CD47 BINDING AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of GB Patent Application No. 1813693.7, filed on August 22, 2018 and GB Patent Application No. 1804860.3, filed on March 27, 2018, the disclosure of which is hereby incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: ULTW_001_02WO_SeqList_ST25.txt, date recorded: March 26, 2019, file size 145,923 bytes).

FIELD OF THE INVENTION

The invention relates to antibody molecules binding specifically to CD47 (Cluster of Differentiation 47, also known as integrin associated protein [IAP]) and medical uses thereof.

BACKGROUND OF THE INVENTION

CD47 (also known as integrin associated protein [IAP]) is a transmembrane protein that belongs to the immunoglobulin superfamily and binds to several known partners, including: membrane integrins, thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα). CD47 is associated with a range of cellular processes, including apoptosis, proliferation, adhesion, and migration of cells and, importantly, it plays a key role in immune and angiogenic responses. CD47-SIRPα signalling is a critical molecular interaction that inhibits the activation of phagocytosis by macrophages and other myeloid cells. This promotes the survival of tumour cells and therefore acts as a myeloid lineage-specific immune checkpoint.

Preclinical evidence suggests that blocking CD47-SIRPα signalling can enhance the phagocytic activity of macrophages and inhibit the growth of xenografts in numerous experimental models of both haematological and solid malignancies. As macrophage activity is also a recognised factor in the biology of inflammation-associated tissue remodelling such as tissue fibrosis and the formation of atherosclerotic plaques, the CD47-SIRPα signalling axis is also of considerable therapeutic potential in non-cancerous diseases. Hence, anti-CD47 mAbs have the potential to act as immunotherapeutic agents in cancer and other settings, and to amplify the effectiveness of currently established therapies.

The majority of currently approved antibody therapeutics are derived from immunized rodents. Many of those antibodies have undergone a process known as “humanization”, via the “grafting” of murine CDRs into human v-gene framework sequences (see Nelson *et al.*, 2010, Nat Rev Drug Discov 9: 767-774). This process is often inaccurate and leads to a reduction in target binding affinity of the resulting antibody. To return the binding affinity of the original antibody, murine residues are usually introduced at key positions in the variable domain frameworks of the grafted v-domains (also known as “back-mutations”).

While antibodies humanized via CDR grafting and back mutations have been shown to induce lower immune response rates in the clinic in comparison to those with fully murine v-domains, antibodies humanized using this basic grafting method still carry significant clinical development risks due to the potential physical instability and immunogenicity motifs still housed in the grafted CDR loops. Antibodies such as anti-CD47, which potentially engage immune effector functions as part of their mechanism of action, are at particularly high risk for immunogenicity as they can encourage phagocytosis of CD47+ target cells, leading to antigen processing of the antibody along with the target cell. These anti-drug antibody responses in the patient can reduce drug half-life, potency and safety during clinical use. As animal testing of protein immunogenicity is often non-predictive of immune responses in man, antibody engineering for therapeutic use focuses on minimizing predicted human T-cell epitope content, non-human germline amino acid content and aggregation potential in the purified protein.

The ideal humanized agonistic anti-CD47 antibody would therefore have as many identical residues as possible in the v-domains to those found in both the frameworks and CDRs of well-characterized human germline sequences. Townsend *et al.* (2015; PNAS 112: 15354-15359) describe a method for generating antibodies in which CDRs derived from rat, rabbit and mouse antibodies were grafted into preferred human frameworks and then subject to a human germ-lining approach termed “Augmented Binary Substitution”. Although the approach demonstrated a fundamental plasticity in the original antibody paratopes, in the absence of highly accurate antibody-antigen co-crystal structural data, it is still not possible to reliably predict which individual residues in the CDR loops of any given antibody can be converted to human germline, and in what combination. Additionally, the Townsend et al. study did not address the addition of mutagenesis beyond the residues found in the human germline at positions where the removal of development risk motifs might be beneficial. This is a technological limitation which renders the process inherently inefficient, leaving antibodies at high risk of development difficulties. In addition, it cannot currently be

accurately predicted what modifications in distal positions of the protein sequence of an individual v-domain, or even on the partner v-domain, might facilitate the removal of risk motifs while maintaining antigen binding affinity and specificity.

CDR germ-lining and development quality optimisation is thus a complex, multifactorial problem, as multiple functional properties of the molecule should preferably be maintained, including in this instance: target binding specificity, affinity to CD47 from both human and animal test species (e.g. cynomolgus monkey, also known as the crab-eating macaque, i.e. *Macaca fascicularis*), v-domain biophysical stability and/or IgG expression yield. Antibody engineering studies have shown that mutation of even single residue positions in key CDRs can have dramatic effects on all of these desired molecular properties.

WO2011143624A2 describes an antagonistic murine anti-CD47 IgG molecule termed "5F9G4", and also the preparation of humanized forms of 5F9G4 (h5F9G4). Those humanized forms of 5F9G4 were produced using classical humanization techniques, i.e. by grafting of Kabat-defined murine CDRs into human heavy and light chain framework sequences, with some of the human framework residues being potentially back-mutated to the correspondingly positioned 5F9G4 murine residues. For reasons noted above, such humanized forms of 5F9G4 described in WO2011143624A2 are not ideal.

SUMMARY OF THE INVENTION

The present invention provides a number of anti-CD47 antibodies and medical uses thereof.

According to one aspect of the invention, there is provided an antibody molecule or an antigen-binding portion thereof, which specifically binds to human CD47, and optionally also to cynomolgus monkey CD47, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region. In some embodiments, the VH region comprises an IGHV5-51-based or an IGHV1-3-based framework. In some embodiments, the VL region comprises an IGKV2-28-based framework.

In the IGHV5-51 background, the anti-CD47 antibody molecule or antigen-binding portion may comprise a heavy chain variable region with:

an HCDR1 having amino acids in sequence in the following order: G-Y-S-F-T-N or a conservative substitution of N (such as S)-Y-N or a conservative substitution of N (such as S)-M or a conservative substitution of M (such as I)-H or any amino acid (such as G) (SEQ ID NO:28);

an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M (for example, I)-G-T or any amino acid (such as I)-I-Y-P-G-N or any amino acid (such as D)-D or any amino acid (such as S)-D-T-S or any amino acid (such as R)-Y-N or any amino acid (such as S, H)-Q or any amino acid (such as P)-K or any amino acid (such as S)-F-Q/K-G/D (SEQ ID NO:29); and

an HCDR3 having amino acids in sequence in the following order: G or any amino acid (such as Q)-G-Y or any amino acid (such as A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W)-R-A or any amino acid (such as I, T, V, W)-M or any amino acid (such as A, E, K, L, P, Q, R, S, T, V, W)-D-Y (SEQ ID NO:30).

In the IGHV1-3 background, the anti-CD47 antibody molecule or antigen-binding portion may comprise a heavy chain variable region with:

an HCDR1 having amino acids in sequence in the following order: G-Y-T-F-T-N or any amino acid (such as D)-Y-N or any amino acid (such as A)-M-H (SEQ ID NO:31);

an HCDR2 having amino acids in sequence in the following order: M-G-T-I-Y-P-G-N-D-D or any amino acid (such as N)-T-S or any amino acid (such as K)-Y-N or any amino acid (such as S)-Q-K-F-Q-G (SEQ ID NO:32); and

an HCDR3 having amino acids in sequence in the following order: G or any amino acid (such as Q)-G-Y or any amino acid (such as A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W)-R-A or any amino acid (such as I, T, V, W)-M or any amino acid (such as A, E, K, L, P, Q, R, S, T, V, W)-D-Y (SEQ ID NO:30).

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTNYNMH (SEQ ID NO:33; 5F9G4 murine/humanized antibody HCDR1 disclosed in WO2011143624A2; US20130142786A1), the HCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence MGTIYPGNDDTSYNQKFKD (SEQ ID NO:34; 5F9G4 murine/humanized antibody HCDR2 disclosed in WO2011143624A2; US20130142786A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYRAMDY (SEQ ID NO:35; 5F9G4 murine/humanized antibody HCDR3 disclosed in WO2011143624A2; US20130142786A1).

The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:

an LCDR1 having amino acids in sequence in the following order: R-S-S-Q-S-I or a conservative substitution of I (such as L)-V or a conservative substitution of V (such as L)-Y or a conservative substitution of Y (such as H)-S-N or any amino acid (such as G, S, K)-G or

any amino acid (such as A, Y)-N or any amino acid (such as Q, Y)-T or any amino acid (such as N)-Y-L-G or any amino acid (such as D) (SEQ ID NO:36);
an LCDR2 having amino acids in sequence in the following order: K-V or any amino acid (such as G)-S-N-R-F or any amino acid (such as A, S)-S (SEQ ID NO:37); and
an LCDR3 having amino acids in sequence in the following order: F or any amino acid (such as L, M)-Q-G or any amino acid (such as A)-S or any amino acid (such as T, L, M)-H or any amino acid (such as Q, R)-V or any amino acid (such as I, T)-P-Y-T or any amino acid (such as I) (SEQ ID NO:38).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence RSSQSIVYSNGNTYLG (SEQ ID NO:39; 5F9G4 murine/humanized antibody LCDR1 disclosed in WO2011143624A2; US20130142786A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSNRFS (SEQ ID NO:40; 5F9G4 murine/humanized antibody LCDR2 disclosed in WO2011143624A2; US20130142786A1), and/or the LCDR3 of the antibody molecule or antigen binding portion may exclude the sequence FQGSHVPYT (SEQ ID NO:41).

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the HCDR1 comprises the amino acid sequence G-Y- X_1-F-T-X_2-Y-X_3-X_4-X_5, wherein X_1 is S or a conservative substitution of S, X_2 is N or a conservative substitution of N, X_3 is N or any other amino acid, X_4 is M or a conservative substitution of M and X_5 is H or any other amino acid (SEQ ID NO:42);

(b) the HCDR2 comprises the amino acid sequence X_1-X_2-X_3-I-Y-P-G-X_4-X_5-X_6-T-X_7-Y-X_8-X_9-X_{10}-F-Q-G, wherein X_1 is M or a conservative substitution of M, X_2 is G or a conservative substitution of G, X_3 is T or any other amino acid, X_4 is N or a conservative substitution of N, X_5 is D or any other amino acid, X_6 is D or any other amino acid, X_7 is S or any other amino acid, X_8 is N or any other amino acid, X_9 is Q or any other amino acid and X_{10} is K or any other amino acid (SEQ ID NO:43);

(c) the HCDR3 comprises the amino acid sequence X_1-G-X_2-R-X_3-X_4-D-Y, wherein X_1 is G or any other amino acid, X_2 is Y or any other amino acid, X_3 is A or any other amino acid and X_4 is M or any other amino acid (SEQ ID NO:44);

(d) the LCDR1 comprises the amino acid sequence R-S-S-Q-S-X_1-X_2-X_3-S-X_4-X_5-X_6-X_7-Y-L-X_8, wherein X_1 is I or a conservative substitution of I, X_2 is V or a conservative substitution of V, X_3 is Y or a conservative substitution of Y, X_4 is N or any other amino

acid, X_5 is G or any other amino acid, X_6 is N or any other amino acid, X_7 is T or any other amino acid and X_8 is G or any other amino acid (SEQ ID NO:45);

(e) the LCDR2 comprises the amino acid sequence K-X_1-S-N-R-X_2-S, wherein X_1 is V or any other amino acid and X_2 is F or any other amino acid (SEQ ID NO:46); and

(f) the LCDR3 comprises the amino acid sequence X_1-Q-X_2-X_3-X_4-X_5-P-Y-X_6, wherein X_1 is F or any other amino acid, X_2 is G or any other amino acid, X_3 is S or any other amino acid, X_4 is H or any other amino acid, X_5 is V or any other amino acid, X_6 is T or any other amino acid (SEQ ID NO:47).

In some aspects, the invention provides an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(g) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGNSDTSYNPSFQG (SEQ ID NO: 61) and HCDR3 of GGVRAMDY (SEQ ID NO:62); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQASQVPYT (SEQ ID NO:63); or

(h) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58) and HCDR3 of GGYRAEDY (SEQ ID NO:59); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQGSHVPY (SEQ ID NO:60).

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

the VH region amino acid sequence comprises:

(a) HCDR1 of SEQ ID NO: 48 or SEQ ID NO: 57;

(b) HCDR2 of SEQ ID NO: 49 or SEQ ID NO: 61; and

(c) HCDR3 of SEQ ID NO: 50 or SEQ ID NO: 62; and

the VL region amino acid sequence comprises:

(a') LCDR1 of SEQ ID NO: 51, SEQ ID NO: 55 or SEQ ID NO: 56;

(b') LCDR2 of SEQ ID NO: 52; and

(c') LCDR3 of SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 63.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises SEQ ID NO:1 and the VL region amino acid sequence comprises SEQ ID NO:2;

(b) the VH region amino acid sequence comprises SEQ ID NO:3 and the VL region amino acid sequence comprises SEQ ID NO:4;

(c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6;

(d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8;

(e) the VH region amino acid sequence comprises SEQ ID NO:9 and the VL region amino acid sequence comprises SEQ ID NO:10;

(f) the VH region amino acid sequence comprises SEQ ID NO:11 and the VL region amino acid sequence comprises SEQ ID NO:12;

(g) the VH region amino acid sequence comprises SEQ ID NO:13 and the VL region amino acid sequence comprises SEQ ID NO:14; or

(h) the VH region amino acid sequence comprises SEQ ID NO:15 and the VL region amino acid sequence comprises SEQ ID NO:16.

Also provided according to the invention is an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof as defined herein linked to a therapeutic agent.

In another aspect the invention provides a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof as defined herein.

Further provided is a vector comprising the nucleic acid molecule of the invention.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein.

In a further aspect there is provided a method of producing an anti-CD47 antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

Further provided is a method for enhancing an immune response in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein,

or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

Further provided herein is an antibody molecule or antigen-binding portion thereof as defined herein, or the scFv as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use as a medicament. The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or simultaneous use in a combination with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an autoimmune disease or an inflammatory disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

The autoimmune disease or inflammatory disease may be selected in all aspects from the group consisting of: arthritis, asthma, multiple sclerosis, psoriasis, Crohn's disease, inflammatory bowel disease, lupus, Grave's disease and Hashimoto's thyroiditis, and ankylosing spondylitis.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an autoimmune disease or an inflammatory disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease or an inflammatory disease.

The invention also provides a method for treating or preventing a cardiovascular disease or a fibrotic disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of a cardiovascular disease or a fibrotic disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease, an inflammatory disease or a fibrotic disease.

The cardiovascular disease in any aspect of the invention may for example be coronary heart disease or atherosclerosis.

The fibrotic disease in any aspect of the invention may for example be myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, cystic fibrosis, bronchitis or asthma.

The invention also provides a method of producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47, or an antigen-binding portion thereof, comprising the steps of:

(1) grafting anti-CD47 CDRs from a non-human source into a human v-domain framework to produce a humanized anti-CD47 antibody molecule or antigen-binding portion thereof;

(2) generating a phage library of clones of the humanized anti-CD47 antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;

(3) screening the phage library for binding to human CD47 and optionally also to cynomolgus monkey CD47;

(4) selecting clones from the screening step (3) having binding specificity to human CD47 and optionally also to cynomolgus monkey CD47; and

(5) producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A – FIG. 1F. HTRF-based competition screening of library-derived anti-CD47 scFvs against h5F9G4 IgG1 and hSIRPα-Fc binding to human and cyno CD47-Fc proteins. Clones were derived from separate phage selection branches on biotinylated human and/or cynomolgus monkey CD47-Fc proteins in each round. After multiple rounds of selection in two branches, library-derived clones (black circles) were screened for binding against both human and cyno CD47-Fc and epitope blocking (measured via signal reduction) of h5F9G4 IgG1 and hSIRPα-Fc. Round 2 outputs (Fig. 1A, Fig. 1B). Round 3 outputs (Fig. 1C, Fig. 1D). Round 4 outputs (Fig. 1E, Fig. 1F). Negative control (non-CD47-binding) and positive control h5F9G4 scFvs are represented in grey crossed circle and black circle with grey border, respectively. In addition, an IGHV5-51/IGKV2-28 graft scFv was also included on each plate (grey hexagon).

