

CONDITIONALLY ACTIVE ANTI-CD46 ANTIBODIES, ANTIBODY FRAGMENTS, THEIR IMMUNOCONJUGATES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/040,913, filed June 18, 2020, the entire disclosure of which is specifically incorporated herein by reference.

INCORPORATION OF MATERIAL OF ASCII TEXT SEQUENCE LISTING BY REFERENCE

The sequence listing submitted herewith as a text file named "BIAT-1032WO_ST25" created on May 25, 2021, which is 13,000 bytes in size, is hereby incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0001] This disclosure relates anti-CD46 antibodies, antibody fragments and immunoconjugates of such antibodies and antibody fragments and uses of the antibodies, antibody fragments and immunoconjugates in diagnostic and therapeutic methods.

BACKGROUND OF THE DISCLOSURE

[0002] CD46 is a membrane cofactor protein or MCP. It is a type I transmembrane protein that is widely expressed but has a number of isoforms as a result of alternate exon splicing and glycosylation. Recently Karosi et al., Laryngoscope 118: 1669-1676 (September 2008), reported detecting fourteen isoforms of the molecule. The mRNA is transcribed from a single gene located at chromosome 1q32 and undergoes extensive alternative splicing to produce multiple transcripts encoding the various protein isoforms. Of the 14 exons comprising the gene, it appears that exons 1-6 are conserved in all CD46 protein isoforms, whereas exons 7 to 9 encode variably utilized serine-threonine-proline ("STP") rich regions, leading to the hypervariability in the protein isoforms. Exons 11 and 12 encode the transmembrane region of CD46, while exons 13 and 14 encode the cytoplasmic tail of the protein.

[0003] The longest mRNA transcript, variant A (NM_002389), contains sequences from all fourteen exons of the gene. Variable splicing of exons 7, 8, 9, and 13 is believed to yield the majority of CD46's fourteen isoforms, with the predominant observed protein isoforms of 66 and 56 kDa arising from alternative inclusion or exclusion of exon 8. Alternate inclusion/exclusion of exon 13 leads to changes in the encoded sequence of the cytoplasmic

tail of the molecule, suggesting that these changes may affect subcellular trafficking, stability, and the signaling properties of the protein.

[0004] As set forth in Karosi et al., CD46 mRNA isoform D comprises exons 1-6, 8-12 and 14 of the CD46 gene (equivalent to the sequence NM_153826, encoding the protein NP_722548), isoform F comprises exons 1-6, 9-12, and 14 (equivalent to the sequence NM_172353, encoding NP_758863), and isoform J comprises exons 1-6, 8, 10-12, and 14 (equivalent to the sequence NM_172356, encoding NP_758866). More specifically the CD46 molecule comprises four N-terminal short consensus repeat (SCR) modules (“Sushi” domains: 4 Cysteines in a 1-3, 2-4 linkage topology), where these SCR domains are encoded by the first six exons of the gene. The SCR2, 3, and 4 modules have the C3b/C4b binding and regulatory activity (discussed below), while the SCR1 module and sequences distal of SCR4 are not essential for complement regulatory function. The membrane-proximal extracellular sequence, encoded by the alternatively utilized exons 7-9 as well as exon 10, is heavily glycosylated, mainly via O-linked carbohydrates.

[0005] For the purposes of the instant disclosure the term “CD46” shall be held to mean any protein as set forth immediately above including any splice variant or immunoreactive fragment thereof as well as any nucleic acid sequence encoding such proteins, splice variants or fragments unless otherwise contextually dictated.

[0006] A number of biological functions have been attributed to CD46, many of which involve regulation of the immune system. One major immunomodulatory function of CD46 involves the regulation of complement proteins to protect host cells from damage by the complement proteins that are a part of the innate immune response of higher eukaryotes. Specifically, CD46 is a cofactor for Factor I mediated proteolytic cleavage of complement proteins C3b and C4b. CD46 has been shown to activate C3 convertases, molecules that cleave C3b into inactive fragments, and thereby protect against inappropriate complement activation. (See also: Liszewski and Atkinson Human Genomics, *Complement regulator CD46: genetic variants and disease associations* (2015)9:7)

[0007] In addition to its role in innate immunity, CD46 also regulates the acquired immune response. Signaling through CD46 leads to T cell proliferation, and differentiation toward a specific class of regulatory T cells, called Tr1, characterized by production of large amounts of IL-10, an anti-inflammatory cytokine. In addition, because spermatozoa express high levels of CD46, it has been suggested that CD46 is involved in reproduction, perhaps in the fusion of sperm to oocyte. CD46 also seems to be highly expressed in the placenta and may help to protect the fetus from immune rejection by the mother.

[0008] CD46 has also been shown to be ubiquitously expressed on most normal human cells, with the exception of red blood cells. For example, there are reports of strong expression of CD46 in epithelial cells, moderate expression in lymphocytes and the endothelium, and weak expression in other cells such as osteoclasts, osteocytes, interstitial cells and muscle cells. Due to its widespread expression, a number of human pathogens have evolved strategies to utilize CD46 as a receptor or co-receptor for binding to cells as a precursor to infection. These pathogens include human herpes virus 6, measles virus, some serotypes of adenoviruses, and pathogenic species from the *Neisseria* family of commensal bacteria. Certain retroviruses are believed to evade complement-mediated immunity by bearing CD46 mimics on their surfaces (Stoiber et al, *Molecular Immunology* 2005; Saifuddin et al, *J Gen Virol*, 1997).

[0009] In addition to its presence on normal cells, CD46 expression levels may be increased in certain cancers. For example, elevated CD46 expression has been reported in breast cancer (Thorsteinsson et al. *APMIS* 106:869-78 (1998); Hofman et al. *Breast Cancer Res. Treat.* 32:213-9 (1994)); colon/colorectal cancer (Andrew et al. *Cancer Res.* 50: 5225-30 (1990); Koretz et al. *Br. J. Cancer* 68:926-31 (1993); Juhl et al. *J. Surg. Oncol.* 64:222-30 (1997); Bjorge et al. *Cancer Immunol. Immunother.* 42:185-92 (1996)); lung cancer (Varsano et al. *Clin. Exp. Immunol.* 113:173-82 (1998); Varsano et al. *Am. J. Respir. Cell. Mol. Bioi.* 19:522-9 (1998)); ovarian cancer (Bjorge et al. *Int. J. Cancer* 70: 14-25 (1997)); renal cancer (Blok et al. *Lab. Invest.* 80:335-44 (2000); Gorter et al. *Lab. Invest.* 74:1039-49 (1996)); pancreatic cancer (Juhl et al. *J. Surg. Oncol.* 64:222-30 (1997)); and prostate cancer (Jarvis et al. *J. Allergy Clin. Immunol* 99 (NO. I, PART 2): S215 (1997); Liu, *Cancer Res.* 60:3429-3434 (2000)); see also, WO 02/18948 AND WO 01/88537.

[0010] The present invention aims at providing anti-CD46 antibodies or antibody fragments with reduced or minimal side effects suitable for therapeutic and diagnostic use, especially for diagnosis and treatment of cancers. Some of these anti-CD46 antibodies or antibody fragments may have a higher binding activity or binding affinity to CD46 in a tumor microenvironment in comparison with the binding activity or binding affinity to CD46 present in a non-tumor microenvironment. These anti-CD46 antibodies or antibody fragments typically have at least comparable efficacy to known anti-CD46 antibodies. In addition, the present anti-CD46 antibodies or antibody fragments may exhibit reduced side effects in comparison with monoclonal anti-CD46 antibodies known in the art as a result of having a relatively lower binding activity or binding affinity to CD46 in the non-tumor microenvironment present in normal tissues. These advantages may provide a more selective

targeting of the CD46 expressed in a tumor and may permit use of higher dosages of these anti-CD46 antibodies or antibody fragments as a result of the selectivity of the antibodies for CD46 present in a tumor microenvironment, whereby more effective therapeutic treatments can be realized without a corresponding increase in undesirable side effects.

SUMMARY OF THE DISCLOSURE

[0011] In one aspect, the present invention provides isolated polypeptides that specifically bind to human CD46. The isolated polypeptides comprise a light chain variable region having three complementary determining regions (CDRs) having sequences L1, L2, and L3, wherein:

the L1 sequence is RAX₁QX₂IX₃NYLN (SEQ ID NO: 1),

the L2 sequence is YTSSLX₄X₅ (SEQ ID NO: 2),

the L3 sequence is QQYIKLPWT (SEQ ID NO: 3); and

a heavy chain variable region having three complementary determining regions (CDRs) having sequences H1, H2, and H3, wherein

the H1 sequence is GGSVSSYDIS (SEQ ID NO: 8);

the H2 sequence is VIWTDGGTNYNSAFMS (SEQ ID NO: 9); and

the H3 sequence is VYDGYPWFA Y (SEQ ID NO: 10);

wherein X₁ is S or L; X₂ is G or W; X₃ is S or A; X₄ is H or F, X₅ is S or E; with the proviso that X₁, X₂, X₃, X₄ and X₅ cannot be S, G, S, H and S, respectively, at the same time.

[0012] The above polypeptides may have an L1 sequence selected from the amino acid sequences RASQGISNYLN (SEQ ID NO: 5), RASQWISNYLN (SEQ ID NO: 12), RASQGIANYLN (SEQ ID NO: 15) and RALQGISNYLN (SEQ ID NO: 22). The above polypeptides may have an L2 sequence selected from the amino acid sequences YTSSLHS (SEQ ID NO: 6), YTSSLFS (SEQ ID NO: 17) and YTSSLHE (SEQ ID NO: 19). In each of the foregoing embodiments, one of the L1 and L2 sequences must be other than the wild type L1 sequence of SEQ ID NO: 5 and the wild type L2 sequence of SEQ ID NO: 6.

[0013] In one embodiment, the above polypeptides comprise a set of 6 CDRs having the amino acid sequences of:

SEQ ID NO: 12, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

[0014] In another embodiment, the isolated polypeptides of the present invention comprise a light chain variable region and a heavy chain variable region, each light chain variable region and heavy chain variable region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13, respectively; and said isolated polypeptides specifically bind to human CD46 protein.

[0015] In another embodiment, the present invention relates to an isolated polypeptide having an amino acid sequence selected from SEQ ID NOS: 11, 13, 14, 16, 18, 20 and 21.

[0016] In another embodiment, the isolated polypeptides of the present invention comprise a light chain variable region and a heavy chain variable region having a pair of sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, and SEQ ID NOS: 21 and 13.

[0017] In another embodiment, the present invention relates to an isolated antibody, or a fragment thereof, comprising a light chain variable region having three complementary determining regions (CDRs) having sequences L1, L2, and L3, wherein

the L1 sequence is RAX₁QX₂IX₃NYLN (SEQ ID NO: 1),
 the L2 sequence is YTSSLX₄X₅ (SEQ ID NO: 2),
 the L3 sequence is QQYIKLPWT (SEQ ID NO: 3); and
 a heavy chain variable region having three complementary determining regions (CDRs)
 having sequences H1, H2, and H3, wherein

the H1 sequence is GGSVSSYDIS (SEQ ID NO: 8);
 the H2 sequence is VIWTDGGTNYNSAFMS (SEQ ID NO: 9); and
 the H3 sequence is VYDGYPWFAFAY (SEQ ID NO: 10);

wherein X₁ is S or L; X₂ is G or W; X₃ is S or A; X₄ is H or F; X₅ is S or E; with the proviso that X₁, X₂, X₃, X₄ and X₅ cannot be S, G, S, H and S, respectively, at the same time.

[0018] In one embodiment, the above antibody or antibody fragment may have an L1 sequence selected from the amino acid sequences RASQGISNYLN (SEQ ID NO: 5), RASQWISNYLN (SEQ ID NO: 12), RASQGIANYLN (SEQ ID NO: 15) and RALQGISNYLN (SEQ ID NO: 22). The above antibody or antibody fragment may have an L2 sequence selected from the amino acid sequences YTSSLHS (SEQ ID NO: 6), YTSSLFS (SEQ ID NO: 17) and YTSSLHE (SEQ ID NO: 19). In each of the foregoing embodiments of the antibody or antibody fragment, one of the L1 and L2 sequences must be other than the wild type L1 sequence of SEQ ID NO: 5 and the wild type L2 sequence of SEQ ID NO: 6.

[0019] In another embodiment, the antibody or antibody fragment may comprise a set of 6 CDRs selected from the following sets of 6 CDR's:

SEQ ID NO: 12, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

[0020] In one embodiment, the antibody or antibody fragment of the present invention may comprise a light chain variable region and a heavy chain variable region, each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13, respectively; and said antibody or antibody fragment specifically binds to human CD46 protein.

[0021] In one embodiment, the antibody or antibody fragment of the present invention may comprise a light chain variable region and a heavy chain variable region having a pair of sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13.

[0022] In one embodiment, an antibody or antibody fragment of the present invention competes with any of the antibodies or antibody fragments described above for binding to human CD46.

[0023] In each of the previous embodiments, the antibody or antibody fragment may have a higher binding activity to CD46 protein at a value of a condition in a tumor microenvironment in comparison with a different value of a same condition that occurs in a non-tumor microenvironment. In one embodiment, the condition is pH. In one embodiment binding activity is measured by binding affinity.

[0024] In each of the previous embodiments, the isolated polypeptide, antibody or antibody fragment may have at least 70% of the antigen binding activity at pH 6.0 as compared to the same antigen binding activity of the parent polypeptide, antibody or antibody fragment at pH 6.0 and the polypeptide, antibody or antibody fragment may have less than 50%, or less than 40%, or less than 30%, or less than 20% or less than 10% of the antigen binding activity at pH 7.4 as compared to the same antigen binding activity of the parent polypeptide, antibody

or antibody fragment at pH 7.4. The antigen binding activity may be binding to CD46 protein.

