

BINDING AGENTS

FIELD OF THE INVENTION

The invention relates to antibody molecules binding specifically to CD47 (Cluster
5 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
10 belongs to the immunoglobulin superfamily and binds to several known partners, including: membrane integrins, thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRP α). 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-SIRP α signalling is a critical molecular interaction that
15 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-SIRP α signalling can enhance the
20 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-SIRP α signalling axis is also of considerable therapeutic potential in non-cancerous diseases. Hence, anti-
25 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
30 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").

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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 CD47 inhibitors that target receptors on immune cells, and whose pharmacological function is to stimulate immune responses via antigen presentation, are at heightened risk of provoking anti-drug antibody responses. 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 antagonistic 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.

CDR germ-lining 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.

WO2014/093678A2 describes an antagonistic murine anti-CD47 IgG molecule termed "VxP037", and also the preparation of humanized forms of VxP037. Those humanized forms of VxP037 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 VxP037 murine residues. For reasons noted above, such humanized forms of VxP037 described in WO2014/093678A2 are not ideal.

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

SUMMARY OF THE INVENTION

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

- an HCDR1 having amino acids in sequence in the following order: G-Y-T or any amino acid (for example, S, N or R)-F-T or a conservative substitution of T-N or a conservative substitution of N-Y-Y-I or a conservative substitution of I-F or any amino acid (for example, V or G) (SEQ ID NO: 1);

- an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M-G-I or any amino acid (for example, N, V or D)-I-N or any amino acid (for example, Y)-P-V or any amino acid (for example, G or F)-D or a conservative substitution of D-G or a conservative substitution of G-D-T-N or a conservative substitution of N (for example, R)-Y or a conservative substitution of Y-N or a conservative substitution of N (for example, S)-P-S-F-Q-G (SEQ ID NO: 2); and

- an HCDR3 having amino acids in sequence in the following order: G-G-Y or any amino acid (for example, H, I, Q or F)-T or any amino acid (for example, V or I)-M or any amino acid (for example, T, R, P, A or L)-D or any amino acid (for example, G)-R or any amino acid (for example, Q, N, Y, S, W, K, A, E, F, H, I, L, M, T or V) (SEQ ID NO: 3).

- In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTNYYVF (SEQ ID NO: 4) (VxP037 murine/humanized antibody HCDR1 disclosed in WO2014/093678A2) and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYTMDY (SEQ ID NO: 5) (VxP037 murine/humanized antibody HCDR3 disclosed in WO2014/093678A2).

- 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 or a conservative substitution of Q-S-L or a conservative substitution of L-L or a conservative substitution of L-H-S-N or any amino acid (for example, Q, S, T, A or G) or a conservative substitution of N (for example, Q, S, T or G)-G or a conservative substitution of G (for example, A)-Y or any amino acid (for example, N or S)-T or a conservative substitution of T (for example, N)-Y-L-H or any amino acid (for example, D) (SEQ ID NO: 6);

an LCDR2 having amino acids in sequence in the following order: K or any amino acid (for example, L or M)-V or any amino acid (for example, G)-S-N or any amino acid (for example, Y)-R-L or any amino acid (for example, F, A or S)-S (SEQ ID NO: 7); and

an LCDR3 having amino acids in sequence in the following order: F or any amino acid (for example, L, M, S, T or V)-Q-Q or any amino acid (for example, N, A, T or S)-T or any amino acid (for example, L, M or I)-H or a conservative substitution of H-T or any amino acid (for example, V, I, A or F)-P or any amino acid (for example, L)-R or any amino acid (for example, W)-T (SEQ ID NO: 8).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence RSSQSLVHSNGNTYLH (SEQ ID NO: 9) (VxP037 murine/humanized antibody LCDR1 disclosed in WO2014/093678A2), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSRYFS (SEQ ID NO: 10) (VxP037 murine/humanized antibody LCDR2 disclosed in WO2014/093678A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence SQNTHVPRT (SEQ ID NO: 11) (VxP037 murine/humanized antibody LCDR3 disclosed in WO2014/093678A2).

The CDR sequences above are defined using the "Unified" definition, as set out in Table 1 and described below. As an alternative, the CDR sequences in the present invention may be defined using the shorter "AHo" definition (see Table 1), which is based on structural biology and aims to unify nomenclature for all immunoglobulin v-domains.

Using the shorter "AHo" CDR definition, the invention in one aspect provides an antibody molecule which specifically binds to human CD47, and optionally also to cynomolgus monkey CD47 and/or to mouse CD47, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with: an HCDR1 having amino acids in sequence in the following order: G-S-G-Y-T or any amino acid (for example, S, N or R)-F-T or a conservative substitution of T-N or a conservative substitution of N-Y-Y (SEQ ID NO: 12);

- an HCDR2 having amino acids in sequence in the following order: I-N or any amino acid (for example, Y)-P-V or any amino acid (for example, G or F)-D or a conservative substitution of D-G or a conservative substitution of G-D-T-N or a conservative substitution of N (for example, R)-Y or a conservative substitution of Y-N or a conservative substitution of N (for example, S)-P-S-F-Q-G (SEQ ID NO: 13); and
- an HCDR3 having amino acids in sequence in the following order: G-G-Y or any amino acid (for example, H, I, Q or F)-T or any amino acid (for example, V or I)-M or any amino acid (for example, T, R, P, A or L)-D or any amino acid (for example, G) (SEQ ID NO: 14).
- Using the AHo definition, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GSGYTFTNYY (SEQ ID NO: 15) (VxP037 murine/humanized antibody HCDR1 disclosed in WO2014/093678A2) and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYTMD (SEQ ID NO: 16) (VxP037 murine/humanized antibody HCDR3 disclosed in WO2014/093678A2).
- The antibody molecule or antigen-binding portion may further comprise a light chain variable region with CDRs defined using the AHo definition as follows:
- an LCDR1 having amino acids in sequence in the following order: S-S-Q or a conservative substitution of Q-S-L or a conservative substitution of L-L or a conservative substitution of L-H-S-N or any amino acid (for example, Q, S, T, A or G) or a conservative substitution of N (for example, Q, S, T or G)-G or a conservative substitution of G (for example, A)-Y or any amino acid (for example, N or S)-T or a conservative substitution of T (for example, N)-Y (SEQ ID NO: 17);
- an LCDR2 having amino acids in sequence in the following order: K or any amino acid (for example, L or M)-V or any amino acid (for example, G)-S-N or any amino acid (for example, Y)-R-L or any amino acid (for example, F, A or S)-S (SEQ ID NO: 7); and
- an LCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example, N, A, T or S)-T or any amino acid (for example, L, M or I)-H or a conservative substitution of H-T or any amino acid (for example, V, I, A or F)-P or any amino acid (for example, L)-R or any amino acid (for example, W) (SEQ ID NO: 18).
- Using the AHo definition, in aspects of the invention the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence SSQSLVHSNGNTY (SEQ ID NO: 19) (VxP037 murine/humanized antibody LCDR1 disclosed in WO2014/093678A2), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSYRFS (SEQ ID NO: 10) (VxP037 murine/humanized antibody LCDR2 disclosed in

WO2014/093678A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence NTHVPR (SEQ ID NO: 20) (VxP037 murine/humanized antibody LCDR3 disclosed in WO2014/093678A2).

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

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

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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.

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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

20 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 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.

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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.

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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

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

5 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.

10 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.

15 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.

20 The invention also provides a method for treating or preventing an ischemia-reperfusion injury, 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.

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

30 The ischemia-reperfusion injury in all aspect may occur in organ transplantation, acute kidney injury, cardiopulmonary bypass surgery, pulmonary hypertension, sickle cell disease,

myocardial infarction, stroke, surgical resections and reconstructive surgery, reattachment of an appendage or other body part, skin grafting or trauma.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein,
5 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 ischemia-reperfusion injury, an autoimmune disease or an
inflammatory disease.

10 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 ischemia-reperfusion
injury, an autoimmune disease or an inflammatory disease.

15 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,
20 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
25 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
30 herein, in the manufacture of a medicament for the treatment of an ischemia-reperfusion
injury, 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.

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The fibrotic disease in any aspect of the invention may be selected from the group consisting of myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, cystic fibrosis, bronchitis and asthma.

- 5 The invention also provides a method of producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47 and/or to mouse 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;
 - 10 (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 and/or to mouse CD47;
 - (4) selecting clones from the screening step (3) having binding specificity to human CD47
 - 15 and optionally also to cynomolgus monkey CD47 and/or to mouse CD47; and
 - (5) producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47 and/or to mouse CD47, or an antigen-binding portion thereof from clones selected from step (4).
- 20 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).
- 25 The method may comprise a further step of assessing immunogenicity of one or more v-domains in the clones selected in step (4) or the antibody molecule produced in step (5), and optionally generating one or more further mutations, for example in a CDR and framework region, to reduce immunogenicity. Immunogenicity may be assessed by identifying the
- 30 location of T cell epitopes, for example using *in silico* technologies as described herein.

BRIEF DESCRIPTION OF THE FIGURES

- 35 **Figure 1. Direct binding ELISA of library-derived anti-CD47 scFvs against human and mouse CD47-Fc proteins.** Clones were derived from 3 separate phage selection branches (A shows Branch A periprep ELISA; B shows Branch B periprep ELISA; and C shows Branch

C periprep ELISA) where phage populations were selected on biotinylated human, mouse and/or cynomolgus monkey CD47-Fc proteins in each round. After each round of selection, library-derived clones (black circles) were screened against both human and mouse CD47-Fc. Mean $\pm$ SD values in each round are represented in grey bars. In each graph, the X-axis shows selection round ("R"), with "H" denoting human and "M" denoting mouse, and the Y-axis shows binding signal (OD 450nm).

Figure 2. 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 854 unique scFv clones is shown for V_H (A) and V_L (B) domains, respectively. Only those residues targeted for human/murine residue mutagenesis are plotted, other than in the HCDR3. In each plot, CDR residues are shown in the X-axis and the Y-axis shows percentage retention of each murine residue. 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 cutoff for tolerance of murine residue replacement by human germline.

Figure 3. Direct titration ELISA for IgG binding to human, mouse and cyno CD47-Fc proteins. Chimeric anti-CD47 (mVH/mVL), library-derived clones in human IgG1null format were titrated (in μ g/ml) in a direct binding ELISA against human, mouse and cyno CD47-Fc proteins (A-H). The mVH/mVL, library-derived clones and designer clone MH demonstrated binding activity against all 3 orthologs of CD47. Clone VH-A1/VL-B1 binds human and cyno CD47 but does not bind to mouse. Clone TTP lost almost all binding function. In each graph, the X-axis shows IgG concentration in μ g/ml and the Y-axis shows binding signal (OD 450 nm).

Figure 4. ELISA-based CD47-Fc-SIRP α competition assay. ELISA binding signal for human (A), cyno (B) and mouse (C) CD47-Fc proteins to plate-bound human SIRP α was examined in the presence of titrated competitor library-derived leads: A-D5, G-B6, D-H3 and VH-A1/VL-B1 in IgG1null format, Isotype IgG1 as a negative control, plus mVH/mVL in IgG1null format as a positive control. All library-derived IgGs and mVH/mVL demonstrated concentration-dependent reduction in binding of the human, murine and cyno CD47-Fc proteins, suggesting maintenance of a shared epitope. Notably, clone A-D5 exhibited significantly increased potency in neutralisation of mouse CD47 in comparison to the mVH/mVL and VH-A1/VL-B1 did not show the capacity to neutralise the activity of murine CD47. Neither designer clone MH nor TTP exhibited any neutralisation signal and are not plotted here, for clarity. In each graph, the X-axis shows antibody concentration in nM and the Y-axis shows binding signal (OD 450 nm). In the figure legends, "IC" refers to isotype

control.

