Full length HC

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYIHWVRQAPGQGLEWMGYTDPRTD
YTEYNQKFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGGRVGLGYWGQGTLT
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSNFGTQTYTCNVDPKPSNTKVDKTKVERKCCVECPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQF

NSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:79).

>Vx9humH14 Full length HC

5 EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYWIHWVRQMPGKGLEWMGYTDPRTDY
TEYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYYCARGGRVGLGYWGQGTTLTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
10 TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:80).

>Vx9humH15 Full length HC

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYWIHWVRQAPGQGLEWMGYTDPRTDY
15 TEYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGRVGLGYWGQGTTLTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
20 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:81).

>Vx4humH02 Full length HC

QVQLVQSGAEVKKPGASVQVSCKASGYTFTNYVIHWLRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTSDTSISTAYMELSSLRSDDTAVYYCARGGYVYDYWGQATLTVS
25 SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO:82).

>Vx9humH13 Full length HC

QVQLVQSGAEVKKPGSSVKV SCKASGYTFTNYWIHWVRQAPGQGLEWMGYTDPRTDY
5 TEYNQKFKDRVTITADESTSTAYMELSSLRSEDTAVYYCARGGRVGLGYWGQGLTVTS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVS VLT TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
10 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSR
WQGGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:83).

>Vx8humH10 Full length HC

EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIHWVRQMPGKGLEWMGYIDPLNGDT
TYS PSFQGGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGKRAMDYWGRGTLTVTS
15 SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFEGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVS VLT TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSRLTVDKSR
20 WQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO:84).

>Vx4humH04 Full length HC

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYYVIHWVRQMPGKGLEWMGYIYPYNDGI
LYNEKFKGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGYVVPDYWGQGT TVTVTS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
25 SGLYSLSSVVTVPSSSLGKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFEGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVS VLT TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO:85).

>Vx4humH05 Full length HC

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYVIHWVRQAPGQGLEWMGYIYPYNDG
ILYNEKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARGGYVDPDYWGQGTTTVT
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
5 SSGLYSLSSVVTVPSSSLGKTYTCNVDPHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID NO:86).

10 >Vx9humH16 Full length HC

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYYWIHWVRQMPGKGLEWMGYTDPRTDY
TEYSPSFQGGVTISADKSISTAYLQWSSLKASDTAMYYCARGGRVGLGYWGQGLTVTS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVDKTVKCCVECPPCPAPPVAGPS
15 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:87).

>Vx8humH06 Full length HC

20 QVQLVQSGAEVKKPGASVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDVAVYYCARGGKRAMDYWGQGLTVT
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGKTYTCNVDPHKPSNTKVDKRVESKYGPPCPPCPAPEFEGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQF
25 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID NO:88).

>Vx8humH07 Full length HC

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTITADESTSTAYMELSSLRSED TAVYYCARGGKRAMDYWGQGTLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTGTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGP
5 SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKS
RWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK (SEQ ID NO:89).

>Vx8humH08 Full length HC

10 EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIHWVRQMPGKGLEWMGYIDPLNGDT
TYNQKFKGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGKRAMDYWGQGTLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTGTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
15 STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKS
RWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK (SEQ ID NO:90).

>Vx8humH09 Full length HC

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
20 TTYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGGKRAMDYWGQGTLVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTGTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
25 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSR
WQEGNVFSCSV MHEALHNHYTQKSLSLGLGK (SEQ ID NO:91).

>Vx8humH06 Full length HC

QVQLVQSGAEVKKPGASVKVSCKASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTRDTSTSTVYMELSSLRSED TAVYYCARGGKRAMDYWGQGTLVT

VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
5 EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLG (SEQ ID NO:92).

>Vx8mur-H03 Full length HC

EVQLQQSGPELMKPGASVKISCKASGYSTNYIHWVNQSHGKSLEWIGYIDPLNGDTT
YNQKFKGKATLTVDKSSSTAYMRLSSLTSADSAVYYCARGGKRAMDYWGQGTSTVTVSS
10 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDKSRW
15 QEGNVFSCSVMEALHNHYTQKSLSLGLG (SEQ ID NO:93).

>Vx9mur-H04 Full length HC

QVQLQQFGAELAKPGASVQMSCKASGYTFTNYWIHWVKQRPQGQGLEWIGYTDPRTDY
TEYNQKFKDKATLAADRSSSTAYMRLSSLTSEDSAVYYCAGGGRVGLGYWGHGSSVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
20 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSGDSFFLYSKLTVDKSR
WQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:94).

25 >Vx8humH06 Full length HC

QVQLVQSGAEVKKPGASVKVSCASGYSTNYIHWVRQAPQGQGLEWMGYIDPLNGD
TTYNQKFKGRVTMTRDTSTSTVYMESSLRSEDTAVYYCARGGKRAMDYWGQGTSLV
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAG

PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:95).

5 >Vx8humH07 Full length HC

QVQLVQSGAEVKKPGSSVKVSCASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYNQKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGKRAMDYWGQGTLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
10 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:96).

>Vx8humH08 Full length HC

15 EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIHWVRQMPGKGLEWMGYIDPLNGDT
TYNQKFKGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGKRAMDYWGQGTLVTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
20 TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:97).

>Vx8humH09 Full length HC

25 QVQLVQSGAEVKKPGSSVKVSCASGYSFTNYYIHWVRQAPGQGLEWMGYIDPLNGD
TTYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGGKRAMDYWGQGTLVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:98).

>Vx4mur-ratL01 Full length LC

DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVSNR
5 SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIK
RADAAPTIVSIFPPSMEQLTSGGATVVCVFNNFYPRDISVKWKIDGSEQRDGVLDSDTDQ
DSKDSTYSMSSTLSLTKVEYERHNLTYTCEVVHKTSSSPVVKSFNRNEC (SEQ ID NO:101).