[0025] In each of the previous embodiments, the antigen binding activity may be measured by an ELISA assay.

[0026] In yet another aspect, the present invention provides an immunoconjugate that includes any of the antibodies or antibody fragments of the invention described above. In the immunoconjugate, the antibody or antibody fragment may be conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0027] In yet another aspect, the present invention provides a pharmaceutical composition that includes any of the polypeptides, the antibodies or antibody fragments, or the immunoconjugates of the invention described above, together with a pharmaceutically acceptable carrier.

[0028] A single dose of the pharmaceutical composition of may include an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate of about 135 mg, 235 mg, 335 mg, 435 mg, 535 mg, 635 mg, 735 mg, 835 mg, 935 mg, 1035 mg, 1135 mg, 1235 mg, or 1387 mg.

[0029] A single dose of the pharmaceutical composition of may include an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate in a range of 135-235 mg, 235-335 mg, 335-435 mg, 435-535 mg, 535-635 mg, 635-735 mg, 735-835 mg, 835-935 mg, 935-1035 mg, 1035-1135 mg, 1135-1235 mg, or 1235-1387 mg.

[0030] Each of the foregoing pharmaceutical compositions may further include an immune checkpoint inhibitor molecule. The immune checkpoint inhibitor molecule may be an antibody or antibody fragment against an immune checkpoint. The immune checkpoint may be selected from LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, CTLA4, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS or the immune checkpoint may be CTLA4, PD-1 or PD-L1.

[0031] Each of the foregoing pharmaceutical compositions may further include an antibody or antibody fragment against an antigen selected from PD1, PD-L1, CTLA4, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein. The WNT protein may be selected from WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11 and WNT16.

[0032] In yet another aspect, the present invention provides a kit for diagnosis or treatment including any of the polypeptides, the antibodies or antibody fragments, the

immunoconjugates or the pharmaceutical compositions of the present invention described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1 shows the binding activities of exemplary conditionally active anti-CD46 antibodies of the present invention conjugated to a linker payload (hereinafter “CAB ADCs”) to human CD46 at pH 6.0, as measured by enzyme linked immunosorbent assay (ELISA). In FIG. 1, BA-133-00-01 is the benchmark (BM) wild type antibody conjugated to the linker payload (hereinafter “WT ADC”).

[0034] FIG. 2 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC tested in FIG. 1 to human CD46 at pH 7.4, as measured by ELISA.

[0035] FIG. 3 shows the binding activities of exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to cyno CD46 at pH 6.0, as measured by ELISA.

[0036] FIG. 4 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC tested in FIG. 3 to cyno CD46 at pH 7.4, as measured by ELISA.

[0037] FIG. 5 shows binding activities of exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to human CD46 under pH titration, as measured by ELISA.

[0038] FIG. 6 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to HEK 293 cells that express human CD46 at pH 6.0, as measured by fluorescence activated cell sorting (FACS).

[0039] FIG. 7 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to HEK 293 cells that express human CD46 at pH 7.4, as measured by FACS.

[0040] FIG. 8 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to Colo205 cells that express CD46 at pH 6.0, as measured by FACS.

[0041] FIG. 9 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to Colo205 cells that express CD46 at pH 7.4, as measured by FACS.

[0042] FIG. 10 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to HEK 293 cells expressing cyno CD46 at pH 6.0, as measured by FACS.

[0043] FIG. 11 shows the binding activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC to HEK 293 cells expressing cyno CD46 at pH 7.4, as measured by FACS.

[0044] FIG. 12 show the cell killing activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC against HEK 293 cells that express human CD46 at pH 6.0.

[0045] FIG. 13 shows the cell killing activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC against HEK 293 cells that express human CD46 at pH 7.4.

[0046] FIG. 14 shows the cell killing activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC against Colo205 cells that express CD46 at pH 6.0.

[0047] FIG. 15 shows the cell killing activities of the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC against Colo205 cells that express CD46 at pH 7.4.

[0048] FIG. 16 shows the effect on tumor volumes of tumor xenograft mice of treatment with the exemplary anti-CD46 CAB ADCs of the present invention and the WT ADC.

[0049] Figure 17 shows protein sequences for representative conditionally active antibodies of the present invention.

DEFINITIONS

[0050] In order to facilitate understanding of the examples provided herein, certain frequently occurring terms are defined herein.

[0051] In connection with a measured quantity, the term "about" as used herein refers to the normal variation in that measured quantity that would be expected by a skilled person making the measurement and exercising a level of care commensurate with the objective of the measurement and the precision of the measuring equipment used. Unless otherwise indicated, "about" refers to a variation of +/- 10% of the value provided.

[0052] The term "affinity" as used herein refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0053] The term “affinity matured” antibody as used herein refers to an antibody with one or more alterations in one or more heavy chain or light chain variable regions, compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0054] The term "amino acid" as used herein refers to any organic compound that contains an amino group ($--NH_2$) and a carboxyl group ($--COOH$); preferably either as free groups or alternatively after condensation as part of peptide bonds. The "twenty naturally encoded polypeptide-forming alpha-amino acids" are understood in the art and refer to: alanine (ala or A), arginine (arg or R), asparagine (asn or N), aspartic acid (asp or D), cysteine (cys or C), glutamic acid (glu or E), glutamine (gin or Q), glycine (gly or G), histidine (his or H), isoleucine (ile or I), leucine (leu or L), lysine (lys or K), methionine (met or M), phenylalanine (phe or F), proline (pro or P), serine (ser or S), threonine (thr or T), tryptophan (trp or W), tyrosine (tyr or Y), and valine (val or V).

[0055] The term “antibody” as used herein refers to intact immunoglobulin molecules, as well as fragments of immunoglobulin molecules, such as Fab, Fab', (Fab')₂, Fv, and SCA fragments, that are capable of binding to an epitope of an antigen. These antibody fragments, which retain some ability to selectively bind to an antigen (e.g., a polypeptide antigen) of the antibody from which they are derived, can be made using well known methods in the art (see, e.g., Harlow and Lane, supra), and are described further, as follows. Antibodies can be used to isolate preparative quantities of the antigen by immunoaffinity chromatography. Various other uses of such antibodies are to diagnose and/or stage disease (e.g., neoplasia) and for therapeutic application to treat disease, such as for example: neoplasia, autoimmune disease, AIDS, cardiovascular disease, infections, and the like. Chimeric, human-like, humanized or fully human antibodies are particularly useful for administration to human patients.

[0056] An Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

[0057] An Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

[0058] An (Fab')₂ fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')₂ fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.

[0059] An Fv fragment is defined as a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

[0060] The term “antibody fragment” as used herein refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

[0061] The terms “anti-CD46 antibody,” “CD46 antibody” and “an antibody that binds to CD46” as used herein refer to an antibody that is capable of binding CD46 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD46. In one embodiment, the extent of binding of an anti-CD46 antibody to an unrelated, non-CD46 protein is less than about 10% of the binding of the antibody to CD46 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD46 has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-CD46 antibody binds to an epitope of CD46 that is conserved among CD46 from different species, for example, the extracellular domain of CD46.

[0062] The term “binding” as used herein refers to interaction of the variable region or an Fv of an antibody with an antigen with the interaction depending upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, an antibody variable region or Fv recognizes and binds to a specific protein structure rather than to proteins generally. As used herein, the term “specifically binding” or “binding specifically” means that an antibody variable region or Fv binds to or associates with more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen than with other proteins. For example, an antibody variable region or Fv specifically binds to its antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens. For another example, an antibody variable region or Fv binds to a cell surface protein (antigen) with materially greater affinity than it does to related proteins or other cell surface proteins or to antigens commonly recognized by polyreactive natural antibodies (i.e., by naturally occurring antibodies known to bind a variety of antigens naturally found in humans). However, “specifically binding” does not necessarily require exclusive binding or non-detectable binding of another antigen, this is meant by the term

"selective binding". In one example, "specific binding" of an antibody variable region or Fv (or other binding region) binds to an antigen, means that the an antibody variable region or Fv binds to the antigen with an equilibrium constant (KD) of 100 nM or less, such as 50nM or less, for example 20nM or less, such as, 15nM or less, or 10 nM or less ,or 5nM or less, 2 nM or less, or 1 nM or less.

[0063] The terms "cancer" and "cancerous" as used herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

[0064] The terms "cell proliferative disorder" and "proliferative disorder" as used herein refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

[0065] The term "chemotherapeutic agent" as used herein refers to a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylmelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a

sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Nicolaou et al., Angew. Chem. Intl. Ed. Engl., 33: 183-186 (1994))); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, athracycline, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), pegylated liposomal doxorubicin (CAELYX®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglutone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2'-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine;

mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (“Ara-C”); thiotepa; taxoid, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin (e.g., ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovorin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMF®); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase I inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (e.g. celecoxib or etoricoxib), proteasome inhibitor (e.g. PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0066] Chemotherapeutic agents as defined herein include “anti-hormonal agents” or “endocrine therapeutics,” which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including,

tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrozole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, and 4(5)-imidazoles; lutenizing hormone-releasing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and triptorelin; sex steroids, including progestins such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretinoic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

[0067] The term “chimeric” antibody as used herein refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0068] The term "conditionally active antibody" as used herein refers to an anti-CD46 antibody which is more active under a condition in the tumor microenvironment compared to under a condition in the non-tumor microenvironment. The conditions in the tumor microenvironment include lower pH, higher concentrations of lactate and pyruvate, hypoxia, lower concentration of glucose, and slightly higher temperature in comparison with non-tumor microenvironment. For example, a conditionally active antibody is virtually inactive at normal body temperature, but is active at a higher temperature in a tumor microenvironment. In yet another aspect, the conditionally active antibody is less active in normal oxygenated blood, but more active under a less oxygenated environment exists in tumor. In yet another aspect, the conditionally active antibody is less active in normal physiological pH 7.2-7.8, but more active under an acidic pH 5.8-7.0, or 6.0-6.8 that exists in a tumor microenvironment. There are other conditions in the tumor microenvironment known to a person skilled in the field may also be used as the condition in the present invention under which the anti-CD46 antibodies to have different binding affinity to CD46.

[0069] The term “cytostatic agent” as used herein refers to a compound or composition which arrests growth of a cell either in vitro or in vivo. Thus, a cytostatic agent may be one which significantly reduces the percentage of cells in S phase. Further examples of cytostatic agents include agents that block cell cycle progression by inducing G0/G1 arrest or M-phase arrest. The humanized anti-Her2 antibody trastuzumab (HERCEPTIN®) is an example of a cytostatic agent that induces G0/G1 arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Certain agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled “Cell cycle regulation, oncogenes, and antineoplastic drugs” by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0070] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to radioactive isotopes (e.g., At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0071] The term “diabodies” as used herein refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

[0072] The term “detectably label” as used herein refers to any substance whose detection or measurement, either directly or indirectly, by physical or chemical means, is indicative of the presence of an antigen in a sample. Representative examples of useful detectable labels, include, but are not limited to the following: molecules or ions directly or indirectly detectable based on light absorbance, fluorescence, reflectance, light scatter, phosphorescence, or luminescence properties; molecules or ions detectable by their radioactive properties; molecules or ions detectable by their nuclear magnetic resonance or paramagnetic properties. Included among the group of molecules indirectly detectable based on light absorbance or fluorescence, for example, are various enzymes which cause appropriate substrates to convert, e.g., from non-light absorbing to light absorbing molecules, or from non-fluorescent to fluorescent molecules.

[0073] The term “diagnostics” as used herein refers to determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, prognosis of a subject affected by a disease or disorder (e. g., identification of pre- metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy), and therapeutics (e. g., monitoring a subject's condition to provide information as to the effect or efficacy of therapy). In some embodiments, the diagnostic method of this invention is particularly useful in detecting early stage cancers.

[0074] The term “diagnostic agent” as used herein refers to a molecule which can be directly or indirectly detected and is used for diagnostic purposes. The diagnostic agent may be administered to a subject or a sample. The diagnostic agent can be provided per se or may be conjugated to a vehicle such as a conditionally active antibody.

[0075] The term “effector functions” as used herein refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0076] The term “effective amount” of an agent as used herein, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0077] The term “Fc region” as used herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG

heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0078] The term “framework” or “FR” as used herein refers to variable domain residues other than complementarity determining regions (CDRs or H1-3 in the heavy chain and L1-3 in the light chain) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in V_H (or V_L): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0079] The term “full length antibody,” “intact antibody,” or “whole antibody” refers to an antibody which comprises an antigen-binding variable region (V_H or V_L) as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof. Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different “classes”. There are five major classes of full length antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0080] The term “function-conservative variants” as used herein refers a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70% to 99% as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGALIGN algorithm. A “function-conservative variant” also includes a polypeptide which has at least 60% amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75%, more preferably at

least 85%, still preferably at least 90%, and even more preferably at least 95%, and which has the same or substantially similar properties or functions as the native or parent protein to which it is compared.

[0081] The terms “host cell,” “host cell line,” and “host cell culture” as used herein are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0082] The term “human antibody” as used herein is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0083] The term “humanized” antibody as used herein refers to a chimeric antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0084] The term “immunoconjugate” as used herein is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0085] The term “individual” or “subject” as used herein refers to a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0086] The term “inhibiting cell growth or proliferation” as used herein means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death.

[0087] The term “isolated” antibody as used herein is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase High Performance Liquid Chromatography (HPLC)). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B*, vol. 848, pp. 79-87, 2007.