FIG. 2A – FIG. 2B. Analysis of CDR residue tolerance for mutation to germline. A plot

of murine amino acid retention frequencies in the CDRs of the ELISA-positive population of 269 unique scFv clones is shown for V_H (SEQ ID NOs: 64-66) (Fig. 1A) and V_L (SEQ ID NOs: 67-69) (Fig. 2B) domains, respectively. Only those residues targeted for human/murine residue mutagenesis are plotted, other than in the HCDR3. CDR residues noted in parentheses on the X-axes were identical to those found in the human germlines used for grafting (IGKV2-28 and IGHV5-51). Those residues in the HCDR2 that are not in parentheses, but whose values are set at 0, were mutated to human germline during the grafting process. In both plots the dashed line in grey at 75% represents the cut off for tolerance of murine residue replacement by human germline.

FIG. 3A – FIG. 3B. Direct titration ELISA for library-derived lead IgGs binding to human and cyno CD47-Fc proteins. Chimeric anti-CD47 (m5F9G4) and library-derived clones in human IgG1null format were titrated (in μg/ml) in a direct binding ELISA against human (Fig. 3A) and cyno (Fig. 3B) CD47-Fc proteins. The m5F9G4 and library-derived clones demonstrated binding activity against both orthologs of CD47.

FIG. 4A – FIG. 4B. ELISA-based CD47-Fc-SIRPα competition assay for library-derived leads. ELISA binding signal for human (Fig. 4A) and cyno (Fig. 4B) CD47-Fc proteins to plate-bound human SIRPα was examined in the presence of titrated competitor IgGs in IgG1null format, D-A6 scFv-Fc, plus Isotype IgG1, IGHV5-51 graft, h5F9G4 and m5F9G4 in IgG1null format.

FIG. 5A – FIG. 5B. HTRF-based solution-phase, high-sensitivity, CD47 epitope competition assay for library-derived leads. HTRF binding signal for the h5F9G4 IgG to human or cyno CD47 was examined in the presence of titrated competitor IgGs including library-derived leads, plus Isotype IgG1 and unlabelled h5F9G4 and m5F9G4. All library-derived leads exhibited concentration-dependent inhibition of h5F9G4 binding to human CD47, suggesting maintenance of a shared epitope, but lower binding affinity (Fig. 5A). Both D-A6 proteins (IgG and scFv-Fc) demonstrated the ability to fully inhibit h5F9G4 binding to human CD47 (Fig. 5B), with the scFv-Fc fusion form of the identical v-domains being higher potency than the IgG.

FIG. 6A – FIG. 6H. Direct titration ELISA for designer lead IgGs binding to human and cyno CD47-Fc proteins. Chimeric anti-CD47 (m5F9G4) and designer clones in human IgG1null format were titrated (in μg/ml) in a direct binding ELISA against human and cyno CD47-Fc proteins. The m5F9G4 and library-derived clones demonstrated binding activity against both orthologs of CD47: VH1.1-VH1.3 families versus human (Fig. 6A) and cyno (Fig. 6B); VH1.4-VH1.6 families versus human (Fig. 6C) and cyno (Fig. 6D); VH1.7, VH1.9 and VH1.10 families against human (Fig. 6E) and cyno (Fig. 6F) and VH1.8 family against human (Fig. 6G) and cyno (Fig. 6H).

FIG. 7A – FIG. 7B. ELISA-based CD47-Fc-SIRPα competition assay for VH1.8 family

designer leads. ELISA binding signal for human (FIG. 7A) and cyno (FIG. 7B) CD47-Fc proteins to plate-bound human SIRPα was examined in the presence of titrated competitor IgGs in IgG1null format, plus m5F9G4 and h5F9G4 in IgG1null format as positive controls.

FIG. 8. ELISA-based CD47-Fc-SIRPα competition assay for further designer leads. ELISA binding signal for human CD47-Fc to plate-bound human SIRPα was examined in the presence of titrated competitor IgGs in IgG1null format, plus Isotype IgG1 and m5F9G4 in IgG1null format.

FIG. 9A – FIG. 9D. HTRF-based solution-phase, high-sensitivity, CD47 epitope competition assay for designer leads. HTRF binding signal for the h5F9G4 IgG to human or cyno CD47 was examined in the presence of titrated competitor IgGs including library-derived leads, plus unlabelled h5F9G4 and m5F9G4 as positive controls. All VH1.8-family designer leads exhibited potent concentration-dependent inhibition of h5F9G4 binding to human (FIG. 9A) and cyno (FIG. 9B) CD47. Other selected designer leads also exhibited concentration-dependent inhibition of h5F9G4 binding to human (FIG. 9C) and cyno (FIG. 9D) CD47, with reduced potency.

FIG. 10. Flow cytometric binding to HL60, human and cyno CD47+ CHO-K1 cells for designer leads. Anti-CD47 controls m5F9G4 and h5F9G4, designer leads and Isotype control (all IgG1null) were examined for specific binding on wild type (WT, i.e. untransfected) CHO-K1 cells, human CD47-transfected CHO-K1 cells, cyno CD47-transfected CHO-K1 cells, and the human AML cell line HL60. IgGs were tested at concentrations ranging from 24-100,000 ng/ml. Concentration-dependent binding was observed against human and cyno cell lines, plus HL60 cells, for all CD47-specific antibodies but not isotype controls. No binding signals above background were observed against wild type CHO-K1 cells.

FIG. 11A – FIG. 11C. Development risk ELISAs. This assay showed that designer leads, h5F9G4 and m5F9G4 antibodies in IgG1 form exhibit equivalent or lower binding (with the exception of VH1.12/VL8) to the negatively charged biomolecules Insulin (FIG. 11A), double-stranded DNA (dsDNA) (FIG. 11B) and single-stranded DNA (ssDNA) (FIG. 11C) than the negative control IgG1 Ustekinumab analogue. Strong off-target binding to these molecules, as observed for Bococizumab and Briakinumab analogues has been shown to be a high-risk indicator of poor pharmacokinetics of therapeutic antibodies.

FIG. 12A – FIG. 12C. Summary analysis of the VH and VL sequences of h5F9G4 using iTope™. Peptides spanning the full v-domain sequences were tested as 9mer peptides in one amino acid increments. Each row indicates the position of a p1 anchor residue of a 9mer peptide which is scored for MHC class II binding (core 9mer). Each box indicates a binding score of >0.6 between the aligned 9mer and MHC class II. A promiscuous binding peptide is defined as binding 17 or more alleles. Regions containing potentially immunogenic peptides are indicated in 'Promiscuous High' (HAF) and 'Promiscuous Moderate' (LAF) columns; dark

grey vertical bars indicate HAF MHC class II binding peptides and light grey vertical bars indicate LAF MHC class II binding peptides. In the 'TCED' column, regions representing closely homologous peptides from the T cell epitope database are shown as vertical grey bars. Example analyses are shown for the key HAF and TCED+ epitopes found in the LCDR1 (SEQ ID NO: 70) (FIG. 12A), HCDR1 (SEQ ID NO: 71) (FIG. 12B) and HCDR3 (SEQ ID NO: 72) (FIG. 12C).

FIG. 13A – FIG. 13B. Summary analysis of the VL sequences of key leads using iTope™. Peptides spanning the full v-domain sequences were tested as 9mer peptides in one amino acid increments across the full v-domains (VL1.1 shown here as an example). Data is represented as in Fig. 13A – FIG. 13B. Example analyses are shown for the key HAF and TCED+ epitopes found in the LCDR2 (SEQ ID NO: 73) (FIG. 13A) and LCDR3 (SEQ ID NO: 74) (FIG. 13B).

FIG. 14A – FIG. 14C. Phagocytosis potency assays using human macrophage and Jurkat cells. Clones h5F9G4, VH1.8/VL1.1, VH1.8/VL1.6, and VH1.8/VL1.6-DI in IgG4(S228P) format were examined for their relative potencies in a flow cytometric assay of phagocytosis, using human monocyte-derived macrophages derived from 3 separate donors and Jurkat human cancer cells as the CD47+ target cells. Phagocytosis was measured as Percentage CFSE+ cells from CD11b+ population for Donors 1 (FIG. 14A), 2 (FIG. 14B) and 3 (FIG. 14C).

DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, there is provided an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

In the IGHV5-51 background; an HCDR1 having amino acids in sequence in the following order: G-Y-S-F-T-N or a conservative substitution of N (such as S)-Y-N or a conservative substitution of N (such as S)-M or a conservative substitution of M (such as I)-H or any amino acid (such as G) (SEQ ID NO:28);

an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M (for example, I)-G-T or any amino acid (such as I)-I-Y-P-G-N or any amino acid (such as D)-D or any amino acid (such as S)-D-T-S or any amino acid (such as R)-Y-N or any amino acid (such as S, H)-Q or any amino acid (such as P)-K or any amino acid (such as S)-F-Q-G (SEQ ID NO:29); and

an HCDR3 having amino acids in sequence in the following order: G or any amino acid (such as Q)-G-Y or any amino acid (such as A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W)-R-

A or any amino acid (such as I, T, V, W)-M or any amino acid (such as A, E, K, L, P, Q, R, S, T, V, W)-D-Y (SEQ ID NO:30).

In some aspects an anti-CD47 antibody or antigen-binding portion provided herein specifically binds to a CD47 protein comprising or consisting of SEQ ID NO:24 or SEQ ID NO:25. In some aspects an anti-CD47 antibody or antigen-binding portion provided herein specifically binds to a CD47 protein having an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:24 or SEQ ID NO:25.

In the IGHV1-3 background; an HCDR1 having amino acids in sequence in the following order: G-Y-T-F-T-N or any amino acid (such as D)-Y-N or any amino acid (such as A)-M-H (SEQ ID NO:31);
an HCDR2 having amino acids in sequence in the following order: M-G-T-I-Y-P-G-N-D-D or any amino acid (such as N)-T-S or any amino acid (such as K)-Y-N or any amino acid (such as S)-Q-K-F-Q-G (SEQ ID NO:32); and
an HCDR3 having amino acids in sequence in the following order: G or any amino acid (such as Q)-G-Y or any amino acid (such as A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W)-R-A or any amino acid (such as I, T, V, W)-M or any amino acid (such as A, E, K, L, P, Q, R, S, T, V, W)-D-Y (SEQ ID NO:30).

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTNYNMH (SEQ ID NO:33; 5F9G4 murine/humanized antibody HCDR1 disclosed in WO2011143624A2; US20130142786A1), the HCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence MGTIYPGNDDTSYNQKFKD (SEQ ID NO:34; 5F9G4 murine/humanized antibody HCDR1 disclosed in WO2011143624A2; US20130142786A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYRAMDY (SEQ ID NO:35; 5F9G4 murine/humanized antibody HCDR3 disclosed in WO2011143624A2; US20130142786A1).

The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:
an LCDR1 having amino acids in sequence in the following order: R-S-S-Q-S-I or a conservative substitution of I (such as L)-V or a conservative substitution of V (such as L)-Y or a conservative substitution of Y (such as H)-S-N or any amino acid (such as G, S, K)-G or

any amino acid (such as A, Y)-N or any amino acid (such as Q, Y)-T or any amino acid (such as N)-Y-L-G or any amino acid (such as D) (SEQ ID NO:36);
an LCDR2 having amino acids in sequence in the following order: K-V or any amino acid (such as G)-S-N-R-F or any amino acid (such as A, S)-S (SEQ ID NO:37); and
an LCDR3 having amino acids in sequence in the following order: F or any amino acid (such as L, M)-Q-G or any amino acid (such as A)-S or any amino acid (such as T, L, M)-H or any amino acid (such as Q, R)-V or any amino acid (such as I, T)-P-Y-T or any amino acid (such as I) (SEQ ID NO:38).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence RSSQSIVYSNGNTYLG (SEQ ID NO:39; 5F9G4 murine/humanized antibody LCDR1 disclosed in WO2011143624A2; US20130142786A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSNRFS (SEQ ID NO:40; 5F9G4 murine/humanized antibody LCDR2 disclosed in WO2011143624A2; US20130142786A1), and/or the LCDR3 of the antibody molecule or antigen binding portion may exclude the sequence FQGSHVPYT (SEQ ID NO:41).

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the HCDR1 comprises the amino acid sequence G-Y- X_1-F-T-X_2-Y-X_3-X_4-X_5, wherein X_1 is S or a conservative substitution of S, X_2 is N or a conservative substitution of N (for example, S), X_3 is N or any other amino acid (for example, S, A), X_4 is M or a conservative substitution of M (for example, I) and X_5 is H or any other amino acid (for example, G) (SEQ ID NO:42);

(b) the HCDR2 comprises the amino acid sequence X_1-X_2-X_3-I-Y-P-G-X_4-X_5-X_6-T-X_7-Y-X_8-X_9-X_{10}-F-Q-G, wherein X_1 is M or a conservative substitution of M (for example, I), X_2 is G or a conservative substitution of G, X_3 is T or any other amino acid (for example, I), X_4 is N or any other amino acid (for example, D), X_5 is D or any other amino acid (for example, S), X_6 is D or any other amino acid, X_7 is S or any other amino acid (for example, R), X_8 is N or any other amino acid (for example, S), X_9 is Q or any other amino acid (for example, P) and X_{10} is K or any other amino acid (for example, S) (SEQ ID NO:75);

(c) the HCDR3 comprises the amino acid sequence X_1-G-X_2-R-X_3-X_4-D-Y, wherein X_1 is G or any other amino acid (for example, Q), X_2 is Y or any other amino acid (for example, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V or W), X_3 is A or any other amino acid (for example, I, T, V or W) and X_4 is M or any other amino acid (for example, A, E, K, L, P, Q, R, S, T, V or W) (SEQ ID NO:44);

(d) the LCDR1 comprises the amino acid sequence R-S-S-Q-S-X_1-X_2-X_3-S-X_4-X_5-X_6-X_7-Y-L-X_8, wherein X_1 is I or a conservative substitution of I (for example, L), X_2 is V or a conservative substitution of V (for example, L), X_3 is Y or a conservative substitution of Y (for example, H), X_4 is N or any other amino acid (for example, G, S or K), X_5 is G or any other amino acid (for example, A or Y), X_6 is N or any other amino acid (for example, Q or Y), X_7 is T or any other amino acid (for example, N) and X_8 is G or any other amino acid (for example, D) (SEQ ID NO:45);

(e) the LCDR2 comprises the amino acid sequence K-X_1-S-N-R-X_2-S, wherein X_1 is V or any other amino acid (for example, G) and X_2 is F or any other amino acid (for example, A or S) (SEQ ID NO:46); and

(f) the LCDR3 comprises the amino acid sequence X_1-Q-X_2-X_3-X_4-X_5-P-Y-X_6, wherein X_1 is F or any other amino acid (for example, L or M), X_2 is G or any other amino acid (for example, A), X_3 is S or any other amino acid (for example, T, L or M), X_4 is H or any other amino acid (for example, Q or R), X_5 is V or any other amino acid (for example, I or T), X_6 is T or any other amino acid (for example, I) (SEQ ID NO:47).

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising, in amino-terminal to carboxyl-terminal order, FR1-HCDR1-FR2-HCDR2-FR3-HCDR3-FR4 and a light chain variable (VL) region comprising, in amino-terminal to carboxyl-terminal order, FR1-LCDR1-FR2-LCDR2-FR3-LCDR3-FR4, wherein the HCDR1 is SEQ ID NO:42, the HCDR2 is SEQ ID NO:75, the HCDR3 is SEQ ID NO:44, the LCDR1 is SEQ ID NO:45, the LCDR2 is SEQ ID NO:46 and the LCDR3 is SEQ ID NO:47, wherein the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences are the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO:141 (see Table 2) and wherein the light chain FR1, FR2, FR3 and FR4 amino acid sequences are the light chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO:144 (see Table 2).

As elaborated herein, the present inventors have succeeded for the first time in generating a number of optimized anti-CD47 antibody molecules using CDR sequences derived from the murine anti-CD47 antibody 5F9G4 disclosed in WO2011143624A2; US20130142786A1. In embodiments of the present invention, these antibody molecules have been selected to have binding specificity to both human CD47 as well as cynomolgus monkey CD47 (to facilitate *in vivo* studies in an appropriate animal test species). Further refining of the optimized antibody molecules as described herein has provided improved variable domain stability, higher expression yields, and/or reduced immunogenicity.

Preferred optimized anti-CD47 antibody molecules of the present invention do not necessarily have the maximum number of human germline substitutions at corresponding murine CDR or other (such as framework) amino acid positions. As elaborated in the experimental section below, we have found that “maximally humanized” antibody molecules are not necessary “maximally optimized” in terms of anti-CD47 binding characteristics and/or other desirable features.

The present invention encompasses modifications to the amino acid sequence of the antibody molecule or antigen-binding portion thereof as defined herein. For example, the invention includes antibody molecules and corresponding antigen-binding portions thereof comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to CD47. Insertions which include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues, are envisaged. Examples of terminal insertions include an antibody molecule with an N-terminal methionyl residue or the antibody molecule fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

The antibody molecule or antigen-binding portion of the invention may include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. The antibody molecule or antigen-binding portion of the invention may be mutated to alter such post-translational modifications, for example by adding, removing or replacing one or more amino acid residues to form or remove a glycosylation site.