>Vx4mur-ratH01 Full length HC

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYYVHWVKRRPGQGLEWIGYIYPYNDGIL
10 YNEKFKGKATVTSKSSSTAYMDLSSLTSEDSAVYYCTRGGYVVPDYWGQGTTTLTVSS
ARTTAPSVYPLVPGCSGTSGSLVTLGCLVKGYFPEPVTVKWNSGALSSGVHTFPAVLQS
GLYTLSSSVTVPSSTWSSQTVTCSVAHPATKSNLIKRIEPRRPKPRPPTDICSCDDNLGRPS
VFIFPPKPKDILMITLTPKVTCTVVVDVSEEEPDVQFSWFVDNVRVFTAQTQPHEEQLNGT
FRVSTLHIHQDWMSGKEFKCKVNNKDLPSPIEKTISKPRGKARTPQVYTIPPPREQMS
15 KKNVSLTCMVTSFYPAISVEWERNGELEQDYKNTLPVLDSDESYFLYSKLSVDTDSW
MRGDIYTCSVVHEALHNHHTQKNLSRSPGK (SEQ ID NO:102).

>Vx4mur-rabL01 Full length LC

DVLMTQTPLSLPVNLGDQASISCRSRQSIVHTNGNTYLGWFLQKPGQSPKLLIYKVSNR
SGVPDRFSGSGSGTDFTLTISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIK
20 RDPVAPTIVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQN
SADCTYNLSSTLTLTSTQYNHKEYTCKVTQGTTSVVQSFNRGDC (SEQ ID NO:103).

>Vx4mur-rabH01 Full length HC

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYYVHWVKRRPGQGLEWIGYIYPYNDGIL
YNEKFKGKATVTSKSSSTAYMDLSSLTSEDSAVYYCTRGGYVVPDYWGQGTTTLTVSS
25 GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSS
GLYSLSSVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPP
KPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVST
LPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSL

TCMINGFYPSDISVEWEKNGKAEDNYKTTTPAVLSDSGSYFLYSKLSVPTSEWQRGDVFT
CSVMHEALHNHYTQKSISRSPGK (SEQ ID NO:104).

Example 2

Production of CD47 Antibodies

5 Chimeric antibodies disclosed herein comprise a mouse heavy chain variable domain and a light chain variable domain combined with a human kappa or human Fc IgG1, IgG1-N297Q, IgG2, IgG4, IgG4 S228P, and IgG4 PE constant domains, respectively. These were designed to incorporate a secretion signal and cloned into a mammalian expression system, and transfected into CHO cells to generate chimeric (murine-human) antibodies. The chimeric variants were
10 expressed as full length IgG molecules, secreted into the medium, and purified using protein A.

As such, the humanized antibodies disclosed herein comprise frameworks derived from the human genome. The collection covers the diversity found in the human germ line sequences, yielding functionally expressed antibodies *in vivo*. The complementarity determining regions (CDRs) in the light and heavy chain variable regions of the murine and chimeric (murine-human)
15 are described herein and were determined by following commonly accepted rules disclosed in “Protein Sequence and Structure Analysis of Antibody Variable Domains”, In: *Antibody Engineering Lab Manual*, eds. S. Duebel and R. Kontermann, Springer-Verlag, Heidelberg (2001)). The human light chain variable domains were then designed. The humanized variable domains were then combined with a secretion signal and human kappa and human Fc IgG1, IgG1-N297Q, IgG2, IgG3, IgG4 S228P and IgG4 PE constant domains, cloned into a mammalian
20 expression system, and transfected into CHO cells to generate humanized mAbs. The humanized variants were expressed as full length IgG molecules, secreted into the medium and purified using protein A.

A non-glycosylated version (IgG1-N297Q) was created by site directed mutagenesis of
25 heavy chain position 297 to change the asparagine to glutamine (Human Fc IgG1-N297Q, SEQ ID NO:54). An IgG4 variant was created by site-directed mutagenesis at position 228 to change the serine to proline thereby preventing *in vivo* Fab arm exchange. An IgG4 double mutant was created by site-directed mutagenesis at positions 228 (serine to proline) and 235 (leucine to glutamate) to prevent Fab arm exchange and to further reduce Fc effector function. IgG2, IgG3,
30 IgG4 S228P, and IgG4 PE isotypes were constructed by cloning the heavy chain variable domain in frame with the human IgG2, IgG3, IgG4 S228P, and IgG4PE constant domains (Human Fc-

IgG2, SEQ ID NO:55 Human Fc-IgG3, SEQ ID NO:56; Human Fc-IgG4 S228P, SEQ ID NO:58; and Human Fc-IgG4 PE, SEQ ID NO:59).

Example 3

Binding of CD47 Monoclonal Antibodies (mAbs)

The binding of chimeric (murine-human) and humanized antibodies of the present disclosure was determined by ELISA using OV10 cells transfected with human CD47 (OV10 hCD47) or using freshly isolated human red blood cells (hRBCs), which display CD47 on their surface (Kamel et al. (2010) *Blood. Transfus.* 8(4):260-266).

Binding activities of VLX4, VLX8, and VLX9 chimeric (murine-human) and humanized mAbs were determined using a cell-based ELISA assay with human OV10 hCD47 cells expressing cell surface human CD47. OV10 hCD47 cells were grown in IMDM medium containing 10% heat inactivated fetal bovine serum (BioWest; S01520). One day before assay, 3×10^4 cells were plated in 96 well cell bind plates (Corning #3300, VWR #66025-626) and were 95-100% confluent at the time of assay. Cells were washed and various concentrations of purified antibodies added in IMDM 37°C for 1 hr in 95%O₂ / 5%CO₂. Cells were then washed with media and incubated for an additional hour at 37°C with HRP labeled secondary anti-human antibody (Promega) diluted 1/2500 in media. Cells were washed three times with PBS, and the peroxidase substrate 3,3', 5,5'-tetramethylbenzidine is added (Sigma; Catalog #T4444). Reactions were terminated by the addition of HCl to 0.7N, and absorbance at 450nm is determined using a Tecan model Infinite M200 plate reader. The apparent binding affinities of these clones to human OV10 hCD47 cells was determined by non-linear fit (Prism GraphPad software).