[0088] The term “isolated nucleic acid encoding an anti-CD46 antibody” as used herein refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0089] The term “metastasis” as used herein refers to all CD46-involving processes that support cancer cells to disperse from a primary tumor, penetrate into lymphatic and/or blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasis) in normal tissues elsewhere in the body. In particular, it refers to cellular events of tumor cells such as proliferation, migration, anchorage independence, evasion of apoptosis, or secretion of angiogenic factors, that underlie metastasis and are stimulated or mediated by CD46.

[0090] The term “microenvironment” as used herein means any portion or region of a tissue or body that has constant or temporal, physical or chemical differences from other regions of the tissue or regions of the body. For tumors, the term “tumor microenvironment” as used herein refers to the environment in which a tumor exists, which is the non-cellular area within the tumor and the area directly outside the tumorous tissue but does not pertain to the intracellular compartment of the cancer cell itself. The tumor and the tumor microenvironment are closely related and interact constantly. A tumor can change its microenvironment, and the microenvironment can affect how a tumor grows and spreads. Typically, the tumor microenvironment has a low pH in the range of 5.0 to 7.0, or in the range of 5.0 to 6.8, or in the range of 5.8 to 6.8, or in the range of 6.2-6.8. On the other hand, a normal physiological pH is in the range of 7.2-7.8. The tumor microenvironment is also known to have lower concentration of glucose and other nutrients, but higher concentration of lactic acid, in comparison with blood plasma. Furthermore, the tumor microenvironment can have a temperature that is 0.3 to 1 °C higher than the normal physiological temperature. The tumor microenvironment has been discussed in Gillies et al., “MRI of the Tumor Microenvironment,” *Journal of Magnetic Resonance Imaging*, vol. 16, pp.430-450, 2002,

hereby incorporated by reference herein its entirety. The term “non-tumor microenvironment” refers to a microenvironment at a site other than a tumor.

[0091] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0092] The term “naked antibody” as used herein refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0093] The term “package insert” as used herein is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0094] The term “percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence as used herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine

appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0095] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0096] The term “pharmaceutical formulation” as used herein refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0097] The term “pharmaceutically acceptable carrier” as used herein refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0098] The terms “purified” and “isolated” used herein refer to an antibody according to the invention or to a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term “purified” as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An “isolated” nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

[0099] The term “recombinant antibody” as used herein refers to an antibody (e.g. a chimeric, humanized, or human antibody or antigen-binding fragment thereof) that is expressed by a recombinant host cell comprising nucleic acid encoding the antibody. Examples of “host cells” for producing recombinant antibodies include: (1) mammalian cells, for example, Chinese Hamster Ovary (CHO), COS, myeloma cells (including Y0 and NS0 cells), baby hamster kidney (BHK), HeLa and Vero cells; (2) insect cells, for example, sf9, sf21 and Tn5; (3) plant cells, for example plants belonging to the genus *Nicotiana* (e.g. *Nicotiana tabacum*); (4) yeast cells, for example, those belonging to the genus *Saccharomyces* (e.g. *Saccharomyces cerevisiae*) or the genus *Aspergillus* (e.g. *Aspergillus niger*); (5) bacterial cells, for example *Escherichia coli* cells or *Bacillus subtilis* cells, etc.

[0100] The term “single chain Fv” (“scFv”) as used herein is a covalently linked V_H::V_L heterodimer which is usually expressed from a gene fusion including V_H and V_L encoding genes linked by a peptide-encoding linker. “dsFv” is a V_H::V_L heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂.

[0101] The term “therapeutically effective amount” of the antibody of the invention is meant a sufficient amount of the antibody to treat said cancer, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the antibodies and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific antibody employed; the specific composition employed, the age, body weight, general health, sex and

diet of the patient; the time of administration, route of administration, and rate of excretion of the specific antibody employed; the duration of the treatment; drugs used in combination or coincidental with the specific antibody employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

[0102] The term “treatment,” “treat,” or “treating” as used herein refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0103] The term “tumor” as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer,” “cancerous,” “cell proliferative disorder,” “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein.

[0104] The term “variable region” or “variable domain” as used herein refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L , respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementarity determining regions (CDRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, e.g., Portolano et al., *J. Immunol.*, vol. 150, pp. 880-887, 1993; Clarkson et al., *Nature*, vol. 352, pp. 624-628, 1991.

[0105] The term “vector” as used herein refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression

of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

DETAILED DESCRIPTION

[0106] For illustrative purposes, the principles of the present invention are described by referencing various exemplary embodiments. Although certain embodiments of the invention are specifically described herein, one of ordinary skill in the art will readily recognize that the same principles are equally applicable to, and can be employed in, other systems and methods. Before explaining the disclosed embodiments of the present invention in detail, it is to be understood that the invention is not limited in its application to the details of any particular embodiment shown. Additionally, the terminology used herein is for the purpose of description and not for limitation. Furthermore, although certain methods are described with reference to steps that are presented herein in a certain order, in many instances, these steps can be performed in any order as may be appreciated by one skilled in the art; the novel method is therefore not limited to the particular arrangement of steps disclosed herein.

[0107] It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Furthermore, the terms “a” (or “an”), “one or more”, and “at least one” can be used interchangeably herein. The terms “comprising”, “including”, “having” and “constructed from” can also be used interchangeably.

[0108] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, percent, ratio, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about,” whether or not the term “about” is present. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0109] It is to be understood that each component, compound, substituent, or parameter disclosed herein is to be interpreted as being disclosed for use alone or in combination with one or more of each and every other component, compound, substituent, or parameter disclosed herein.

[0110] It is also to be understood that each amount/value or range of amounts/values for each component, compound, substituent, or parameter disclosed herein is to be interpreted as also being disclosed in combination with each amount/value or range of amounts/values disclosed for any other component(s), compounds(s), substituent(s), or parameter(s) disclosed herein and that any combination of amounts/values or ranges of amounts/values for two or more component(s), compounds(s), substituent(s), or parameters disclosed herein are thus also disclosed in combination with each other for the purposes of this description.

[0111] It is further understood that each lower limit of each range disclosed herein is to be interpreted as disclosed in combination with each upper limit of each range disclosed herein for the same component, compounds, substituent, or parameter. Thus, a disclosure of two ranges is to be interpreted as a disclosure of four ranges derived by combining each lower limit of each range with each upper limit of each range. A disclosure of three ranges is to be interpreted as a disclosure of nine ranges derived by combining each lower limit of each range with each upper limit of each range, etc. Furthermore, specific amounts/values of a component, compound, substituent, or parameter disclosed in the description or an example is to be interpreted as a disclosure of either a lower or an upper limit of a range and thus can be combined with any other lower or upper limit of a range or specific amount/value for the same component, compound, substituent, or parameter disclosed elsewhere in the application to form a range for that component, compound, substituent, or parameter.

Anti-CD46 Antibodies

[0112] The heavy chain variable regions and the light chain variable regions of the present invention were each obtained from a parent antibody using a method disclosed in U.S. Patent Nos. 8,709,755 and 8,859,467. This method of generating the heavy chain variable regions and the light chain variable regions, as well as the method of generating antibodies and antibody fragments disclosed in U.S. Patent Nos. 8,709,755 and 8,859,467, are hereby incorporated by reference herein.

[0113] In one aspect, the present invention provides isolated polypeptides that specifically bind to human CD46. The isolated polypeptides comprise a light chain variable region

having three complementary determining regions (CDRs) having sequences L1, L2, and L3, wherein:

the L1 sequence is RAX₁QX₂IX₃NYLN (SEQ ID NO: 1),

the L2 sequence is YTSSLX₄X₅ (SEQ ID NO: 2),

the L3 sequence is QQYIKLPWT (SEQ ID NO: 3); and

a heavy chain variable region having three complementary determining regions (CDRs) having sequences H1, H2, and H3, wherein

the H1 sequence is GGSVSSYDIS (SEQ ID NO: 8);

the H2 sequence is VIWTDGGTNYNSAFMS (SEQ ID NO: 9); and

the H3 sequence is VYDGYPWFA Y (SEQ ID NO: 10);

wherein X₁ is S or L; X₂ is G or W; X₃ is S or A; X₄ is H or F, X₅ is S or E; with the proviso that X₁, X₂, X₃, X₄ and X₅ cannot be S, G, S, H and S, respectively, at the same time.

[0114] The above polypeptides may have an L1 sequence selected from the amino acid sequences RASQGISNYLN (SEQ ID NO: 5), RASQWISNYLN (SEQ ID NO: 12), RASQGIANYLN (SEQ ID NO: 15) and RALQGISNYLN (SEQ ID NO: 22). The above polypeptides may have an L2 sequence selected from the amino acid sequences YTSSLHS (SEQ ID NO: 6), YTSSLFS (SEQ ID NO: 17) and YTSSLHE (SEQ ID NO: 19). In each of the foregoing embodiments, one of the L1 and L2 sequences must be other than the wild type L1 sequence of SEQ ID NO: 5 and the wild type L2 sequence of SEQ ID NO: 6.

[0115] In one embodiment, the above polypeptides comprise a set of 6 CDRs having the amino acid sequences of:

SEQ ID NO: 12, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

[0116] In another embodiment, the isolated polypeptides of the present invention comprise a light chain variable region and a heavy chain variable region, each light chain variable region and heavy chain variable region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13, respectively; and said isolated polypeptides specifically bind to human CD46 protein.

[0117] In another embodiment, the present invention relates to an isolated polypeptide having an amino acid sequence selected from SEQ ID NOS: 11, 13, 14, 16, 18, 20 and 21.

[0118] In another embodiment, the isolated polypeptides of the present invention comprise a light chain variable region and a heavy chain variable region having a pair of sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, and SEQ ID NOS: 21 and 13.

[0119] In another embodiment, the present invention relates to an isolated antibody, or a fragment thereof, comprising a light chain variable region having three complementary determining regions (CDRs) having sequences L1, L2, and L3, wherein

the L1 sequence is RAX₁QX₂IX₃NYLN (SEQ ID NO: 1),

the L2 sequence is YTSSLX₄X₅ (SEQ ID NO: 2),

the L3 sequence is QQYIKLPWT (SEQ ID NO: 3); and

a heavy chain variable region having three complementary determining regions (CDRs) having sequences H1, H2, and H3, wherein

the H1 sequence is GGSVSSYDIS (SEQ ID NO: 8);

the H2 sequence is VIWTDGGTNYNSAFMS (SEQ ID NO: 9); and

the H3 sequence is VYDGYPWFAFAY (SEQ ID NO: 10);

wherein X₁ is S or L; X₂ is G or W; X₃ is S or A; X₄ is H or F, X₅ is S or E; with the proviso that X₁, X₂, X₃, X₄ and X₅ cannot be S, G, S, H and S, respectively, at the same time.

[0120] In one embodiment, the above antibody or antibody fragment may have an L1 sequence selected from the amino acid sequences RASQGISNYLN (SEQ ID NO: 5), RASQWISNYLN (SEQ ID NO: 12), RASQGIANYLN (SEQ ID NO: 15) and RALQGISNYLN (SEQ ID NO: 22). The above antibody or antibody fragment may have an L2 sequence selected from the amino acid sequences YTSSLHS (SEQ ID NO: 6), YTSSLFS (SEQ ID NO: 17) and YTSSLHE (SEQ ID NO: 19). In each of the foregoing embodiments of the antibody or antibody fragment, one of the L1 and L2 sequences must be other than the wild type L1 sequence of SEQ ID NO: 5 and the wild type L2 sequence of SEQ ID NO: 6.

[0121] In another embodiment, the antibody or antibody fragment may comprise a set of 6 CDRs selected from the following sets of 6 CDR's:

SEQ ID NO: 12, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 5, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; or

SEQ ID NO: 22, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

[0122] In one embodiment, the antibody or antibody fragment of the present invention may comprise a light chain variable region and a heavy chain variable region, each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13, respectively; and said antibody or antibody fragment specifically binds to human CD46 protein.

[0123] In one embodiment, the antibody or antibody fragment of the present invention may comprise a light chain variable region and a heavy chain variable region having a pair of sequences selected from: SEQ ID NOS: 11 and 13, SEQ ID NOS: 14 and 13, SEQ ID NOS: 16 and 13, SEQ ID NOS: 18 and 13, SEQ ID NOS: 20 and 13, or SEQ ID NOS: 21 and 13.

[0124] In one embodiment, an antibody or antibody fragment of the present invention competes with any of the antibodies or antibody fragments described above for binding to human CD46.

[0125] In each of the previous embodiments, the antibody or antibody fragment may have a higher binding activity to CD46 protein at a value of a condition in a tumor microenvironment in comparison with a different value of a same condition that occurs in a non-tumor microenvironment. In one embodiment, the condition is pH. In one embodiment, binding activity is determined by binding affinity.

[0126] In each of the previous embodiments, the isolated polypeptide, antibody or antibody fragment may have at least 70% of the antigen binding activity at pH 6.0 as compared to the same antigen binding activity of the parent polypeptide, antibody or antibody fragment at pH 6.0 and the polypeptide, antibody or antibody fragment may have less than 50%, or less than 40%, or less than 30%, or less than 20% or less than 10% of the antigen binding activity at pH 7.4 as compared to the same antigen binding activity of the parent polypeptide, antibody or antibody fragment at pH 7.4. The antigen binding activity may be binding to CD46 protein.

[0127] In each of the previous embodiments, the antigen binding activity may be measured by an ELISA assay.

[0128] Antibodies and antibody fragments including these heavy chain variable regions and light chain variable regions can specifically bind to CD46, especially human CD46. Antibodies or antibody fragments comprising a combination of one of these heavy chain variable regions and one of these light chain variable regions have been found to have higher binding activity to CD46 at a pH in the tumor microenvironment (e.g. pH 6.0-6.8) than at a

pH in a non-tumor microenvironment (e.g. pH 7.0-7.6). As a result, the anti-CD46 antibodies or antibody fragments of the present invention have a higher binding activity to CD46 in a tumor microenvironment in comparison with their binding activity to CD46 in a typical normal tissue (non-tumor) microenvironment.