Figure 5. Binding specificity analyses for prioritized lead clones. Off-target homologue binding risk for mVH/mVL in IgG1 (A) and IgG1null (B) format and library-derived leads A-D5 (C), VH-A1/VL-B1 (D), F-E7 (E), D-H3 (F), and G-B6 (G) in IgG1null format was examined by direct ELISA on CD47-Fc orthologs and a panel of 14 human immunoglobulin superfamily proteins as labelled on each X-axis ("B" refers to blank). Binding to human, cyno and murine CD47-Fcs (h/c/mCD47-Fc) was performed at an IgG concentration of 1 µg/ml. Binding to all other proteins was performed at an IgG concentration of 10 µg/ml. In each plot, the Y-axis shows binding signal (OD 450 nm). For almost all IgGs, binding was observed to hCD47-Fc, mCD37-Fc and cCD47-Fc alone. No binding above background was observed for any other human protein. Notably, clone VH-A1/VL-B1 again did not show reactivity to murine CD47.

Figure 6. Flow cytometric binding to human and cyno CD47+ CHO-K1 cells. Commercial anti-CD47 antibody MS1991, human IgG1 ("I IgG1") and IgG4 ("I IgG4") Isotype controls, lead library-derived IgGs in both IgG1null ("IgG1N") and IgG4(S228P) formats were examined for specific binding on cyno-transfected CHO-K1 cells (A), human-transfected CHO-K1 cells (B), and wild type (wt, i.e. untransfected) CHO-K1 cells (C). IgGs were tested at concentrations ranging from 24-100,000 ng/ml. Concentration-dependent binding was observed against both human and cyno cell lines for all CD47-specific antibodies but not Isotype controls. Low-level binding signals above background were observed against wild type CHO-K1 cells for most antibodies, with significantly stronger signal for the mVH/mVL-derived IgGs and particularly low signal for VH-A1/VL-B1 IgGs. In each graph, the X-axis shows each IgG tested and its concentration in ng/ml, and the Y-axis shows mean fluorescence intensity (MFI).

Figure 7. Flow cytometric testing of binding to human HL60 cells. Commercial anti-CD47 antibody MS1991, human IgG1 and IgG4 Isotype controls ("I IgG1" and "I IgG4", respectively), lead library-derived IgGs in both IgG1null ("IgG1N") and IgG4(S228P) formats were examined for specific binding on HL60 cells. IgGs were tested at concentrations ranging from 24-100000 ng/ml. Concentration-dependent binding was observed for all clones other than the Isotype controls. In each graph, the X-axis shows each IgG tested and its concentration in ng/ml, and the Y-axis shows mean fluorescence intensity (MFI).

Figure 8. Development risk ELISAs. This assay showed that the A-D5, G-B6, D-H3, VH-A1/VL-B1 and mVH/mVL antibodies in IgG1null form exhibit little or no binding to the negatively charged biomolecules Insulin (A), double-stranded DNA (dsDNA) (B) and single-stranded DNA (ssDNA) (C). In each graph, the X-axis shows IgG concentration in µg/ml and the Y-axis shows binding signal (OD 450 nm). 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 clinical performance of therapeutic antibodies.

Figure 9. Direct titration ELISA for designer IgGs binding to human, mouse and cyno CD47-Fc proteins. Chimeric anti-CD47 (mVH/mVL), designer A-D5-derived clones in human IgG1null format were titrated (in $\mu\text{g/ml}$) in a direct binding ELISA against human (A), cyno (B) and mouse (C) CD47-Fc proteins. In each graph, the X-axis shows IgG concentration in $\mu\text{g/ml}$ and the Y-axis shows binding signal (OD 450 nm). Most clones demonstrated binding activity against all 3 orthologs of CD47, although clone A-D5.7 only shows weak binding to cyno CD47 and clone A-D5.10 lost almost all binding function to mouse CD47. In the figure legend, "IgG1NI" refers to IgG1 null isotype.

Figure 10. ELISA-based CD47-Fc-SIRP α competition assay for designer IgGs. ELISA binding signal for human (A), cyno (B) and mouse (C) CD47-Fc proteins to plate-bound human SIRP α was examined in the presence of titrated competitor designer IgGs in IgG1null format, plus Isotype IgG1 as a negative control (represented by "IgG1NI") and mVH/mVL in IgG1null format as a positive control. In each graph, the X-axis shows IgG concentration in nM and the Y-axis shows binding signal (OD 450 nm). Notably, several A-D5-derived clones again exhibited significantly increased potency in neutralisation of mouse CD47 in comparison to the mVH/mVL. Neither designer clone A-D5.7 nor A-D5.10 exhibited any neutralisation signal on the orthologs for which they showed weak ELISA binding signal and are not plotted here, for clarity.

Figure 11. Direct titration ELISA for A-D5.4-derived designer IgGs binding to human, mouse and cyno CD47-Fc proteins. Chimeric anti-CD47 (mVH/mVL), designer A-D5.4-derived clones in human IgG1null format were titrated (in $\mu\text{g/ml}$) in a direct binding ELISA against human (A), cyno (B) and mouse (C) CD47-Fc proteins. In each graph, the X-axis shows IgG concentration in $\mu\text{g/ml}$ and the Y-axis shows binding signal (OD 450 nm). All clones demonstrated binding activity against all 3 orthologs of CD47. In the figure legends, "IgG1NI" refers to IgG1 null isotype.

Figure 12. ELISA-based CD47-Fc-SIRP α competition assay for designer IgGs. ELISA binding signal for human (A), cyno (B) and mouse (C) CD47-Fc proteins to plate-bound human SIRP α was examined in the presence of titrated competitor designer IgGs in IgG1null format, plus Isotype IgG1 as a negative control (represented by "IgG1NI") and mVH/mVL in IgG1null format as a positive control. In each graph, the X-axis shown IgG concentration in nM and the Y-axis shows binding signal (OD 450 nm). Notably, several A-D5.4-derived clones again exhibited significantly increased potency in neutralisation of mouse CD47 in comparison to the mVH/mVL.

Figure 13. Binding specificity analyses for designer clones A-D5.4 and A-D5.16. Off-

target homologue binding risk for A-D5.4 (A) and A-D5.16 (B) in IgG1null format was examined by direct ELISA on CD47-Fc orthologs and a panel of 14 human immunoglobulin superfamily proteins (as labelled on each X-axis; "B" refers to blank). Binding to all proteins was performed at an IgG concentration of 10 µg/ml. In each plot, the Y-axis shows binding signal (OD 450 nm). For both IgGs, binding was observed to hCD47-Fc, mCD37-Fc and cCD47-Fc alone. No binding above background was observed for any other human protein.

Figure 14. Development risk ELISAs for designer clones A-D5.4 and A-D5.16. This assay showed that the A-D5.4 and A-D5.16 antibodies in IgG1null form exhibit low (below negative control, Ustekinumab) binding to the negatively charged biomolecules Insulin (A), double-stranded DNA (dsDNA) (B) and single-stranded DNA (ssDNA) (C). In each graph, the X-axis shows IgG concentration in µg/ml and the Y-axis shows binding signal (OD 450 nm). 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 clinical performance of therapeutic antibodies.

Figure 15. Flow cytometric binding of library-derived and designer IgGs to CHO-K1 cells. Commercial anti-CD47 antibody MS1991, human IgG1 and IgG4 Isotype controls (represented by "I IgG1" and "I IgG4" respectively), and lead IgGs A-D5, A-D5.4 and A-D5.16 in both IgG1null and IgG4 formats were examined for specific binding on wild type (i.e. untransfected) CHO-K1 cells. IgGs were tested at concentrations ranging from 24-25,000 ng/ml. Concentration-dependent binding was observed for the parental mVH/mVL antibody in both IgG1null and IgG4 formats, but only weak or no binding was observed for Isotype controls, MS1991 and IgGs A-D5, A-D5.4 and A-D5.16 in both IgG formats. In each graph, the X-axis shows concentration of IgG in ng/ml, and the Y-axis shows MFI.

Figure 16. Flow cytometric testing of binding to human HL60 cells. Commercial anti-CD47 antibody MS1991, human IgG1 and IgG4 Isotype controls (represented by "I IgG1" and "I IgG4", respectively), lead IgGs in both IgG1null and IgG4(S228P) formats were examined for specific binding on HL60 cells. IgGs were tested at concentrations ranging from 24-100000 ng/ml. Concentration-dependent binding was observed for all clones other than the Isotype controls. In each graph, the X-axis shows concentration of IgG in ng/ml, and the Y-axis shows MFI.

Figure 17. T cell epitope peptide content in lead antibody v-domains. The v-domains of mVH/mVL, A-D5, A-D5.4 A-D5.16 and A-D5.16-DI antibodies were examined for the presence of Germline (GE), High Affinity Foreign (HAF), Low Affinity Foreign (LAF) and TCED+ T cell receptor epitopes. Both the VH and VL domains of mVH/mVL were found to contain multiple high risk human T cell epitopes and few germline epitopes. In all lead clones,

the high risk epitope content was significantly reduced and germline epitope content significantly improved.

Figure 18. Direct titration ELISA for A-D5.16 and A-D5.16-DI designer IgGs binding to human, mouse and cyno CD47-Fc proteins. Chimeric anti-CD47 (mVH/mVL), designer A-

5 D5.16 and A-D5.16-DI clones in human IgG1null format were titrated (in $\mu\text{g/ml}$) in a direct binding ELISA against human (A), cyno (B) and mouse (C) CD47-Fc proteins. All clones demonstrated binding activity against all 3 orthologs of CD47. In each graph, the X-axis shows concentration of IgG in $\mu\text{g/ml}$, and the Y-axis shows binding signal (OD 450 nm).

Figure 19. ELISA-based CD47-Fc-SIRP α competition assay for designer IgGs. ELISA binding signal for human (A), cyno (B) and mouse (C) CD47-Fc proteins to plate-bound human SIRP α was examined in the presence of titrated competitor designer IgGs in IgG1null format, plus Isotype IgG1 as a negative control and mVH/mVL in IgG1null format as a positive control. In each graph, the X-axis shows antibody concentration in nM, and the Y-axis shows binding signal (OD 450 nm).

15 **Figure 20. Flow cytometry phagocytosis analyses.** (A) Flow cytometric analysis of the phagocytosis of CFSE-labelled HL60 cells by human CD14+ macrophages was performed at multiple concentrations (as shown on the X-axis) for clones A-D5, A-D5.4, A-D5.16 and mVH/mVL in IgG4 (S228P) format and additionally A-D5 in IgG1null format (represented by "IgG1 A-D5N"). The X-axis shows antibody concentration ($\mu\text{g/ml}$), and the Y-axis shows % cells that are CFSE+ and CD14+. (B) The analysis was then repeated across multiple human macrophage donors for A-D5 and mVH/mVL in IgG4 format, at a standard concentration of 10 $\mu\text{g/ml}$. The X-axis shows donor number, and the Y-axis shows % cells that are CFSE+ and CD14+. "V" denotes vehicle.