Binding activities of chimeric (murine-human) and humanized VLX4, VLX8, and VLX9 mAbs to human CD47 on hRBCs were also determined using flow cytometry. hRBCs were incubated for 60 min on at 37°C with various concentrations of the chimeric or humanized antibodies in a solution of phosphate buffered saline, pH 7.2, 2.5 mM EDTA (PBS+E). Cells were then washed with cold PBS+E, and incubated for an additional hour on ice with FITC labeled donkey anti-human antibody (Jackson Immuno Research Labs, West Grove, PA; Catalogue # 709-096-149) in PBS +E. Cells were washed with PBS+E, antibody binding analyzed using a C6 Accuri Flow Cytometer (Becton Dickinson) and apparent binding affinities determined by non-

linear fit (Prism GraphPad software) of the median fluorescence intensities at the various antibody concentrations.

All of the VLX4 chimeric (murine-human) mAbs bound to human OV10 hCD47 tumor cells with apparent affinities in the picomolar (pM) range (Table 1).

5 Similarly, the humanized VLX4 mAbs bound to human OV10 hCD47 tumor cells in a concentration-dependent manner (FIG. 1A and FIG. 1B) with apparent affinities ranging from the picomolar to low nanomolar range (Table 2).

All of the chimeric VLX4 mAbs bound to human RBCs with apparent K_d values in the picomolar range and these were similar to the K_d values obtained for OV10 hCD47 tumor cells by
10 ELISA (Table 1).

The humanized VLX4 mAbs VLX4hum_01 IgG1 N297Q, VLX4hum_02 IgG1 N297Q, VLX4hum_01 IgG4 PE, VLX4hum_02 IgG4 PE, VLX4hum_12 IgG4 PE, and VLX4hum_13 IgG4 bound to human RBCs with K_d values similar to the values obtained for OV10 hCD47 tumor cells whereas VLX4hum_06 IgG4 PE and VLX4hum_07 IgG4 PE exhibited
15 reduced binding to hRBCs (FIG. 2A, FIG. 2B, and Table 2). This differential binding of the humanized mAbs to tumor cells and RBCs was unexpected as the VLX4 IgG4PE chimeric mAb bound with similar apparent K_d values to both tumor and RBC CD47 (Table 1).

As shown in Table 1, all of the VLX8 chimeric (murine-human) mAbs bound to human OV10 hCD47 tumor cells in a concentration-dependent manner with apparent affinities in the
20 picomolar (pM) range.

Similarly, the humanized VLX8 mAbs bound to human OV10 hCD47 tumor cells in a concentration-dependent manner (FIG. 3A and FIG. 3B) with apparent affinities in the picomolar range (Table 2).

All of the VLX8 chimeric mAbs bound to hRBCs with apparent K_d values in the picomolar
25 range and these were similar to the K_d values obtained for OV10 hCD47 tumor cells by ELISA (Table 1).

The VLX8 humanized mAbs VLX8hum_01 IgG4 PE, VLX8hum_02 IgG4 PE, VLX8hum_03 IgG4 PE, VLX8hum_04 IgG4 PE, VLX8hum_05 IgG4 PE, and VLX8hum_06 IgG4 PE, VLX8hum_07 IgG4 PE, VLX8hum_08 IgG4 PE, VLX8hum_09 IgG4 PE,
30 VLX8hum_11 IgG4 PE, VLX8hum_06 IgG2, VLX8hum_07 IgG2, VLX8hum_08 and VLX8hum_09 IgG2 bound to human RBCs with K_d values similar to the values obtained

for OV10 hCD47 tumor cells whereas VLX8hum_10 IgG4 PE exhibited reduced, but measurable binding to hRBCs (FIG. 4A, FIG. 4B, and Table 2). This differential binding of the humanized mAbs to tumor cells and RBCs was unexpected as the VLX8 IgG4PE chimeric mAb bound with similar apparent K_d values to both tumor and RBC CD47 (Table 1).

Table 1 shows the apparent binding affinities of VLX9 murine-human chimeric mAbs to human OV10 hCD47 cells and to human RBCs. All of the chimeric mAbs bound to OV10 hCD47 tumor cells with apparent K_d values in the picomolar range. Similarly, the humanized VLX9 mAbs bound to human OV10 hCD47 tumor cells in a concentration-dependent manner (FIG. 5A and FIG. 5B) with apparent affinities in the picomolar to nanomolar range (Table 2).

All of the VLX9 chimeric mAbs bound to hRBCs with apparent K_d values in the picomolar range and these were similar to the K_d values obtained for OV10 hCD47 tumor cells by ELISA (Table 1). In contrast to the chimeric mAbs, the VLX9 humanized mAbs VLX9hum_01 IgG2, VLX9hum_02 IgG2 and VLX9hum_07 IgG2 exhibited reduced but measurable binding to human RBCs (FIG. 6, Table 2). Humanized mAbs VLX9hum_03, 04, 05, 06, 08, 09 and 10 IgG2 exhibited no measurable binding to RBCs (Table 2). This differential binding of the humanized mAbs to tumor cells and RBCs was unexpected as the VLX9 IgG2 chimeric mAbs all bound with similar apparent K_d values to both tumor and RBC CD47 (Table 1).

Table 1: Binding of VLX4, VLX8, and VLX9 Chimeric (xi) mAbs to OV10 hCD47 Cells and Human Red Blood Cells (hRBCs).