[0129] Anti-CD46 antibodies or antibody fragments of the present invention are thus expected to exhibit reduced side-effects, relative to non-conditionally active anti-CD46 antibodies, due to their reduced binding to CD46 in normal tissue such as non-tumor microenvironments. Anti-CD46 antibodies or antibody fragments of the present invention are also expected to have a comparable efficacy to monoclonal anti-CD46 antibodies known in the art. This combination of features permits use of a higher dosage of these anti-CD46 antibodies or antibody fragments due to the reduced side effects, which may provide a more effective therapy option.

[0130] In other embodiments, the amino acid sequence of the heavy and light chain variable regions outside of the complementarity determining regions may be mutated in accordance with the principles of substitution, insertion and deletion, as discussed in this application to provide these variants. In still further embodiments, the constant regions may be modified to provide these variants. In still further embodiments, both the amino acid sequence of the heavy and light chain variable regions outside of the complementarity determining regions and the constant regions may be modified to provide these variants.

[0131] In deriving these variants, one is guided by the process as described herein. The variants of the heavy chain and light chain variable regions may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the heavy and light chain variable regions, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the heavy and light chain variable regions. Any combination of deletion, insertion, and substitution can be made to arrive at the antibodies or antibody fragments of the present invention, provided that they possess the desired characteristics, e.g., antigen-binding to human CD46 and/or conditional activity.

Substitution, Insertion, and Deletion Variants

[0132] In certain embodiments, antibody or antibody fragment variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and framework regions (FRs). Conservative substitutions are shown in Table 1 under the heading of “conservative substitutions.” More substantial changes are provided in

Table 1 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody or antibody fragment of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, or decreased immunogenicity.

Table 1: Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0133] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0134] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0135] One type of substitutional variant involves substituting one or more complementarity determining region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0136] Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR “hotspots,” i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.*, vol. 207, pp. 179-196, 2008), and/or SDRs (a-CDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology*, vol. 178, pp. 1-37, 2001). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0137] In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody or antibody fragment to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding activity or binding affinity may be made in CDRs. Such alterations may be outside of CDR “hotspots” or SDRs. In certain embodiments of the variant V_H and V_L sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0138] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells, *Science*, vol. 244, pp. 1081-1085, 1989. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody or antibody fragment with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody or antibody fragment and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0139] Amino acid sequence insertions 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. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0140] Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding, activity, affinity and/or other biological properties of the antibody. It is known that when a humanized antibody is produced by simply grafting only CDRs in V_H and V_L of an antibody derived from a non-human animal in FRs of the V_H and V_L of a human antibody, the antigen binding activity is reduced in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the V_H and V_L of the non-human antibody, not only in CDRs but also in FRs, are directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the V_H and V_L of the human antibody would reduce of the binding activity. In order to resolve the problem, in antibodies grafted with human CDR, attempts have to be made to identify, among amino acid sequences of the FR of the V_H and V_L of human antibodies, an amino acid residue which is directly associated with binding to the antigen, or which interacts with an amino acid residue of CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding

to the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal.

[0141] Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still obtain a functional molecule that encodes an antibody with desirable characteristics.

[0142] In making the changes in the amino sequences, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophane (−0.9); tyrosine (−1.3); proline (−1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

[0143] A further object of the present invention also encompasses function-conservative variants of the antibodies of the present invention.

[0144] Two amino acid sequences are “substantially homologous” or “substantially similar” when greater than 80%, preferably greater than 85%, preferably greater than 90% of the amino acids are identical, or greater than about 90%, preferably greater than 95%, are similar (functionally identical) over the whole length of the shorter sequence. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wis.) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

[0145] For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a protein define the protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and, of course, in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the sequences of the antibodies or antibody fragments of the

invention, or corresponding DNA sequences which encode said antibodies or antibody fragments, without appreciable loss of their biological activity.

[0146] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

[0147] As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Glycosylation Variants

[0148] In certain embodiments, the anti-CD46 antibodies or antibody fragments provided herein are altered to increase or decrease the extent to which the antibodies or antibody fragments are glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0149] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH*, vol. 15, pp. 26-32, 1997. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0150] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the

asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., U.S. Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.*, vol. 336, pp. 1239-1249, 2004; Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.*, vol. 249, pp. 533-545, 1986; US Pat Appl No US 2003/0157108 A; and WO 2004/056312 A1, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004; Kanda, Y. et al., *Biotechnol. Bioeng.*, vol. 94, pp. 680-688, 2006; and WO2003/085107).

[0151] Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878; U.S. Pat. No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

Fc Region Variants

[0152] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of the anti-CD46 antibodies or antibody fragments provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0153] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.*, vol. 9, pp. 457-492, 1991. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see also, e.g. Hellstrom et al. *Proc. Nat'l Acad. Sci. USA*, vol. 83, pp. 7059-7063, 1986) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA*, vol. 82, pp. 1499-1502, 1985; U.S. Pat. No. 5,821,337 (see also Bruggemann et al., *J. Exp. Med.*, vol. 166, pp. 1351-1361, 1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA*, vol. 95, pp. 652-656, 1998. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods*, vol. 202, pp.163-171, 1996; Cragg, M. S. et al., *Blood*, vol. 101, pp. 1045-1052, 2003; and Cragg, M. S. and M. J. Glennie, *Blood*, vol. 103, pp. 2738-2743, 2004). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al., *Int'l. Immunol.*, vol. 18, pp. 1759-1769, 2006).

[0154] The variants of the antibodies or antibody fragments with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including

the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0155] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.*, vol. 9, pp. 6591-6604, 2001).

[0156] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0157] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.*, vol. 164, pp. 4178-4184, 2000.

[0158] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, vol. 117, pp. 587-593, 1976 and Kim et al., *J. Immunol.*, vol. 24, p. 249, 1994), are described in US2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include/e those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826). See also Duncan & Winter, *Nature*, vol. 322, pp. 738-740, 1988; U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Cysteine Engineered Antibody Variants

[0159] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., “thioMAbs,” in which one or more residues of the anti-CD46 antibodies or antibody fragments are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and 5400 (EU

numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Pat. No. 7,521,541.

Antibody Derivatives

[0160] In certain embodiments, the anti-CD46 antibodies or antibody fragments provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody or antibody fragment include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody or antibody fragment may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody or antibody fragment to be improved, whether the derivative will be used in a therapy under defined conditions, etc.

[0161] In another embodiment, conjugates of the antibodies or antibody fragments and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA*, vol. 102, pp. 11600-11605, 2005). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0162] The anti-CD46 antibodies or antibody fragments of the invention, or their variants, have a higher binding activity or binding affinity to CD46 under a condition in a tumor microenvironment than under a condition in a non-tumor microenvironment. In one embodiment, the condition in tumor microenvironment and the condition in a non-tumor

microenvironment are both pH. The anti-CD46 antibodies or antibody fragments of the invention thus can selectively bind to CD46 at a pH about 5.0-6.8 but will have a lower binding activity or binding affinity to CD46 at a pH about 7.2-7.8 encountered in a normal non-tumor microenvironment. As shown Examples 3 and 6, the anti-CD46 antibodies or antibody fragments have higher binding activity or binding affinity to CD46 at pH 6.0 than at pH 7.4.

[0163] In certain embodiments, the anti-CD46 antibodies or antibody fragments of the present invention have a dissociation constant (K_d) with CD46 under a condition in tumor microenvironment of about $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8}M or less, or from 10^{-8}M to 10^{-13}M , or from 10^{-9}M to 10^{-13}M). In one embodiment, the ratio of the K_d of the antibody or antibody fragment with CD46 at the condition in the non-tumor microenvironment to the K_d at the same condition in the tumor microenvironment is at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

[0164] In another embodiment, the ratio of the binding activity of the antibody or antibody fragment with CD46 at the condition in the tumor microenvironment to the binding activity at the same condition in the non-tumor microenvironment is at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

[0165] In one embodiment, K_d is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen using the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 $\mu\text{g/ml}$ of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C.). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-

4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0166] According to another embodiment, K_d is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C. with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C. at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0167] The anti-CD46 antibodies of the invention may be a chimeric, humanized or human antibody. In one embodiment, an anti-CD46 antibody fragment is employed, e.g., a Fv, Fab,

Fab', Fab'-SH, scFv, a diabody, a triabody, a tetrabody or an F(ab')₂ fragment and multispecific antibodies formed from antibody fragments. In another embodiment, the antibody is a full-length antibody, e.g., an intact IgG antibody or other antibody class or isotype as defined herein. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.*, vol. 9, pp. 129-134, 2003. For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Pat. No. 5,869,046.

[0168] The diabodies of the invention may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, vol. 90, pp. 6444-6448, 1993 for examples of diabodies. Examples of triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.*, vol. 9, pp. 129-134, 2003.

[0169] In some embodiments, the invention comprises single-domain antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1).

[0170] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

[0171] In some embodiments, the anti-CD46 antibodies of the invention may be chimeric antibodies. Certain chimeric antibodies are described, e.g., in U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, vol. 81, pp. 6851-6855, 1984). In one example, the chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, the chimeric antibody is a "class switched" antibody in which the class or subclass of the antibody has been changed relative to the class or subclass of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0172] In certain embodiments, the chimeric antibody of the invention is a humanized antibody. Typically, such a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody.

Generally, a humanized antibody comprises one or more variable domains in which CDRs (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody may optionally also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0173] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.*, vol. 13, pp. 1619-1633, 2008, and are further described, e.g., in Riechmann et al., *Nature*, vol. 332, pp. 323-329, 1988; Queen et al., *Proc. Nat'l Acad. Sci. USA*, vol. 86, pp. 10029-10033, 1989; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods*, vol. 36, pp. 25-34, 2005 (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.*, vol. 28, pp. 489-498, 1991 (describing "resurfacing"); Dall'Acqua et al., *Methods*, vol. 36, pp. 43-60, 2005 (describing "FR shuffling"); and Osbourn et al., *Methods*, vol. 36, pp. 61-68, 2005 and Klimka et al., *Br. J. Cancer*, vol. 83, pp. 252-260, 2000 (describing the "guided selection" approach to FR shuffling).

[0174] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.*, vol. 151, p. 2296, 1993); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, vol. 89, p. 4285, 1992; and Presta et al. *J. Immunol.*, vol. 151, p. 2623, 1993); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.*, vol. 13, pp. 1619-1633, 2008); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.*, vol. 272, pp. 10678-10684, 1997 and Rosok et al., *J. Biol. Chem.*, vol. 271, pp. 22611-22618, 1996).

[0175] In some embodiments, the anti-CD46 antibodies of the invention are multispecific, e.g. bispecific antibodies. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for CD46 and the other is for another antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD46. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD46. Bispecific antibodies can be prepared as full-length antibodies or antibody fragments.

[0176] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature*, vol. 305, pp. 537-540, 1983), WO 93/08829, and Traunecker et al., *EMBO J.* vol. 10, pp. 3655-3659, 1991), and “knob-in-hole” engineering (see, e.g., U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980, and Brennan et al., *Science*, vol. 229, pp. 81-83, 1985); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, vol. 148, pp. 1547-1553, 1992); using “diabody” technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, vol. 90, pp. 6444-6448, 1993); and using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, vol. 152, pp. 5368-5374, 1994); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.*, vol. 147, pp. 60-69, 1991.

[0177] Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (see, e.g. US 2006/0025576A1).

[0178] The anti-CD46 antibodies or antibody fragments of the invention may be produced using recombinant methods and compositions, which are described in detail in US 2016/0017040.

[0179] The physical/chemical properties and/or biological activities of the anti-CD46 antibodies or antibody fragments of the invention may be tested and measured by various assays known in the art. Some of these assays are described in U.S. Patent No. 8,853,369.

B. Immunoconjugates

[0180] In another aspect, the invention also provides immunoconjugates comprising an anti-CD46 antibody or antibody fragment conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), and radioactive isotopes.

[0181] In one embodiment, the immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody or antibody fragment is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a

calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.*, vol. 53, pp. 3336-3342, 1993; and Lode et al., *Cancer Res.*, vol. 58, pp. 2925-2928, 1998); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.*, vol. 13, pp. 477-523, 2006; Jeffrey et al., *Bioorganic & Med. Chem. Letters*, vol. 16, pp. 358-362, 2006; Torgov et al., *Bioconj. Chem.*, vol. 16, pp. 717-721, 2005; Nagy et al., *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 829-834, 2000; Dubowchik et al., *Bioorg. & Med. Chem. Letters*, vol. 12, pp. 1529-1532, 2002; King et al., *J. Med. Chem.*, vol. 45, pp. 4336-4343, 2002; and U.S. Pat. No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0182] In another embodiment, an immunoconjugate comprises an antibody or antibody fragment as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the trichothecenes.

[0183] In another embodiment, an immunoconjugate comprises an antibody or antibody fragment as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese and iron.

[0184] In some embodiments, the immunoconjugate comprises a radioactive agent, which may be selected from an alpha emitter, a beta emitter and a gamma emitter. Examples of alpha emitters are ²¹¹At, ²¹⁰Bi, ²¹²Bi, ²¹¹Bi, ²²³Ra, ²²⁴Ra, ²²⁵Ac and ²²⁷Th. Examples of beta-emitters are ⁶⁷Cu, ⁹⁰Y, ¹³¹I, ¹⁵³Sm, ¹⁶⁶Ho and ¹⁸⁶Re. Examples of gamma emitters are ⁶⁰Co, ¹³⁷Ce, ⁵⁵Fe, ⁵⁴Mg, ²⁰³Hg, and ¹³³Ba.