25 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 and/or to mouse CD47, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

30 an HCDR1 having amino acids in sequence in the following order: G-Y-T or any amino acid (for example, S, N or R)-F-T or a conservative substitution of T-N or a conservative substitution of N-Y-Y-I or a conservative substitution of I-F or any amino acid (for example, V or G) (SEQ ID NO: 1);

an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M-G-I or any amino acid (for example, N, V or D)-I-N or any amino acid (for example, Y)-P-V or any amino acid (for example, G or F)-D or a conservative substitution of

- D-G or a conservative substitution of G-D-T-N or a conservative substitution of N (for example, R)-Y or a conservative substitution of Y-N or a conservative substitution of N (for example, S)-P-S-F-Q-G (SEQ ID NO: 2); and
- an HCDR3 having amino acids in sequence in the following order: G-G-Y or any amino acid (for example, H, I, Q or F)-T or any amino acid (for example, V or I)-M or any amino acid (for example, T, R, P, A or L)-D or any amino acid (for example, G)-R or any amino acid (for example, Q, N, Y, S, W, K, A, E, F, H, I, L, M, T or V) (SEQ ID NO: 3).

- In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTNYYVF (SEQ ID NO: 4) (VxP037 murine/humanized antibody HCDR1 disclosed in WO2014/093678A2) and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYTMDY (SEQ ID NO: 5) (VxP037 murine/humanized antibody HCDR3 disclosed in WO2014/093678A2).

- The antibody molecule or antigen-binding portion thereof according to the invention may further comprise a light chain variable region with:

- an LCDR1 having amino acids in sequence in the following order: R-S-S-Q or a conservative substitution of Q-S-L or a conservative substitution of L-L or a conservative substitution of L-H-S-N or any amino acid (for example, Q, S, T, A or G) or a conservative substitution of N (for example, Q, S, T or G)-G or a conservative substitution of G (for example, A)-Y or any amino acid (for example, N or S)-T or a conservative substitution of T (for example, N)-Y-L-H or any amino acid (for example, D) (SEQ ID NO: 6);
- an LCDR2 having amino acids in sequence in the following order: K or any amino acid (for example, L or M)-V or any amino acid (for example, G)-S-N or any amino acid (for example, Y)-R-L or any amino acid (for example, F, A or S)-S (SEQ ID NO: 7); and
- an LCDR3 having amino acids in sequence in the following order: F or any amino acid (for example, L, M, S, T or V)-Q-Q or any amino acid (for example, N, A, T or S)-T or any amino acid (for example, L, M or I)-H or a conservative substitution of H-T or any amino acid (for example, V, I, A or F)-P or any amino acid (for example, L)-R or any amino acid (for example, W)-T (SEQ ID NO: 8).

- In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence RSSQSLVHSNGNTYLH (SEQ ID NO: 9) (VxP037 murine/humanized antibody LCDR1 disclosed in WO2014/093678A2), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSYRFS (SEQ ID NO: 10) (VxP037 murine/humanized antibody LCDR2 disclosed in

WO2014/093678A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence SQNTHVPRT (SEQ ID NO: 11) (VxP037 murine/humanized antibody LCDR3 disclosed in WO2014/093678A2).

- 5 The CDR sequences above are defined using the "Unified" definition, as set out in Table 1. As an alternative, the CDR sequences in the present invention may be defined using the shorter "AHo" definition (see Table 1), which is based on structural biology and aims to unify nomenclature for all immunoglobulin v-domains.
- 10 Using the shorter "AHo" definition, the invention in one aspect provides an antibody molecule which specifically binds to human CD47, and optionally also to cynomolgus monkey CD47 and/or to mouse CD47, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:
 - an HCDR1 having amino acids in sequence in the following order: G-S-G-Y-T or any amino acid (for example, S, N or R)-F-T or a conservative substitution of T-N or a conservative substitution of N-Y-Y (SEQ ID NO: 12);
 - an HCDR2 having amino acids in sequence in the following order: I-N or any amino acid (for example, Y)-P-V or any amino acid (for example, G or F)-D or a conservative substitution of D-G or a conservative substitution of G-D-T-N or a conservative substitution of N (for example, R)-Y or a conservative substitution of Y-N or a conservative substitution of N (for example, S)-P-S-F-Q-G (SEQ ID NO: 13); and
 - an HCDR3 having amino acids in sequence in the following order: G-G-Y or any amino acid (for example, H, I, Q or F)-T or any amino acid (for example, V or I)-M or any amino acid (for example, T, R, P, A or L)-D or any amino acid (for example, G) (SEQ ID NO: 14).
- 25 Using the AHo definition, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GSGYTFTNYY (SEQ ID NO: 15) (VxP037 murine/humanized antibody HCDR1 disclosed in WO2014/093678A2) and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GGYTMD (SEQ ID NO: 16) (VxP037 murine/humanized antibody HCDR3 disclosed in WO2014/093678A2).
- 30

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: S-S-Q or a conservative substitution of Q-S-L or a conservative substitution of L-L or a conservative substitution of L-H-S-N or any amino acid (for example, Q, S, T, A or G) or a conservative substitution of N
- 35

(for example, Q, S, T or G)-G or a conservative substitution of G (for example, A)-Y or any amino acid (for example, N or S)-T or a conservative substitution of T (for example, N)-Y (SEQ ID NO: 17);

- an LCDR2 having amino acids in sequence in the following order: K or any amino acid (for example, L or M)-V or any amino acid (for example, G)-S-N or any amino acid (for example, Y)-R-L or any amino acid (for example, F, A or S)-S (SEQ ID NO: 7); and
- an LCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example, N, A, T or S)-T or any amino acid (for example, L, M or I)-H or a conservative substitution of H-T or any amino acid (for example, V, I, A or F)-P or any amino acid (for example, L)-R or any amino acid (for example, W) (SEQ ID NO: 18).

- Using the AHo definition, in aspects of the invention the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence SSQSLVHSNGNTY (SEQ ID NO: 19) (VxP037 murine/humanized antibody LCDR1 disclosed in WO2014/093678A2), and/or the
- LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence KVSYRFS (SEQ ID NO: 10) (VxP037 murine/humanized antibody LCDR2 disclosed in WO2014/093678A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence NTHVPR (SEQ ID NO: 20) (VxP037 murine/humanized antibody LCDR3 disclosed in WO2014/093678A2).

- 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 VxP037 disclosed in WO2014/093678A2. 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 and, for some clones, also to mouse CD47 (to facilitate studies in an animal test species). Further refining of the optimized antibody molecules as described herein has provided improved binding to the mouse orthologue of CD47, improved potency in neutralisation of mouse CD47-SIRP α signalling, improved variable domain stability, high expression yields, and/or reduced immunogenicity. For example, we demonstrate herein that the progenitor molecule for the murine anti-CD47 antibody VxP037 carries two major immunogenicity risks in the LCDR1 and LCDR2 that will be carried through with classical humanization techniques (as used in WO2014/093678A2) but are ameliorated in optimized antibody molecules as described herein.

The antibody molecule or antigen-binding portion of the present invention may have improved *in silico* immunogenicity compared with an antibody molecule comprising the CDR sequences of SEQ ID NOs: 4 (HCDR1), 123 (HCDR2), 5 (HCDR3), 9 (LCDR1), 10 (LCDR2) and 11 (LCDR3).

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The antibody molecule or antigen-binding portion of the present invention may exhibit no binding to hamster CD47, or exhibit reduced binding to hamster CD47 compared with an antibody molecule comprising the CDR sequences of SEQ ID NOs: 4 (HCDR1), 123 (HCDR2), 5 (HCDR3), 9 (LCDR1), 10 (LCDR2) and 11 (LCDR3). For example, the antibody molecule or antigen-binding portion of the present invention may exhibit no binding to CHO cells as measured by flow cytometry, or reduced binding to CHO cells as measured by flow cytometry, compared with an antibody comprising the CDR sequences of SEQ ID NOs: 4 (HCDR1), 123 (HCDR2), 5 (HCDR3), 9 (LCDR1), 10 (LCDR2) and 11 (LCDR3). As shown in Fig. 15, representative antibody molecules of the present invention have little or no cross-reactivity with CHO cells, whereas the original murine v-domains of clone mVH/mVL in either IgG1null or IgG4 format drive strong, concentration-dependent binding to CHO cells.

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.

5 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.

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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.

15 In the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-Y-T/S/N/R-F-T/N-N/S-Y-Y-I/V-F/V/G (SEQ ID NO: 21); the HCDR2 may have the amino acid sequence: M/I-G-V/N/I/D-I-N/Y-P-V/G/F-N/D-G/S-D-T-N/R/K-F/Y-N/S-P-S-F-Q-G (SEQ ID NO: 22); and the HCDR3 may have the amino acid sequence: G-G-F/H/I/Q/Y-T/V/I-M/T/R/P/A/L-D/G-Y/Q/N/R/S/W/K/A/E/F/H/I/L/M/T/V (SEQ ID NO: 23). Alternatively, using the AHo definition, in the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-S-G-Y-T/S/N/R-F-T/N-N/S-Y-Y (SEQ ID NO: 24); the HCDR2 may have the amino acid sequence: I-N/Y-P-V/G/F-N/D-G/S-D-T-N/R/K-F/Y-N/S-P-S-F-Q-G (SEQ ID NO: 25); and the HCDR3 may have the amino acid sequence: G-G-F/H/I/Q/Y-T/V/I-M/T/R/P/A/L-D/G (SEQ ID NO: 26).

25 For example, the HCDR1 may have the amino acid sequence: G-Y-T/S-F-T-N-Y-Y-I-F (SEQ ID NO: 27); the HCDR2 may have the amino acid sequence: M/I-G-I/D-I-N-P-V-N/D-G-D-T-N/R-F/Y-N/S-P-S-F-Q-G (SEQ ID NO: 28); and the HCDR3 may have the amino acid sequence: G-G-F/Y-T-M/P-D-Y/R/K/I (SEQ ID NO: 29). Alternatively, using the AHo definition, the HCDR1 may have the amino acid sequence: G-S-G-Y-T/S-F-T-N-Y-Y (SEQ ID NO: 30); the HCDR2 may have the amino acid sequence: I-N-P-V-N/D-G-D-T-N/R-F/Y-N/S-P-S-F-Q-G (SEQ ID NO: 31); and the HCDR3 may have the amino acid sequence: G-G-F/Y-T-M/P-D (SEQ ID NO: 32).

35 In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: R-S-S-Q/H-S-F/L-L/V-H-S-N/Q/A-G/A-Y/N/S-N/T-Y-L-H/D (SEQ ID NO: 33); the LCDR2 may have the amino acid sequence: L/K/M-V/G-S-N/Y-R-A/F/L/S-S (SEQ ID NO: 34).

34); and the LCDR3 may have the amino acid sequence: F/L/M/S/T/V-Q-Q/N/A/T/S-T/L/M/I-Q/H-T/V//I/A/F-P/L-R/W-T (SEQ ID NO: 35). Alternatively, using the AHo definition, in the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: S-S-Q/H-S-F/L-L/V-H-S-N/Q/A-G/A-Y/N/S-N/T-Y (SEQ ID NO: 36); the LCDR2
 5 may have the amino acid sequence: L/K/M-V/G-S-N/Y-R-A/F/L/S-S (SEQ ID NO: 34); and the LCDR3 may have the amino acid sequence: Q/N/A/T/S-T/L/M/I-Q/H-T/V//I/A/F-P/L-R/W (SEQ ID NO: 37).