The antibody molecule or antigen-binding portion of the invention may be modified for example by amino acid substitution to remove potential proteolytic sites in the antibody.

In the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-Y-T/S-F-T-N/S/D-Y-N/S/A-M/I-H/G (SEQ ID NO:76); the HCDR2 may have the amino acid sequence: M/I/V-G/A/S-T/I-I-Y-P-G-N/D-D/S-D/N-T-S/K/R-Y-N/S/H-Q/P/H-K/S-F-K/Q-D/G (SEQ ID NO:77); and the HCDR3 may have the amino acid sequence: G/Q-G-Y/A/C/D/E/F/G/H/I/K/L/M/N/Q/R/S/T/V/W-R-A/I/T/V/W-M/A/E/K/L/P/Q/R/S/T/V/W-D-Y (SEQ ID NO:78).

For example, the HCDR1 may have the amino acid sequence: G-Y-T/S-F-T-N/D-Y-N/A-M/I-H (SEQ ID NO:79); the HCDR2 may have the amino acid sequence: M-G-T-I-Y-P-G-N/D-D/S-D/N-T-S/K/R-Y-N/S-Q/P/H-K/S-F-Q-G (SEQ ID NO:80); and the HCDR3 may have the amino acid sequence: G-G-Y/E/F/I/K//N/V-R-A-M/E/Q-D-Y (SEQ ID NO:81).

In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: R-S-S-Q-S-L-V/L-Y/H-S-N/G/S/K-G/A/Y-N/Y-T/N-Y-L-G/D (SEQ ID NO:82); the LCDR2 may have the amino acid sequence: K-G-S-N-R-F/A-S (SEQ ID NO:83); and the LCDR3 may have the amino acid sequence: F/M-Q-G/A-S-H/Q-V-P-Y-T/I (SEQ ID NO:84).

For example, the LCDR1 may have the amino acid sequence: R-S-S-Q-S-L-L-H-S-N/K-G/A/Y-N/Q/Y-T/N-Y-L-G (SEQ ID NO:85); the LCDR2 may have the amino acid sequence: K-V/G-S-N-R-F/A/S-S (SEQ ID NO:86); and the LCDR3 may have the amino acid sequence: F/L/M-Q-G/A-S/T/L/M-H/Q/R-V/I/T-P-Y-T/I (SEQ ID NO:87).

In specific embodiments of the invention, the antibody molecule or antigen-binding portion may comprise:

(a) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.8/VL1.1]; or

(b) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.8/VL1.2]; or

(c) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.8/VL1.3]; or

(d) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNAYNYLG (SEQ ID NO:56; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.8/VL1.4]; or

(e) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50;

HCDR3), RSSQSLLHSNAYNYLG (SEQ ID NO:56; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.8/VL1.5]; or

(f) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.8/VL1.6]; or

(g) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.1/VL1.1]; or

(h) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.1/VL1.2]; or

(i) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.1/VL1.3]; or

(j) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.1/VL1.6]; or

(k) the amino acid sequences GYTFTDYNMH (SEQ ID NO:88; HCDR1), MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.5/VL1.1]; or

(l) the amino acid sequences GYTFTNYNIH (SEQ ID NO:89; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.7/VL1.1]; or

(m) the amino acid sequences GYTFTNYNIH (SEQ ID NO:89; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.7/VL1.2]; or

(n) the amino acid sequences GYTFTNYNIH (SEQ ID NO:89; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGFRAMDY (SEQ ID NO:50;

HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.7/VL1.3]; or

(o) the amino acid sequences GYTFTNYNIH (SEQ ID NO:89; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNAYNYLG (SEQ ID NO:56; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.7/VL1.5]; or

(p) the amino acid sequences GYTFTNYNIH (SEQ ID NO:89; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNANTYLG (SEQ ID NO:55; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.7/VL1.6]; or

(q) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.6/VL1.1]; or

(r) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.6/VL1.2]; or

(s) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASHVPYT (SEQ ID NO:53; LCDR3), [Clone VH1.9/VL1.1]; or

(t) the amino acid sequences GYTFTNYAMH (SEQ ID NO:48; HCDR1), MGTIYPGNDDTKYSQKFQG (SEQ ID NO:91; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQASQVPYT (SEQ ID NO:54; LCDR3), [Clone VH1.9/VL1.2]; or

(u) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGNSDTSYNPSFQG (SEQ ID NO:61; HCDR2), GGVRAMDY (SEQ ID NO:62; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQASQVPYT (SEQ ID NO:63; LCDR3), [Clone D6]; or

(v) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGNSDTSYNPKFQG (SEQ ID NO:92; HCDR2), GGKRAMDY (SEQ ID NO:93; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQASQVPYT (SEQ ID NO:63; LCDR3), [Clone F-E5]; or

(w) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94; HCDR2), GGYRAQDY (SEQ ID NO:95;

HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQASHVPYT (SEQ ID NO:96; LCDR3), [Clone E-C5]; or

(x) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQGSHVPYT (SEQ ID NO:97; LCDR3), [Clone E-D6]; or

(y) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGDSDTRYNQKFQG (SEQ ID NO:98; HCDR2), GGIRAMDY (SEQ ID NO:99; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), FQGSHVPYI (SEQ ID NO:100; LCDR3), [Clone B-A6]; or

(z) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGNSDTRYNPKFQG (SEQ ID NO:101; HCDR2), GGFRAMDY (SEQ ID NO:50; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQGSHVPYT (SEQ ID NO:97; LCDR3), [Clone D-C12]; or

(zi) the amino acid sequences GYSFTNYNIH (SEQ ID NO:57; HCDR1), MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58; HCDR2), GGYRAEDY (SEQ ID NO:59; HCDR3), RSSQSLLHSNGNTYLG (SEQ ID NO:51; LCDR1), KGSNRFS (SEQ ID NO:52; LCDR2), MQGSHVPYT (SEQ ID NO:97; LCDR3), [Clone E-A1].

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54);

(g) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGNSDTSYNPSFQG (SEQ ID NO: 61) and HCDR3 of GGVRAMDY (SEQ ID NO:62); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQASQVPYT (SEQ ID NO:63); or

(h) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58) and HCDR3 of GGYRAEDY (SEQ ID NO:59); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQGSHVPY (SEQ ID NO:60).

In some aspects, disclosed herein is anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region comprises any one of the VH region amino acid sequences in Table 7 and the VL region comprises any one of the VL region amino acid sequences in Table 7.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises SEQ ID NO:1 and the VL region amino acid sequence comprises SEQ ID NO:2;

(b) the VH region amino acid sequence comprises SEQ ID NO:3 and the VL region amino acid sequence comprises SEQ ID NO:4;

(c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6;

(d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8;

(e) the VH region amino acid sequence comprises SEQ ID NO:9 and the VL region amino acid sequence comprises SEQ ID NO:10;

(f) the VH region amino acid sequence comprises SEQ ID NO:11 and the VL region amino acid sequence comprises SEQ ID NO:12;

(g) the VH region amino acid sequence comprises SEQ ID NO:13 and the VL region amino acid sequence comprises SEQ ID NO:14; or

(h) the VH region amino acid sequence comprises SEQ ID NO:15 and the VL region amino acid sequence comprises SEQ ID NO:16.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:1 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:2;

(b) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:3 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:4;

(c) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:5 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least

about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:6;

(d) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:7 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:8;

(e) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:9 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:10;

(f) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:11 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:12;

(g) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:13 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:14; or

(h) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:15 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:16.

In some aspects, the CDR amino acid sequences of an anti-CD47 antibody are 100% identical to the CDR amino acid sequences in the recited sequences while the FR amino acid sequences are less than 100% identical to the FR amino acid sequences in the recited sequences.

In some aspects, the antibody or antigen-binding portion as defined herein may be isolated.

The antibody molecule or antigen-binding portion as defined herein may cross-compete for binding to CD47 with an antibody or antigen-binding portion thereof comprising the sets of CDRs disclosed herein. In some embodiments, the invention provides an isolated anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to CD47 with the antibody or antigen-binding portion comprising the sets of CDRs disclosed herein; and (a) comprises fully germline human framework amino acid sequences; and/or (b) does not comprise an 'NG' high-risk deamidation site in the LCDR1; (c) does not comprise an 'NT' deamidation site in the LCDR1; and/or (d) comprises a human germline peptide sequence with high MHC class II binding affinity in HCDR1 and/or HCDR2; and/or (e) does not comprise a human T cell epitope sequence in the LCDR1; and/or (f) does not comprise a human T cell epitope sequence in the HCDR1 or in the HCDR1/Framework 2 region of the VH domain. In some embodiments, the human germline peptide sequence with high MHC class II binding affinity in the HCDR2 is FQGQVTISA (SEQ ID NO:102) or FQGRVTITA (SEQ ID NO:103).

The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or portion thereof to interfere with the binding directly or indirectly through allosteric modulation of the anti-CD47 antibodies of the invention to the target CD47 (e.g., human CD47). The extent to which an antibody or portion thereof is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block or cross-compete according to the invention, can be determined using competition binding assays. One example of a binding competition assay is Homogeneous Time Resolved Fluorescence (HTRF). One particularly suitable quantitative cross-competition assay uses a FACS- or an AlphaScreen-based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) antibody or portion thereof and the other antibody or portion thereof in terms of their binding to the target. In general, a cross-competing antibody or portion thereof is, for example, one which will bind to the target in the cross-competition assay such that, during the assay and in the presence of a second antibody or portion thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide

according to the invention is up to 100% (e.g. in a FACS based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (e.g. unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the potentially cross-blocking antibody or fragment thereof that is present in a given amount. Preferably, cross-competing antibodies or portions thereof have a recorded displacement that is between 10% and 100%, or between 50% and 100%.

The antibody molecule or antigen-binding portion as defined herein may comprise one or more substitutions, deletions and/or insertions which remove a post-translational modification (PTM) site, for example a glycosylation site (N-linked or O-linked), a deamination site, a phosphorylation site or an isomerisation/fragmentation site.

More than 350 types of PTM are known. Key forms of PTM include phosphorylation, glycosylation (*N*- and *O*-linked), sumoylation, palmitoylation, acetylation, sulfation, myristoylation, prenylation and methylation (of K and R residues). Statistical methods to identify putative amino acid sites responsible for specific PTMs are well known in the art (see Zhou *et al.*, 2016, Nature Protocols 1: 1318-1321). Removal of such a site for example by substitution, deletion and/or insertion and then optionally testing (experimentally and/or theoretically) for (a) binding activity and/or (b) loss of the PTM is contemplated.

For example, the 5F9G4 murine LCDR1 (as defined herein, i.e. the amino acid sequence RSSQSIVYSNGNTYLG (SEQ ID NO:39)) has been identified to have a putative 'high-risk' deamidation motif at residues 10 and 11 (NG). Removal this site at equivalent positions in an LCDR1 of the invention, for example by substitution of residue 10 (such as to G, S or K), is envisaged (as for example in multiple clones in Table 3). Removal this site at equivalent positions in an LCDR1 of the invention, for example by substitution of residue 11 (such as to A or Y), is also envisaged (as for example in clones containing the VL1.4 v-domain sequence and multiple other clones found in Tables 3 and 4).

In a further example, the 5F9G4 murine LCDR1 (as defined herein, i.e. the amino acid sequence RSSQSIVYSNGNTYLG (SEQ ID NO:39)) has been identified to have a putative 'high-risk' deamidation motif at residues 12 and 13 (NT). Removal this risk site at equivalent positions in an LCDR1 of the invention, for example by substitution of residue 12 (such as to Q, Y), is envisaged (as for example in clones containing the VL1.4 v-domain sequence and multiple other clones found in Tables 3 and 4).

The antibody molecule or antigen-binding portion thereof may be human, humanized or chimeric.

The antibody molecule or antigen-binding portion thereof may comprise one or more human variable domain framework scaffolds into which the CDRs have been inserted. For example, the VH region, the VL region, or both the VH and the VL region may comprise one or more human framework region amino acid sequences.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV5-51 human germline scaffold into which the corresponding HCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV5-51 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-3 human germline scaffold into which the corresponding HCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-3 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

The antibody molecule or antigen-binding portion thereof may comprise an IGKV2-28 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VL region that comprises an IGKV2-28 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV5-51 human germline scaffold into which the corresponding HCDR sequences have been inserted and an IGKV2-28 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV5-51 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted and a VL region that comprises an IGKV2-28 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences may be the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of any one of the clones in Table 4. In some embodiments, all six

CDR sequences are from the same clone in Table 4. In some embodiments, the HCDR1, HCDR2 and HCDR3 sequences are from a first clone in Table 4, and the LCDR1, LCDR2 and LCDR3 sequences are from a second clone in Table 4.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-3 human germline scaffold into which the corresponding HCDR sequences have been inserted and an IGKV2-28 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-3 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted and a VL region that comprises an IGKV2-28 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences may be the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of any one of the clones in Table 4. In some embodiments, all six CDR sequences are from the same clone in Table 4. In some embodiments, the HCDR1, HCDR2 and HCDR3 sequences are from a first clone in Table 4, and the LCDR1, LCDR2 and LCDR3 sequences are from a second clone in Table 4.

In some aspects, the antibody molecule or antigen-binding portion thereof may comprise an immunoglobulin constant region. In some embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. In additional embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG1null, IgG4(S228P), IgA1 or IgA2. The antibody molecule or antigen-binding portion thereof may comprise an immunologically inert constant region. In some aspects, an anti-CD47 antibody or antigen-binding portion thereof may comprise an immunoglobulin constant region comprising a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S. In some aspects, an anti-CD47 antibody or antigen-binding portion thereof may comprise an immunoglobulin constant region comprising a wild-type human IgG2 constant region or a wild-type human IgG4 constant region. In some aspects, an anti-CD47 antibody may comprise an immunoglobulin constant region comprising any one of the amino acid sequences in Table 8. The Fc region sequences in Table 8 begin at the CH1 domain. In some aspects, an anti-CD47 antibody may comprise an immunoglobulin constant region comprising an amino acid sequence of an Fc region of human IgG4, human IgG4(S228P), human IgG2, human IgG1, human IgG1-3M or human IgG1-4M. For example, the human IgG4(S228P) Fc region comprises the

following substitution compared to the wild-type human IgG4 Fc region: S228P. For example, the human IgG1-3M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A and G237A, while the human IgG1-4M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A, G237A and P331S. In some aspects, a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). In some aspects, an immunoglobulin constant region may comprise an RDELT (SEQ ID NO:26) motif or an REEM (SEQ ID NO:27) motif (underlined in Table 8). The REEM (SEQ ID NO:27) allotype is found in a smaller human population than the RDELT (SEQ ID NO:26) allotype. In some aspects, an anti-CD47 antibody may comprise an immunoglobulin constant region comprising any one of SEQ ID NOS:17-23. In some aspects, an anti-CD47 antibody may comprise the six CDR amino acid sequences of any one of the clones in Table 4 and any one of the Fc region amino acid sequences in Table 8. In some aspects, an anti-CD47 antibody may comprise an immunoglobulin heavy chain constant region comprising any one of the Fc region amino acid sequences in Table 8 and an immunoglobulin light chain constant region that is a kappa light chain constant region or a lambda light chain constant region.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and

LCDR3 of FQASHVPYT (SEQ ID NO:53); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNAYNYLG (SEQ ID NO:56), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASQVPYT (SEQ ID NO:54); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(g) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGNSDTSYNPSFQG (SEQ ID NO: 61) and HCDR3 of GGVRAMDY (SEQ ID NO:62); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQASQVPYT (SEQ ID NO:63); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23; or

(h) the VH region amino acid sequence comprises HCDR1 of GYSFTNYNIH (SEQ ID NO:57), HCDR2 of MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58) and HCDR3 of GGYRAEDY (SEQ ID NO:59); the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of MQGSHVPY (SEQ ID NO:60); and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

a) the VH region amino acid sequence comprises or consists of SEQ ID NO:1; the VL region amino acid sequence comprises or consists of SEQ ID NO:2; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:3; the VL region amino acid sequence comprises or consists of SEQ ID NO:4; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:5; the VL region amino acid sequence comprises or consists of SEQ ID NO:6; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:7; the VL region amino acid sequence comprises or consists of SEQ ID NO:8; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:9; the VL region amino acid sequence comprises or consists of SEQ ID NO:10; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:11; the VL region amino acid sequence comprises or consists of SEQ ID NO:12; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(g) the VH region amino acid sequence comprises or consists of SEQ ID NO:13; the VL region amino acid sequence comprises or consists of SEQ ID NO:14; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A; or

(h) the VH region amino acid sequence comprises or consists of SEQ ID NO:15; the VL region amino acid sequence comprises or consists of SEQ ID NO:16; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A.