	V _H (SEQ ID NO:)	V _L (SEQ ID NO:)	K _d (pM) OV10 hCD47 Cell-based ELISA	K _d (pM) hRBC	HA hRBC
VLX4 IgG1 (xi)	21	41	315	104	Yes
VLX4 IgG1 N297Q (xi)	21	41	258	92	Yes
VLX4 IgG2 (xi)	21	41	431	184	Yes

VLX4 IgG4 S228P (xi)	21	41	214	99	No	
VLX4 IgG4 PE(xi)	21	41	225	303	No	
VLX8 IgG1 N297Q (xi)	28	46	42	91	Yes	
VLX8 IgG4 PE (xi)	28	46	56	77	Yes	
VLX9 IgG1 (xi)	35	50	280	381	Yes	
VLX9 IgG1 N297Q (xi)	35	50	275	190	Yes	
VLX9 IgG2 (xi)	35	50	880	742	Yes	
VLX9 IgG4 PE (xi)	35	50	293	126	Yes	

Table 2. Binding of VLX4, VLX8, and VLX9 Humanized mAbs to Human OV10 hCD47 and Human Red Blood Cells (hRBCs).

	Kd (pM)	Kd (pM)	HA
	OV10	hRBC	hRBC
	hCD47		

	Cell-based ELISA		
VLX4hum_01 IgG1	73	23	Yes
VLX4hum_02 IgG1	80	70	Yes
VLX4hum_01 IgG4 PE	82	63	No
VLX4hum_02 IgG4 PE	95	75	R***
VLX4hum_06 IgG4 PE	196	>66,000**	Yes
VLX4hum_07 IgG4 PE	209	>66,000**	Yes
VLX4hum_12 IgG4 PE	56	263	Yes
VLX4hum_13 IgG4 PE	62	340	Yes
VLX8hum_01 IgG4 PE	54	209	No
VLX8hum_02 IgG4 PE	50	221	No
VLX8hum_03 IgG4 PE	67	183	No
VLX8hum_04 IgG4 PE	49	119	No
VLX8hum_05 IgG4 PE	68	264	No
VLX8hum_06 IgG4 PE	61	274	Yes
VLX8hum_07 IgG4 PE	24	241	Yes

VLX8hum_08 IgG4 PE	97	217	Yes
VLX8hum_09 IgG4 PE	82	336	Yes
VLX8hum_10 IgG4 PE	183	>33,000**	Yes
VLX8hum_11 IgG4 PE	90	18	No
VLX8hum_06 IgG2	403	246	Yes
VLX8hum_07 IgG2	460	671	Yes
VLX8hum_08 IgG2	464	820	Yes
VLX8hum_09 IgG2	680	1739	Yes
VLX9hum_01 IgG2	162	1653**	N
VLX9hum_02 IgG2	227	4103**	N
VLX9hum_03 IgG2	606	*NB	N
VLX9hum_04 IgG2	823	*NB	N
VLX9hum_05 IgG2	6372	*NB	N
VLX9hum_06 IgG2	547	*NB	N
VLX9hum_07 IgG2	341	>66,000**	***R

VLX9hum_08 IgG2	688	*NB	N
VLX9hum_09 IgG2	8340	*NB	N
VLX9hum_10 IgG2	12232	*NB	N

*NB - No binding detected at mAb concentration up to 100 µg/mL.

** - Reduced RBC binding.

***R - Reduced hemagglutination.

Cross-species binding of humanized VLX4, VLX8, and VLX9 mAbs was determined using flow cytometry. Mouse, rat, rabbit or cynomolgus monkey RBCs were incubated for 60 min on at 37°C with various concentrations of the humanized antibodies in a solution of phosphate buffered saline, pH 7.2, 2.5 mM EDTA (PBS+E). Cells were then washed with cold PBS+E, and incubated for an additional hr on ice with FITC labeled donkey anti-human antibody (Jackson Immuno Research Labs, West Grove, PA; Catalogue # 709-096-149) in PBS +E. Cells were washed with PBS+E, and antibody binding analyzed using a C6 Accuri Flow Cytometer (Becton Dickinson).

Table 3 shows the apparent binding affinities of the humanized VLX4 and VLX8 mAbs to RBCs from mouse, rat, and cynomolgus monkey determined by non-linear fit (Prism GraphPad software) of the median fluorescence intensities at various antibody concentrations. This data demonstrates that humanized VLX4 and VLX8 mAbs bind to mouse, rat, rabbit (data not shown) and cynomolgus monkey RBCs with apparent K_d values in the picomolar to nanomolar range (Table 4).

Table 3. Binding of VLX4 and VLX8 Humanized mAbs to mouse and rat RBCs.

	K _d (pM) Mouse RBC	K _d (pM) Rat RBC	K _d (pM) Cynomolgus Monkey RBC
VLX4hum_01 IgG4 PE	16166	29917	23
VLX4hum_07 IgG4 PE	21340	17610	4313

VLX8hum_11 IgG4 PE	2473	10921	76
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Example 4

CD47 Antibodies Block CD47/SIRP α binding

To assess the effect of humanized CD47 mAbs on binding of CD47 to SIRP α *in vitro* the following method is employed using the binding of fluorescently-labelled SIRP α -Fc fusion protein to CD47 expressing Jurkat T cells.

SIRP α -Fc fusion protein (R&D Systems, cat #4546-SA) was labelled using an Alexa Fluor® antibody labelling kit (Invitrogen Cat No. A20186) according to the manufacturers specifications. 1.5 x 10⁶ Jurkat T cells were incubated with humanized mAbs (5µg/ml), a human control antibody in RPMI containing 10% media or media alone for 30 min at 37°C. An equal volume of fluorescently labeled SIRP α -Fc fusion protein was added and incubated for an additional 30 min at 37°C. Cells were washed once with PBS and the amount of labelled SIRP α -Fc bound to the Jurkat T cells analyzed by flow cytometry.

As shown in FIG. 7, the humanized VLX4, VLX8 and VLX9 mAbs, blocked the interaction of CD47 expressed on the Jurkat T cells with SIRP α , while the human control antibody (which does not bind to CD47) or media alone, did not block the CD47/SIRP α interaction.

Example 5

CD47 Antibodies Increase Phagocytosis

To assess the effect of chimeric (murine-human) and humanized VLX4, VLX8, and VLX9 CD47 mAbs on phagocytosis of tumor cells by macrophages *in vitro* the following method is employed using flow cytometry (Willingham et al. (2012) *Proc Natl Acad Sci USA* 109(17):6662-7 and Tseng et al. (2013) *Proc Natl Acad Sci U S A* 110(27):11103-8).