For example, the LCDR1 may have the amino acid sequence: R-S-S-Q-S-L-L/V-H-S-N/Q/A-G-Y/N-N/T-Y-L-H/D (SEQ ID NO: 38); the LCDR2 may have the amino acid sequence: L/K-V/G-S-N/Y-R-A/F/L-S (SEQ ID NO: 39); and the LCDR3 may have the amino acid sequence: F/S-Q-Q/N/A-T/L-Q/H-T/V-P-R-T (SEQ ID NO: 40). Alternatively, using the AHo definition, the LCDR1 may have the amino acid sequence: S-S-Q-S-L-L/V-H-S-N/Q/A-G-Y/N-N/T-Y (SEQ ID NO: 41); the LCDR2 may have the amino acid sequence: L/K-V/G-S-N/Y-R-A/F/L-S
 15 S (SEQ ID NO: 39); and the LCDR3 may have the amino acid sequence: Q/N/A-T/L-Q/H-T/V-P-R (SEQ ID NO: 42).

In specific embodiments of the invention as defined using the Unified CDR definition, the antibody molecule or antigen-binding portion may comprise:

- 20 (a) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGDINPVNGDTNYSFQGG (SEQ ID NO: 44) (HCDR2), GGYTPDY (SEQ ID NO: 45) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRFS (SEQ ID NO: 47) (LCDR2) and SQNLHVPRT (SEQ ID NO: 48) (LCDR3) [Clone D-H3]; or
- 25 (b) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYNPSFQGG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5]; or
- (c) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
 30 IGDINPVNGDTNFSFQGG (SEQ ID NO: 55) (HCDR2), GGYTMDK (SEQ ID NO: 56) (HCDR3), RSSQSLVHSNGYTYLH (SEQ ID NO: 57) (LCDR1), KGSYRAS (SEQ ID NO: 58) (LCDR2) and SQNTQTPRT (SEQ ID NO: 59) (LCDR3) [Clone G-B6]; or
- (d) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVNGDTNYNPSFQGG (SEQ ID NO: 60) (HCDR2), GGYTMGK (SEQ ID NO: 61)
 35 (HCDR3), RSSQSLVHSNGNTYLD (SEQ ID NO: 62) (LCDR1), KGSYRFS (SEQ ID NO: 63) (LCDR2) and SQATHTPRT (SEQ ID NO: 64) (LCDR3) [Clone F-E7]; or

- (e) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGFTMDY (SEQ ID NO: 66) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRAS (SEQ ID NO: 67) (LCDR2) and SQNTHTPRT (SEQ ID NO: 68) (LCDR3) [Clone VH-A1/VL-B1]; or
- 5 (f) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), IGIINPVDGDTRYSPSFQG (SEQ ID NO: 69) (HCDR2), GGYTMDI (SEQ ID NO: 70) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), LGSNRFS (SEQ ID NO: 71) (LCDR2) and SQNTQTPRT (SEQ ID NO: 59) (LCDR3) [Clone MH]; or
- (g) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
10 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDI (SEQ ID NO: 70) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), LGSNRAS (SEQ ID NO: 72) (LCDR2) and SQATQTPRT (SEQ ID NO: 73) (LCDR3) [Clone TTP]; or
- (h) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRSL (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.1]; or
- (i) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 74) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRSL (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.2]; or
- 20 (j) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRSL (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.3]; or
- (k) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KVSNRSL (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.4]; or
- (l) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1),
30 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRSL (SEQ ID NO: 75) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.5]; or
- (m) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRSL (SEQ ID NO: 75) (LCDR2) and FQNTQTPRT (SEQ ID NO: 76) (LCDR3) [Clone A-D5.6]; or
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- (n) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), LGSNRLS (SEQ ID NO: 77) (LCDR2) and FQNTQTPRT (SEQ ID NO: 76) (LCDR3) [Clone A-D5.7]; or
- 5 (o) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSQGYTYLH (SEQ ID NO: 78) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.8]; or
- (p) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1),
10 MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQQTHTPRT (SEQ ID NO: 79) (LCDR3) [Clone A-D5.9]; or
- (q) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51)
15 (HCDR3), RSSQSLLHSQGYTYLH (SEQ ID NO: 78) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQQTHTPRT (SEQ ID NO: 79) (LCDR3) [Clone A-D5.10]; or
- (r) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KVSNRLS (SEQ ID NO: 53)
20 (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.11]; or
- (s) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYNPSFQG (SEQ ID NO: 74) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.12]; or
- 25 (t) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.13]; or
- (u) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
30 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSSGYNYLH (SEQ ID NO: 80) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.14]; or
- (v) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51)
35 (HCDR3), RSSQSLLHSGGYNYLH (SEQ ID NO: 81) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.15]; or

- (w) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSAGYNYLH (SEQ ID NO: 82) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.16]; or
- 5 (x) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSTGYNYLH (SEQ ID NO: 83) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.17]; or
- (y) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
- 10 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNAYNYLH (SEQ ID NO: 84) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.18]; or
- (z) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51)
- 15 (HCDR3), RSSQSLLHSAGYNYLH (SEQ ID NO: 82) (LCDR1), KVSNRFS (SEQ ID NO: 85) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.16-DI]; or
- (z.1) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPSPFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRFS (SEQ ID NO: 85)
- 20 (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5-DI].

In the above specific embodiments of the invention, the CDRs may alternatively be defined using the AHo CDR definition such that the antibody molecule or antigen-binding portion comprises:

- 25 (a) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVNGDTNYPSPFQG (SEQ ID NO: 87) (HCDR2), GGYTPD (SEQ ID NO: 88) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), KGSNRFS (SEQ ID NO: 47) (LCDR2) and NLHVPR (SEQ ID NO: 90) (LCDR3) [Clone D-H3]; or
- (b) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1),
- 30 INPVDGDTNYPSPFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5]; or
- (c) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVNGDTNFPSPFQG (SEQ ID NO: 94) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
- 35 SSQSLVHSNGYTY (SEQ ID NO: 95) (LCDR1), KGSYRAS (SEQ ID NO: 58) (LCDR2) and NTQTPR (SEQ ID NO: 96) (LCDR3) [Clone G-B6]; or

- (d) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVNGDTNYPSPFQG (SEQ ID NO: 97) (HCDR2), GGYTMG (SEQ ID NO: 98) (HCDR3), SSQSLVHSNGNTY (SEQ ID NO: 19) (LCDR1), KGSYRFS (SEQ ID NO: 63) (LCDR2) and ATHTPR (SEQ ID NO: 99) (LCDR3) [Clone F-E7]; or
- 5 (e) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGFTMD (SEQ ID NO: 101) (HCDR3), SSQSLHNSNGYNY (SEQ ID NO: 89) (LCDR1), KGSNRAS (SEQ ID NO: 67) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone VH-A1/VL-B1]; or
- (f) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1),
10 INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLHNSNGYNY (SEQ ID NO: 89) (LCDR1), LGSNRFS (SEQ ID NO: 71) (LCDR2) and NTQTPR (SEQ ID NO: 96) (LCDR3) [Clone MH]; or
- (g) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
15 SSQSLHNSNGYNY (SEQ ID NO: 89) (LCDR1), LGSNRAS (SEQ ID NO: 72) (LCDR2) and ATQTPR (SEQ ID NO: 102) (LCDR3) [Clone TTP]; or
- (h) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLHNSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and
20 NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.1]; or
- (i) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 103) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLHNSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.2]; or
- 25 (j) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLHNSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.3]; or
- (k) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1),
30 INPVDGDTRYSPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLHNSNGYNY (SEQ ID NO: 89) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.4]; or
- (l) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
35 SSQSLHNSNGYNY (SEQ ID NO: 89) (LCDR1), KGSNRLS (SEQ ID NO: 75) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.5]; or

- (m) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), KGSNRLS (SEQ ID NO: 75) (LCDR2) and NTQTPR (SEQ ID NO: 96) (LCDR3) [Clone A-D5.6]; or
- 5 (n) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), LGSNRLS (SEQ ID NO: 77) (LCDR2) and NTQTPR (SEQ ID NO: 96) (LCDR3) [Clone A-D5.7]; or
- (o) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1),
10 INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSQGYTY (SEQ ID NO: 104) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.8]; or
- (p) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
15 SSQSLLHSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and QTHTPR (SEQ ID NO: 105) (LCDR3) [Clone A-D5.9]; or
- (q) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSQGYTY (SEQ ID NO: 104) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and
20 QTHTPR (SEQ ID NO: 105) (LCDR3) [Clone A-D5.10]; or
- (r) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.11]; or
- 25 (s) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 103) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.12]; or
- (t) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1),
30 INPVDGDTNYPNPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYNY (SEQ ID NO: 89) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.13]; or
- (u) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTNYPNPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
35 SSQSLLHSNGYNY (SEQ ID NO: 106) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.14]; or

- (v) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSGGYNY (SEQ ID NO: 107) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.15]; or
- 5 (w) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSAGYNY (SEQ ID NO: 108) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.16]; or
- (x) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1),
10 INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSTGYNY (SEQ ID NO: 109) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.17]; or
- (y) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3),
15 SSQSLLHSNAYNY (SEQ ID NO: 110) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.18]; or
- (z) the amino acid sequences GSGYSFTNYY (SEQ ID NO: 86) (HCDR1), INPVDGDTRYSPSFQG (SEQ ID NO: 100) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSAGYNY (SEQ ID NO: 108) (LCDR1), KVSNRFS (SEQ ID NO: 85) (LCDR2) and
20 NTHTPR (SEQ ID NO: 93) (LCDR3) [Clone A-D5.16-DI]; or
- (z.1) the amino acid sequences GSGYTFTNYY (SEQ ID NO: 15) (HCDR1), INPVDGDTNYPSPFQG (SEQ ID NO: 91) (HCDR2), GGYTMD (SEQ ID NO: 16) (HCDR3), SSQSLLHSNGYTY (SEQ ID NO: 92) (LCDR1), KVSNRFS (SEQ ID NO: 85) (LCDR2) and NTHTPR (SEQ ID NO: 93) LCDR3) [Clone A-D5-DI].
- 25
- The antibody molecule or antigen-binding portion of the invention (defined using the AHo definition) may comprise: an HCDR1 having the amino acid sequence GSGYTFTNYY (SEQ ID NO: 15) or GSGYSFTNYY (SEQ ID NO: 86);
- an HCDR2 having the amino acid sequence INPVDGDTNYPSPFQG (SEQ ID NO: 91) or
30 INPVDGDTRYSPSFQG (SEQ ID NO: 100); and
- an HCDR3 having the amino acid sequence GGYTMD (SEQ ID NO: 16), and optionally further comprising:
- an LCDR1 having the amino acid sequence SSQSLLHSNGYNY (SEQ ID NO: 89) or SSQSLLHSNGYTY (SEQ ID NO: 92) or SSQSLLHSAGYNY (SEQ ID NO: 108);
- 35 an LCDR2 having the amino acid sequence KVSNRLS (SEQ ID NO: 53) or KVSNRFS (SEQ ID NO: 85); and

an LCDR3 having the amino acid sequence NTHTPR (SEQ ID NO: 93).

The above antibody molecule or antigen-binding portion may alternatively defined using the equivalent Unified CDR definitions as disclosed herein.

5

In particular embodiments of the invention, the antibody molecule or antigen-binding portion may comprise the six CDR sequences of Clone A-D5, Clone A-D5.4 or Clone A-D5.16 or Clone A-D5.16-DI or Clone A-D5-DI as defined above, or a suitable combination of the CDR sequences from each of these clones.