In some aspects, disclosed herein is an anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

a) the VH region amino acid sequence comprises or consists of SEQ ID NO:1; the VL region amino acid sequence comprises or consists of SEQ ID NO:2; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:3; the VL region amino acid sequence comprises or consists of SEQ ID NO:4; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:5; the VL region amino acid sequence comprises or consists of SEQ ID NO:6; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:7; the VL region amino acid sequence comprises or consists of SEQ ID NO:8; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:9; the VL region amino acid sequence comprises or consists of SEQ ID NO:10; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:11; the VL region amino acid sequence comprises or consists of SEQ ID NO:12; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23;

(g) the VH region amino acid sequence comprises or consists of SEQ ID NO:13; the VL region amino acid sequence comprises or consists of SEQ ID NO:14; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23; or

(h) the VH region amino acid sequence comprises or consists of SEQ ID NO:15; the VL region amino acid sequence comprises or consists of SEQ ID NO:16; and the heavy chain constant region comprises any one of SEQ ID NOS: 17-23.

In some aspects, an anti-CD47 antibody may be immune effector null. In some aspects, an anti-CD47 antibody or an antigen-binding portion thereof does not induce immune effector function and, optionally, suppresses immune effector function. In some aspects, an anti-CD47 may lack measurable binding to human FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb receptors but maintain binding to human FcγRIIb receptor and optionally maintain binding to human FcRn receptor. FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb are examples of activating receptors. FcγRIIb is an example of an inhibitory receptor. FcRn is an example of a recycling receptor. In some aspects, binding affinity of an anti-CD47 antibody or an antigen-binding portion thereof for human Fc receptors may be measured by BIACORE® analysis. In some aspects, Homogeneous Time Resolved Fluorescence (HTRF) can be used to study binding of an anti-CD47 antibody to human Fc receptors. In one example of HTRF, human IgG1 (wild type) is labelled, as is the full suite of Fc gamma receptors and then antibodies with engineered Fc fragments are used in titration competition. In some aspects, CD47-positive cells may be mixed with human white blood cells and anti-CD47 antibodies, and cell killing by CDC, ADCC and/or ADCP may be measured. In some aspects, an anti-CD47 antibody comprising an amino acid sequence of an Fc region of human IgG1-3M (see Table 8) is effector null. In some aspects, an anti-CD47 antibody comprising an amino acid sequence of an Fc region of human IgG1-3M (see Table 8) is not effector null.

The antibody molecule or antigen-binding portion thereof may be a Fab fragment, a $F(ab)_2$ fragment, an Fv fragment, an scFv (single-chain Fv) fragment, a tetrameric antibody, a tetravalent antibody, a multispecific antibody (for example, a bispecific antibody), a domain-specific antibody, a single domain antibody, a monoclonal antibody or a fusion protein. In one embodiment, an antibody may be a bispecific antibody that binds specifically to a first antigen and a second antigen, wherein the first antigen is CD47 and the second antigen is not CD47. Antibody molecules and methods for their construction and use are described, in for example Holliger & Hudson (2005, Nature Biotechnol. 23(9): 1126-1136).

In some aspects, the invention provides a heavy chain variable (VH) region sequence and a light chain variable (VL) region sequence that neutralizes the CD47-SIRPα interaction more potently in the single-chain Fv (scFv) format than in the IgG format. In some embodiments, the VH regions of such molecules are in the IGHV5-51 framework. In some embodiments, the invention encompasses a single-chain Fv (scFv) comprising a heavy

chain variable (VH) region and a light chain variable (VL) region, wherein (a) the HCDR1 comprises G-Y- T-F-T-X_1-Y-X_2-X_3-X_4, wherein X_1 is N or a conservative substitution of N, X_2 is N or any other amino acid, X_3 is M or a conservative substitution of M and X_4 is H or any other amino acid (SEQ ID NO:104); (b) the HCDR2 comprises M-G-X_1-I-Y-P-G-X_2-X_3-X_4-T-X_5-Y-X_6-X_7-X_8-F-Q-G, wherein X_1 is T or any other amino acid, X_2 is N or a conservative substitution of N, X_3 is D or any other amino acid, X_4 is D or any other amino acid, X_5 is S or any other amino acid, X_6 is N or any other amino acid, X_7 is Q or any other amino acid and X_8 is K or any other amino acid (SEQ ID NO:105); (c) the HCDR3 comprises ; X_1-G-X_2-R-X_3-X_4-D-Y, wherein X_1 is G or any other amino acid, X_2 is Y or any other amino acid, X_3 is A or any other amino acid and X_4 is M or any other amino acid (SEQ ID NO:44) (d) the LCDR1 comprises R-S-S-Q-S-X_1-X_2-X_3-S-X_4-X_5-X_6-X_7-Y-L-X_8, wherein X_1 is I or a conservative substitution of I, X_2 is V or a conservative substitution of V, X_3 is Y or a conservative substitution of Y, X_4 is N or any other amino acid, X_5 is G or any other amino acid, X_6 is N or any other amino acid, X_7 is T or any other amino acid and X_8 is G or any other amino acid (SEQ ID NO:45); (e) the LCDR2 comprises K-X_1-S-N-R-X_2-S, wherein X_1 is V or any other amino acid and X_2 is F or any other amino acid (SEQ ID NO:46); and (f) the LCDR3 comprises X_1-Q-X_2-X_3-X_4-X_5-P-Y-X_6, wherein X_1 is F or any other amino acid, X_2 is G or any other amino acid, X_3 is S or any other amino acid, X_4 is H or any other amino acid, X_5 is V or any other amino acid, X_6 is T or any other amino acid (SEQ ID NO:47); and wherein the VH region comprises an IGHV5-51 human germline scaffold amino acid sequence into which the HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted. In one embodiment, an scFv of the invention may comprise (a) the VH amino acid sequence comprises the HCDR1 of GYSFTNYNIH (SEQ ID NO:57), the HCDR2 of MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94) and the HCDR3 of GGYRAEDY (SEQ ID NO:59); and (b) the VH amino acid sequence comprises the LCDR1 of RSSQSLLHSNGNTYLG (SEQ ID NO:51), the LCDR2 of KGSNRFS (SEQ ID NO:52) and the LCDR3 of MQGSHVPYT (SEQ ID NO:97). In some aspects, an scFv comprises, in amino-terminal to carboxyl-terminal order, VH-VL or VL-VH. In some aspects, an scFv comprises a linker between the VH sequence and the VL sequence. In some embodiments, an scFv further comprises an immunoglobulin constant region or a portion of an immunoglobulin constant region. In some embodiments, the invention provides a multispecific (e.g., bispecific) CD47-binding molecule comprising one or more scFv disclosed herein. In some cases, the immunoglobulin constant region comprises any one of SEQ ID NOS:17-23.

In another aspect of the invention, there is provided an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein linked to a therapeutic agent.

Examples of suitable therapeutic agents include cytotoxins, radioisotopes, chemotherapeutic agents, immunomodulatory agents, anti-angiogenic agents, antiproliferative agents, pro-apoptotic agents, and cytostatic and cytolytic enzymes (for example RNAses). Further therapeutic agents include a therapeutic nucleic acid, such as a gene encoding an immunomodulatory agent, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent. These drug descriptors are not mutually exclusive, and thus a therapeutic agent may be described using one or more of the above terms.

Examples of suitable therapeutic agents for use in immunoconjugates include the taxanes, maytansines, CC-1065 and the duocarmycins, the calicheamicins and other enediynes, and the auristatins. Other examples include the anti-folates, vinca alkaloids, and the anthracyclines. Plant toxins, other bioactive proteins, enzymes (i.e., ADEPT), radioisotopes, photosensitizers may also be used in immunoconjugates. In addition, conjugates can be made using secondary carriers as the cytotoxic agent, such as liposomes or polymers, Suitable cytotoxins include an agent that inhibits or prevents the function of cells and/or results in destruction of cells. Representative cytotoxins include antibiotics, inhibitors of tubulin polymerization, alkylating agents that bind to and disrupt DNA, and agents that disrupt protein synthesis or the function of essential cellular proteins such as protein kinases, phosphatases, topoisomerases, enzymes, and cyclins.

Representative cytotoxins include, but are not limited to, doxorubicin, daunorubicin, idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifluhdine, pentostatin, broxuhdine, capecitabine, cladhbine, decitabine, floxuhdine, fludarabine, gougerotin, puromycin, tegafur, tiazofuhn, adhamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone, bleomycin, mechlorethamine, prednisone, procarbazine, methotrexate, flurouracils, etoposide, taxol, taxol analogs, platins such as cis-platin and carbo-platin, mitomycin, thiotepa, taxanes, vincristine, daunorubicin, epirubicin, actinomycin, authramycin, azaserines, bleomycins, tamoxifen, idarubicin, dolastatins/auristatins, hemiasterlins, esperamicins and maytansinoids.

Suitable immunomodulatory agents include anti-hormones that block hormone action on tumors and immunosuppressive agents that suppress cytokine production, down-regulate self-antigen expression, or mask MHC antigens.

Also provided is a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof of the invention as defined herein. A nucleic acid molecule may encode (a) the VH region amino acid sequence; (b) the VL region amino acid sequence; or (c) both the VH and the VL region amino acid sequences of an anti-CD47 antibody or an antigen-binding portion thereof described herein. In some aspects, the nucleic acid molecule as defined herein may be isolated.

Further provided is a vector comprising the nucleic acid molecule of the invention as defined herein. The vector may be an expression vector.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein. The host cell may be a recombinant host cell.

In a further aspect there is provided a method of producing an anti-CD47 antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

Further provided is a method for enhancing an immune response in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering to the subject an effective amount of the antibody molecule or

antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

For example, the cancer may be Gastrointestinal Stromal cancer (GIST), pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or simultaneous use in a combination combined with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an autoimmune disease or an inflammatory disease in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

For example, the autoimmune disease or inflammatory disease may be arthritis, asthma, multiple sclerosis, psoriasis, Crohn's disease, inflammatory bowel disease, lupus, Grave's disease and Hashimoto's thyroiditis, or ankylosing spondylitis.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an autoimmune disease or an inflammatory disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease or an inflammatory disease.

The invention also provides a method for treating or preventing a cardiovascular disease or a fibrotic disease in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of a cardiovascular disease or a fibrotic disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of a cardiovascular disease or a fibrotic disease.

The cardiovascular disease in any aspect of the invention may for example be coronary heart disease or atherosclerosis.

For example, the fibrotic disease in any aspect of the invention may be myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis or bronchitis.

In one embodiment, the invention provides an anti-CD47 antibody or an antigen-binding portion thereof comprising the amino acid sequences disclosed herein for use in therapy.

The pharmaceutical composition of the invention may comprise a pharmaceutically acceptable excipient, carrier or diluent. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not provoke secondary reactions and which allows, for example, facilitation of the administration of the anti-CD47 antibody molecule, an increase in its lifespan and/or in its efficacy in the body or an increase in its solubility in solution. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the mode of administration of the anti-CD47 antibody molecule.

In some embodiments, the anti-CD47 antibody molecule may be provided in a lyophilised form for reconstitution prior to administration. For example, lyophilised antibody molecules may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

The anti-CD47 antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule. Thus pharmaceutical compositions may comprise, in addition to the anti-CD47 antibody molecule, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the anti-CD47 antibody molecule. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

For parenteral, for example sub-cutaneous or intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the anti-CD47 antibody molecule may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringe's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives

(such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

A pharmaceutical composition comprising an anti-CD47 antibody molecule may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

An anti-CD47 antibody molecule as described herein may be used in a method of treatment of the human or animal body, including prophylactic or preventative treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering the anti-CD47 antibody molecule to an individual in need thereof.

Administration is normally in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann J.A. *et al.*, 1991, Int. J. Cancer 47: 659-664; Bagshawe K.D. *et al.*, 1991, Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of an antibody molecule may be determined by comparing its *in vitro* activity and *in vivo* activity in

an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the antibody is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment) and the nature of any detectable label or other molecule attached to the antibody.

A typical antibody dose will be in the range 100 µg to 1 g for systemic applications, and 1 µg to 1 mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG1 or IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. The treatment schedule for an individual may be dependent on the pharmocokinetic and pharmacodynamic properties of the antibody composition, the route of administration and the nature of the condition being treated.

Treatment may be periodic, and the period between administrations may be about two weeks or more, e.g. about three weeks or more, about four weeks or more, about once a month or more, about five weeks or more, or about six weeks or more. For example, treatment may be every two to four weeks or every four to eight weeks. Treatment may be given before, and/or after surgery, and/or may be administered or applied directly at the anatomical site of surgical treatment or invasive procedure. Suitable formulations and routes of administration are described above.

In some embodiments, anti-CD47 antibody molecules as described herein may be administered as sub-cutaneous injections. Sub-cutaneous injections may be administered using an auto-injector, for example for long or short-term prophylaxis/treatment.

In some preferred embodiments, the therapeutic effect of the anti-CD47 antibody molecule may persist for several multiples of the antibody half-life in serum, depending on the dose. For example, the therapeutic effect of a single dose of the anti-CD47 antibody molecule may persist in an individual for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, or 6 months or more.

The invention also provides a method of producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47 or an antigen-binding portion thereof, comprising the steps of:

(1) grafting anti-CD47 CDRs from a non-human source into a human v-domain framework to produce a humanized anti-CD47 antibody molecule or antigen-binding portion thereof;

(2) generating a phage library of clones of the humanized anti-CD47 antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;

(3) selecting the phage library for binding to human CD47 and optionally also to cynomolgus monkey CD47;

(4) screening clones from the selection step (3) having binding specificity to human CD47 and optionally also to cynomolgus monkey CD47; and

(5) producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

Refinements applicable to the above method are as described in Example 1 below.

As used herein, the term “CD47” refers to IAP (Integrin Associated Protein) and variants thereof that retain at least part of the biological activity of CD47. As used herein, CD47 includes all mammalian species of native sequence CD47, including human, rat, mouse and chicken. The term “CD47” is used to include variants, isoforms and species homologs of human CD47. Antibodies of the invention may cross-react with CD47 from species other than human, in particular CD47 from cynomolgus monkey (*Macaca fascicularis*). Examples of human and cynomolgus CD47 amino acid sequences are provided in Table 9. In certain embodiments, the antibodies may be completely specific for human CD47 and may not exhibit non-human cross-reactivity.

As used herein, an “antagonist” as used in the context of the antibody of the invention or an “anti-CD47 antagonist antibody” (interchangeably termed “anti-CD47 antibody”) refers to an antibody which is able to bind to CD47 and inhibit CD47 biological activity and/or downstream pathway(s) mediated by CD47 signalling. An anti-CD47 antagonist antibody encompasses

antibodies that can block, antagonize, suppress or reduce (including significantly) CD47 biological activity, including downstream pathways mediated by CD47 signalling, such as receptor binding and/or elicitation of a cellular response to CD47. For the purposes of the present invention, it will be explicitly understood that the term “anti-CD47 antagonist antibody” encompass all the terms, titles, and functional states and characteristics whereby CD47 itself, and CD47 biological activity (including but not limited to its ability to enhance the activation of phagocytosis by cells of the myeloid lineage), or the consequences of the activity or biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree.

The antibody “specifically binds” “specifically interacts”, “preferentially binds”, “binds” or “interacts” with CD47 if it binds with greater affinity, avidity, more readily and/or with greater duration than it binds to other receptors.

An “antibody molecule” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody molecule” encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (for example, an “antigen-binding portion”) or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (for example, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

An “antibody molecule” encompasses an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term “antigen binding portion” of an antibody molecule, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to CD47.

Antigen binding functions of an antibody molecule can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding portion” of an antibody molecule include Fab; Fab'; F(ab')2; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).

The term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The “Fc region” may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. When choosing FR to flank CDRs, for example when humanizing or optimizing an antibody, FRs from antibodies which contain CDR sequences in the same canonical class are preferred.

The CDR definitions used in the present application combine the domains used in the many disparate, often conflicting schemes that have been created in the field, which are based on the combination of immunoglobulin repertoire analyses and structural analyses of antibodies in isolation and in their co-crystals with antigens (see review by Swindells *et al.*, 2016, abYsis: Integrated Antibody Sequence and Structure-Management, Analysis, and Prediction. J Mol Biol. [PMID: 27561707; Epub 22 August 2016]). The CDR definition used herein (a “Unified” definition) incorporates the lessons of all such prior insights and includes all appropriate loop positions required to sample the full residue landscape that potentially mediates target-binding complementarity.