Human derived macrophages were derived from leukapheresis of healthy human peripheral blood and incubated in AIM-V media (Life Technologies) for 7-10 days. For the *in vitro* phagocytosis assay, macrophages were re-plated at a concentration of 1x10⁴ cells per well in 100 µl of AIM-V media in a 96-well plate and allowed to adhere for 24 hrs. Once the effector macrophages adhered to the culture dish, the target human cancer cells (Jurkat) were labeled with

1 μ M 5(6)-Carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE; Sigma Aldrich) and added to the macrophage cultures at a concentration of 5×10^4 cells in 1ml of AIM-V media (5:1 target to effector ratio). VLX4, VLX8, and VLX9 CD47 mAbs (1 μ g/ml) were added immediately upon mixture of target and effector cells and allowed to incubate at 37°C for 2-3 hours. After 2-3 hrs,
5 all non-phagocytosed cells were removed and the remaining cells washed three times with phosphate buffered saline (PBS; Sigma Aldrich). Cells were then trypsinized, collected into microcentrifuge tubes, and incubated in 100ng of allophycocyanin (APC) labeled CD14 antibodies (BD Biosciences) for 30 minutes, washed once, and analyzed by flow cytometry (Accuri C6; BD Biosciences) for the percentage of CD14⁺ cells that were also CFSE⁺ indicating complete
10 phagocytosis.

As shown in FIG. 8, the VLX4 chimeric (murine-human) mAbs VLX4 IgG1, VLX4 IgG1 N297Q, VLX4 IgG4 PE, and VLX4 IgG4 S228P increased phagocytosis of Jurkat cells by human macrophages by blocking the CD47/SIRP α interaction and this enhanced phagocytosis is independent of Fc function.

15 Similarly, as shown in FIG. 9A and FIG. 9B, VLX4hum_01 IgG1, VLX4hum_01 IgG4 PE, VLX4hum_06 IgG4 PE, VLX4hum_07 IgG4 PE, VLX4hum_12 IgG4 PE, and VLX4hum_13 IgG4 PE increased phagocytosis of Jurkat cells by human macrophages by blocking the CD47/SIRP α interaction.

As shown in FIG. 10A, the VLX8 chimeric (murine-human) mAbs VLX8 IgG1 N297Q
20 and VLX8 IgG4 PE increase phagocytosis of Jurkat T cells by human macrophages via blocking the CD47/SIRP α interaction and this enhanced phagocytosis is independent of Fc function.

Similarly, as shown in FIG. 10B, VLX8hum_01 IgG4 PE, VLX8hum_03 IgG4 PE, VLX8hum_07 IgG4 PE, VLX8hum_08 IgG4 PE, and VLX8hum_09 IgG4 PE increased phagocytosis of Jurkat cells by human macrophage by blocking the CD47/SIRP α interaction and
25 this enhanced phagocytosis is independent of Fc function

As shown in FIG. 11A, the VLX9 IgG1N297Q, VLX9 IgG2 and VLX9 IgG4 PE chimeric mAbs all increased phagocytosis of Jurkat T cells by human macrophages by blocking the CD47/SIRP α interaction and this enhanced phagocytosis is independent of Fc effector function. Similarly as shown in FIG. 11B, all of the humanized VLX9 IgG2 mAbs increased phagocytosis
30 of Jurkat T cells.

Example 6

Induction of Cell Death by Soluble CD47 Antibodies

Some soluble CD47 antibodies have been shown to induce selective cell death of tumor cells. This additional property of selective toxicity to cancer cells is expected to have advantages compared to mAbs that only block SIRP α binding to CD47.

Induction of cell death by soluble anti-CD47 mAbs is measured *in vitro* (Manna et al. (2003) *J. Immunol.* 107 (7): 3544-53). For the *in vitro* cell death assay, 1×10^5 transformed human T cells (Jurkat T cells) were incubated with soluble humanized VLX4, VLX8, and VLX9 CD47 mAbs (1 μ g/ml) for 24 hrs at 37°C. As cell death occurs, mitochondrial membrane potential is decreased, the inner leaflet of the cell membrane is inverted, exposing phosphatidylserines (PS), and propidium iodide (PI) is able to incorporate into nuclear DNA. In order to detect these cellular changes, cells were then stained with fluorescently labeled annexin V and PI or 7-aminoactinomycin D (7-AAD) (BD Biosciences) and the signal detected using an Accuri C6 flow cytometer (BD Biosciences). The increase in PS exposure is determined by measuring the percent increase in annexin V signal and the percent of dead cells by measuring the percent increase in PI or 7-AAD signal. Importantly for therapeutic purposes, these mAbs induce cell death of tumor cells directly and do not require complement or the intervention of other cells (e.g., NK cells, T cells, or macrophages) to kill. Thus, the mechanism is independent of both other cells and of Fc effector function. Therefore, therapeutic antibodies developed from these mAbs can be engineered to reduce Fc effector functions such as ADCC and CDC and thereby limit the potential for side effects common to humanized mAbs with intact Fc effector functions.

As shown in FIG. 12A and FIG. 12B, the soluble VLX4 humanized mAbs induced cell death of Jurkat T ALL cells as measured by increased annexin V staining and 7-AAD staining (not shown). The humanized mAbs VLX4hum_01 IgG1, VLX4hum_01 IgG4 PE, VLX4hum_02 IgG1, VLX4hum_02 IgG4 PE, VLX4hum_06 IgG4 PE, VLX4hum_07 IgG4 PE, VLX4hum_12 IgG4 PE, and VLX4hum_13 IgG4 PE caused cell death. In contrast, the humanized mAbs VLX4hum_08 IgG4 PE and VLX4hum_11 IgG4 PE did not cause cell death of Jurkat T cells. Induction of cell death and the promotion of phagocytosis of susceptible cancer cells imparts an additional desirable antibody property and therapeutic benefit in the treatment of cancer.