10

For example, in the antibody molecule or antigen-binding portion as defined using the Unified CDR definition, the HCDR1 may have the amino acid sequence: G-Y-T/S-F-T-N-Y-Y-I-F (SEQ ID NO: 27); the HCDR2 may have the amino acid sequence: M-G-I-I-N-P-V-D-G-D-T-N/R-Y-N/S-P-S-F-Q-G (SEQ ID NO: 111); the HCDR3 may have the amino acid sequence: G-G-Y-T-M-D-R (SEQ ID NO: 51); the LCDR1 may have the amino acid sequence: R-S-S-Q-S-L-L-H-S-N/A-G-Y-N/T-Y-L-H (SEQ ID NO: 112); the LCDR2 may have the amino acid sequence: K-V-S-N-R-L/F-S (SEQ ID NO: 113); and the LCDR3 may have the amino acid sequence: F-Q-N-T-H-T-P-R-T (SEQ ID NO: 54).

Alternatively, in the antibody molecule or antigen-binding portion as defined using the AHO definition, the HCDR1 may have the amino acid sequence: G-S-G-Y-T/S-F-T-N-Y-Y (SEQ ID NO: 30); the HCDR2 may have the amino acid sequence: I-N-P-V-D-G-D-T-N/R-Y-N/S-P-S-F-Q-G (SEQ ID NO: 114); the HCDR3 may have the amino acid sequence: G-G-Y-T-M-D (SEQ ID NO: 16); the LCDR1 may have the amino acid sequence: S-S-Q-S-L-L-H-S-N/A-G-Y-N/T-Y (SEQ ID NO: 115); the LCDR2 may have the amino acid sequence: K-V-S-N-R-L/F-S (SEQ ID NO: 113); and the LCDR3 may have the amino acid sequence: N-T-H-T-P-R (SEQ ID NO: 93).

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.

5

For example, the VxP037 murine LCDR1 (as defined herein, i.e. the amino acid sequence RSSQSLVHSNGNTYLH (SEQ ID NO: 9)) has been identified to have a putative deamidation site at residue 10 (N) and/or 12 (N). Removal of either of both of these sites at equivalent positions in an LCDR1 of the invention, for example by conservative substitution (such as to S, A, Q or D), is envisaged (as for example in clone A-D5.8, clone A-D5 and other clones in Tables 3 and 4, or clones A-D5.11 to A-D5.18 in Table 5).

10

Similarly, the VxP037 murine LCDR3 (as defined herein, i.e. the amino acid sequence SQNTHVPRT (SEQ ID NO: 11)) has been identified to have a putative deamidation site at residue 3 (N). Removal of this site at an equivalent position in an LCDR3 of the invention, for example by conservative or non-conservative substitution (such as to A, S, H, D, T, K, G, E, Q or R), is envisaged (as for example in clone F-E7 and other clones in Tables 3 and 4).

15

Similarly, the VxP037 murine HCDR3 (as defined herein, i.e. the amino acid sequence GGYTMDY (SEQ ID NO: 5)) has been identified to have a putative oxidation site at residue 5 (M). Removal of this site at an equivalent position in an HCDR3 of the invention, for example by conservative or non-conservative substitution (such as to P, A, T, S, L, F, W, V, I, Y or R), is envisaged (as for example in clone D-H3 and other clones in Tables 3 and 4).

20

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

25

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.

30

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 an IGKV2-28 human germline scaffold into which the corresponding LCDR sequences have been inserted.

35

The antibody molecule or antigen-binding portion thereof may comprise an immunologically inert constant region.

5 The antibody molecule or antigen-binding portion thereof may be a Fab fragment, a F(ab)₂ fragment, an Fv fragment, a tetrameric antibody, a tetravalent antibody, a multispecific antibody (for example, a bivalent antibody), a single domain antibody (for example, a shark antibody [V_{NAR} antibody], or a fragment thereof, or a camelid antibody [V_HH antibody], or a fragment thereof), a monoclonal antibody or a fusion protein. Antibody molecules and methods for their construction and use are described, in for example Holliger & Hudson
10 (2005, Nature Biotechnol. 23(9): 1126-1136).

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
15 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
20 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,
25 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.
30

35

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, doxifludine, pentostatin, broxuhdine, capecitabine, cladhbine, 5 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, 10 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 15 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.

20 Further provided is a vector comprising the nucleic acid molecule of the invention as defined herein.

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

25 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 30 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 35 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.

The cancer may for example be selected from the group consisting of: 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, and 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.

5 The invention also provides a method for treating or preventing an ischemia-reperfusion injury, 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
10 herein.

The ischemia-reperfusion injury in all aspect may occur in organ transplantation, acute kidney injury, cardiopulmonary bypass surgery, pulmonary hypertension, sickle cell disease, myocardial infarction, stroke, surgical resections and reconstructive surgery, reattachment
15 of an appendage or other body part, skin grafting or trauma.

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

20 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 ischemia-reperfusion injury, an autoimmune disease or an
25 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
30 herein, in the manufacture of a medicament for the treatment of an ischemia-reperfusion injury, 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
35 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,
5 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
10 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.

15 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 be selected from the group consisting
20 of myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis and bronchitis.

The pharmaceutical composition of the invention may comprise a pharmaceutically acceptable excipient. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not
25 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.

30 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.

35

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
5 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.

10 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
15 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
20 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 dextrans; chelating
25 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 TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

A pharmaceutical composition comprising an anti-CD47 antibody molecule may be
30 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
35 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 pharmacokinetic 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 term prophylaxis/treatment.

In some preferred embodiments, the therapeutic effect of the anti-CD47 antibody molecule may persist for several half-lives, 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 and/or to mouse 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 and/or to mouse CD47;
- (4) selecting clones from the screening step (3) having binding specificity to human CD47 and optionally also to cynomolgus monkey CD47 and/or to mouse CD47; and
- (5) producing an antibody molecule which specifically binds to human CD47 and optionally also to cynomolgus monkey CD47 and/or to mouse 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, minimise

human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

5 The method may comprise a further step of assessing immunogenicity of one or more v-domains in the clones selected in step (4) or the antibody molecule produced in step (5), and optionally generating one or more further mutations, for example in a CDR and framework region, to reduce immunogenicity. Immunogenicity may be assessed by identifying the location of T cell epitopes, for example using *in silico* technologies as described herein.

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

As used herein, the term "CD47" refers to Integrin Associated Protein (IAP) 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
15 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*). In certain embodiments, the antibodies may be completely specific for human CD47 and may not exhibit non-human cross-reactivity.

20

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
25 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
30 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.

CD47 “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.

5 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 antibodies [V_{NAR} antibodies], or a fragment thereof, and camelid antibodies [V_HH antibodies], or a fragments thereof), maxibodies, minibodies, intrabodies, diabodies, triabodies, 10 tetrabodies, 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 20 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. 25

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 30 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 V_H and CH₁ domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).

35

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
5 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.

- 10 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, contribute to the formation of the antigen binding site of antibodies.
- 15 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
20 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"
25 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 VxP037 murine anti-CD47 antibody CDRs
30 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
35 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
5	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
10	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
15	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
20	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
25	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 subsequences 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
5 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 and is conjugated to cytotoxic, cytostatic and/or therapeutic agents.

10 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.

15 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
20 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
25 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).

30 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 μ M indicates weak binding affinity compared to a K_D of 1 nM.
35 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.

5 The term "potency" is a measurement of biological activity and may be designated as IC₅₀, 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.

10 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.

15 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.

20 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
25 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
30 condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

35 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
5 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
10 "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
15 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
20 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
25 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
30 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 and characterisation 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 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 forms of engineered human IgG: IgG4 with the S228P mutation to stabilise the IgG4 hinge, and IgG1null – IgG1 with the lower hinge mutations L234A/L235A/G237A, which minimise Fc γ receptor-driven effector functions. IgGs were expressed in HEK-293expi 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 μ g/ml in carbonate buffer and added at 100 μ l per well, at 4°C, o/n. Coated plates were washed 3x with PBS pH7.4, blocked with 1% BSA in PBS (380 μ l/ well) for 1 hr at RT, then washed 3x with PBS-Tween 20 (PBST). CD47 antibodies (100 μ l/well; diluted in PBST) were then added and then incubated 1hr at RT. Plates were then washed 3x with PBST and goat anti-human kappa chain-HRP added (100 μ l/well) at RT, for 1 hr. Plates were then washed 3x with PBST and twice with PBS before the addition of 100 μ l TMB per well. Reactions were stopped by adding 100 μ l 2M H₂SO₄/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 H₂SO₄ and absorbances were read at 450 nm. IgG binding analysis via ELISA on negatively charged biomolecular surfaces were performed
5 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 oligo synthesis and PCR. The amplified scFv repertoire was then cloned via restriction-ligation into a phagemid vector, transformed
10 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
15 protein in round 1 of selection, followed by 100, 50 and 10 nM in subsequent rounds.

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,
20 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 IgGs. Plates were then
25 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 H₂SO₄/well and OD was read on a plate reader at 450nm.

30 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
35 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. 5 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 10 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 15 against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

Cancer cell phagocytosis analysis

Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation. CD14+ PBMCs were subsequently isolated via magnetic cell 20 isolation using CD14 microbeads. In parallel, HL-60 cells were labelled using a green CFSE (carboxy-fluorescein diacetate, succinimidyl ester) cell tracer dye. A total of 1.25×10^6 labelled HL-60 cells were pre-incubated in the presence of anti-CD47 antibodies in 24 well plates for 1 hour at 37°C in a humidified atmosphere containing 5% CO₂. Following 25 incubation, 5×10^5 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, fixed using ice-cold 4% paraformaldehyde for 10 minutes and then blocked with an Fc receptor binding inhibitor monoclonal antibody for 10 minutes. Following the blocking step cells were incubated with an Alexa Fluor 647 (AF647) conjugated anti-human CD14 antibody at room 30 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 1×10^4 AF647 positive events were recorded. Data were analysed 35 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 CD14 double positives cells were gated using a quadrant gate placed based on the population of CD14 positive cells in the vehicle treated test. The percentage of CFSE positive cells from the CD14 positive population was calculated and plotted using
5 GraphPad Prism software (version 7.0a).

Results and Discussion

10 CDR grafting onto preferred human germline v-genes

The CDRs of an antagonistic murine anti-CD47 IgG VP037 (mVH/mVL; see WO2014/093678 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
15 graft the CDRs of the parental antibody onto "preferred" germline scaffolds IGHV5-51 and IGKV2-28, which are known to have good solubility and are used at high frequency in the expressed human antibody repertoire.

Those scaffolds and grafted CDR definitions are outlined in Table 2. The heavy and light
20 chain sequences for murine anti-CD47 antibody 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 IGHV5-51/IGKV2-
25 28 graft was therefore taken forward as the template for CDR mutagenesis and selection of improved clones.

Library generation and screening

The CDR-grafted IGHV5-51/IGKV2-28 v-domain sequences were combined into a VL-VH
30 scFv format and a mutagenesis library cassette was generated by mass oligo synthesis and assembly. The final scFv library was ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 1.3×10^9 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
35 sampled at a frequency of approximately 50%. Libraries were rescued using helper phage M13 and selections performed on biotinylated human, mouse and cynomolgus monkey CD47-Fc proteins in three separate branches A, B and C.

Post-selection screening (Fig. 1) and DNA sequencing revealed the presence of 854 unique, human and mouse CD47-binding scFv clones with significantly increased human content within the CDRs, while the framework sequences remained fully germline. Amongst these
5 854 clones, germ-lining mutations were observed in all CDRs (Table 3). Lead clones were ranked based on level of CDR germ-lining versus ELISA signal for binding to both human and mouse CD47-Fc (Fig. 1). The v-domains of the 4 top clones from this ranking, plus a 5th clone ('VH-A1/VL-B1') that combined the two most humanized observed heavy and light chain v-domains were then sub-cloned into IgG expression vectors for further testing as
10 below (Table 4).