[0185] Conjugates of an antibody/antibody fragment and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio)

propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science*, vol. 238, pp. 1098-, 1987. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.*, vol. 52, pp. 127-131, 1992; U.S. Pat. No. 5,208,020) may be used.

[0186] The immunoconjugates herein expressly contemplate, but are not limited to, conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SLAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, Ill., U.S.A).

[0187] An exemplary embodiment of an ADC includes an antibody or antibody fragment (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine.

[0188] An exemplary ADC has Formula I as $Ab-(L-D)_p$, where p is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein.

Exemplary ADC's of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon et al., *Methods in Enzym.*, vol. 502, pp. 123-138, 2012). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is

exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

[0189] Linkers are used to conjugate a moiety to the antibody to form an immunoconjugate such as an ADC. Suitable linkers are described in WO 2017/180842.

[0190] Some drug moieties that may be conjugated to the antibodies are described in WO 2017/180842.

[0191] Drug moieties also include compounds with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease).

[0192] In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc⁹⁹ or I¹²³, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983).

[0193] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc⁹⁹, I¹²³, Re¹⁸⁶, Re¹⁸⁸ and In¹¹¹ can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al., *Biochem. Biophys. Res. Commun.*, vol. 80, pp. 49-57, 1978) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

[0194] In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are

useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as *serratia* protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β -lactamase, which is useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. See, e.g., Neuberger et al., *Nature*, vol. 312, pp. 604-608, 1984.

[0195] Drug loading in the conjugates is represented by p , the average number of drug moieties per antibody. Drug loading may range from 1 to 20 drug moieties per antibody. The conjugates of the present invention may have a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody use in the preparation of the conjugates from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC.

[0196] For some antibody-drug conjugates (ADC), the drug loading may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (U.S. Pat. No. 7,498,298).

[0197] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety. Indeed, most cysteine thiol residues in

antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarboylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0198] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

C. Methods and Compositions for Diagnostics and Detection

[0199] In certain embodiments, any of the anti-CD46 antibodies or antibody fragments provided herein may be used for detecting the presence of CD46 in a biological sample, either quantitatively or qualitatively. In certain embodiments, a biological sample comprises a cell or tissue, such as breast, pancreas, esophagus, lung and/or brain cells or tissue.

[0200] A further aspect of the invention relates to an anti-CD46 antibody or antibody fragment of the invention for diagnosing and/or monitoring a cancer or another disease in which CD46 expression levels are increased or decreased from a normal physiological level at least one location in the body.

[0201] In a preferred embodiment, antibodies or antibody fragments of the invention may be labelled with a detectable molecule or substance, such as a fluorescent molecule, a radioactive molecule or any other label known in the art as above described. For example, an antibody or antibody fragment of the invention may be labelled with a radioactive molecule. For example, suitable radioactive molecules include but are not limited to radioactive atoms used for scintigraphic studies such as ^{123}I , ^{124}I , ^{111}In , ^{186}Re , and ^{188}Re . Antibodies or antibody fragments of the invention may also be labelled with a spin label for nuclear magnetic resonance (NMR) imaging, such as iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Following administration of the antibody, the distribution of the radiolabeled antibody within the patient is detected. Any suitable known method can be used. Some non-limiting examples include, computed tomography (CT), position emission tomography (PET), magnetic resonance imaging (MRI), fluorescence, chemiluminescence and sonography.

[0202] Antibodies or antibody fragments of the invention may be useful for diagnosing and staging of cancer and diseases associated with CD46 overexpression. Cancers associated with

CD46 overexpression may include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer or other CD46 expressing or overexpressing hyperproliferative diseases.

[0203] Antibodies or antibody fragments of the invention may be useful for diagnosing diseases other than cancers for which CD46 expression is increased or decreased. Both the (soluble or cellular CD46 forms can be used for such diagnoses. Typically, such diagnostic methods involve use of a biological sample obtained from the patient. The biological sample encompasses a variety of sample types obtained from a subject that can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or a tissue culture or cells derived therefrom, and the progeny thereof. For example, biological samples include cells obtained from a tissue sample collected from an individual suspected of having a cancer associated with CD46 overexpression, and in preferred embodiments from glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial. Biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0204] In a particular embodiment, the invention is a method of diagnosing a cancer associated with CD46 overexpression in a subject by detecting CD46 on cells from the subject using the antibody of the invention. In particular, said method may include steps of:

- 1) contacting a biological sample of a subject with an antibody or antibody fragment according to the invention under conditions suitable for the antibody or antibody fragment to form complexes with cells in the biological sample that express CD46; and
- (b) detecting and/or quantifying said complexes, whereby detection of said complexes is indicative of a cancer associated with CD46 overexpression.

[0205] In order to monitor the progress of a cancer, the method according to the invention may be repeated at different times, in order to determine if antibody binding to the samples increases or decreases, wherefrom it can be determined if the cancer has progressed, regressed or stabilized.

[0206] In a particular embodiment, the invention is a method of diagnosing a disease associated with the expression or overexpression of CD46. Examples of such diseases may include cancers, human immune disorders, thrombotic diseases (thrombosis and atherothrombosis), and cardiovascular diseases

[0207] In one embodiment, an anti-CD46 antibody or antibody fragment for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of CD46 in a biological sample is provided. In a further aspect, a method of quantifying the amount of CD46 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-CD46 antibody or antibody fragment as described herein under conditions permissive for binding of the anti-CD46 antibody or antibody fragment to CD46, and detecting whether a complex is formed between the anti-CD46 antibody or antibody fragment and CD46. Such a method may be carried out *in vitro* or *in vivo*. In one embodiment, an anti-CD46 antibody or antibody fragment is used to select subjects eligible for therapy. In some embodiments, the therapy will include administration of an anti-CD46 antibody or antibody fragment to the subject.

[0208] In certain embodiments, labeled anti-CD46 antibodies or antibody fragments are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

D. Pharmaceutical Formulations

[0209] The anti-CD46 antibodies or antibody fragments have cell killing activity. This cell killing activity extends to multiple different types of cell lines. Further, these antibodies or antibody fragments, once conjugated to a cytotoxic agent, can reduce tumor size and may

exhibit reduced toxicity. Thus, the anti-CD46 antibodies, fragments or immunoconjugates thereof may be useful for treating proliferative diseases associated with CD46 expression. The antibodies, fragments or immunoconjugates may be used alone or in combination with any suitable agent or other conventional treatments.

[0210] The anti-CD46 antibody or antibody fragment may be used to treat diseases associated with CD46 expression, overexpression or activation. There are no particular limitations on the types of cancer or tissue that can be treated other than the requirement for CD46 expression. Examples include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer. More preferable cancers are glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial cancer.

[0211] Anti-CD46 antibodies or antibody fragments are potential activators of the innate immune response and thus may be used in the treatment of human immune disorders, such as sepsis. The anti-CD46 antibody or antibody fragment of the invention may also be used as adjuvants for immunization such as for vaccines and as anti-infection agents against, for example, bacteria, viruses and parasites.

[0212] Anti-CD46 antibody or antibody fragment may be used to protect against, prevent or treat thrombotic diseases such as venous and arterial thrombosis and atherothrombosis. Anti-CD46 antibody or antibody fragment may also be used to protect against, prevent or treat cardiovascular diseases as well as to prevent or inhibit the entry of viruses such as Lassa and Ebola viruses and to treat viral infections.

[0213] In each of the embodiments of the treatment methods described herein, the anti-CD46 antibody, antibody fragment or anti-CD46 antibody or antibody fragment immunoconjugate may be delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the antibody, antibody fragment or immunoconjugate is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder. Thus, an aspect of the invention relates to a method for treating a disease associated with the expression of CD46

comprising administering to a subject in need thereof with a therapeutically effective amount of an antibody, antibody fragment or immunoconjugate of the invention.

[0214] For administration, the anti-CD46 antibody, antibody fragment or immunoconjugate may be formulated as a pharmaceutical composition. The pharmaceutical composition including anti-CD46 antibody, antibody fragment or immunoconjugate can be formulated according to known methods for preparing pharmaceutical compositions. In such methods, the therapeutic molecule is typically combined with a mixture, solution or composition containing a pharmaceutically acceptable carrier.

[0215] A pharmaceutically acceptable carrier is a material that can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable pharmaceutically acceptable carriers are well-known to those in the art. (See, e.g., Gennaro (ed.), Remington's Pharmaceutical Sciences (Mack Publishing Company, 19th ed. 1995)) Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc.

[0216] The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc. These considerations can be taken into account by a skilled person to formulate suitable pharmaceutical compositions. The pharmaceutical compositions of the invention can be formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

[0217] Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition of, for example, sterilized water or physiological saline, permit the constitution of injectable solutions.

[0218] In some embodiments, tonicity agents, sometimes known as "stabilizers" are present to adjust or maintain the tonicity of a liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter- and intra-molecular interactions. Tonicity agents can be present in any amount of from 0.1% to 25% by weight, preferably 1 to 5% of the pharmaceutical composition. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0219] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients may include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, myoinositol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose); disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0220] Non-ionic surfactants or detergents (also known as “wetting agents”) may be employed to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants may be present in a concentration range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0221] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0222] The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment. To prepare pharmaceutical compositions, an effective amount of the antibody or antibody fragment may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0223] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol;

and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0224] Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in a water suitably mixed with a surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0225] The anti-CD46 antibody or antibody fragment can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0226] The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0227] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with one or more of the other ingredients enumerated above, as may be required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients

from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0228] The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of dimethyl sulfoxide (DMSO) as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

[0229] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

[0230] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0231] The antibodies or antibody fragments may be formulated within a therapeutic mixture to deliver about 0.0001 to 10.0 milligrams, or about 0.001 to 5 milligrams, or about 0.001 to 1 milligram, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose. Multiple doses can also be administered at selected time intervals.

[0232] In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently used.

[0233] In certain embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of antibodies or antibody fragments into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

[0234] Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) are generally designed using polymers able to degrade *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made.

[0235] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm . Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations

[0236] Pharmaceutical formulations containing an anti-CD46 antibody or antibody fragment as described herein are prepared by mixing such antibody or antibody fragment having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0237] Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos.

2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0238] Exemplary lyophilized antibody formulations are described in U.S. Patent No. 6,267,958. Aqueous antibody formulations include those described in U.S. Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0239] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated. Preferably, ingredients with complementary activities that do not adversely affect each other may be combined into a single formulation. For example, it may be desirable to provide an EGFR antagonist (such as erlotinib), an anti-angiogenic agent (such as a VEGF antagonist which may be an anti-VEGF antibody) or a chemotherapeutic agent (such as a taxoid or a platinum agent) in addition to the anti-CTLA4 antibody, antibody fragment or immunoconjugate of the present invention. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0240] In one embodiment, the anti-CD46 antibody, antibody fragment or immunoconjugate of the present invention is combined in a formulation with another antibody or antibody fragment against an antigen selected from CTLA4, PD1, PD-L1, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein including WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16. The combination may be in the form of two separate molecules: the anti-CD46 antibody, antibody fragment or immunoconjugate of the present invention, and the other antibody or antibody fragment. Alternatively, the combination may also be the form of a single molecule with binding activity or binding affinity to both CD46 and the other antigen, thus forming a multispecific (e.g. bispecific) antibody.

[0241] Active ingredients may be encapsulated in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization. For example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions may be employed. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0242] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers

containing the antibody or antibody fragment, which matrices may be in the form of shaped articles, e.g. films, or microcapsules.

[0243] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

E. Therapeutic Methods and Compositions

[0244] Any of the anti-CD46 antibodies or antibody fragments provided herein may be used in therapeutic methods. In one aspect, an anti-CD46 antibody or antibody fragment for use as a medicament is provided. In further aspects, an anti-CD46 antibody or antibody fragment for use in treating cancer (e.g., breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, osteosarcoma, and/or melanoma) is provided. In certain embodiments, an anti-CD46 antibody or antibody fragment for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-CD46 antibody or antibody fragment for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-CD46 antibody or antibody fragment. In certain embodiments, the invention provides an anti-CD46 antibody or antibody fragment for use in a method of treating an individual having an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-CD46 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-CD46 antibody or antibody fragment for use in inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function.

[0245] In certain embodiments, the invention provides an anti-CD46 antibody or antibody fragment for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual comprising administering to the individual an effective of the anti-CD46 antibody or antibody

fragment to inhibit angiogenesis, inhibit cell proliferation, inhibit immune function, inhibit inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An “individual” according to any of the above embodiments is preferably a human.

[0246] In a further aspect, the invention provides for the use of an anti-CD46 antibody or antibody fragment in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer (in some embodiments, breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of the pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, osteosarcoma, and/or melanoma). In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-CD46 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function. In a further embodiment, the medicament is for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual comprising administering to the individual an amount effective of the medicament to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An “individual” according to any of the above embodiments may be a human.

[0247] In a further aspect, the invention provides a method for treating a cancer. In one embodiment, the method comprises administering to an individual having such cancer an

effective amount of an anti-CD46 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

[0248] In a further aspect, the invention provides a method for treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

[0249] In a further aspect, the invention provides a method for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-CD46 antibody or antibody fragment to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine section (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. In one embodiment, an “individual” is a human.

[0250] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-CD46 antibodies or antibody fragments provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-CD46 antibodies or antibody fragments provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-CD46 antibodies or antibody fragments provided herein and at least one additional therapeutic agent, e.g., as described below.