As shown in FIG. 13A and FIG. 13B, the soluble VLX8 chimeric and humanized mAbs induced cell death of Jurkat T ALL cells as measured as measured by increased annexin V staining and 7-AAD staining (not shown). The chimeric mAbs, VLX8 IgG1 N297Q (xi) and VLX8 IgG4

PE, and the humanized mAbs, VLX8hum_07 IgG4 PE and VLX8hum_08 IgG4 PE, induced cell death of Jurkat T ALL cells. In contrast, the humanized mAbs VLX8hum_02 IgG4 PE and VLX8hum_04 IgG4 PE did not cause cell death of Jurkat T cells. Induction of cell death and the promotion of phagocytosis of susceptible cancer cells imparts an additional desirable antibody property and therapeutic benefit in the treatment of cancer.

As shown in FIG. 14A, the soluble VLX9 chimeric antibodies induced cell death of Jurkat cells as measured by increased annexin V staining and 7-AAD staining (not shown). In addition as shown in FIG. 14B, the chimeric VLX9 IgG2xi mAb and the humanized mAbs VLX9hum_06 IgG2, VLX9hum_07 IgG2, VLX9hum_08 IgG2, and VLX9hum_09 IgG2 induced cell death of Jurkat cells (greater than 2-fold increase in annexin V staining). In contrast, the humanized mAbs VLX9hum_01 IgG2, VLX9hum_02 IgG2, VLX9hum_03 IgG2, VLX9hum_04 IgG2, VLX9hum_05 IgG2 and VLX9hum_010 IgG2 did not cause cell death of Jurkat cells. . Induction of cell death and the promotion of phagocytosis of susceptible cancer cells imparts an additional desirable antibody property and therapeutic benefit in the treatment of cancer.

Example 7

Hemagglutination of Human Red Blood Cells (hRBCs)

Many CD47 antibodies, including B6H12, BRIC126, MABL1, MABL2, CC2C6, 5F9, have been shown to cause hemagglutination (HA) of washed RBCs *in vitro* or *in vivo* (Petrova P. et al. *Cancer Res* 2015; 75(15 Suppl): Abstract nr 4271; US Patent 9,045,541; Uno et al. *Oncol Rep.* 17: 1189-94, 2007; Kikuchi et al. *Biochem Biophys Res. Commun.* 315: 912-8, 2004; Sikic B. et al. *J Clin Oncol* 2016;34 (suppl; abstract 3019)). Hemagglutination of hRBCs was assessed following incubation of hRBCs with various concentrations of chimeric and humanized VLX4, VLX8, and VLX9 mAbs *in vitro* essentially as described by Kikuchi et al. *Biochem Biophys Res. Commun* (2004) 315:912-918. Blood was obtained from healthy donors, diluted (1:50) in PBS/1 mM EDTA/BSA and washed 3 times with PBS/EDTA/BSA. hRBCs were added to U-bottomed 96 well plates with equal volumes of the antibodies (75 µl of each) and incubated for 3 hrs at 37°C and overnight at 4°C.

As shown in FIG. 15A and Tables 1 and 2, The VLX4hum_01 IgG1 N297Q caused hemagglutination of hRBCs, whereas the humanized VLX4hum_01 IgG4 PE mAb did not (mAb concentrations 50 µg/ml to 0.3 ng/ml). The lack of hemagglutination by VLX4hum_01 IgG4 PE

imparts an additional desirable antibody property and therapeutic benefit in the treatment of cancer.

As shown in FIG. 15B and Tables 1 and 2, the chimeric antibody VLX8 IgG4 PE (xi) and the humanized antibodies VLX8hum_08 IgG4 PE, VLX8hum_09 IgG4 PE, and VLX8hum_010
5 IgG4 PE caused hemagglutination of hRBCs, whereas the VLX8 humanized Abs VLX8hum_01 IgG4 PE, VLX8hum_02 IgG4 PE, VLX8hum_03 IgG4 PE and VLX8hum_11 IgG4 PE did not (mAb concentrations 50 µg/ml to 0.3 ng/ml).

The lack of hemagglutination by humanized antibodies VLX4hum_01 IgG4 PE, VLX8hum_01 IgG4 PE, VLX8hum_02 IgG4 PE, VLX8hum_03 IgG4 PE and VLX8hum_11
10 IgG4 PE imparts an additional desirable antibody property and a therapeutic benefit in the treatment of cancer.

As shown in FIG. 16A and FIG. 16B, The chimeric antibody VLX9 IgG2 caused hemagglutination of hRBCs, whereas all of the humanized VLX9 mAbs except for VLX9hum_07 IgG2, did not (at concentrations from 50 ug/ml to 0.3 pg/ml). However, the amount of
15 hemagglutination caused by VLX9hum_07 was reduced compared to the VLX9 IgG2 chimeric mAb. Again, the lack of hemagglutination by the VLX9 humanized mAbs imparts an additional desirable antibody property and a therapeutic benefit in the treatment of cancer.

Example 8

Anti-Tumor Activity *in Vivo*

The purpose of this experiment was to demonstrate that VLX4, VLX8 and VLX9 humanized antibodies, exemplified by VLX4_07 IgG4PE, VLX8_10 IgG4PE and VLX9hum_08 IgG2, reduce tumor burden *in vivo* in a mouse xenograft model of lymphoma.

Raji human Burkitt's lymphoma cells (ATCC #CCL-86, Manassas, VA) were maintained
25 in RPMI-1640 (Lonza; Walkersville, MD) supplemented with 10% Fetal Bovine Serum (FBS; Omega Scientific; Tarzana, CA) within a 5% CO₂ atmosphere. Cultures were expanded in tissue culture flasks.