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. The 854 sequence-unique hits with
15 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&B). Murine residues with RF < 75% were regarded as positions that are possibly not essential to the target-binding paratope and are likely to be
20 open to germ-lining, in a series of combinatorial designs.

A design containing only those murine residues with RF > 75% was designated "MH" (MH = Maximally Humanized). Another designer clone ('TTP' = Total Theoretically Possible) was also created that combined the 5 most humanized CDRs observed in the population. The
25 MH and TTP clones were generated by gene synthesis and (along with the 4 library-derived clones outlined above and positive control mVH/mVL and negative control isotype-matched non-CD47-reactive v-domains), cloned into human expression vectors for production as IgG1null and IgG4(S228P). All IgGs were readily expressed and purified from transient transfections of HEK-293 cells.

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Lead IgG specificity and potency characteristics

The purified IgGs described above were then tested for binding to human, mouse and cyno CD47-Fc in direct titration ELISA format (Fig. 3). Surprisingly, this analysis demonstrated that while clones MH, A-D5, and D-H3 retained binding affinity for all 3 orthologues of CD47,
35 two clones showed reduced binding to mouse CD47 (G-B6 and F-E7), one clone (VHA-1/VL-

B1) maintained comparable binding to both human and mouse CD47, but had lost cross-reactivity to mouse CD47, and one clone (TTP) had lost almost all binding function.

In a CD47-SIRP α blockade assay (Fig. 4), A-D5, G-B6, F-E7 and D-H3 all exhibited concentration-dependent blockade of human, mouse and cyno CD47 interaction with SIRP α -Fc in similar concentration ranges as the mVH/mVL IgG1 antibody. Notably, the VH-A1/VL-B1 IgG1 exhibited potent blockade of human and cyno CD47, but no blockade of mouse CD47. Interestingly, clone MH, despite having demonstrated binding to all 3 orthologues of CD47 in ELISA, showed no blockade signal in any assay. Clone TTP was also negative in all blockade assays.

To ensure that that lead clones had not suffered from loss of target specificity during the mutation and reselection process; lead and control IgG1 clones were tested for binding to a panel of 14 purified human proteins from the immunoglobulin superfamily (Fig. 5). All five IgGs exhibited binding signals at 1 μ g/ml to CD47-Fc (human OD450 nm > 2.0, cyno > 2.0, mouse > 1.25), and no detectable binding (OD450 nm < 0.1) against any other protein. One notable exception was the VH-A1/VI-B1 clone, which again showed strong binding to human and cyno CD47, but no signal against mouse CD47.

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

Antibodies to CD47 were analysed for concentration-dependent binding at the cell surface via flow cytometry. CHO-K1 cells were stably transfected with either human, mouse or cyno CD47 full-length cDNAs. Anti-CD47 IgGs mVH/mVL, VH-A1/VL-B1, A-D5, G-B6, F-E7 and D-H3, and an isotype control IgG1 were then all tested in IgG1null and IgG4(S228P) formats, alongside a commercial mouse anti-human CD47 monoclonal MS1991, over a concentration range of 100,000-24 ng/ml for binding to human, cyno or wild type control ('wt', i.e. untransfected) CHO-K1. All IgGs other than the isotype control showed concentration-dependent binding to human and cyno CD47+ cells, with a maximum MFI in each case being >10-fold higher than observed signals for binding to untransfected CHO-K1 (Fig. 6). Measurable binding to CHO-K1 wt cells was observed for all clones other than VH-A1/VL-B1, but only at high antibody concentrations. The mVH/mVL IgGs, however, showed the strongest reactivity with > 10-fold higher signal than the 'no antibody' negative control at concentrations as low as 24 ng/ml. This background binding may be indicative of the original mouse antibody VxP037 having not just cross-reactivity to mouse CD47, but also hamster. The minimisation of this cross-reactivity to hamster CD47 is preferable for a therapeutic protein, as antibodies will typically be produced in CHO cell culture for clinical

use. High IgG binding affinity for hamster CD47 protein may potentially lead to a significant increase in the content of unwanted co-purified CD47 host cell protein in production runs, which must be removed from the drug product to avoid immunogenicity in patients.

- 5 To examine the binding of the lead IgGs to CD47+ human cancer cells, HL60 cells (derived from human acute myeloid leukaemia) were also used in flow cytometry analyses, as above. All antibodies other than the Isotype control IgGs showed strong, concentration-dependent binding to the HL60 cells (Fig. 7).

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. These three substrates were therefore used to coat ELISA plates and examine
- 15 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
- 20 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. 8, the positive control antibodies exhibited the expected strong reactivity to all 3 substrates, while the negative control showed low reactivity. Importantly, all of the IgG1null lead proteins tested showed binding $\leq$ the negative control against all 3 substrates. This finding underlined the
- 25 maintenance of highly specific, target-driven binding in the optimised clones A-D5, G-B6, D-H3 and VH-A1/VL-B1.

Analyses of designer IgGs based on the lead clone A-D5

- As described above, clone A-D5 had proven to have highly specific binding to human, cyno
- 30 and mouse CD47, low off-target binding potential, improved neutralisation of mouse CD47, reduced background binding to CHO cells and multiple human germlining mutations in the CDRs. As a library-derived clone, however, the A-D5 sequence still retained a number of non-germline (mouse-derived) residues that were suggested to be potentially superfluous by the data found in Fig. 2A and 2B. The A-D5 sequence and all other library-derived clones
- 35 also retained an 'NG' motif in the CDR-L1 that posed high risk for deamidation, as it was found to exhibit high solvent exposure at the apex of the long, flexible, IGKV2-28 germline-

template CDR-L1 loop. In an attempt to simultaneously maximise human germline sequence in the variable domains of A-D5 and to minimise high-risk deamidation motif content in the protein, a series of designer clones were created. This effort was carried out in two phases, with the A-D5.1 to A-D5.10 clones from the first phase containing the CDR sequences outlined in Table 4. These clones were expressed and purified in IgG1null format and examined for target binding by ELISA on all CD47 orthologs (Fig. 9A, B, C) and neutralisation of CD47-SIRP α interaction for all orthologs (Fig. 10A, B, C). In this phase, it was found that the human germline and these improvements could be combined with a moderate loss of potency. In addition, the initial mutations attempting to remove the 'NG' motif in the CDR-L1 (via conservative substitution of N to Q) were successful, albeit with associated reductions in potency in both target-binding ELISAs and neutralisation of CD47-SIRP α interaction (Fig. 10A, B, C).

In the second phase, a series of further mutants were designed on the highest-performing mutant from phase 1: A-D5.4 (Table 5). These 9 mutants sampled further humanization of the CDR-H2 of A-D.4, with or without also replacing the 'N' in the 'NG' deamidation risk motif with conservative and non-conservative mutations such as S, G, A and T, or replacing the 'G' residue with A. These clones were again expressed and purified in IgG1null format and examined for target binding by ELISA on all CD47 orthologs (Fig. 11A, B, C) and neutralisation of CD47-SIRP α interaction for all orthologs (Fig. 12A, B, C). In this phase, it was found that the human germline content of the CDR-H2 could be raised by 2 further residues, without loss of potency in comparison to clone A-D5. In addition, the mutations attempting to remove the 'NG' motif in the CDR-L1 via substitution of N were successful, leading to identification of the clone A-D5.16 which contained the non-conservative N to A mutation, plus the maximally humanized CDR-H2, with only minor (approximately 3-fold) reductions in potency in either target-binding ELISAs or neutralisation of CD47-SIRP α , versus both A-D5 and mVL/mVH. The CDR-L1 sequence of A-D.16 'RSSQSLLHSAGYNYLH' (SEQ ID NO: 82) (and clones A-D5.14, A-D5.15, A-D5.17 and A-D5.28) contained non-germline residues at only two positions (underlined) and achieved an optimized balance between maximum human germline content and maximum stability characteristics.

The A-D5 derivatives A-D5.4 and A-D5.16 were subsequently also tested in IgG1null format for maintenance of binding specificity to ensure that there had been no loss of target specificity during the mutation and reselection process; both clones were tested for binding to a panel of 14 purified human proteins from the immunoglobulin superfamily (Fig. 13). Both

IgGs exhibited binding signals at 10 µg/ml to CD47-Fc (human, cyno and mouse all > 2.0 OD450 nm), and no detectable binding (OD450 nm <0.1) against any other protein. In the 'Developability' ELISA analyses shown in Fig. 14, the positive control antibodies exhibited the expected strong reactivity to all 3 substrates, while the negative control showed low reactivity. Importantly, both A-D5.4 and A-D5.16 IgG1null lead proteins showed binding ≤ the negative control against all 3 substrates. This finding underlined the maintenance of highly specific, target-driven binding in the optimised clones A-D5, A-D5.4 and A-D5.16.

Finally, the A-D5 derivative mutant A-D5.4 and A-D5.16 were examined for their binding to wild type CHO (Fig. 15) and HL60 (Fig. 16) cells by flow cytometry. These analyses confirmed that the original murine v-domains of clone mVH/mVL in either IgG1null or IgG4 format drive strong, concentration-dependent binding to CHO cells, whereas clones A-D5, A-D5.4 and A-D5.16 mediate little or no binding signal in either IgG format (Fig. 15). In contrast, clones mVH/mVL, A-D5, A-D5.4 and A-D5.16 all demonstrated strong binding to human CD47+ HL60 cells (Fig. 16), demonstrating that human CD47 binding at the cell membrane had indeed been retained in our optimized clones, but hamster CD47 reactivity had been ameliorated.

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 mVH/mVL 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.
- As shown in Figure 17, key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to mVH/mVL. As the v-domain engineering process undertaken here had successfully selected for antibodies that maintained anti-CD47 potency without the need for any murine residues being included in the frameworks (Table 2), multiple HAF and LAF epitopes found in the frameworks of both the heavy and light chain v-domains of mVH/mVL were absent in all library-derived and designer leads (Fig. 17). GE epitope content was also found to be significantly increased (from 3 to ≥ 14 in all leads), particularly in the VH regions of lead clones where GE content increased from 0 to 9 in all leads, and TCED+ epitopes were reduced from in all leads from 3 to 2 (Table 8). Importantly, however, multiple foreign epitopes were also eliminated by germlining mutations found in the CDRs of lead clones. For example, a TCED+ peptide 'LVHSNGNTY' (SEQ ID NO: 116) found in the LCDR-1 of mVH/mVL (and, therefore, in any forms of VxP037 previously humanized by CDR grafting) was eliminated in the majority of lead clones by the mutation V>L at position 2 and N>Y at position 7 (Tables 4 and 5). Similarly, the LCDR2 from the mVH/mVL sequence encoded for three foreign epitope peptides spanning the VL Framework 2-LCDR2-Framework 3. These included two HAF peptides ('LLIYKVSyr' (SEQ ID NO: 117) and 'YRFSGVPDR' (SEQ ID NO: 118)) and one LAF peptide ('LIYKVSyrf' (SEQ ID NO: 119)). Insertion of the LCDR2 into the human germline framework IGKV2-28 did not fully remove this issue, as the total sequence 'LLIYKVSyrfSGVPDR' (SEQ ID NO: 120) from mVH/mVL maintains 100% identity in the IGKV2-28 grafted sequence (Table 2). One HAF and two LAF peptides were still found in this region for clones A-D5, A-D5.4 and A-D5.16, while the HAF sequence 'YRFSGVPDR'

(SEQ ID NO: 118) was deleted by the mutation Y>N at position 1. Surprisingly however, the LCDR2 sequence KVSNRFS (SEQ ID NO: 85) that had been identified in the functional binding population during library screening (Table 3), and contained the single human germlining mutation Y>N at position 4, was found to fully ameliorate all predicted foreign epitopes in this region (no HAF, LAF or TCED+ peptides predicted), while also generating two further GE sequences (Fig. 18). It was also observed that the lead clones A-D5 and all of its designer derivatives (Tables 4 and 5) retained an HAF peptide 'VGYYCFQN' (SEQ ID NO: 121) that was also TCED+, spanning the VL Framework 3 and LCDR3 regions. The mutation of V at position 1 of this peptide to A, T or F were all found to disrupt predicted epitope structure and remove the immunogenicity risk of this peptide.