[0251] In each and every treatment described above, the antibodies or antibody fragments of the invention can be used alone, as immunoconjugates or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is an anti-angiogenic agent. In certain embodiments, an additional therapeutic agent is a VEGF antagonist (in some embodiments, an anti-VEGF antibody, for example bevacizumab). In certain embodiments, an additional therapeutic agent is an EGFR antagonist (in some

embodiment, erlotinib). In certain embodiments, an additional therapeutic agent is a chemotherapeutic agent and/or a cytostatic agent. In certain embodiments, an additional therapeutic agent is a taxoid (e.g., paclitaxel) and/or a platinum agent (e.g., carboplatinum). In certain embodiments the additional therapeutic agent is an agent that enhances the patient's immunity or immune system.

[0252] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or antibody fragment can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or antibody fragments can also be used in combination with radiation therapy.

[0253] The anti-CD46 antibodies or antibody fragments may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder 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 agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or antibody fragment need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or antibody fragment present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0254] For the prevention or treatment of disease, the appropriate dosage of an antibody or antibody fragment (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody or antibody fragment, the severity and course of the disease, whether the antibody or antibody fragment is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or antibody fragment, and the discretion of the attending physician. The antibody or antibody fragment is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg of antibody or antibody fragment/kg bodyweight of the patient to 40 mg of antibody or antibody fragment/kg bodyweight of the patient can be an initial candidate

dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg of antibody or antibody fragment/kg bodyweight of the patient to 100 mg of antibody or antibody fragment/kg bodyweight of the patient or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or antibody fragment). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0255] Specific dosages of the anti-CD46 antibody or antibody fragment of the present invention that may be administered for the prevention or treatment of a disease in a subject may be about 0.3, 0.6, 1.2, 18, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4, 6.0, 6.6, 7.2, 7.8, 8.4, 9.0, 9.6 or 10.2 mg of antibody or antibody fragment/kg bodyweight of the patient. In certain embodiments, the dosage may be in a range of 0.3-2.4, 2.4-4.2, 4.2-6.0, 6.0-7.8, 7.8-10.2, 10.2-12, 12-14, 14-16, 16-18 or 18-20 mg of antibody or antibody fragment/kg bodyweight of the patient. The dosage of the antibody or antibody fragment will remain the same if administered in the form of a bispecific antibody, in combination with another immune checkpoint inhibitor or another antibody or antibody fragment or as an immunoconjugate. Further, a polypeptide having anti-CD46 activity will be administered in the same amounts as the antibody or antibody fragment.

[0256] A single dose of pharmaceutical formulation of the present invention may contain an amount of the anti-CD46 antibody or antibody fragment of the present invention of from about 45 µg of antibody or antibody fragment from about 13,600 mg, or from about 45 µg of antibody or antibody fragment from about 5440 mg. In some embodiments, a single dose of pharmaceutical formulation of the present invention may contain an amount of the anti-CD46 antibody or antibody fragment of the present invention of from to 135 mg to 1,387 mg, or an amount such as 135, 235, 335, 435, 535, 635, 735, 835, 935, 1035, 1135, 1235, 1387 mg. In certain embodiments, the amount of the anti-CD46 antibody or antibody fragment of the present invention in a single dose of the pharmaceutical formulation is in the range of 135-235, 235-335, 335-435, 435-535, 535-635, 635-735, 735-835, 835-935, 935-1035, 1035-1135, 1135-1235, 1235-1387 mg. The amount of the antibody or antibody fragment in the single dose of the pharmaceutical formulation will remain the same if administered in the

form of a bispecific antibody, in combination with another immune checkpoint inhibitor or as an immunoconjugate, or in combination with another antibody or antibody fragment against another antigen as disclosed herein. Further, a polypeptide having anti-CD46 activity will be included in the single dose of the pharmaceutical formulation in the same amounts as the antibody or antibody fragment.

[0257] In one example, the anti-CD46 antibody or antibody fragment may be conjugated to an immune checkpoint inhibitor molecule or may form part of a bispecific antibody with an immune checkpoint inhibitor.

[0258] The combination can be the anti-CD46 antibody or antibody fragment disclosed in this application and the immune checkpoint inhibitor molecule administered as separate molecules or as a bispecific antibody. Such a bispecific antibody has a binding activity to CD46 and a second binding activity to the immune checkpoint.

[0259] The immune checkpoint may be selected from CTLA4, LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, PD-1, PD-L1, and GITR (Zahavi and Weiner, *International Journal of Molecular Sciences*, vol. 20, 158, 2019). Additional immune checkpoints include B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS (Manni et al., Immune checkpoint blockade and its combination therapy with small-molecule inhibitors for cancer treatment, *Bbacan*, <https://doi.org/10.1016/j.bbcan.2018.12.002>, 2018).

[0260] The immune checkpoint is preferably CTLA4, PD-1 or PD-L1.

[0261] It is understood that any of the above formulations or therapeutic methods may be carried out using an antibody fragment or an immunoconjugate of the invention in place of or in addition to an anti-CD46 antibody.

[0262] Enhancing the host's immune function to combat tumors is the subject of increasing interest. Conventional methods include (i) APC enhancement, such as (a) injection into the tumor of DNA encoding foreign MHC alloantigens, or (b) transfecting biopsied tumor cells with genes that increase the probability of immune antigen recognition (e.g., immune stimulatory cytokines, GM-CSF, co-stimulatory molecules B7.1, B7.2) of the tumor, (iii) adoptive cellular immunotherapy, or treatment with activated tumor-specific T-cells. Adoptive cellular immunotherapy includes isolating tumor-infiltrating host T-lymphocytes, expanding the population in vitro, such as through stimulation by IL-2 or tumor or both. Additionally, isolated T-cells that are dysfunctional may be also be activated by in vitro application of the anti-PD-L1 antibodies of the invention. T-cells that are so-activated may then be readministered to the host. One or more of these methods may be used in

combination with administration of the antibody, antibody fragment or immunoconjugate of the present invention.

[0263] Traditional therapies for cancer include the following: (i) radiation therapy (e.g., radiotherapy, X-ray therapy, irradiation) or the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered either externally via external beam radiotherapy (EBRT) or internally via brachytherapy; (ii) chemotherapy, or the application of cytotoxic drug which generally affect rapidly dividing cells; (iii) targeted therapies, or agents which specifically affect the deregulated proteins of cancer cells (e.g., tyrosine kinase inhibitors imatinib, gefitinib; monoclonal antibodies, photodynamic therapy); (iv) immunotherapy, or enhancement of the host's immune response (e.g., vaccine); (v) hormonal therapy, or blockade of hormone (e.g., when tumor is hormone sensitive), (vi) angiogenesis inhibitor, or blockade of blood vessel formation and growth, and (vii) palliative care, or treatment directed to improving the quality of care to reduce pain, nausea, vomiting, diarrhea and hemorrhage. Pain medication such as morphine and oxycodone, anti-emetics such as ondansetron and aprepitant, can permit more aggressive treatment regimens.

[0264] In the treatment of cancer, any of the previously described conventional treatments for the treatment of cancer immunity may be conducted, prior, subsequent or simultaneous with the administration of the anti-CD46 antibodies or antibody fragments. Additionally, the anti-CD46 antibodies or antibody fragments may be administered prior, subsequent or simultaneous with conventional cancer treatments, such as the administration of tumor-binding antibodies (e.g., monoclonal antibodies, toxin-conjugated monoclonal antibodies) and/or the administration of chemotherapeutic agents.

F. Articles of Manufacture and Kits

[0265] In another aspect of the invention, an article of manufacture containing an anti-CD46 antibody or antibody fragment and other materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or

antibody fragment of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or antibody fragment; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0266] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-CD46 antibody or antibody fragment.

[0267] Finally, the invention also provides kits comprising at least one antibody or antibody fragment of the invention. Kits containing polypeptide, antibodies or antibody fragments, or antibody drug conjugate of the invention find use in detecting CD46 expression (increase or decrease), or in therapeutic or diagnostic assays. Kits of the invention can contain an antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantification of CD46 *in vitro*, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

[0268] The kits further contain instructions on the use thereof. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Administration for *in vitro* diagnostic kits. In some embodiments, the kits further comprise instructions for diagnosing the presence or absence of cerebrospinal fluid in a sample based on the presence or absence of CD46 in said sample. In some embodiments, the kits comprise one or more antibodies or antibody fragments. In other embodiments, the kits further comprise one or more enzymes, enzyme inhibitors or enzyme activators. In still other embodiments, the kits further comprise one or more chromatographic compounds. In yet other embodiments, the kits further comprise one or more compounds used to prepare the sample for spectroscopic assay. In further embodiments, the kits further comprise comparative reference material to

interpret the presence or absence of CD46 according to intensity, color spectrum, or other physical attributes of an indicator.

[0269] The following examples are illustrative, but not limiting, of the anti-CD46 antibodies of the present disclosure. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in the field, and which are obvious to those skilled in the art, are within the scope of the disclosure.

EXAMPLES

[0270] Antibodies or antibody fragments of the present invention having the following sets of 6 CDR's have been tested in the working examples:

SEQ ID NO: 12, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10;

SEQ ID NO: 15, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10;

SEQ ID NO: 5, SEQ ID NO: 17, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10;

SEQ ID NO: 5, SEQ ID NO: 19, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; and

SEQ ID NO: 22, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

Antibody	Light Chain Vk	Heavy Chain Vh
Benchmark (BM) BA-133-00-01	SEQ ID NO: 4	SEQ ID NO: 7
BA-133-04-01	SEQ ID NO: 11	SEQ ID NO: 13
BA-133-04-02	SEQ ID NO: 14	SEQ ID NO: 13
BA-133-04-03	SEQ ID NO: 16	SEQ ID NO: 13
BA-133-04-04	SEQ ID NO: 18	SEQ ID NO: 13
BAP133-2-03-03-SL	SEQ ID NO: 20	SEQ ID NO: 13
BAP133-2-02-12	SEQ ID NO: 21	SEQ ID NO: 13

Example 1: Binding activity of conditionally active anti-CD46 antibodies to huCD46

[0271] The binding activities of conditionally active anti-CD46 antibodies to human CD46 were measured by ELISA, using the BM (benchmark) antibody as a control. The EC50 values of the conditionally active anti-CD46 antibodies for binding to human CD46 at pH 6.0 and pH 7.4 are summarized in Table 1 and the binding activities are shown in FIGS. 1-2.

Table 1

hit name	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
EC50 pH6.0	8.135	13.98	13.62	7.188	10.42	12.01	15.75
EC50 pH7.4	8.061	46.2	51.72	16.05	37.82	38.79	110.6
ratio (pH7.4/pH6.0)	1.31	3.46	3.80	2.23	3.63	3.23	7.02

Table 2

hit name	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
EC50 pH6.0	11.1	24.64	26.88	9.695	19.93	22.13	55.42
EC50 pH7.4	14.72	236.7	239.9	69.21	259.8	244.8	739.9
ratio (pH7.4/pH6.0)	1.33	9.20	9.00	7.14	13.03	11.01	13.35

Table 3

hit name	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
pH infection point	n/a	6.611	6.638	n/a	6.702	6.801	6.575

Table 4

	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
EC50 (ng/ml)							
pH 6.0	147.5	324.4	319.5	146.9	241.1	335.6	675.9
pH 7.4	367.7	1340	1092	426.9	646.6	748.4	2232
Ratio (pH7.4/6.0)	2.5	4.1	3.4	2.9	2.7	2.2	3.3

Example 2: Binding activity of conditionally active anti-CD46 antibodies to cyno CD46

[0272] The binding activities of conditionally active anti-CD46 antibodies to cyno CD46 were measured by ELISA and are shown in FIGS. 3-4. The EC50 values for binding to the cyno CD46 at pH 6.0 and pH 7.4 for the conditionally active anti-CD46 antibodies are summarized in Table 2.

Example 3: Binding activity of conditionally active anti-CD46 antibodies to human CD46

[0273] The binding activities of conditionally active anti-CD46 antibodies to human CD46 with pH titration were similarly measured by ELISA. See FIG. 5 The pH inflection points of the conditionally active anti-CD46 antibodies to human CD46 are summarized in Table 3

Example 4: Binding activity of conditionally active anti-CD46 antibodies measured by FACS

[0274] FACS analysis was carried out using 293 cells expressing human CD46. The conditionally active anti-CD46 antibodies consistently showed higher binding activity to the 293 cells expressing human CD46 at pH 6.0 than at pH 7.4. See FIGS. 6-7. The EC50 values for binding to the 293 cells expressing human CD46 by the humanized conditionally active anti-CD46 antibodies are summarized in Table 4.

Example 5. Binding activity of conditionally active anti-CD46 antibodies measured by FACS

[0275] Binding activities of conditionally active anti-CD46 antibodies to Colo205 cells that express CD46 were measured by FACS at pH 6.0 and pH 7.4. The conditionally active anti-CD46 antibodies consistently showed higher binding activities to the Colo205 cells at pH 6.0 than at pH 7.4. See FIGS. 8-9. The EC50 values for binding to the Colo205 cells that express CD46 by the conditionally active anti-CD46 antibodies are summarized in Table 5.

Table 5

EC50 (ng/mL)	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
pH 6.0	201	359.8	370.9	150.8	245.8	340.6	704.2
pH 7.4	513.3	1660	1567	1467	959.9	1483	3627
Ratio (pH 7.4/5.0)	2.6	4.6	4.2	9.7	3.9	4.4	5.2

Table 6

EC50 (ng/mL)	BM (bench mark)	BA-133-04-01	BA-133-04-02	BA-133-04-03	BA-133-04-04	BAP133-2-03-03-SL	BAP133-2-02-12
pH 6.0	33.63	114.3	131.6	41.08	94.67	104	197.9
pH 7.4	49.29	957.7	1368	330.2	943.7	1148	2924
Ratio (pH 7.4/6.0)	1.5	8.4	10.4	8.0	10.0	11.0	14.8

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Table 7

IC50 (ng/mL)	BM (bench mark)	BA133-04-04	BAP133-2-03-03-SL
pH 6.0	15.75	13.71	20.64
pH 7.4	23.56	39.94	90.36
Ratio (pH 7.4/pH 6.0)	1.50	2.91	4.38

Table 8

IC50 (ng/mL)	BM (bench mark)	BA133-04-04	BAP133-2-03-03-SL
pH 6.0	4.91	6.584	33.32
pH 7.4	11.64	56.04	253.3
Ratio (pH 7.4/pH 6.0)	2.37	8.51	7.60

[0276] A similar FACS analysis was also carried out using 293 cells expressing cyno CD46. The conditionally active anti-CD46 antibodies also consistently showed higher binding to the 293 cells expressing cyno CD46 at pH 6.0 than at pH 7.4. See FIGS. 10-11. The EC50 values for binding to the 293 cells expressing cyno CD46 by the conditionally active anti-CD46 antibodies are summarized in Table 6.