Table 1 shows the amino acid sequences of the 5F9G4 murine anti-CD47 antibody CDRs as defined herein (a “Unified” scheme), in comparison to well-known alternative systems for defining the same CDRs.

As used herein the term “conservative substitution” refers to replacement of an amino acid with another amino acid which does not significantly deleteriously change the functional activity. A preferred example of a “conservative substitution” is the replacement of one amino acid with another amino acid which has a value ≥ 0 in the following BLOSUM 62 substitution matrix (see Henikoff & Henikoff, 1992, PNAS 89: 10915-10919):

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4.

The term “monoclonal antibody” (Mab) refers to an antibody, or antigen-binding portion thereof, 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. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

A “humanized” antibody molecule refers to a form of non-human (for example, murine) antibody molecules, or antigen-binding portion thereof, that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding sub-sequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies may be human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR

of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

"Human antibody" or "fully human antibody" refers to an antibody molecule, or antigen-binding portion thereof, derived from transgenic mice carrying human antibody genes or from human cells.

The term "chimeric antibody" is intended to refer to an antibody molecule, or antigen-binding portion thereof, in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody molecule in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

"Antibody-drug conjugate" and "immunoconjugate" refer to an antibody molecule, or antigen-binding portion thereof, including antibody derivatives that binds to CD47, which is conjugated to cytotoxic, cytostatic and/or therapeutic agents.

Antibody molecules of the invention, or antigen-binding portion thereof, can be produced using techniques well known in the art, for example recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

The term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

The term “epitope” refers to that portion of a molecule capable of being recognized by and bound by an antibody molecule, or antigen-binding portion thereof, at one or more of the antibody molecule's antigen-binding regions. Epitopes can consist of defined regions of primary secondary or tertiary protein structure and includes combinations of secondary structural units or structural domains of the target recognised by the antigen binding regions of the antibody, or antigen-binding portion thereof. Epitopes can likewise consist of a defined 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. The term “antigenic epitope” as used herein, is defined as a portion of a polypeptide to which an antibody molecule can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays, antibody competitive binding assays or by x-ray crystallography or related structural determination methods (for example NMR).

The term “binding affinity” or “KD” refers to the dissociation rate of a particular antigen-antibody interaction. The KD is the ratio of the rate of dissociation, also called the “off-rate (k_{off})”, to the association rate, or “on-rate (k_{on})”. Thus, K_D equals k_{off} / k_{on} and is expressed as a molar concentration (M). It follows that the smaller the K_D, the stronger the affinity of binding. Therefore, a K_D of 1 μM indicates weak binding affinity compared to a K_D of 1 nM. KD values for antibodies can be determined using methods well established in the art. One method for determining the KD of an antibody is by using surface plasmon resonance (SPR), typically using a biosensor system such as a Biacore® system.

The term “potency” is a measurement of biological activity and may be designated as IC_{50}, or effective concentration of an antibody or antibody drug conjugate to the antigen CD47 to inhibit 50% of activity measured in a CD47 activity assay as described herein.

The phrase “effective amount” or “therapeutically effective amount” as used herein refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount is at least the minimal amount, but less than a toxic amount, of an active agent which is necessary to impart therapeutic benefit to a subject.

The term “inhibit” or “neutralize” as used herein with respect to bioactivity of an antibody molecule of the invention means the ability of the antibody to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse for example

progression or severity of that which is being inhibited including, but not limited to, a biological activity or binding interaction of the antibody molecule to CD47.

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As used herein, “vector” means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The term “treating”, as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, delaying the progression of, delaying the onset of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term “treatment”, as used herein, unless otherwise indicated, refers to the act of treating as defined above. The term “treating” also includes adjuvant and neoadjuvant treatment of a subject. For the avoidance of doubt, reference herein to “treatment” includes reference to curative, palliative and prophylactic treatment. For the avoidance of doubt, references herein to “treatment” also include references to curative, palliative and prophylactic treatment.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term “e.g.” or “for example” is not meant to be exhaustive or limiting.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

Particular non-limiting embodiments of the present invention will now be described with reference to accompanying drawings.

EXAMPLE 1. Generation of optimized anti-CD47 therapeutic antibodies

Introduction

In this example, we successfully generate a panel of antagonistic, optimized anti-CD47 antibodies. These anti-CD47 antibodies are well expressed, biophysically stable, highly soluble and of maximized amino acid sequence identity to preferred human germlines.

Materials and methods

IgG cloning, transient expression, purification

Antibody v-domain encoding DNA sequences were cloned via restriction-ligation cloning into separate IgG heavy and light-chain expression cassettes in separate plasmid vectors. Antibodies were expressed in two human IgG1 formats: IgG4(S228P) and IgG1null – IgG1 with the lower hinge mutations L234A/L235A/G237A, which minimise Fcγ receptor-driven effector functions. IgGs were expressed in HEK-293expi or CHO cells after transient transfection with endotoxin-free IgG expression plasmid preparations, per manufacturer’s protocols. IgGs were purified using a single-step protocol: Conditioned media were loaded (neat) onto a 1 ml ProA sepharose column, pre-equilibrated in PBS pH7.4. The column was washed with 5 column volumes of PBS pH7.4, before the protein was eluted with 100mM glycine, pH 2.7 and subjected to dialysis in PBS pH 7.4 using 30 kDa cutoff dialysis membrane.

IgG titration binding ELISAs

To coat Greiner Bio-One High bind ELISA plates, target proteins were diluted to 1 μg/ml in carbonate buffer and added at 100 µl per well, at 4°C, o/n. Coated plates were washed 3x with PBS pH7.4, blocked with 1% BSA in PBS (380 µl/ well) for 1 hr at RT, then washed 3x with PBS-Tween 20 (PBST). CD47 antibodies (100 µl/well; diluted in PBST) were then added and then incubated 1 hr at RT. Plates were then washed 3x with PBST and goat anti-human kappa chain-HRP added (100 µl/well) at RT, for 1 hr. Plates were then washed 3x with PBST and twice with PBS before the addition of 100 µl TMB per well. Reactions were stopped by adding 100 µl 2M H_2SO_4/well and OD was read on a plate reader at 450nm.

Anti-CD47 antibodies were tested for polyreactivity by ELISA. Purified, recombinant, target and non-target antigens were coated in 96-well Nunc maxisorp plates at 100 ng per well in carbonate buffer, at 4°C overnight. Plates were then washed 3x with PBS, blocked with 1% BSA in PBS, then washed 3x with PBS-Tween20. A dilution series of primary antibodies was then applied, plates were washed 3x with PBS-Tween20 followed by application of goat anti-human kappa chain-HRP 1:4,000 secondary antibody. Wells were then washed 3x with PBS-Tween20 and 2x with PBS, 100 µl TMB peroxidase substrate was added per well, the reaction was stopped by adding 100 µl 2M H_2SO_4 and absorbances were read at 450 nm. IgG binding analysis via ELISA on negatively charged biomolecular surfaces were performed as previously described (see Mouquet *et al.*, 2010, Nature 467: 591-595).

CD47 library generation and selection

The CD47 scFv repertoire was assembled by mass oligonucleotide synthesis and PCR. The amplified scFv repertoire was then cloned via restriction-ligation into a phagemid vector, transformed into *E.coli* TG-1 cells, and the phage repertoire rescued essentially as previously described in detail (Finlay *et al.*, 2011, Methods Mol Biol 681: 383-401).

Phage selections were performed by coating streptavidin magnetic microbeads with CD47-Fc protein (either human or cyno), washing the beads thrice with PBS and resuspending in PBS pH7.4 plus 5% skim milk protein (MPBS). These beads were coated at 200 nM target protein in round 1 of selection, followed by 100, 50 and 10 nM in subsequent rounds.

HTRF binding competition assay

A competition HTRF assay was established to examine epitope competition against h5F9G4 IgG binding to human and/or cyno CD47-Fc by grafted and library-derived clones. The purified h5F9G4 IgG1 was labelled with terbium using a labelling kit (CisBio) per the manufacturer's instructions. The final reaction mix contained biotinylated human or cyno

CD47–Fc, SA-XL665 (CisBio), terbium-labelled parental h5F9G4 and competitor scFv or IgG of interest, prepared as described above, in a total reaction volume of 20 µl in 1× assay buffer [50 mM sodium phosphate, pH 7.5, 400 mM potassium fluoride, and 0.1% BSA (w/v)]. Reagents were added sequentially into 384-well low-volume black plates (Nunc). Reactions proceeded for 1 h at room temperature, and plates were subsequently read on a plate reader with excitation at 340 nm and two emission readings at 615 nm (measuring input donor fluorescence from h5F9G4-terbium) and 665 nm (measuring output acceptor fluorescence from SAXL665). Readings were expressed as 665 nm/615 nm ratios.

CD47-SIRPα binding competition assay

A competition ELISA assay was established to examine the capacity of optimized leads to block the binding interaction of CD47 with SIRPα. To coat Greiner Bio-One High bind ELISA plates, 10 µg/ml human SIRPα-Fc in carbonate coating buffer was added at 100 µl per well, at 4°C, o/n. Coated plates were washed 3x with PBS pH7.4, blocked with 1% BSA in PBS (380 µl/ well) for 1 hr at RT, then washed 3x with PBS-Tween 20 (PBST). Biotinylated human, mouse or cyno CD47-Fc was then added at 0.2 µg/ml in PBS, 100 µl per well, at room temperature for 60 minutes with or without the addition of competing scFv or IgGs. Plates were then washed 3x with PBST and Streptavidin-HRP added (100 µl/well) at room temperature, for 1 hr. Plates were then washed 3x with PBST and twice with PBS before the addition of 100 µl TMB per well. Reactions were stopped by adding 100 µl 2M H_2SO_4/well and OD was read on a plate reader at 450nm.

Antibody v-domain T cell epitope content: *in silico* analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing potential immunogenicity in antibody v-domains. iTope™ was used to analyse the VL and VH sequences of key leads for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software predicts favourable interactions between amino acid side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles. These alleles represent the most common HLA-DR alleles found world-wide with no weighting attributed to those found most prevalently in any particular ethnic population. Twenty of the alleles contain the 'open' p1 configuration and 14 contain the 'closed' configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino

acids spanning the test protein sequence. This process successfully discriminates with high accuracy between peptides that either bind or do not bind MHC class II molecules.

In addition, the sequences were analysed using TCED™ (T Cell Epitope Database™) search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

Phagocytosis potency assays using human macrophages

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation. CD14 positive PBMCs were subsequently isolated via magnetic cell isolation using CD14 microbeads. In parallel, Jurkat cells were labelled using a green CFSE (carboxy-fluorescein diacetate, succinimidyl ester) cell tracer dye. A total of 6.25 x 10^5 labelled Jurkat cells were pre incubated in the presence of UH2 antibodies in 96 well plates for 1 hour at 37 degrees C in a humidified atmosphere containing 5% CO_2. Following incubation, 2.5 x 10^5 CD14 positive cells were added to each well and incubated for a further hour under the same culture conditions.

Cells were harvested by vigorous pipetting, stained with viability dye, fixed using ice-cold 4% paraformaldehyde for 10 minutes. Following fixation, cells were blocked with an Fc receptor binding inhibitor monoclonal antibody for 10 minutes and then incubated with an Alexa Fluor 647 (AF647) conjugated anti-human CD11b antibody at room temperature for 30 minutes and fixed a further time in 4% paraformaldehyde for 5 minutes.

Cells were analysed on the BD Fortessa flow cytometer recording side scatter and forward scatter properties along with CFSE and AF647 fluorescence intensity data. Data was captured until at least 1 x 10^4 AF647 positive events were recorded. Data were analysed post-acquisition using FlowJo software (version 10.4.2). Briefly, cell debris was gated out by scatter properties (SSC-Area by FSC-Area). Single cells were also gated for by SSC-Area by SSC-Height and then by FSC-Area by FSC-Height. From the remaining single cell population, CFSE and CD11b double positives cells were gated using a quadrant gate placed based on the population of CD11b positive cells in the vehicle treated test. The percentage of CFSE positive cells from the CD11b positive population was calculated and plotted.

Results and Discussion

CDR grafting onto preferred human germline v-genes

The CDRs of an antagonistic murine anti-CD47 IgG 5F9G4 (5F9G4; see WO2011143624A2 and Table 2) were initially introduced to human germline immunoglobulin v-domain framework sequence scaffolds using CDR grafting. To bias our engineering efforts towards final lead therapeutic IgG compounds with optimal drug-like properties, we chose to graft the CDRs of the parental antibody onto "preferred" germline scaffolds IGHV5-51 and IGKV2-28, which are known to be very well displayed and expressed in scFv format in phage display, have good solubility, high physical stability and are used at high frequency in the expressed human antibody repertoire. IGHV5-51 was also the preferred germline framework for grafting of the VH as it showed high sequence and structural motif homology with the m5F9G4 HCDR2 (Fig. 2).

Those scaffolds and grafted CDR definitions are outlined in Table 2. The heavy and light chain sequences for chimeric anti-CD47 antibody m5F9G4 and humanized h5F9G4 are also shown in Table 2. While this process of CDR grafting is well known, it is still problematic to predict whether a given set of human v-domain sequences will act as suitable acceptor frameworks for non-human CDR grafting. The use of unsuitable frameworks can lead to the loss of target binding function, protein stability issues or even impaired expression of the final IgG. The IGKV2-28/IGHV5-51 graft was therefore taken forward as the template for CDR mutagenesis and selection of improved clones.

Library generation and screening

The CDR-grafted IGKV2-28/IGHV5-51 v-domain sequences were combined into a VL-VH scFv format and a mutagenesis library cassette was generated by mass oligonucleotide synthesis and assembly. The final scFv library was ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 6.0 x 10^8 independent clones. Library build quality was verified by sequencing 96 clones. This sequencing data showed that the positions encoding either the murine or human germline residue at each position of variance had been effectively sampled at a frequency of approximately 50%. Libraries were rescued using helper phage M13 and selections performed on biotinylated human and cynomolgus monkey CD47-Fc proteins in multiple separate branches.

Post-selection screening (as shown in Fig. 1A-F) and DNA sequencing revealed the presence of 269 unique, human and mouse CD47-binding scFv clones that retained epitope binding competition with h5F9G4 IgG1, blocked the binding of hCD47 to hSIRPα and contained significantly increased human content within the CDRs, while the framework

sequences remained fully germline. Amongst these 269 clones, germ-lining mutations were observed in all CDRs (Table 3). Lead clones were ranked based on the level of CDR germ-lining versus HTRF signals for both human CD47-Fc and h5F9G4 (Fig. 1). The v-domains of the 11 top clones from this ranking were then sub-cloned into IgG expression vectors for further testing as below (Table 4). Unexpectedly however, in all analyses of both CD47-SIRPα and CD47-h5F9G4 neutralisation, none of the library derived clones, nor the IGKV2-28/IGHV5-51 graft scFv achieved the same potency as the IGKV2-28/IGHV1-3-grafted h5F9G4 scFv (Fig. 1A-F).

While germ-lining mutations were observed in all CDRs for the lead clones derived directly from library selections, it remained possible that sequence analyses might allow further clones to be designed to have maximal humanization and retain maximal CD47 binding affinity and antagonism potency. The 269 sequence-unique hits with binding signals against human and mouse protein were therefore used to analyse the retention frequency for murine amino acids in the CDRs of this functionally characterized population. Positional amino acid retention frequency was expressed as a percentage found in the V_H and V_L domains (Fig. 2A, 2B). Murine residues with RF < 75% were regarded as positions that are possibly not essential to the target-binding paratope and are likely to be open to germ-lining, in a series of combinatorial designs. Importantly, as observed in the scFv screening data outlined above, the potency of the IGHV5-51-grafted VH domains was deemed likely to be lower than those on a IGHV1-3 framework. To examine whether or not this was the case, the paratope sampling information outlined in Fig. 2 A and B was used to construct designer clones in the IGKV2-28 and IGHV1-3 frameworks for comparison alongside the heavily-humanized clones derived from the initial library (IGHV5-51 framework in the VH domain).

Ten VH designs containing principally those murine residues with RF > 75%, in a number of combinations, were designated VH1.1-VH1.10. Six VL designs were also created (VL1.1-VL1.6) that combined the most humanized CDRs observed in the high-functioning population of scFv sequences, plus further-humanizing and deamidation motif-disrupting mutations in the LCDR1 (Table 4). These LCDR1 humanizing and stabilising mutations sampled positions 11, 12 and 13 (residues GNT), which had been observed to be variable positions (Fig. 2A, Table 3), but had not explicitly demonstrated that these potentially optimal sequences (e.g. the 'ANT' and especially 'AYN' motifs found in designer chain VL1.4 and others outlined in Table 4) could be tolerated while retaining target binding specificity and the ability to block CD47-SIRPα interaction.