Female NSG (NOD-Cg-Prkdc^{scid}I12rg^{tm1Wjl}/SzJ) were obtained from Jackson Laboratory (Bar Harbor, ME) at 5-6 weeks of age. Mice were acclimated prior to handling and housed in
30 microisolator cages (Lab Products, Seaford, DE) under specific pathogen-free conditions. Mice were fed Teklad Global Diet[®] 2920x irradiated laboratory animal diet (Envigo, Formerly Harlan;

Indianapolis, IN) and provided autoclaved water *ad libitum*. All procedures were carried out under Institutional Animal Care and Use guidelines.

Female NSG mice were inoculated subcutaneously in the right flank with 0.1 mL of a 30% RPMI / 70% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of 5x10⁶ Raji tumor cells. Five days following inoculation, digital calipers were used to measure width and length diameters of the tumor. Tumor volumes were calculated utilizing the formula: tumor volume (mm³) = (a x b²/2) where 'b' is the smallest diameter and 'a' is the largest diameter. Mice with palpable tumor volumes of 31-74 mm³ were randomized into 8-10/group and VLX9hum_08 or PBS (control) administration was initiated at this time. Mice were treated with 5 mg/kg of antibody 5X/week for 4 weeks by intraperitoneal injection. Tumor volumes and body weights were recorded twice weekly.

As shown in FIG. 17, treatment with the humanized VLX4hum_07 IgG4 PE significantly reduced tumor growth of the Raji tumors (p < 0.05, two-way ANOVA), demonstrating anti-tumor efficacy *in vivo*.

As shown in FIG. 18, treatment with the humanized anti-CD47 mAb, VLX8hum_10 IgG4 PE significantly reduced (p < 0.0001, two-way ANOVA) tumor growth of the Raji tumors, demonstrating anti-tumor efficacy *in vivo*.

As shown in FIG. 19, treatment with the humanized anti-CD47 mAb, VLX9hum_08 IgG2 significantly reduced (p < 0.05, ANOVA) tumor growth of the Raji tumors, demonstrating anti-tumor efficacy *in vivo*.

Example 9

Effect on Circulating Red Blood Cell Parameters

The purpose of this experiment is to demonstrate that VLX9 humanized antibodies that do not bind to human RBC *in vitro* (Table 2), exemplified by hum1017_08 IgG2, do not cause a reduction in either hemoglobin (Hg) or circulating RBCs following administration to cynomolgus monkeys.

Female Chinese cynomolgus monkeys (Charles River Laboratories, Houston, TX) 2.5 – 3 kg were used in accordance with the Institutional Animal Care and Use guidelines. VLX9hum_08 IgG2 or vehicle (PBS) was administered as a 1 hour intravenous infusion on day 1 at a dose of 5 mg/kg and on day 18 at a dose of 15 mg/kg (3 animals/group). Hematological parameters were measured throughout the study on days -7, -3, 3, 8, 12, 18 (pre-dose), 20, 25, 29, 35 and 41 and

compared/normalized to the means values of control animals. The pre-treatment RBC and Hg values on day 0 in the VLX9hum_08 IgG2 group were lower than the control group. Following treatment with either dose of VLX9hum_08 IgG2, there were minimal changes (< 10%) in Hg (FIG. 20A) or RBC counts (FIG. 20B) compared to the control demonstrating that antibodies that do not bind to human RBCs in vitro do not cause a reduction in RBC hematological parameters when administered to cynomolgus monkeys.

Example 10

Immunohistochemical Staining of CD47

Localization of CD47 expression was determined in formalin-fixed, paraffin embedded (FFPE) blocks from patients with a number of types of cancer (obtained from commercial sources) using mouse/rabbit chimeric anti-CD47 mAbs. 3-4 micron sections were cut from FFPE blocks, deparaffinized and treated with antigen retrieval solution. Sections were then incubated with 4 µg/ml of the primary anti-CD47 mouse/rabbit chimeric mAbs for 1 hr and with an anti-rabbit HRP labeled secondary antibody for 20 minutes. The anti-CD47 antibody bound to human CD47 was visualized using the peroxidase substrate, 3,3',5,5'-tetramethylbenzidine. Sections were counterstained with hematoxylin and evaluated using standard light microscopy. As shown in FIG. 21, high CD47 expression was detected in human breast cancer tissue, as shown by dark areas denoted by arrows, using CD47 mouse/rabbit chimeric mAbs, exemplified by the VLX4 mouse/rabbit chimeric mAb. This demonstrates that these mAbs can be used for immunohistochemical localization of human CD47 in tumor tissue sections obtained from FFPE blocks in diagnostic assays.

Example 11

Antibodies to CD47 Regulate Nitric Oxide Signaling

TSP1 binding to CD47 activates the heterotrimeric G protein Gi, which leads to suppression of intracellular cyclic AMP (cAMP) levels. In addition, the TSP1/CD47 pathway opposes the beneficial effects of the nitric oxide (NO) pathway in all vascular cells. The NO pathway consists of any of three nitric oxide synthase enzymes (NOS I, NOS II and NOS III) that generate bioactive gas NO using arginine as a substrate. NO can act within the cell in which it is produced or in neighboring cells, to activate the enzyme soluble guanylyl cyclase that produces the messenger molecule cyclic GMP (cGMP). The proper functioning of the NO/cGMP pathway is essential for protecting the cardiovascular system against stresses including, but not limited to,

those resulting from wounding, inflammation, hypertension, metabolic syndrome, ischemia, and ischemia-reperfusion injury (IRI). In the context of these cellular stresses, the inhibition of the NO/cGMP pathway by the TSP1/CD47 system exacerbates the effects of stress. This is a particular problem in the cardiovascular system where both cGMP and cAMP play important protective roles. There are many cases in which ischemia and reperfusion injury cause or contribute to disease, trauma, and poor outcomes of surgical procedures.

The purpose of these experiment will be to demonstrate that humanized anti-CD47 mAbs of the present disclosure exhibit the ability to reverse TSP1-mediated inhibition of NO-stimulated cGMP synthesis as, for example, described previously using mouse monoclonal antibodies to CD47 as disclosed by Isenberg et al. (2006) J. Biol. Chem. 281:26069-80, or alternatively other downstream markers of or effects resulting from NO signaling, for example smooth muscle cell relaxation or platelet aggregation as described previously by Miller et al. (2010) Br J. Pharmacol. 159: 1542-1547.