Example 6: In vitro cell killing of 293 cells expressing human CD46.

[0277] In vitro cell killing of 293 cells expressing human CD46 was analyzed using 293 cells expressing human CD46 at pH values of 6.0 and 7.4. The *in vitro* killing of the 293 cells by the conditionally active anti-CD46 antibodies is shown in FIGS. 12-13. The IC50 values for the cell killing of 293 cells by the conditionally active anti-CD46 antibodies are shown in Table 7.

Example 7: Cytotoxicity of conditionally active anti-CD46 antibodies in inhibition of Colo205 cells that express CD46

[0278] The conditionally active antibodies were used to treat Colo205 cells that express CD46 at the tumor microenvironment pH of 6.0 and a normal physiological pH of 7.4. The conditionally active antibodies induced greater inhibition rates (IR%) at the tumor microenvironment pH than at the normal physiological pH. FIGS. 14-15. The IC50 values are given in Table 8 below.

Example 8: In vivo efficacy test of the conditionally active antibodies in the subcutaneous Colo 205 CDX model

[0279] The objective of this study was to evaluate the in vivo anti-tumor efficacy of test articles in the subcutaneous Colo 205 human colorectal cancer xenograft model in female BALB/c nude mice.

Abbreviations

Abbreviation	Definition
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
IACUC	Institutional Animal Care and Use Committee
ATCC	American Type Culture Collection
Q4D	Once every 4 days
GLP	Good Laboratory Practice
IV	Intravenous
SEM	Standard error of the mean
TGI	Tumor growth inhibition
TV	Tumor volume
RTV	Relative tumor volume

Experimental Design

Table 1-1. Description of experimental design

Group	N _a	Treatment	Dose (mg/kg)	Dose volume (mL/kg ^b)	Conc. mg/mL	Route	Schedule
1	8	Vehicle	--	10	--	IV	Q4D × 4 doses
2	8	BA-133-LP1 or BA-133-00-01 (BM)	3	10	0.3	IV	Q4D × 4 doses
3	8	BA-133-04-01 LP1	3	10	0.3	IV	Q4D × 4 doses
4	8	BA-133-04-02 LP1	3	10	0.3	IV	Q4D × 4 doses
5	8	BA-133-04-03 LP1	3	10	0.3	IV	Q4D × 4 doses
6	8	BA-133-04-04 LP1	3	10	0.3	IV	Q4D × 4 doses
7	8	BAP133-2-03-03-SL-LP1	3	10	0.3	IV	Q4D × 4 doses
8	8	BAP133-2-02-12 LP-1	3	10	0.3	IV	Q4D × 4 doses
9	8	B12-LP1	3	10	0.3	IV	Q4D × 4 doses

Note:

a. N: number of animals per group.

b. Dose volume: dosing volume was adjusted to 10 µL/g body weight.

Experimental Methods and Procedures

Cell Culture

The Colo 205 tumor cells (ATCC, Manassas, VA, cat # ATCC® CCL-222TM) were maintained in vitro as a monolayer culture in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C with 5% CO₂ in air. The tumor cells were routinely subcultured twice weekly by trypsin-EDTA treatment. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

Tumor Inoculation and Animal Grouping

Each mouse was inoculated subcutaneously at the right flank with Colo 205 tumor cells (5 × 10⁶) in 0.2 mL of PBS for tumor development. Treatments were started on day 11 after tumor inoculation when the average tumor size reached approximately 204 mm³. Animals were assigned into groups according to their tumor volume using an Excel-based stratified randomization program. Each group consisted of 8 tumor-bearing mice. The testing articles were administered according to the experimental design shown in Table 1-1.

Testing Article Preparation

Table 2-1. Description of test article preparation

Compounds	Stock (mg/mL)	Preparation	Concentration (mg/mL)	Storage
Vehicle	--	PBS	--	4°C
BA-133-LP1 or BA-133-00-01 (BM)	1.60	Add 0.450 mL stock to 1.950 mL PBS	0.3	4°C
BA-133-04-01 LP1	0.90	Add 0.800 mL stock to 1.600 mL PBS	0.3	4°C
BA-133-04-02 LP1	1.00	Add 0.720 mL stock to 1.680 mL PBS	0.3	4°C
BA-133-04-03 LP1	1.60	Add 0.450 mL stock to 1.950 mL PBS	0.3	4°C
BA-133-04-04 LP1	1.00	Add 0.720 mL stock to 1.680 mL PBS	0.3	4°C
BAP133-2-03-03-SL-LP1	1.00	Add 0.720 mL stock to 1.680 mL PBS	0.3	4°C
BAP133-2-02-12 LP-1	1.42	Add 0.507 mL stock to 1.893 mL PBS	0.3	4°C
B12-LP1	1.12	Add 0.643 mL stock to 1.757 mL PBS	0.3	4°C

Note: The test article formulation was prepared before each dosing.

Observations

All the procedures related to animal handling, care and the treatment in the study were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). At the time of routine monitoring, the animals were checked daily for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption (by looking only), body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect as stated in the protocol. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

Tumor Measurements and Endpoints

The major endpoint was to assess whether the tumor growth could be delayed. Tumor size was measured twice weekly in two dimensions using a caliper and was calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was then used for calculations of T/C, TGI and RTV values. The T/C value (in percent) is an indication of antitumor effectiveness; T and C are the mean volumes of the treated and control groups, respectively, on a given day.

TGI for each treatment group was calculated using the formula: $TGI (\%) = [1 - (T_i - T_0) / (V_i - V_0)] \times 100$; T_i is the average tumor volume of a treatment group on a given day, T_0 is the average tumor volume of the treatment groups on day 0, V_i is the average tumor volume of vehicle control group or isotype group on the same day with T_i , and V_0 is the average tumor volume of vehicle group or isotype group on day 0.

Individual RTV (relative tumor volume) was calculated by dividing the tumor volume on a specific day by its volume on day 0. The RTV value of each mouse was calculated individually which was then used for mean RTV calculation for each group.

Sampling

50~60 μ L serum was collected from 3 mice per group at 24 hours and 96 hours (right before the 2nd dose) post the first dose, respectively.

Statistical Analysis

The mean tumor volume of each group and SEM at different time points were calculated (Table 3-1). Statistical analysis of differences in tumor volume between vehicle group and test article groups were conducted on the data obtained on Day 25 after the start of treatment, which was the last day when all groups still had all mice. Meanwhile, statistical analysis of differences in tumor volume between isotype group and other test article groups were conducted on the data obtained on Day 32 after the start of treatment, which was the last day when all the test article groups still had all mice.

One-way ANOVA was performed to compare the mean tumor volumes and RTVs among groups. Significant F-statistics were obtained, and comparisons between groups were carried out with Games-Howell test. All data were analyzed using IBM® SPSS Statistics® software (version 17.0.). p value < 0.05 was considered to be statistically significant.

Tumor Volume

The mean tumor volumes of different groups are shown in Table 3-1.

Table 3-1. Tumor volume

Days	Tumor volume (mm ³) ^a								
	G1	G2	G3	G4	G5	G6	G7	G8	G9 ^b
0	204±17	204±19	204±18	204±18	204±18	204±20	204±21	204±21	204±17
4	382±44	232±27	261±24	308±34	244±32	227±28	252±25	305±30	302±30
7	476±61	174±25	246±27	312±37	245±36	206±28	233±37	377±46	454±44
11	620±87	51±4	152±30	180±29	127±27	87±19	120±19	346±44	574±50
14	789±108	26±3	114±34	110±22	90±24	50±9	66±10	273±47	713±62
18	933±125	16±3	88±38	53±13	53±19	25±4	31±6	205±50	824±70
21	1,074±13	13±3	77±40	40±12	45±18	18±3	20±6	193±55	903±79
25 ^c	1,288±14	8±2	78±48	60±25	55±29	8±2	17±6	274±88	1093±83
28	1,347±13	5±1	95±65	71±33	69±39	7±2	16±6	355±120	1,368±12
32	1,576±14	2±1	107±76	87±41	81±49	3±1	14±6	447±158	1,602±14
35	--	2±1	127±97	126±66	123±71	2±1	22±16	553±180	--
39	--	1±1	146±110	165±84	166±93	2±1	35±30	715±228	--
42	--	1±1	166±126	223±113	201±115	2±1	35±28	896±286	--

Note:

- Mean ± SEM
- G1: Vehicle, G2: BAP133-LP1 or BA-133-00-01 (BM) (3 mg/kg), G3: BA-133-04-01 LP1 (3 mg/kg), G4: BA-133-04-02 LP1 (3 mg/kg), G5: BA-133-04-03 LP1 (3 mg/kg), G6: BA-133-04-04 LP1 (3 mg/kg), G7: BAP133-2-03-03-SL-LP1 (3 mg/kg), G8: BAP133-2-02-12 LP-1 (3 mg/kg), G9: B12-LP1 (3 mg/kg).
- Mouse #34329 in group 1 was euthanized on PG-D25 when tumor volume reached 2,000 mm³. All the mice in G1 and G9 were euthanized on PG-D32 when their mean tumor volume reached 1,500 mm³.

Tumor Growth Inhibition Analysis

Table 3-2 Tumor growth inhibition when compared to vehicle group (based on Day 25 data)

Treatment	Tumor Size (mm ³) ^a	T/C ^b (%)	TGI (%)	RTV ^a	<i>p</i> ^c	<i>p</i> ^d
Vehicle	1,288±149	--	--	6.43±0.69	--	--
BAP133-LP1 or BA-133-00-01 (BM) (3 mg/kg)	8±2	0.65	118.07	0.04±0.01	0.001	0.001
BA-133-04-01 LP1 (3 mg/kg)	78±48	6.08	111.63	0.42±0.26	0.001	<0.001
BA-133-04-02 LP1 (3 mg/kg)	60±25	4.68	113.31	0.29±0.12	0.001	0.001
BA-133-04-03 LP1 (3 mg/kg)	55±29	4.27	113.76	0.22±0.11	0.001	0.001
BA-133-04-04 LP1 (3 mg/kg)	8±2	0.64	118.08	0.04±0.01	0.001	0.001
BAP133-2-03-03-SL-LP1 (3 mg/kg)	17±6	1.33	117.27	0.09±0.04	0.001	0.001
BAP133-2-02-12 LP-1 (3 mg/kg)	274±88	21.25	93.60	1.44±0.52	0.002	0.002
B12-LP1 (3 mg/kg)	1,093±83	84.86	17.99	5.44±0.41	0.954	0.932

Note:

- Mean ± SEM.
- Tumor Growth Inhibition is calculated by dividing the group average tumor volume for the treated group by the group average tumor volume for the vehicle control group (T/C).
- p* value calculated based on tumor size.
- p* value calculated based on RTV.

Table 3-3 Tumor growth inhibition when compared to isotype group (based on Day 32 data)

Treatment	Tumor Size (mm ³) ^a	T/C ^b (%)	TGI (%)	RTV ^a	<i>p</i> ^c	<i>p</i> ^d
Isotype (B12-LP1, 3 mg/kg)	1,602±146	--	--	7.95±0.72	--	--
BAP133-LP1 or BA-133-00-01 (BM) (3 mg/kg)	2±1	0.12	114.47	0.01±0.00	<0.001	<0.001
BA-133-04-01 LP1 (3 mg/kg)	107±76	6.65	107.00	0.57±0.40	<0.001	<0.001
BA-133-04-02 LP1 (3 mg/kg)	87±41	5.43	108.40	0.41±0.19	<0.001	<0.001
BA-133-04-03 LP1 (3 mg/kg)	81±49	5.08	108.77	0.32±0.18	<0.001	<0.001
BA-133-04-04 LP1 (3 mg/kg)	3±1	0.19	114.39	0.01±0.01	<0.001	<0.001
BAP133-2-03-03-SL-LP1 (3 mg/kg)	14±6	0.90	113.58	0.08±0.03	<0.001	<0.001
BAP133-2-02-12 LP-1 (3 mg/kg)	447±158	27.91	82.62	2.35±0.90	0.002	0.005

Note:

- Mean ± SEM.
- Tumor Growth Inhibition is calculated by dividing the group average tumor volume for the treated group by the group average tumor volume for the vehicle control group (T/C).
- p* value calculated based on tumor size.
- p* value calculated based on RTV.

Tumor Growth Curve

Tumor growth curves are shown in Figure 16. In Figure 16, data presented are mean ± SEM.

Summary and Discussion

In this study, the therapeutic efficacy of conditionally active antibodies was evaluated using the Colo205 human colorectal xenograft model. The tumor sizes of different groups at different time points after treatment are shown in Table 3-1, Table 3-2 and Figure 16. The mean tumor size of the vehicle group reached 1,288 mm³ on Day 25 (RTV= 6.43 ± 0.69) after the start of treatment. All the mice in vehicle and isotype groups were euthanized at PG-D32. Observation of other groups was prolonged until 4 weeks after the 4th dose.