The VH and VL clones were generated by gene synthesis (along with the 11 library-derived clones outlined above, positive controls IGHV5-51 graft, h5F9G4 and m5F9G4, and negative control non-CD47-reactive v-domains), then cloned into expression vectors for production as human IgG1null. To maximise the opportunity to find functional optimised sequences, the designer VH and VL clones were matrixed to combine all potential combinations of VH and VL sequences. To examine whether or not the observed reduced potency of the IGHV5-51-based scFvs observed above was due to scFv formatting and not the framework choice, the scFv of lead clone D6 was cloned as both IgG1 and scFv-Fc fusion formats. All IgGs and the D6 scFv-Fc fusion protein were readily expressed and purified from transient transfections of HEK-293 cells.

Library-derived lead IgG specificity and potency characteristics

The purified IgGs described above were then tested for binding to human and cyno CD47-Fc in direct titration ELISA format. This analysis demonstrated that while all library derived clones clones retained binding affinity for human CD47, none fully recapitulated the binding of m5F9G4 IgG1 (Fig. 3A). This reduced binding was more pronounced on cyno CD47, with clone F-G3 showing particularly weak reactivity (Fig. 3B).

As direct ELISA binding signal is influenced by avidity and does not prove the maintenance a specific epitope, all IgGs were then examined in a CD47-SIRPα binding blockade assay (Fig 4) and a solution-phase HTRF competition assay of 5F9G4 binding to CD47 (Fig. 5). In the CD47-SIRPα binding blockade assay, all library-derived IgGs exhibited significantly reduced potency in comparison to h5F9G4, m5F9G4 and the IGHV5-51 grafted IgG, whether binding to human (Fig. 4A) or cyno CD47 (Fig. 4B). Importantly, the IGHV5-51-grafted IgG demonstrated the ability to fully block the binding of both human and cyno CD47 to SIRPα, but with demonstrably reduced potency in comparison to h5F9G4. This suggested that the grafting process of placing the 5F9G4 CDRs onto the IGHV5-51 framework had specifically reduced the potency of the molecule in comparison to h5F9G4, as the CDR sequences are maintained across both frameworks. In a further unexpected finding, the D6 scFv-Fc fusion protein maintained the starting CD47 blocking potency of the IGHV5-51 graft IgG against both orthologs, while the D-A6 IgG did not and was incapable of fully blocking either ortholog (Fig. 4A, 4B).

To ensure that the observed loss of CD47 blockade potency by some IGHV5-51-framework clones was not driven by the phenomenon of ‘epitope shift’, an HTRF assay was performed. This assay examined the ability of these clones to compete for the binding of h5F9G4 to CD47. These analyses showed that almost all clones (F-G3 excepted) could cross-compete

with h5F9G4, with most clones demonstrating full inhibition of binding at the highest concentration (Fig 5A). Potency of inhibition was again reduced in comparison to h5F9G4 self-competition, however. This finding suggested maintenance of a shared epitope, but reduced binding affinity in the IGHV5-51 framework clones. Importantly, the improved function of the D6 scFv-Fc over the D6 IgG was again demonstrated, with the D6 scFv-Fc showing equivalent potency to the IGHV5-51 graft IgG, while the D-A6 IgG was clearly reduced potency (Fig 5B).

The findings regarding D6 scFv-Fc fusion potency versus D6 IgG strongly suggested that the process of re-grafting the CDRs, coupled with scFv display and screening had unexpectedly selected for clones which are preferentially functional in scFv format, which are then impaired when reconfigured into IgG1. This observation renders such clones particularly attractive in bispecific antibody generation as scFv fragments can be successfully used as modular building blocks of multi-specific antibodies. In totality, however, these findings also illustrate that the original methods of Townsend *et al.* had failed to generate an improved version of h5F9G4

Designer IgG specificity and potency characteristics

The purified designer IgGs described above were then tested for binding to human and cyno CD47-Fc in direct titration ELISA format. This analysis demonstrated that while many clones retained binding affinity for human and cyno CD47, the range of binding potencies was highly heterogeneous, with some clones being significantly reduced in binding signal, but with a subset of IgGs fully recapitulating the binding of m5F9G4 IgG1 (Fig. 6A-H). The family of clones containing the VH1.8 VH domain in combination with the VL1.1-1.6 domains demonstrated the most consistent recapitulation of the full binding potency of m59G4 and are shown separately in FIG 6G and H.

As direct ELISA binding signal is influenced by avidity and does not prove the maintenance a specific epitope, all IgGs were then examined in a CD47-SIRPα binding blockade assay (Fig. 7, Fig. 8). In the CD47-SIRPα binding blockade assay, all VH1.8 family IgGs exhibited high potency, with functional equivalency for all clones other than VH1.8/VL1.4, in comparison to h5F9G4 and m5F9G4, whether binding to human (Fig. 7A) or cyno CD47 (Fig. 7B). Several other designer clones which had performed well in ELISA also exhibited the ability to block hCD47-SIRPα binding (Fig. 8), albeit with lower potency than the VH1.8 family clones.

To ensure that the observed maintenance of CD47 blockade potency by high-functioning designer clones was coupled with maintained epitope specificity, a solution-phase HTRF competition assay of 5F9G4 binding to CD47 was performed (Fig. 9). This assay examined the ability of the key designer clones to compete for the binding of h5F9G4 to CD47. These analyses showed that all VH1.8 family clones could cross-compete with h5F9G4, with multiple clones demonstrating equivalent (Table 5) potency against human (Fig 9A) and cyno (Fig. 9B) CD47. The potency of inhibition for VH1.8/VL1.4, however, was again reduced in comparison to h5F9G4 self-competition. This finding suggested maintenance of a shared epitope, but reduced binding affinity for the VL1.4 domain-containing clones. Further HTRF analyses showed that all non-VH1.8 family clones which demonstrated hCD47 blockade (Fig. 8) could also cross-compete with h5F9G4, but with all clones demonstrating reduced potency (Table 5) against human (Fig 9C) and cyno (Fig. 9D) CD47. In an attempt to further sample CDR residues in the VH1.8 background, two further designs (VH1.11 and VH1.12) were generated, along with 3 more designer descendants of the VL1.5 sequence (VL1.7, VL1.8 and VL1.9). These designs were co-expressed in a matrix to produce 6 further IgGs, then examined in the CD47-SIRPα blockade assay. This analysis showed that all of the clones had lost all potency in antagonising CD47, other than VH1.12/VL1.8, which maintained minimal blocking (<50%) at 10 μg/ml. These findings showed that the mutation of F>A at position 6 of the LCDR2, or the mutation F>M at position 1 of the LCDR3 both led to the full ablation of CD47 antagonism.

Flow cytometric analyses of lead IgG binding specificity at the cell membrane

Antibodies shown to retain binding to CD47 by ELISA were analysed for concentration-dependent binding at the cell surface via flow cytometry. CHO-K1 cells were stably transfected with either human or cyno CD47 full-length cDNAs. Anti-CD47 IgGs and an isotype control IgG1 were then all tested in IgG1null format, over a concentration range of 100-0.024 μg/ml for binding to human, cyno, or wild type control ('wt', i.e. untransfected) CHO-K1 (Fig. 10). All IgGs other than the isotype control showed concentration-dependent binding to human and cyno CD47+ cells, and also to human AML cell line HL60, with a maximum MFI in each case being >50-fold higher than observed background signals for binding to untransfected CHO-K1. Anti-CD47 antibodies exhibited no measurable background binding on untransfected CHO-K1 cells, comparable to the Isotype control IgG1 (Fig. 10).

Lead IgG analyses in 'Developability' ELISA assays

It is known in the art that the binding of IgGs intended for therapeutic use to several indicative biological substrates is an indicator of high risk for poor performance in patients due to poor

bioavailability and short *in vivo* half-life. Three such biological substrates are Insulin, dsDNA and ssDNA (Avery *et al.* Mabs; 10:2; 244-255; 2018). These three substrates were therefore used to coat ELISA plates and examine the binding of the IgG1null versions of the optimised lead antibodies. Binding signals for these human IgG-based antibodies was compared to 'positive control' human IgG antibodies that have been found to have polyreactivity and poor performance, which stopped their progress in clinical trials (Bococizumab and Briakinumab human IgG1 analogues). For a negative control human IgG1 antibody, an IgG1 Ustekinumab analogue was used as it reacts with the same therapeutic target as Briakinumab, but has longer pK and was successfully approved as a therapeutic product. In the ELISA analyses shown in Fig. 11A, B and C, the positive control antibodies exhibited the expected strong reactivity to all 3 substrates, while the negative control Ustekinumab showed low reactivity. Importantly, all of the IgG1 lead clones tested showed binding ≤ the negative control against all 3 substrates. This finding underlined the maintenance of highly specific, target-driven binding in the optimised clones VH1.8/VL1.1, VH1.8/VL1.2, VH1.8/VL1.3, VH1.8/VL1.4, VH1.8/VL1.5, VH1.8/VL1.6, VH1.7/VL1.4, VH1.6/VL1.9 and VH1.12/VL1.8.

Antibody v-domain T cell epitope analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing the immunogenicity of both the h5F9G4 and lead antibody v-domains. Analysis of the v-domain sequences was performed with overlapping 9mer peptides (with each overlapping the last peptide by 8 residues) which were tested against each of the 34 MHC class II allotypes. Each 9mer was scored based on the potential 'fit' and interactions with the MHC class II molecules. The peptide scores calculated by the software lie between 0 and 1. Peptides that produced a high mean binding score (>0.55 in the iTope™ scoring function) were highlighted and, if >50% of the MHC class II binding peptides (i.e. 17 out of 34 alleles) had a high binding affinity (score >0.6), such peptides were defined as 'high affinity' MHC class II binding peptides which are considered a high risk for containing CD4+ T cell epitopes. Low affinity MHC class II binding peptides bind a high number of alleles (>50%) with a binding score >0.55 (but without a majority >0.6). Further analysis of the sequences was performed using the TCED™. The sequences were used to interrogate the TCED™ by BLAST search in order to identify any high sequence homology between peptides (T cell epitopes) from unrelated proteins/antibodies that stimulated T cell responses in previous in vitro T cell epitope mapping studies performed at Abzena Ltd.

Peptides were grouped into four classes: High Affinity Foreign ('HAF' – high immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), TCED+ (previously identified

epitope in TCED™ database), and Germline Epitope ('GE' - human germline peptide sequence with high MHC Class II binding affinity). Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance (i.e. these peptides are recognised as 'self' in the host), as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic. On the other end of the scale, TCED+ peptides are very high risk for immunogenicity in the clinic, as they have the characteristics of experimentally-proven peptide epitopes that can be presented on MHC molecules and activate human t cells.

Key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to h5F9G4. The v-domain engineering process undertaken here had therefore successfully selected for antibodies that maintained anti-CD47 potency, while removing multiple HAF and LAF epitopes that were TCED+ and found in both the heavy and light chain v-domains of h5F9G4. Importantly, these foreign epitopes were specifically eliminated by germlining mutations found in the CDRs of lead clones. For example, a TCED+ peptide 'IVYSNGNTY' (SEQ ID NO:106) (Fig. 12A) found in the LCDR-1 of h5F9G4 (and, therefore, in any forms of h5F9G4 humanized by CDR grafting) was eliminated in the majority of lead clones by the mutation I>L and V>L at positions 1 and 2, as found in all lead clones in Table 4. This finding illustrated that germlining and non-germlining mutations in the LCDR1 alone are sufficient to remove not only asparagine deamidation risk motifs, but also a proven human T cell epitope.

Similarly, the HCDR1/Framework 2 region from the h5F9G4 sequence encoded for a HAF and TCED+ peptide 'YNMHWVRQA' (SEQ ID NO:107) (Fig. 12B). This epitope was ablated in a number of lead sequences found in Table 4, such as antibodies containing the sequence 'YAMHWVRQA' (SEQ ID NO:108) (e.g. VH1.1, VH1.6, VH1.8, VH1.9, VH1.11, VH1.12), or 'YNIHWVRQA' (SEQ ID NO:109) (e.g. VH1.7). Importantly, the mutations N>A at position 2 and M>I at position 3 not only ablated the TCED+ epitope, but converted this epitope into GE peptides. In addition, the germlining of the last residues of the HCDR2 of all leads described in Table 4 (mutation D>G at the final c-terminal HCDR2 position as defined in Table 2), also reinstated GE peptides that are found in the germlines of both IGHV5-51 ('FQGQVTISA' SEQ ID NO:102) and IGHV1-3 ('FQGRVTITA' SEQ ID NO:103).

In the HCDR3/Framework 4 sequence of h5F9G4 VH domain, a further TCED+ peptide 'YRAMDYWGQ' (SEQ ID NO:110) was identified (Fig. 12C). This epitope was ablated in a number of lead sequences found in Table 4, such as antibodies containing the sequence 'YRAEDYWGQ' (SEQ ID NO:111) (e.g. VH1.1, VH1.2, VH1.3, VH1.6, VH1.9).

Critically, non-germline and germline mutations that were predicted not to be tolerated in CD47-binding clones according to the data in Fig. 2A and 2B were included in several key leads, as exploratory, novel substitutions. For example, in the LCDR-1 of h5F9G4 the development risk motif-removing mutations found in light chain sequences VL1.3, VL 1.4, VL 1.5 and VL 1.6 (Table 4) should not be tolerated (Fig. 2B). Similarly, the single, deimmunising, non-conservative, N>A mutation in the HCDR1 of clones based on the VH1.8 sequence and others (Table 4) was strongly predicted to be not tolerated in the data of Fig 2A). These findings illustrate that beneficial mutations are contextual, v-gene framework-dependent and in this case could not be fully predicted either *a priori*, or by the method of Townsend *et al.*

Finally, two remaining HAF peptides were found in the VL sequences VL1.1-1.9. The first HAF peptide 'LIYKGSNRF' (SEQ ID NO:112), spanning the Framework2/LCDR2 regions was identified in the analysis shown in Fig. 13A. The second HAF peptide 'VGVYYCFQA' (SEQ ID NO:113), spanning the Framework3/LCDR3 regions was identified in the analysis shown in Fig. 13A. Both HAF peptides could be fully ablated by mutation of the residues at position 1 to alanine (L>A and V>A, respectively). To sample these substitutions, a new clone 'VH1.8/VL1.6-DI' was constructed, containing both alanine substitutions in the VL domain.

Potency and affinity analyses of key leads

Clones h5F9G4, VH1.8/VL1.1, VH1.8/VL1.6, and VH1.8/VL1.6-DI were readily expressed and purified in the human IgG4(S228P) format. For all clones the Fab domains were also expressed as soluble monomers and purified for binding affinity analyses. These Fabs were then examined for affinity to human and cyno CD47 by Biacore, using steady state affinity analysis. Steady state was used as the binding affinity analysis as the on and off rates of all Fabs were too fast for reliable 1:1 modelling. As shown in Table 6, all 4 clones exhibited accurate, well-fitted data, as evidenced by very low Chi2 values. All clones were found to have binding affinities in the low nM range, within 2-fold of h5F9G4.

As final analysis of retained potency, clones h5F9G4, VH1.8/VL1.1, VH1.8/VL1.6, and VH1.8/VL1.6-DI in IgG4(S228P) format were examined for their relative potencies in a flow cytometric assay of phagocytosis, using human monocyte-derived macrophages derived

from 3 separate donors and Jurkat human cancer cells as the CD47+ target cells. For all clones analysed against cells from Donor 1 (Fig. 14A), Donor 2 (Fig. 14B) and Donor 3 (Fig. 14C), all clones had fully retained or improved over the potency of h5F9G4, as evidenced by fully overlapping response curves.

Although the present invention has been described with reference to preferred or exemplary embodiments, those skilled in the art will recognize that various modifications and variations to the same can be accomplished without departing from the spirit and scope of the present invention and that such modifications are clearly contemplated herein. No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.

No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Table 1. Amino acid sequences murine anti-CD47 CDRs as defined here ("Unified" scheme) in comparison to alternative definitions.