The findings above allowed the design of a maximally deimmunised light chain sequence which was paired with the A-D5.16 VH sequence (Table 4) to form the clone 'A-D5.16-DI'. A-D5.16-DI contained the LCDR sequence 'RSSQSLLSAGYNYLH' (SEQ ID NO: 82), the LCDR2 sequence 'KVSNRFS' (SEQ ID NO: 85) and the Framework 2-LCDR3 sequence 'AGVYYCFQNTHTPRT' (SEQ ID NO: 122) (Framework 2 residues underlined). This clone was readily expressed in IgG1null format and was found to retain target binding affinity against human, cyno and mouse CD47 (Fig 18. A-C) comparable to the mVH/mVL IgG (reduced on mouse Fig. 18C). A-D5.16-DI was also found to exhibit improved CD47-SIRP α blockade in comparison to A-D5.16 and comparable blockade to mVH/mVL against human and cyno CD47, slightly reduced on mouse (Fig. 19). These findings thereby led to a deimmunized clone A-D5.4-DI with a light chain design containing the LCDR sequence "RSSQSLLSNGYTYLH" (SEQ ID NO: 52) (or SSQSLLSNGYTY [SEQ ID NO: 92]) using the AHo definition), the LCDR2 sequence "KVSNRFS" (SEQ ID NO: 85) and the Framework 2-LCDR3 sequence "AGVYYCFQNTHTPRT" (SEQ ID NO: 12s2) (Framework 2 residues underlined).

Phagocytosis of cancer cells

Studies were performed to examine the relative potency of CD47 blockade in driving the phagocytosis of HL60 human cancer cells by human primary macrophages. As shown in Fig. 20A, in IgG4 (S228P) format - mVH/mVL, A-D5, A-D5.4 and A-D5.16 all drove significant phagocytosis at all concentrations tested. A-D5 in IgG1null format did not show any significant potency at 20 μ g/ml, demonstrating the necessity of Fc gamma receptor 1 binding affinity to provoke phagocytosis. Unexpectedly, IgG4 A-D5, A-D5.4 and A-D5.16 all drove significantly more potent phagocytosis at both 1 and 10 μ g/ml, when compared to mVH/mVL. This phenomenon was then examined for IgG4 A-D5 and mVH/mVL across 4 separate

donors of human macrophage (Fig. 20B). This analysis showed that the higher potency shown in Fig. 20A was correct, with A-D5 being significantly more potent in all donors (Fig. 20B).

- 5 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
10 is intended nor should any be inferred.

All documents cited herein are incorporated by reference in their entirety.

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

<u>Scheme</u>	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>	<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>
Unified	GYTFTNYYVF (4)	IGDINPVNGDTNENEKPKN (123)	GGYTMDY (5)	RSSQSLVHSNGNTYLH	KVSYRES (10)	QNTHVPRT (11)
Kabat	NYVVF (124)	DINPVNGDTNENEKPKN (125)	GGYTMDY (5)	RSSQSLVHSNGNTYLH	KVSYRES (10)	QNTHVPRT (11)
Chotia	GYTFTNY	NPVNGD	GGYTMDY (5)	RSSQSLVHSNGNTYLH	KVSYRES (10)	QNTHVPRT (11)
IMGT	GYTFTNYY	INPVNGDT	TRGGYTMDY (130)	QSLVHSNGNTY	KVS	QNTHVPRT (11)
AHo	GSgyTFTNYY (15)	INPVNGDTNENEKPKN (132)	GGYTMD	SSQSLVHSNGNTY	KVSYRES (10)	NTHVPR
AbM	GYTFTNYYVF (4)	DINPVNGDTN	GGYTMDY (5)	RSSQSLVHSNGNTYLH	KVSYRES (10)	QNTHVPRT (11)
Contact	TNYYVF (134)	IGDINPVNGDTN	TRGGYTMD	HSNGNTYLHWY (137)	LLIYKVSYRF	QNTHVPR (139)

Table 2. Amino acid sequence of VXP037 murine anti-CD47 v-domains (mVH/mVL) and human germline CDR grafts (VH1/VL1). SEQ ID NOs are shown in brackets.

V-DOMAIN	Human germline ¹	Amino acid sequence ²
CD47-mVH	n/a	EVQLQQFGAEILVKPGASMKLSCKASGYFTFTNYVFWVKORPGGLEWIGDINPVNGDINFNKKFNKAPLTVDKSSSTFTYIQLSSLTSEDSAVYYCTRGGYTMDYWGQGLVTVSS (140)
CD47-VH1	IGHV5-51	EVQLVQSGAEVKKPGESLKISCKGSGYFTFTNYVFWVRQMPGKGLEWIGDINPVNGDINFNESFGQVTSADKSSISTAYLQWSSLKASDTAMYYCARGGYTMDYWGQGLVTVSS (141)
CD47-mVL	n/a	DVMTQTPLSLTSLVSIGDQASISCRSSQSLVHSNGNTYLHWYLDKPKGSPKLLIYKVSIRFSGVPRFSGSGSGTDFTLKISRVEAEDLGVYFCSONTHYPRTFGGGTKEIK (142)
CD47-VL1	IGKV2-28	DIVMTQSPFLSLPVTPGEPAISICRSSQSLVHSNGNTYLHWYLDKPKGSPQLLIYKVSIRFSGVPRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHYPRTFGGGTKEIK (143)

¹Human germline definitions used for grafting, based on IMGT system. ²CDR 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.

Table 3. Amino acid sequences of unique CDRs (using Unified definition) from 854 unique anti-CD47 v-domains. SEQ ID NOs are shown in brackets.

LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
RSSHSLVHSGNGNTYLH (144)	KGSNRAS (67)	FQNTHTPRT (54)	GYNFTNYYIF (145)	MGDINPENGDTNFNPSFQG (146)	GGFTMDY (66)
RSSQSFLHSGNGNNYLH (147)	KGSNRFS (47)	LQNTHTPRT (148)	GYRFTNYYIF (149)	MGDINPGDGTNFNPSFQG (150)	GGHTMDY (151)
RSSQSLLHSGNGNNYLD (152)	KGSNRSS (153)	MQALHTPWT (154)	GYRFTNYYVF (155)	MGDINPGNGDTNFNPSFQG (156)	GGITMDY (157)
RSSQSLLHSGNGNNYLH (158)	KGSYRAS (58)	MQALHVPRT (159)	GYSFNYYIF (160)	MGDINPGNGDTNPNPSFQG (161)	GGQTMQD (162)
RSSQSLLHSGNGNTYLD (163)	KGSYRFS (63)	MQALQVPWT (164)	GYSFTNYYIF (165)	MGDINPGNGDTNYSFQG (166)	GGYIMDY (166)
RSSQSLLHSGNGNTYLH (167)	KGSYRLS (168)	MQATHVPWT (169)	GYSFTSYIF (170)	MGDINPGNGDTRFNPSFQG (171)	GGYTADY (172)
RSSQSLLHSGNSNYLH (173)	KVSNRAS (174)	MQATQVPWT (175)	GYSFTSYIV (176)	MGDINPGNGDTRFSPSFQG (177)	GGYTLDY (178)
RSSQSLLHSGNGNYLH (46)	KVSNRFS (85)	MQNLQTPRT (179)	GYSFTSYVF (180)	MGDINPGNGDTRYNPSFQG (181)	GGYTMDA (182)
RSSQSLLHSGNGTYLD (183)	KVSNRLS (53)	MQNTHIPRT (184)	GYSFTSYVG (185)	MGDINPGNGDTRYSPSFQG (186)	GGYTMDE (187)
RSSQSLLHSGNGTYLH (52)	KVSYRAS (188)	MQNTHLRT (189)	GYFTNYYIF (49)	MGDINPGNSDTNFNPSFQG (190)	GGYTMDF (191)
RSSQSLLHSGNGNNYLD (192)	KVSYRLS (193)	MQNTHPRT (194)	GYFTSYIF (195)	MGDINPGNSDTNPNPSFQG (196)	GGYTMDH (197)
RSSQSLLHSGNGNNYLH (201)	LGSNRAS (72)	MQNTHVPRT (198)	GYFTSYVF (199)	MGDINPVDGDKYNPSFQG (200)	GGYTMDI (70)
RSSQSLLHSGNGNTYLD (62)	LGSNRFS (71)	MQNTQTPRT (202)		MGDINPVDGDTNFNPSFQG (203)	GGYTMDK (56)

RSSQSLVHSNGYNYLH (207)	LGSNRLS (77)	MQTHTPRT (204)		MGDINPVDGDTNFPSPFQ (205)	GGYTMDL (206)
RSSQSLVHSNGYTYLD (212)	LGSYRAS (208)	MQTQIPRT (209)		MGDINPVDGDTNYPSPFQ (210)	GGYTMDM (211)
RSSQSLVHSNGYTYLH (57)	LGSYRFS (213)	SQATHFPRT (214)		MGDINPVDGDTNYPSPFQ (215)	GGYTMDN (216)
	LGSYRLS (217)	SQATQTPRT (73)		MGDINPVDGDTNFPSPFQ (218)	GGYTMDQ (219)
	LVSNRAS (220)	SQNIQTPRT (221)		MGDINPVDGDTNYPSPFQ (222)	GGYTMDR (51)
	LVSNRFS (223)	SQNLHTPRT (224)		MGDINPVDGDTNYPSPFQ (225)	GGYTMDT (226)
	LVSNRLS (227)	SQNLQTPRT (228)		MGDINPVDSDTKFNPSFQ (229)	GGYTMDV (230)
	LVSYRAS (231)	SQNMHTPRT (232)		MGDINPVDSDTNFNPSFQ (233)	GGYTMDW (234)
	MGSNRFS (235)	SQNTHPRT (236)		MGDINPVDSDTNYPSPFQ (237)	GGYTMGK (61)
	MGSYRLS (238)	SQNTHPWT (239)		MGDINPVDSDTNYPSPFQ (240)	GGYTPDY (45)
	MVSNRFS (241)	SQNTQAPRT (242)		MGDINPVDSDTNFPSPFQ (243)	GGYTRDY (244)
		SQNTQTPRT (59)		MGDINPVDSDTNYPSPFQ (245)	GGYTDS (246)
		SQNTQTPWT (247)		MGDINPVDSDTNYPSPFQ (248)	GGYTDDW (249)
		SQNTQVPRT (250)		MGDINPVNGDTKNPSFQ (251)	GGYTDDY (252)