The method employed that will be to measure cGMP as described by the manufacturer (CatchPoint Cyclic-GMP Fluorescent Assay Kit, Molecular Devices, Sunnyvale, CA). Jurkat JE6.1 cells (ATCC, Manassas, VA; Catalog # TIB-152) or other cells types that retain the NO/cGMP signaling pathway when grown in culture and exhibit a robust and reproducible inhibitory response to TSP1 ligation of CD47 will be used. Cells will be grown in Iscove's modified Dulbeccco's medium containing 5% (v/v) heat inactivated fetal bovine serum (BioWest; Catalogue # S01520), 100 units/mL penicillin, 100 µg mL streptomycin (Sigma; Catalogue # P4222) at densities less than 1×10^6 cells/mL. For the cGMP assay, cells will be plated in 96 well tissue culture plates at a density of 1×10^5 cells/ml in Iscoves modified Dulbecco's medium containing 5% (v/v) heat inactivated fetal bovine serum (BioWest; Catalog # S01520), 100 units/mL penicillin, 100 µg/mL streptomycin (Sigma; #P4222) for 24 hours and then transferred to serum free medium overnight.

The humanized antibodies as disclosed herein, purified from transient transfections in CHO cells as described above in Example 3, as well as the control chimeric antibody, will then be added at a final concentration of 20 ng/ml, followed 15 minutes later by 0 or 1 µg/ml human TSP1 (Athens Research and Technology, Athens, GA, Catalogue # 16-20-201319). After an additional 15 minutes, the NO donor, diethylamine (DEA) NONOate (Cayman Chemical, Ann Arbor, MI, Catalog # 82100), will be added to half the wells at a final concentration of 1 µM. Five minutes

later, the cells will be lysed with buffer supplied in the cGMP kit, and aliquots of each well assayed for cGMP content.

It is anticipated that some of the chimeric or humanized antibodies will reverse TSP1 inhibition of cGMP. Reversal will be complete (>80 %) or intermediate (20% -80%). This reversal of TSP1 inhibition of cGMP will demonstrate that they have the ability to increase NO signaling and suggest utility in protecting the cardiovascular system against stresses including, but not limited to, those resulting from wounding, inflammation, hypertension, metabolic syndrome, ischemia, and ischemia-reperfusion injury (IRI). Additional assay systems, for example smooth muscle cell contraction, will also be expected to show that some of the chimeric or humanized antibody clones reverse the inhibitory actions of TSP on downstream effects resulting from the activation of NO signaling.

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CLAIMS

What is claimed is:

1. A monoclonal antibody or antigen-binding fragment thereof that specifically binds CD47 and comprises:
 - a variable heavy chain CDR1 amino acid sequence (HCDR1) amino acid sequence set forth in SEQ ID NO:3;
 - a variable heavy chain CDR2 amino acid sequence (HCDR2) amino acid sequence set forth in SEQ ID NO:6;
 - a variable heavy chain CDR3 amino acid sequence (HCDR3) amino acid sequence set forth in SEQ ID NO:10;
 - a variable light chain CDR1 amino acid sequence (LCDR1) amino acid sequence set forth in SEQ ID NO:14;
 - a variable light chain CDR2 amino acid sequence (LCDR2) amino acid sequence set forth in SEQ ID NO:17; and
 - a variable light chain CDR3 amino acid sequence (LCDR3) amino acid sequence set forth in SEQ ID NO:18.
2. The monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the monoclonal antibody, or antigen-binding fragment thereof is chimeric or humanized.
3. The monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the monoclonal antibody, or antigen-binding fragment thereof specifically binds human CD47.
4. The monoclonal antibody or antigen-binding fragment thereof of claim 3, wherein the monoclonal antibody, or antigen-binding fragment thereof specifically binds at least one of cynomolgus monkey, rat, or mouse CD47.
5. The monoclonal antibody or antigen-binding fragment thereof of claim 1, further comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), selected from:
 - (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51;
 - (ii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
 - (iii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51;
 - (iv) a heavy chain variable domain comprising the amino acid sequence SEQ ID NO:37 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52;
 - (v) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:51; and
 - (vi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence SEQ ID NO:52.

6. The monoclonal antibody or antigen-binding fragment thereof of claim 5, wherein the monoclonal antibody or antigen-binding fragment thereof further comprises an IgG isotype selected from IgG1, IgG1-N297Q, IgG2, IgG4, IgG4 S228P, and IgG4 PE.
7. The monoclonal antibody or antigen binding fragment thereof of claim 5, comprising one heavy chain and one light chain selected from:
 - (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a light chain comprising the amino acid sequence SEQ ID NO:70;
 - (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:79 and a light chain comprising the amino acid sequence SEQ ID NO:70;
 - (iii) a heavy chain comprising the amino acid sequence of SEQ ID NO:79 and a light chain comprising the amino acid sequence SEQ ID NO:73;
 - (iv) a heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a light chain comprising the amino acid sequence SEQ ID NO:73.
8. The monoclonal antibody or antigen binding fragment thereof of claim 7 comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a light chain comprising the amino acid sequence SEQ ID NO:70.
9. A pharmaceutical composition, comprising the monoclonal antibody, or antigen-binding fragment thereof, of claim 1, and a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.
10. A pharmaceutical composition, comprising the monoclonal antibody, or antigen-binding fragment thereof, of claim 5, and a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.
11. A pharmaceutical composition, comprising the monoclonal antibody, or antigen-binding fragment thereof, of claim 6, and a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.
12. A pharmaceutical composition, comprising the monoclonal antibody, or antigen-binding fragment thereof, of claim 7, and a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.
13. A pharmaceutical composition, comprising the monoclonal antibody, or antigen-binding fragment thereof, of claim 8, and a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.
14. The pharmaceutical composition of claim 13, wherein the pharmaceutical composition is to be administered intravenously.