Scheme	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
Unified	GYTFTNYNMH (SEQ ID NO:114)	MGTIYPGNDDTSYNQKFKD (SEQ ID NO:119)	GGYRAMDY (SEQ ID NO:126)	RSSQSIVYSNGNTYLG (SEQ ID NO:130)	KVSNRFS (SEQ ID NO:134)	FQGSHVPYT (SEQ ID NO:136)
Kabat	NYNMH (SEQ ID NO:115)	TIYPGNDDTSYNQKFKD (SEQ ID NO:120)	GGYRAMDY (SEQ ID NO:126)	RSSQSIVYSNGNTYLG (SEQ ID NO:130)	KVSNRFS (SEQ ID NO:134)	FQGSHVPYT (SEQ ID NO:136)
Chotia	GYTFTNY (SEQ ID NO:116)	YPGNDD (SEQ ID NO:121)	GGYRAMDY (SEQ ID NO:126)	RSSQSIVYSNGNTYLG (SEQ ID NO:130)	KVSNRFS (SEQ ID NO:134)	FQGSHVPYT (SEQ ID NO:136)
IMGT	GYTFTNYN (SEQ ID NO:117)	IYPGNDDT (SEQ ID NO:122)	ARGGYRAMDY (SEQ ID NO:127)	QSIVYSNGNTY (SEQ ID NO:131)	KVS	FQGSHVPYT (SEQ ID NO:136)
AHo	GYTFTNYNMH (SEQ ID NO:114)	IYPGNDDTSYNQKFKD (SEQ ID NO:123)	GGYRAMD (SEQ ID NO:128)	SSQSIVYSNGNTY (SEQ ID NO:132)	KVSNRFS (SEQ ID NO:134)	GSHVPY (SEQ ID NO:137)
AbM	GYTFTNYNMH (SEQ ID NO:114)	TIYPGNDDTS (SEQ ID NO:124)	GGYRAMDY (SEQ ID NO:126)	RSSQSIVYSNGNTYLG (SEQ ID NO:130)	KVSNRFS (SEQ ID NO:134)	FQGSHVPYT (SEQ ID NO:136)
Contact	TNYNMH (SEQ ID NO:118)	MGTIYPGNDDTS (SEQ ID NO:125)	ARGGYRAMD (SEQ ID NO:129)	VYSNGNTYLGWY (SEQ ID NO:133)	ALIYKVSNRF (SEQ ID NO:135)	FQGSHVPY (SEQ ID NO:138)

Table 2. Amino acid sequence of 5F9G4 murine anti-CD47 v-domains (m5F9G4) and human germline CDR grafts (h5F9G4).

V DOMAIN	Human germline[1]	Amino acid sequence[2]
m5F9G4-VH	na	QVQLQQPGAELVKPGASVMMSCKAS**GYTFTNYNMH**WVKQTPGQGLEW**IGTIYPGNDDTSYNQKFKD**KATLTADKSSSAAYMQLSSLTSEDSAVYYCAR**GGYRAMDY**WGQGTSVTVSS (SEQ ID NO:139)
h5F9G4-VH	IGHV1-3	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYNMH**WVRQAPGQRLEW**MGTIYPGNDDTSYNQKFKD**RVTITADTSASTAYMELSSLRSEDTAVYYCAR**GGYRAMDY**WGQGTLVTVSS (SEQ ID NO:140)
VH graft	IGHV5-51[3]	EVQLVQSGAEVKKPGESLKISCKGS**GYSFTNYNMH**WVRQMPGKGLEW**MGTIYPGNDDTSYNQKFQG**QVTISADKSISTAYLQWSSLKASDTAMYYCAR**GGYRAMDY**WGQGTLVTVSS (SEQ ID NO:141)
m5F9G4-VL	na	DVLMTQTPLSLPVSLGDQASISC**RSSQSIVYSNGNTYLG**WYLQKPGQSPKLLIY**KVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDLGVYHC**FQGSHVPYT**FGGGTKVEIK (SEQ ID NO:142)
h5F9G4-VL	IGKV2-28	DIVMTQSPLSLPVTPGEPASISC**RSSQSIVYSNGNTYLG**WYLQKPGQSPQLLIY**KVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHVPYT**FGQGTKLEIK (SEQ ID NO:143)
VL graft	IGKV2-28[3]	DIVMTQSPLSLPVTPGEPASISC**RSSQSIVYSNGNTYLG**WYLQKPGQSPQLLIY**KVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHVPYT**FGQGTKLEIK (SEQ ID NO:144)

[1]Human germline definitions used for grafting, based on IMGT system. [2]CDR residues are in bold and underlined. As noted above, the "Unified" CDR definitions used in this manuscript are an expanded definition in comparison to the classical Kabat definition. Each sequence above shows the framework regions (FRs) and the CDRs in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. [3]Grafts are fully germline in the framework regions, used as the template for CDR mutant library construction.

Table 3. Amino acid sequences of unique CDRs from 269 unique anti-CD47 v-domains in the IGKV2-28/ IGHV5-51 background.

LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR2	HCDR2	HCDR3
RSSQSLLHSGGNTYLD (SEQ ID NO:145)	KGSNRFS (SEQ ID NO:52)	FQALHVPYT (SEQ ID NO:165)	GYSFTNYNIG (SEQ ID NO:195)	IGTIYPGDDDTRYNPKFQG (SEQ ID NO:200)	MGTIYPGDDDTSYSQKFQG (SEQ ID NO:232)	MGTIYPGNDDTSYNQSFQG (SEQ ID NO:262)	GGARAMDY (SEQ ID NO:289)
RSSQSLLHSGGNTYLG (SEQ ID NO:146)	KVSNRAS (SEQ ID NO:163)	FQAMQTPYT (SEQ ID NO:166)	GYSFTNYNIH (SEQ ID NO: 57)	MGIIYPGDDDTRYNPKFQG (SEQ ID NO:201)	MGTIYPGDGDTSYNPSFQG (SEQ ID NO:233)	MGTIYPGNDDTSYSPKFQG (SEQ ID NO:263)	GGCRALDY (SEQ ID NO:290)
RSSQSLLHSGYNTYLG (SEQ ID NO:147)	KVSNRSS (SEQ ID NO:164)	FQASHIPYT (SEQ ID NO:167)	GYSFTNYNMH (SEQ ID NO:196)	MGIIYPGDDDTRYNPSFQG (SEQ ID NO:202)	MGTIYPGDNDTSYNPSFQG (SEQ ID NO:234)	MGTIYPGNDDTSYSPRFQG (SEQ ID NO:264)	GGCRAMDY (SEQ ID NO:291)
RSSQSLLHSNGNTYLD (SEQ ID NO:148)		FQASHTPYT (SEQ ID NO:168)	GYSFTNYSIH (SEQ ID NO:197)	MGIIYPGDDDTSYNPKFQG (SEQ ID NO:203)	MGTIYPGDSDTKYNPKFQG (SEQ ID NO:235)	MGTIYPGNDDTSYSPSFQG (SEQ ID NO:265)	GGCRAMGY (SEQ ID NO:292)
RSSQSLLHSNGNTYLG (SEQ ID NO:51)		FQASHVPYT (SEQ ID NO:53)	GYSFTSYNIH (SEQ ID NO:198)	MGIIYPGDDDTSYNQKFQG (SEQ ID NO:204)	MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58)	MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90)	GGDRAMDY (SEQ ID NO:293)
RSSQSLLHSNYNTYLG (SEQ ID NO:149)		FQASQIPYT (SEQ ID NO:169)	GYSFTSYNMH (SEQ ID NO:199)	MGIIYPGDSDTRYNPKFQG (SEQ ID NO:205)	MGTIYPGDSDTRYNPSFQG (SEQ ID NO:236)	MGTIYPGNDDTSYSQSFQG (SEQ ID NO:266)	GGERAMDY (SEQ ID NO:294)
RSSQSLLQSNGNTYLG (SEQ ID NO:150)		FQASQTPYT (SEQ ID NO:170)		MGIIYPGDSDTRYNPSFQG (SEQ ID NO:206)	MGTIYPGDSDTRYNQKFQG (SEQ ID NO:98)	MGTIYPGNDGTRYNQKFQG (SEQ ID NO:267)	GGFRAEDY (SEQ ID NO:295)
RSSQSLLYSGGNTYLG (SEQ ID NO:151)		FQASQVPYT (SEQ ID NO:54)		MGIIYPGDSDTRYNQKFQG (SEQ ID NO:207)	MGTIYPGDSDTRYSPKFQG (SEQ ID NO:237)	MGTIYPGNGDTSYNHKFQG (SEQ ID NO:268)	GGFRAMDY (SEQ ID NO:50)
RSSQSLLYSNGNTYLG (SEQ ID NO:152)		FQASRVPYT (SEQ ID NO:171)		MGIIYPGDSDTSYNQSFQG (SEQ ID NO:208)	MGTIYPGDSDTRYSPRFQG (SEQ ID NO:238)	MGTIYPGNGDTSYNQKFQG (SEQ ID NO:269)	GGFRAQDY (SEQ ID NO:296)
RSSQSLVHSGGNTYLG (SEQ ID NO:153)		FQATQVPYT (SEQ ID NO:172)		MGIIYPGNDDTRYNPKFQG (SEQ ID NO:209)	MGTIYPGDSDTRYSPSFQG (SEQ ID NO:239)	MGTIYPGNNDTSYNPKFQG (SEQ ID NO:270)	GGGRAMDY (SEQ ID NO:297)
RSSQSLVHSGYNTYLG (SEQ ID NO:154)		FQGLQVPYT (SEQ ID NO:173)		MGIIYPGNDDTRYNPSFQG (SEQ ID NO:210)	MGTIYPGDSDTRYSQKFQG (SEQ ID NO:240)	MGTIYPGNSDTRYNHSFQG (SEQ ID NO:271)	GGHRAEDY (SEQ ID NO:298)
RSSQSLVHSNGNTYLD (SEQ ID NO:155)		FQGSHIPYT (SEQ ID NO:174)		MGIIYPGNDDTRYNQKFQG (SEQ ID NO:211)	MGTIYPGDSDTRYSQSFQG (SEQ ID NO:241)	MGTIYPGNSDTRYNPKFQG (SEQ ID NO:101)	GGHRAMDY (SEQ ID NO:299)

RSSQSLVHSNGNTYLG (SEQ ID NO:156)		FQGSHTPYT (SEQ ID NO:175)		MGIIYPGNSDTRYNPKFQG (SEQ ID NO:212)	MGTIYPGDSDTSYNHKFQG (SEQ ID NO:242)	MGTIYPGNSDTRYNPSFQG (SEQ ID NO:272)	GGIRAMDY (SEQ ID NO:99)
RSSQSLVHSNGQTYLG (SEQ ID NO:157)		FQGSHVPYI (SEQ ID NO:100)		MGIIYPGNSDTRYNPSFQG (SEQ ID NO:213)	MGTIYPGDSDTSYNPKFHG (SEQ ID NO:243)	MGTIYPGNSDTRYNQKFQG (SEQ ID NO:273)	GGKRAMDY (SEQ ID NO:93)
RSSQSLVHSNYNTYLG (SEQ ID NO:158)		FQGSQTPYT (SEQ ID NO:176)		MGIIYPGNSDTRYNQKFQG (SEQ ID NO:214)	MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94)	MGTIYPGNSDTRYNQSFQG (SEQ ID NO:274)	GGLRAMDY (SEQ ID NO:300)
RSSQSLVHSSGNTYLG (SEQ ID NO:159)		FQGSQVPYT (SEQ ID NO:177)		MGIIYPGNSDTRYNQRFQG (SEQ ID NO:215)	MGTIYPGDSDTSYNPRFQG (SEQ ID NO:244)	MGTIYPGNSDTRYSPKFQG (SEQ ID NO:275)	GGLRTMDY (SEQ ID NO:301)
RSSQSLVQSNGNTYLG (SEQ ID NO:160)		LQGSHIPYT (SEQ ID NO:178)		MGIIYPGNSDTRYNQSFQG (SEQ ID NO:216)	MGTIYPGDSDTSYNPSFQG (SEQ ID NO:245)	MGTIYPGNSDTRYSPSFQG (SEQ ID NO:276)	GGMRAMDY (SEQ ID NO:302)
RSSQSLVYSNGNTYLG (SEQ ID NO:161)		LQGSHTPYT (SEQ ID NO:179)		MGIIYPGNSDTRYSPKFQG (SEQ ID NO:217)	MGTIYPGDSDTSYNQKFQG (SEQ ID NO:246)	MGTIYPGNSDTRYSQKFQG (SEQ ID NO:277)	GGNRAKDY (SEQ ID NO:303)
RSSQSLVYSNYNTYLG (SEQ ID NO:162)		LQGSHVPYT (SEQ ID NO:180)		MGIIYPGNSDTRYSPSFQG (SEQ ID NO:218)	MGTIYPGDSDTSYNQSFQG (SEQ ID NO:247)	MGTIYPGNSDTSYNPKFQG (SEQ ID NO:92)	GGNRAMDY (SEQ ID NO:304)
		LQGSQTPYT (SEQ ID NO:181)		MGIIYPGNSDTSYNPKFQG (SEQ ID NO:219)	MGTIYPGDSDTSYSPKFQG (SEQ ID NO:248)	MGTIYPGNSDTSYNPRFQG (SEQ ID NO:278)	GGQRAMDY (SEQ ID NO:305)
		LQGSQVPYT (SEQ ID NO:182)		MGIIYPGNSDTSYNPRFQG (SEQ ID NO:220)	MGTIYPGDSDTSYSPSFQG (SEQ ID NO:249)	MGTIYPGNSDTSYNPSFQG (SEQ ID NO:61)	GGRRAMDY (SEQ ID NO:306)
		MQALQVPYT (SEQ ID NO:183)		MGIIYPGNSDTSYNPSFQG (SEQ ID NO:221)	MGTIYPGDSDTSYSQKFQG (SEQ ID NO:250)	MGTIYPGNSDTSYNQKFQG (SEQ ID NO:279)	GGSRAKDY (SEQ ID NO:307)
		MQASHIPYT (SEQ ID NO:184)		MGIIYPGNSDTSYNQKFQG (SEQ ID NO:222)	MGTIYPGNDDTKYSPKFQG (SEQ ID NO:251)	MGTIYPGNSDTSYNQSFQG (SEQ ID NO:280)	GGSRAMDY (SEQ ID NO:308)
		MQASHTPYT (SEQ ID NO:185)		MGIIYPGNSDTSYSPKFQG (SEQ ID NO:223)	MGTIYPGNDDTRYNPKFQG (SEQ ID NO:252)	MGTIYPGNSDTSYSPKFQG (SEQ ID NO:281)	GGTRAMDY (SEQ ID NO:309)
		MQASHVPYT (SEQ ID NO:96)		MGTIYPGDDDTRYNPKFQG (SEQ ID NO:224)	MGTIYPGNDDTRYNPSFQG (SEQ ID NO:253)	MGTIYPGNSDTSYSPSFQG (SEQ ID NO:282)	GGVRAMDY (SEQ ID NO:62)

		MQASQIPYT (SEQ ID NO:186)		MGTIYPGDDDTRYNPSFQG (SEQ ID NO:225)	MGTIYPGNDDTRYNQKFQG (SEQ ID NO:254)	MGTIYPGNSDTSYSQKFQG (SEQ ID NO:283)	GGVRATDY (SEQ ID NO:310)
		MQASQTPYT (SEQ ID NO:187)		MGTIYPGDDDTRYNQKFQG (SEQ ID NO:226)	MGTIYPGNDDTRYNQSFQG (SEQ ID NO:255)	MGTIYSGNSDTSYSPSFQG (SEQ ID NO:284)	GGWRAMDY (SEQ ID NO:311)
		MQASQVPYT (SEQ ID NO:63)		MGTIYPGDDDTRYNQNFQG (SEQ ID NO:227)	MGTIYPGNDDTRYSPKFQG (SEQ ID NO:256)	MGTIYPGNDDTRYSQSFQG (SEQ ID NO:285)	GGYHAMDY (SEQ ID NO:312)
		MQGLHVPYT (SEQ ID NO:188)		MGTIYPGDDDTRYNQSFQG (SEQ ID NO:228)	MGTIYPGNDDTRYSPSFQG (SEQ ID NO:257)	MGTIYPGNDDTSYNHKFQG (SEQ ID NO:286)	GGYKAMDY (SEQ ID NO:313)
		MQGLQVPYT (SEQ ID NO:189)		MGTIYPGDDDTRYSPKFQG (SEQ ID NO:229)	MGTIYPGNDDTRYSQKFQG (SEQ ID NO:258)	MGTIYPGNDDTSYNPKFQG (SEQ ID NO:287)	GGYRAADY (SEQ ID NO:314)
		MQGSHIPYT (SEQ ID NO:190)		MGTIYPGDDDTRYSPSFQG (SEQ ID NO:230)	MGTIYPGDDDTSYNPKFQG (SEQ ID NO:259)	MGTIYPGNDDTSYNPSFQG (SEQ ID NO:288)	GGYRAEDY (SEQ ID NO:59)
		MQGSHTPYT (SEQ ID NO:191)		MGTIYPGDDDTRYSQKFQG (SEQ ID NO:231)	MGTIYPGDDDTSYNPSFQG (SEQ ID NO:260)		GGYRALDY (SEQ ID NO:315)
		MQGSHVPYT (SEQ ID NO:97)			MGTIYPGDDDTSYNQKFQG (SEQ ID NO:261)		GGYRAPDY (SEQ ID NO:316)
		MQGSQIPYT (SEQ ID NO:192)					GGYRAQDY (SEQ ID NO:95)
		MQGSQTPYT (SEQ ID NO:193)					GGYRARDY (SEQ ID NO:317)
		MQGSQVPYT (SEQ ID NO:194)					GGYRASDY (SEQ ID NO:318)
							GGYRATDY (SEQ ID NO:319)
							GGYRAVDY (SEQ ID NO:320)

GGYRIMDY (SEQ ID NO:321)						
GGYRTEDY (SEQ ID NO:322)						
GGYRTMDY (SEQ ID NO:323)						
GGYRTTDY (SEQ ID NO:324)						
GGYRVMDY (SEQ ID NO:325)						
GGYRWMDY (SEQ ID NO:326)						
QGYRAMDY (SEQ ID NO:327)						

Table 4. Amino acid sequences of CDRs of unique, library-derived and designer, human/cyno cross-reactive anti-CD47 IgGs.