		SQSTHVPRT (253)		MGDINPVNGDTNFSFQ (254)	GGYVMDY (255)
		SQTHIPRT (256)		MGDINPVNGDTNPNPSFQ (257)	
		SQTHVPRT (258)		MGDINPVNGDTNYSFQ (44)	
		SQTQTPRT (259)		MGDINPVNGDTNPNPSFQ (260)	
		TQNTHTPRT (261)		MGDINPVNGDTNPNPSFQ (262)	
		VQNTQVPT (263)		MGDINPVNGDTNPNPSFQ (264)	
				MGDINPVNGDTNPNPSFQ (265)	
				MGDINPVNSDTNPNPSFQ (266)	
				MGDINPVNSDTNPNPSFQ (267)	
				MGDINPVNSDTNPNPSFQ (268)	
				MGDINPVNSDTNPNPSFQ (269)	
				MGDINPVNSDTNPNPSFQ (270)	
				MGDINPVNSDTNPNPSFQ (271)	
				MGDINPVNSDTNPNPSFQ (272)	

					MGDIYPGNSDTKYNPSFQG (273)	
					MGDIYPVNGDTRYNPSFQG (274)	
					MGIIINPGNGDTRYNPSFQG (275)	
					MGIIINPVDGDTKYNPSFQG (276)	
					MGIIINPVDGDTNYSFQG (277)	
					MGIIINPVDGDTRENPSFQG (278)	
					MGIIINPVDGDTREFSFQG (279)	
					MGIIINPVDGDTRYNPSFQG (74)	
					MGIIINPVDGDTRYSPSFQG (65)	
					MGIIINPVDSDTNYNPSFQG (280)	
					MGIIINPVDSDTRENPSFQG (281)	
					MGIIINPVDSDTRYNPSFQG (282)	
					MGIIINPVDSDTRYSPSFQG (283)	
					MGIIINPVGDTKFNPSFQG (284)	

				MGIINPVNGDTKYNPSFQG (285)	
				MGIINPVNGDTKYSPSFQG (286)	
				MGIINPVNGDTNENPSFQG (287)	
				MGIINPVNGDTNENPSFQG (288)	
				MGIINPVNGDTNENPSFQG (60)	
				MGIINPVNGDTNENPSFQG (289)	
				MGIINPVNGDTNENPSFQG (290)	
				MGIINPVNGDTNENPSFQG (291)	
				MGIINPVNGDTNENPSFQG (292)	
				MGIINPVNGDTNENPSFQG (293)	
				MGIINPVNSDTKYNPSFQG (294)	
				MGIINPVNSDTNENPSFQG (295)	
				MGIINPVNSDTNENPSFQG (296)	
				MGIINPVNSDTNENPSFQG (297)	

					MGIINPVNSDTRYNPSFQG (298)	
					MGIINPVNSDTRYSPSFQG (299)	
					MGNINPVDGDTRYNPSFQG (300)	
					MGVINPVNSDTNYNPSFQG (301)	

Table 4. Amino acid sequences of CDRs (using Unified definition) of unique, library-derived and designer, CD47-SIRP α interaction-blocking anti-CD47 IgGs. SEQ ID NOs are shown in brackets.

Clone name	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
D-H3	RSSQSLHHSNGYNYLH (46)	KGSNRFS (47)	SQNLHVPRT (48)	GYSFTNYIIF (43)	MGLINPVNGDNTNPSFQG (44)	GGYTPDY (45)
A-D5	RSSQSLHHSNGYTYLH (52)	KVSNRLS (53)	FQNTHTPRT (54)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
G-B6	RSSQSLVHSNGYTYLH (57)	KGSYRAS (58)	SQNTQTPT (59)	GYSFTNYIIF (43)	IGDINPVNGDNTNPSFQG (55)	GGYTMDK (56)
F-E7	RSSQSLVHSNGNTYLD (62)	KGSYRFS (63)	SQATHTPRT (64)	GYSFTNYIIF (43)	MGLINPVNGDNTNPNPSFQG (60)	GGYTMGK (61)
VH-A1/VL-B1	RSSQSLHHSNGYNYLH (46)	KGSNRAS (67)	SQNTHTPRT (68)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGFTMDY (66)
MH	RSSQSLHHSNGYNYLH (46)	LGSNRFS (71)	SQNTQTPT (59)	GYSFTNYIIF (43)	IGLINPVDGDTNPNPSFQG (69)	GGYTMDI (70)
TTP	RSSQSLHHSNGYNYLH (46)	LGSNRAS (72)	SQATQTPT (73)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDI (70)
A-D5.1	RSSQSLHHSNGYTYLH (52)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.2	RSSQSLHHSNGYTYLH (52)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (74)	GGYTMDR (51)
A-D5.3	RSSQSLHHSNGYTYLH (52)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.4	RSSQSLHHSNGYNYLH (46)	KVSNRLS (53)	FQNTHTPRT (54)	GYFTNYIIF (49)	MGLINPVDGDTNPNPNPSFQG (50)	GGYTMDR (51)
A-D5.5	RSSQSLHHSNGYNYLH (46)	KGSNRLS (75)	FQNTHTPRT (54)	GYFTNYIIF (49)	MGLINPVDGDTNPNPNPSFQG (50)	GGYTMDR (51)

A-D5.6	RSSQSLHHSNGYNYLH (46)	KGSNRLS (75)	FQNTQTPT (76)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.7	RSSQSLHHSNGYNYLH (46)	LGSNRLS (77)	FQNTQTPT (76)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.8	RSSQSLHHSQGYTYLH (78)	KVSNRLS (53)	FQNTHTPT (54)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.9	RSSQSLHHSNGYTYLH (52)	KVSNRLS (53)	FQQHTPT (79)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.10	RSSQSLHHSQGYTYLH (78)	KVSNRLS (53)	FQQHTPT (79)	GYFTNYIIF (49)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)

Table 5. Amino acid sequences of CDRs (using Unified definition) of unique, A-D5-derived, CD47-SIRP α interaction-blocking, designer anti-CD47 IgGs. SEQ ID NOs are shown in brackets.

<u>Clone name</u>	<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>
A-D5.11	RSSQSLHHSNGYNYLH (46)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (50)	GGYTMDR (51)
A-D5.12	RSSQSLHHSNGYNYLH (46)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (74)	GGYTMDR (51)
A-D5.13	RSSQSLHHSNGYNYLH (46)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.14	RSSQSLHHSNGYNYLH (80)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.15	RSSQSLHHSNGYNYLH (81)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.16	RSSQSLHHSAGYNYLH (82)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.17	RSSQSLHSTGYNYLH (83)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)
A-D5.18	RSSQSLHHSNAYNAYNYLH (84)	KVSNRLS (53)	FQNTHTPRT (54)	GYSFTNYIIF (43)	MGLINPVDGDTNPNPSFQG (65)	GGYTMDR (51)

CLAIMS

1. An antibody molecule which specifically binds to human CD47 (Cluster of Differentiation 47) and cynomolgus monkey CD47, and optionally also binds to mouse CD47, or an antigen-binding portion thereof, in which the antibody molecule or antigen-binding
 5 portion comprises:

(a) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGDINPVNGDTNYSFQ (SEQ ID NO: 44) (HCDR2), GGYTPDY (SEQ ID NO: 45) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRFS (SEQ ID NO: 47) (LCDR2) and SQNLHVPRT (SEQ ID NO: 48) (LCDR3) [Clone D-H3]; or

10 (b) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPFQ (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVSNRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5]; or

15 (c) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), IGDINPVNGDTNFSFQ (SEQ ID NO: 55) (HCDR2), GGYTMDK (SEQ ID NO: 56) (HCDR3), RSSQSLVHSNGYTYLH (SEQ ID NO: 57) (LCDR1), KGSYRAS (SEQ ID NO: 58) (LCDR2) and SQNTQTPRT (SEQ ID NO: 59) (LCDR3) [Clone G-B6]; or

20 (d) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVNGDTNYPFQ (SEQ ID NO: 60) (HCDR2), GGYTMGK (SEQ ID NO: 61) (HCDR3), RSSQSLVHSNGNTYLD (SEQ ID NO: 62) (LCDR1), KGSYRFS (SEQ ID NO: 63) (LCDR2) and SQATHTPRT (SEQ ID NO: 64) (LCDR3) [Clone F-E7]; or

25 (e) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1), MGIINPVDGDTRYSPFQ (SEQ ID NO: 65) (HCDR2), GGFTMDY (SEQ ID NO: 66) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KGSNRAS (SEQ ID NO: 67) (LCDR2) and SQNTHTPRT (SEQ ID NO: 68) (LCDR3) [Clone VH-A1/VL-B1]; or

(f) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1), MGIINPVDGDTNYPFQ (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO:

51) (HCDR3), RSSQSLLHSNGYNYLH (SEQ ID NO: 46) (LCDR1), KVS NRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.4];
or

(g) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
5 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSAGYNYLH (SEQ ID NO: 82) (LCDR1), KVS NRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.16].

2. An antibody molecule or antigen-binding portion according to Claim 1, comprising:

(a) the amino acid sequences GYTFTNYYIF (SEQ ID NO: 49) (HCDR1),
10 MGIINPVDGDTNYPNPSFQG (SEQ ID NO: 50) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSNGYTYLH (SEQ ID NO: 52) (LCDR1), KVS NRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5]; or

(b) the amino acid sequences GYSFTNYYIF (SEQ ID NO: 43) (HCDR1),
15 MGIINPVDGDTRYSPSFQG (SEQ ID NO: 65) (HCDR2), GGYTMDR (SEQ ID NO: 51) (HCDR3), RSSQSLLHSAGYNYLH (SEQ ID NO: 82) (LCDR1), KVS NRLS (SEQ ID NO: 53) (LCDR2) and FQNTHTPRT (SEQ ID NO: 54) (LCDR3) [Clone A-D5.16].

3. An antibody molecule or antigen-binding portion according to Claim 1 or 2, which is humanized or chimeric.

4. An antibody molecule or antigen-binding portion according to Claim 1, 2 or 3,
20 comprising one or more human variable domain framework scaffolds into which the CDRs have been inserted.

5. An antibody molecule or antigen-binding portion according to any preceding claim, comprising an IGHV5-51 human germline scaffold into which the corresponding HCDR sequences have been inserted.

25 6. An antibody molecule or antigen-binding portion according to any preceding claim, comprising an IGKV2-28 human germline scaffold into which the corresponding LCDR sequences have been inserted.

7. An antibody molecule or antigen-binding portion according to any preceding claim, comprising an immunologically inert constant region.
8. An antibody molecule or antigen-binding portion according to any preceding claim, which is a Fab fragment, a F(ab)₂ fragment, an Fv fragment, a tetrameric antibody, a
- 5 tetraivalent antibody, a multispecific antibody (for example, a bivalent antibody), a single domain antibody (for example, V_HH or V_{NAR}, or a fragment of either), a monoclonal antibody or a fusion protein.
9. An immunoconjugate comprising an antibody molecule or antigen-binding portion thereof according to any preceding claim linked to a therapeutic agent.
- 10 10. A nucleic acid molecule encoding an antibody molecule or antigen-binding portion thereof according to any of Claims 1 to 8.
11. A vector comprising a nucleic acid molecule according to Claim 10.
12. A host cell comprising a nucleic acid molecule according to Claim 10 or a vector according to Claim 11.
- 15 13. A pharmaceutical composition comprising an antibody molecule or antigen-binding portion thereof according to any of Claims 1 to 8, an immunoconjugate according to Claim 9, a nucleic acid molecule according to Claim 10 or a vector according to Claim 11.