Clone name	LCDR1	LCDR2	LCDR3	HCRD1	HCDR2	HCDR3
F-E5	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQASQVPYT (SEQ ID NO:63)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTSYNPKFQG (SEQ ID NO:92)	GGKRAMDY (SEQ ID NO:93)
A-H3	RSSQSLLHSKGNTYLG (SEQ ID NO:328)	KGSNRFS (SEQ ID NO:52)	MQASQVPYT (SEQ ID NO:63)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTSYNPSFQG (SEQ ID NO:61)	GGVRAMDY (SEQ ID NO:62)
C-D5	RSSQSLLHSNYNTYLG (SEQ ID NO:329)	KGSNRFS (SEQ ID NO:52)	FQGSQTPYT (SEQ ID NO:176)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58)	GGIRAMDY (SEQ ID NO:99)
E-C5	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQASHVPYT (SEQ ID NO:96)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94)	GGYRAQDY (SEQ ID NO:95)
E-D6	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQGSHVPYT (SEQ ID NO:97)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGDSDTSYNPKFQG (SEQ ID NO:94)	GGYRAEDY (SEQ ID NO:59)
B-A6	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	FQGSHVPYI (SEQ ID NO:100)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGDSDTRYNQKFQG (SEQ ID NO:98)	GGIRAMDY (SEQ ID NO:99)
D-C12	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQGSHVPYT (SEQ ID NO:97)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTRYNPKFQG (SEQ ID NO:101)	GGFRAMDY (SEQ ID NO:50)
B-D2	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	FQASQVPYT (SEQ ID NO:54)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTRYNPSFQG (SEQ ID NO:272)	GGERAMDY (SEQ ID NO:294)
F-G3	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQASQVPYT (SEQ ID NO:63)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTSYNHKFQG (SEQ ID NO:331)	GGNRAEDY (SEQ ID NO:333)
E-A1	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQGSHVPYT (SEQ ID NO:97)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGDSDTRYNPKFQG (SEQ ID NO:58)	GGYRAEDY (SEQ ID NO:59)
D6	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	MQASQVPYT (SEQ ID NO:63)	GYSFTNYNIH (SEQ ID NO:57)	MGTIYPGNSDTSYNPSFQG (SEQ ID NO:61)	GGVRAMDY (SEQ ID NO:62)
VH1.1				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49)	GGYRAEDY (SEQ ID NO:59)
VH1.2				GYTFTDYNMH (SEQ ID NO:88)	MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49)	GGYRAEDY (SEQ ID NO:59)
VH1.3				GYTFTDYNMH (SEQ ID NO:88)	MGTIYPGNDDTKYSQKFQG (SEQ ID NO:91)	GGYRAEDY (SEQ ID NO:59)
VH1.4				GYTFTDYNMH (SEQ ID NO:88)	MGTIYPGNDDTKYSQKFQG (SEQ ID NO:91)	GGFRAMDY (SEQ ID NO:50)
VH1.5				GYTFTDYNMH (SEQ ID NO:88)	MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49)	GGFRAMDY (SEQ ID NO:50)
VH1.6				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90)	GGYRAEDY (SEQ ID NO:59)

VH1.7				GYTFTNYNIH (SEQ ID NO:89)	MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90	GGFRAMDY (SEQ ID NO:50)
VH1.8				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49)	GGFRAMDY (SEQ ID NO:50)
VH1.9				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDDTKYSQKFQG (SEQ ID NO:91)	GGYRAEDY (SEQ ID NO:59)
VH1.10				GYTFTDYNMH (SEQ ID NO:88)	MGTIYPGNDDTSYSQKFQG (SEQ ID NO:90)	GGFRAMDY (SEQ ID NO:50)
VH1.11				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDDTKYSQKFQG (SEQ ID NO:91)	GGFRAMDY (SEQ ID NO:50)
VH1.12				GYTFTNYAMH (SEQ ID NO:48)	MGTIYPGNDNTKYNQKFQG (SEQ ID NO:332)	GGFRAMDY (SEQ ID NO:50)
VL1.1	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	FQASHVPYT (SEQ ID NO:53)			
VL1.2	RSSQSLLHSNGNTYLG (SEQ ID NO:51)	KGSNRFS (SEQ ID NO:52)	FQASQVPYT (SEQ ID NO:54)			
VL1.3	RSSQSLLHSNANTYLG (SEQ ID NO:55)	KGSNRFS (SEQ ID NO:52)	FQASQVPYT (SEQ ID NO:54)			
VL1.4	RSSQSLLHSNAYNYLG (SEQ ID NO:56)	KGSNRFS (SEQ ID NO:52)	FQASQVPYT (SEQ ID NO:54)			
VL1.5	RSSQSLLHSNAYNYLG (SEQ ID NO:56)	KGSNRFS (SEQ ID NO:52)	FQASHVPYT (SEQ ID NO:53)			
VL1.6	RSSQSLLHSNANTYLG (SEQ ID NO:55)	KGSNRFS (SEQ ID NO:52)	FQASHVPYT (SEQ ID NO:53)			
VL1.7	RSSQSLLHSNAYNYLG (SEQ ID NO:56)	KGSNRAS (SEQ ID NO:330)	FQASHVPYT (SEQ ID NO:53)			
VL1.8	RSSQSLLHSNAYNYLG (SEQ ID NO:56)	KGSNRFS (SEQ ID NO:52)	MQASHVPYT (SEQ ID NO:96)			
VL1.9	RSSQSLLHSNAYNYLG (SEQ ID NO:56)	KGSNRAS (SEQ ID NO:330)	MQASHVPYT (SEQ ID NO:96)			

Table 5. HTRF epitope competition IC50 values for designer lead IgGs.

	IC50 µg/ml	
Antibody	**hCD47**	**cCD47**
m5F9G4	0.601	0.809
h5F9G4	0.464	0.899
VH1.8/VL1.1	0.459	0.629
VH1.8/VL1.2	0.586	0.824
VH1.8/VL1.3	0.928	1.327
VH1.8/VL1.4	2.944	5.169
VH1.8/VL1.5	0.911	1.36
VH1.8/VL1.6	0.56	0.626
VH1.1/VL1.6	6.963	1.818
VH1.6/VL1.6	13.981	4.457
VH1.7/VL1.4	22.624	13.241
VH1.7/VL1.5	9.534	4.203
VH1.7/VL1.6	4.637	1.422
VH1.9/VL1.6	11.051	3.26
VH1.12/VL1.8	16.259	5.843

Table 6. Steady State KD binding affinities for monomeric Fabs to human and cyno CD47.

	Cyno CD47		Human CD47	
Analyte	**KD (nM)**	**Chi2**	**KD (nM)**	**Chi2**
FAB h5F9G4	17.1	0.00827	11.9	0.0265
FAB VH1.8/1.1	17.9	0.0366	7.4	0.0126
FAB VH1.8/1.6	25.9	0.0564	15.9	0.00446
FAB VH1.8/1.6DI	27.8	0.00741	20.3	0.00696

Table 7. Examples of antibody variable region amino acid sequences.

VH1.8/VL1.6
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:1)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNANTYLGWYLQKPGQSPQLLIYKGSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASHVPYTFGQGTKLEIK (SEQ ID
NO:2)

VH1.8/VL1.6-DI
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:3)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNANTYLGWYLQKPGQSPQLAIYKGSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDAGVYYCFQASHVPYTFGQGTKLEIK (SEQ ID
NO:4)

VH1.8/VL1.1
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:5)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGNTYLGWYLQKPGQSPQLLIYKGSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASHVPYTFGQGTKLEIK (SEQ ID
NO:6)

VH1.8/VL1.5
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:7)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNAYNYLGWYLQKPGQSPQLLIYKGSNRFS

GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASHVPYTFGQGTKLEIK (SEQ ID NO:8)

VH1.8/VL1.4
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:9)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNAYNYLGWYLQKPGQSPQLLIYKGSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASQVPYTFGQGTKLEIK (SEQ ID NO:10)

VH1.8/VL1.3
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:11)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNANTYLGWYLQKPGQSPQLLIYKGSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASQVPYTFGQGTKLEIK (SEQ ID NO:12)

VH1.8/VL1.2
heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYAMHWVRQAPGQRLEWMGTIYPGNDDT
KYNQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARGGFRAMDYWGQGTLVTVSS
(SEQ ID NO:13)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGNTYLGWYLQKPGQSPQLLIYKGSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQASQVPYTFGQGTKLEIK (SEQ ID NO:14)

D6
heavy chain variable (VH) region
EVQLVQSGAEVKKPGESLKISCKGSGYSFTNYNIHWVRQMPGKGLEWMGTIYPGNSDTS
YNPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGGVRAMDYWGQGTLVTVSS
(SEQ ID NO:15)

light chain variable (VL) region
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGNTYLGWYLQKPGQSPQLLIYKGSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQASQVPYTFGQGTKLEIK (SEQ ID NO:16)

Table 8. Examples of antibody Fc region amino acid sequences.

Human IgG4 wild type
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:17)

Human IgG4(S228P)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:18)

Human IgG1 wild type
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:19)

Human IgG1-3M
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:20)

Human IgG2 wild type
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:21)

Human IgG1 wild type “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:22)

Human IgG1-3M “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:23)

Table 9. Examples of CD47 protein amino acid sequences.

Human CD47 sequence
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRD
IYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIE
LKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIV
GAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGL
SLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMM
NDE (SEQ ID NO:24)

Cynomolgus monkey CD47 sequence
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRD
IYTFDGALNKSTAPANFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIE
LKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLMITVIVIV
GAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGL
SLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMM
NDE (SEQ ID NO:25)

CLAIMS

1. An anti-CD47 antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

the VH region amino acid sequence comprises HCDR1 of GYTFTNYAMH (SEQ ID NO:48), HCDR2 of MGTIYPGNDDTKYNQKFQG (SEQ ID NO:49) and HCDR3 of GGFRAMDY (SEQ ID NO:50); and the VL region amino acid sequence comprises LCDR1 of RSSQSLLHSNANTYLG (SEQ ID NO:55), LCDR2 of KGSNRFS (SEQ ID NO:52) and LCDR3 of FQASHVPYT (SEQ ID NO:53).

2. The antibody or antigen-binding portion of claim 1, wherein

(a) the VH region amino acid sequence comprises SEQ ID NO:1 and the VL region amino acid sequence comprises SEQ ID NO:2; or

(b) the VH region amino acid sequence comprises SEQ ID NO:3 and the VL region amino acid sequence comprises SEQ ID NO:4.

3. The antibody or antigen-binding portion of claim 1 or 2, wherein the antibody is humanized or chimeric.

4. The antibody or antigen-binding portion of any one of claims 1-3, wherein the VH region, the VL region, or both the VH and the VL region comprise one or more human framework region amino acid sequences.

5. The antibody or antigen-binding portion of any one of claims 1-4, wherein the VH region, the VL region, or both the VH and the VL region comprise a human variable region framework scaffold amino acid sequence into which the CDRs have been inserted.

6. The antibody or antigen-binding portion of claim 1, wherein the VH region comprises an IGHV5-51 or IGHV1-3 human germline scaffold amino acid sequence into which the HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

7. The antibody or antigen-binding portion of claim 1 or 6, wherein the VL region comprises an IGKV2-28 human germline scaffold amino acid sequence into which the LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

8. The antibody or antigen-binding portion of any one of claims 1-7, wherein the antibody or antigen-binding portion comprises an immunoglobulin constant region.

9. The antibody or antigen-binding portion of claim 8, wherein the immunoglobulin constant region is IgG, IgE, IgM, IgD, IgA or IgY.

10. The antibody or antigen-binding portion of claim 9, wherein the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2.

11. The antibody or antigen-binding portion of claim 8, wherein the immunoglobulin constant region is immunologically inert.

12. The antibody or antigen-binding portion of claim 8, wherein the immunoglobulin constant region is a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a wild-type human IgG2 constant region, wherein numbering is according to the EU index as in Kabat.

13. The antibody or antigen-binding portion of claim 8, wherein the immunoglobulin constant region comprises any one of SEQ ID NOS:17-23.

14. The antibody or antigen-binding portion of any one of claims 1-13, wherein the antibody or antigen-binding portion is an Fab, an Fab', an $F(ab')_2$, an Fv, an scFv, a maxibody, a minibody, a diabody, a triabody, a tetrabody, or a bis-scFv.

15. The antibody or antigen-binding portion of any one of claims 1-14, wherein the antibody or antigen-binding portion is monoclonal.

16. The antibody or antigen-binding portion of any one of claims 1-15, wherein the antibody or antigen-binding portion is tetrameric, tetravalent or multispecific.

17. The antibody or antigen-binding portion of any one of claims 1-16, wherein the antibody or antigen-binding portion is a bispecific antibody or antigen-binding portion that binds specifically to a first antigen and a second antigen, wherein the first antigen is CD47 and the second antigen is not CD47.

18. The antibody or antigen-binding portion of any one of claims 1-17, wherein the antibody or antigen-binding portion binds specifically to (a) human CD47 or (b) human CD47 and cynomolgus CD47.

19. An immunoconjugate comprising the antibody or antigen-binding portion of any one of claims 1-18 linked to a therapeutic agent.

20. The immunoconjugate of claim 19, wherein the therapeutic agent is a cytotoxin, a radioisotope, a chemotherapeutic agent, an immunomodulatory agent, a cytostatic enzyme, a cytolytic enzyme, a therapeutic nucleic acid, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent.

21. A pharmaceutical composition comprising the antibody or antigen-binding portion of any one of claims 1-18 or the immunoconjugate of claim 19 or 20, and a pharmaceutically acceptable carrier, diluent or excipient.

22. A nucleic acid molecule encoding
the VH region and the VL region amino acid sequences
of the antibody or antigen-binding portion of any one of claims 1-18.

23. An expression vector comprising the nucleic acid molecule of claim 22.

24. A recombinant host cell comprising the nucleic acid molecule of claim 22 or the expression vector of claim 23.

25. A method of producing an anti-CD47 antibody or an antigen-binding portion thereof, the method comprising:
culturing the recombinant host cell of claim 24 under conditions whereby the nucleic acid molecule is expressed, thereby producing the antibody or antigen-binding portion; and
isolating the antibody or antigen-binding portion from the host cell or culture.

26. Use of a therapeutically effective amount of the antibody or antigen-binding portion of any one of claims 1-18, the immunoconjugate of claim 19 or 20 or the pharmaceutical

composition of claim 21 in the manufacture of a medicament for the treatment of cancer in a subject, wherein the medicament is to be administered to the subject.

27. The use of claim 26, wherein the cancer is Gastrointestinal Stromal cancer (GIST), pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma or cancer of hematological tissues.

28. The antibody or antigen-binding portion of any one of claims 1-18, the immunoconjugate of claim 19 or 20 or the pharmaceutical composition of claim 21 for use in the treatment of cancer.

29. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 28, wherein the cancer is Gastrointestinal Stromal cancer (GIST), pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma or cancer of hematological tissues.

30. The antibody or antigen-binding portion of any one of claims 1-18, the immunoconjugate of claim 19 or 20 or the pharmaceutical composition of claim 21, for use as a medicament.