All of the test articles showed significant anti-tumor activity (TGI>93%, p value<0.002, PG-D25). The test articles BAP133-LP1 or BA-133-00-01 (BM) and BA-133-04-04 LP1 at 3 mg/kg dose level exhibited dramatic anti-tumor activities leading to complete remission in most treated mice. Obvious tumor volume decreases were observed using test materials BA-133-04-01 LP1, BA-133-04-02 LP1, BA-133-04-03 LP1, BAP133-2-02-12 LP-1, but several mice in these groups were then found with tumors regrown after 10 days post the final dose. BAP133-2-02-12 LP-1 treatment delayed tumor growth at the beginning of the study, but tumor growth speed reversed within several days after dosing suspension. (Figure 16).

Isotype treatment (B12-LP1) produced nearly no efficacy when compared with vehicle treatment (T/C=84.86%, TGI=17.99%, p value=0.954, PG-D25).

No severe body weight loss or death/morbidity event was observed during the whole treatment and observation period. Thus, no obvious toxicity was observed in association with the administration of the test articles using the specified dose regimen.

EXAMPLE 9: Binding Activity of Conditionally Active Anti-CD46 Antibodies Measured by SPR Analysis

Binding kinetics of anti-CD46 antibodies were measured by surface plasmon resonance on a SPR2/4 instrument (Sierra Sensors, Hamburg, Germany) and flat amine sensor chips. The SPR sensor contained four flow cells (FC1-FC4), each of which can be addressed individually or in groups. huCD46-His was immobilized in FC2, and cynoCD46-His was immobilized in FC4. No protein was immobilized in FC1 and FC3 which were used as control surfaces for FC2 and FC4, respectively.

All injections were done at a flow rate of 25µL/min and 25°C. The sensor surface was activated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (200mM/50mM) for 480 seconds. Human CD46-His (2µg/mL in 10mM NaAc, pH5.0) was injected for 480s and the surface was inactivated by injecting 1M ethanolamine-HCl for 480s. cynoCD46-His was immobilized using the same conditions as

described for huCD46-His, except that it was diluted into 10 mM NaAc buffer pH4.5. The control surface was activated and deactivated using the same conditions, but without injecting protein. PBST buffer (PBS pH7.4 with 0.05% TWEEN20™) was used as running buffer for the surface preparation. The running solution was switched to PBST with 30 mM sodium bicarbonate with the pH adjusted as indicated in the figures before the analyte injections. The instrument was equilibrated with the running solution for one hour before the first analyte injection.

100μL analyte diluted in the corresponding running solution (34.25nM, 13.70nM, 6.85nM, 3.42nM, 1.37nM, and 0.0nM) was injected over flow cells 1 and 2 or 3 and 4. The off-rate was measured for 360s. The chip surface was regenerated after each cycle of interaction by injecting 6μL of 10mM glycine (pH 2.0). Flow cells 1 and 3 without immobilized protein were used as control surfaces for reference subtraction.

In addition, data with buffer only as analyte (0nM analyte) was subtracted from each run. Double subtracted data was fitted with the provided analysis software Analyzer R2 (Sierra Sensors) using a 1:1 binding model. A molecular weight of 200 kDa was used to calculate the molar concentrations of the analytes.

Binding activities at pH 6.0, pH. 6.5 and and pH 7.4 of conditionally active anti-CD46 antibodies to human CD46 and cyno CD46 were measured by SPR analysis and are shown in Tables 9 and 10, respectively, below.

Table 9

human CD46	pH6.0			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	6.82E+05	1.58E-03	2.32E-09	
	1.14E+06	1.13E-03	9.91E-10	
	6.11E+05	1.43E-03	2.33E-09	
	1.18E+06	1.79E-03	1.52E-09	
	8.24E+05	2.07E-03	2.51E-09	
	3.88E+05	9.15E-04	2.36E-09	
	5.39E+05	4.04E-03	7.51E-09	
	BA-133-00-01(BM)			
	pH6.5			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	6.21E+05	1.33E-03	2.15E-09	
	6.96E+05	1.23E-03	1.77E-09	
	5.47E+05	1.27E-03	2.31E-09	
	9.41E+05	1.95E-03	2.07E-09	
	9.22E+05	1.76E-03	1.91E-09	
	5.71E+05	7.36E-04	1.29E-09	
	4.88E+05	2.82E-03	5.78E-09	
	BA133-04-01			
	pH7.4			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	5.55E+05	1.19E-03	2.13E-09	
	3.85E+05	1.54E-03	3.99E-09	
	4.17E+05	1.74E-03	4.17E-09	
	5.19E+05	2.49E-03	4.80E-09	
	4.90E+05	2.41E-03	4.93E-09	
	8.74E+05	7.78E-04	8.90E-10	
	2.56E+05	2.25E-03	8.79E-09	
	BAP133-02-03-03-SL			
	pH6.0			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	1.45E+06	1.15E-03	7.95E-10	
	1.63E+06	7.57E-04	4.65E-10	
	1.61E+06	8.35E-04	5.19E-10	
	2.79E+06	1.43E-03	5.14E-10	
	1.42E+06	1.46E-03	1.02E-09	
	1.00E+06	2.55E-03	2.55E-09	
	1.27E+06	1.84E-03	1.45E-09	
	BA-133-00-01(BM)			
cynoCD46	pH6.5			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	1.51E+06	1.17E-03	7.78E-10	
	1.19E+06	6.62E-04	5.58E-10	
	1.38E+06	9.43E-04	6.83E-10	
	2.28E+06	1.42E-03	6.24E-10	
	1.33E+06	1.45E-03	1.09E-09	
	8.49E+05	1.98E-03	2.33E-09	
	1.16E+06	1.70E-03	1.47E-09	
	BA-133-04-01			
	pH7.4			
	Ka [M·s]	Kd[s ⁻¹]	KD[M]	
	1.21E+06	1.10E-03	9.05E-10	
	6.90E+05	8.93E-04	1.29E-09	
	8.52E+05	9.04E-04	1.06E-09	
	1.21E+06	1.53E-03	1.26E-09	
	6.73E+05	1.54E-03	2.28E-09	
	4.42E+05	2.42E-03	5.47E-09	
	4.95E+05	1.92E-03	3.88E-09	
	BAP133-02-02-12			

Table 10

Experimental Protocols for the Examples

Examples 1 and 2

ABBREVIATIONS

C	Celsius
ECD	Extracellular Domain
ELISA	enzyme-linked immunosorbent assay
g	gram
HCl	hydrochloric acid
hr	hour
HRP	horseradish peroxidase
huCD46	human CD46 extracellular domain
cynoCD46	cynomolgus CD46 extracellular domain
min	minute
mL	milliliter
NA	not applicable
ng	nanogram
nm	nanometers
OD	optical density
PBS	phosphate buffered saline
rpm	revolutions per minute
SD	standard deviation
TMB	3, 3', 5, 5' tetramethylbenzidine
μg	microgram
μL	microliter

TEST ARTICLES

Benchmark (BM)
 CPE hit BA133-04-01
 CPE hit BA133-04-02
 CPE hit BA133-04-03
 CPE hit BA133-04-04
 CPE hit BAP133-2-03-03-SL
 CPE hit BAP133-2-02-12
 B12 (isotype control)

FORMULATIONS

Test articles were first diluted to 300 ng/mL in pH 6.0 or pH 7.4 ELISA incubation buffer. Then 3000 ng/mL of test articles were 3-fold serially diluted in pH 6.0 or pH 7.4 ELISA incubation buffer.

PH AFFINITY ELISA ASSAY

- 1) Coat ELISA plates with 100 μL of 1 μg/mL recombinant human CD46 antigen or cyno CD46 antigen in carbonate-bicarbonate coating buffer.
- 2) Cover plates with sealing film and incubate overnight at 4°C.
- 3) Decant plates and tap out residual liquid on a stack of paper towels.

- 4) Wash wells twice by dispensing 200 μ L of pH 6.0 or pH 7.4 ELISA incubation buffer to the wells and completely aspirate the contents.
- 5) Add 200 μ L of pH 6.0 or pH 7.4 ELISA incubation buffer to the wells. Cover with sealing film and place the plate onto a plate shaker set to 50 rpm for 60 minutes at room temperature.
- 6) Decant plates and tap out residual liquid on a stack of paper towels.
- 7) Serially dilute test articles in 3-fold dilutions starting at 3000 ng/mL in pH 6.0 or pH 7.4 ELISA incubation buffer.
- 8) Add 100 μ L/well of diluted test articles to the plates
- 9) Cover with sealing film and place the plates onto a plate shaker set to 50 rpm for 60 minutes at room temperature.
- 10) Decant plates and tap out residual liquid on a stack of paper towels.
- 11) Wash wells three times by dispensing 200 μ L of pH 6.0 or pH 7.4 ELISA wash buffer to the wells and completely aspirate the contents.
- 12) Dilute the HRP secondary antibody at 1:2500 in pH 6.0 or pH 7.4 ELISA incubation buffer.
- 13) Add 100 μ L HRP secondary antibody diluted in pH 6.0 or pH 7.4 ELISA incubation buffer to each well
- 14) Cover with sealing film and place the plates onto a plate shaker set to 50 rpm for 60 minutes at room temperature.
- 15) Decant plates and tap out residual liquid on a stack of paper towels.
- 16) Wash wells three times by dispensing 200 μ L of pH 6.0 or pH 7.4 ELISA wash buffer to the wells and completely aspirate the contents.
- 17) Dispense 50 μ L per well of the TMB substrate solution into all wells of the plates. Incubate at room temperature for about 2 minutes 15 seconds or 2 minutes.
- 18) Add 50 μ L per well of 1N HCl into all wells of the plates. Read plates at 450 nm using PerkinElmer, EnSpire 2300 Multilabel Reader.

Example 3

ABBREVIATIONS

C	Celsius
ECD	Extracellular Domain
ELISA	enzyme-linked immunosorbent assay
g	gram
HCl	hydrochloric acid
hr	hour
HRP	horseradish peroxidase
huCD46	human CD46 extracellular domain
min	minute
mL	milliliter
NA	not applicable
ng	nanogram
nm	nanometers

OD	optical density
PBS	phosphate buffered saline
rpm	revolutions per minute
TMB	3, 3', 5, 5' tetramethylbenzidine
µg	microgram
µL	microliter

TEST ARTICLES

Benchmark (BM)
CPE hit BA133-04-01
CPE hit BA133-04-02
CPE hit BA133-04-03
CPE hit BA133-04-04
CPE hit BAP133-2-03-03-SL
CPE hit BAP133-2-02-12
B12 isotype control

FORMULATIONS

Test articles were diluted to 10 ng/mL in various pH ELISA incubation buffer range from pH 5.5 to pH 7.4.

PH RANGE ELISA ASSAY

- 1) Coat ELISA plates with 100 µL of 1µg/mL recombinant human CD46 antigen in carbonate-bicarbonate coating buffer
- 2) Cover plates with sealing film and incubate overnight at 4°C
- 3) Decant plates and tap out residual liquid on a stack of paper towels
- 4) Wash wells twice by dispensing 200 µL of various pH incubation buffer to the wells and completely aspirate the contents
- 5) Add 200 µL of various pH incubation buffers (pH5.5, 6.0, 6.2, 6.5, 6.7, 7.0 and 7.4) to the wells. Cover with sealing film and place the plate onto a plate shaker (set to 200 rpm) for 60 minutes at room temperature
- 6) Decant plates and tap out residual liquid on a stack of paper towels
- 7) Serially dilute test substances in various pH incubation buffers (pH5.5, 6.0, 6.2, 6.5, 6.7, 7.0 and 7.4) to 30 ng/mL
- 8) Add 100 µL/well of diluted test substances to the plates
- 9) Cover with sealing film and place the plates onto a plate shaker (set to 200 rpm) for 60 minutes at room temperature.
- 10) Decant plates and tap out residual liquid on a stack of paper towels.
- 11) Wash wells three times by dispensing 200 µL of various pH wash buffers (pH5.5, 6.0, 6.2, 6.5, 6.7, 7.0 and 7.4) to the wells and completely aspirate the contents
- 12) Dilute the HRP secondary antibody at 1:2500 in various pH incubation buffers (pH5.5, 6.0, 6.2,

- 6.5, 6.7, 7.0 and 7.4)
- 13) Add 100 μ L HRP secondary antibody diluted in various pH incubation buffers (pH5.5, 6.0, 6.2, 6.5, 6.7, 7.0 and 7.4) to each well.
 - 14) Cover with sealing film and place the plates onto a plate shaker (set to 200 rpm) for 60 minutes at room temperature.
 - 15) Decant plates and tap out residual liquid on a stack of paper towels.
 - 16) Wash wells three times by dispensing 200 μ L of various pH wash buffer (pH5.5, 6.0, 6.2, 6.5, 6.7, 7.0 and 7.4) to the wells and completely aspirating the contents
 - 17) Dispense 50 μ L per well of the TMB substrate solution into all wells of plates. Incubate at room temperature for 3 minutes.
 - 18) Add 50 μ L per well of 1N HCl into all wells of the plates. Read plates at 450 nm using PerkinElmer EnSpire 2300 Multilabel Reader.

Examples 4 and 5

ABBREVIATIONS

AF488	Alexa Fluor 488
°C	degree Celsius
ECD	Extracellular Domain
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
g	gram
huCD46	human CD46 extracellular domain
cynoCD46	cynomolgus CD46 extracellular domain
HCl	hydrochloric acid
hr	hour
MFI	median fluorescence intensity
min	minute
mL	milliliter
NA	not applicable
ng	nanogram
PBS	phosphate buffered saline
PFA	paraformaldehyde
rpm	revolutions per minute
R.T.	room temperature
SD	standard deviation
μ g	microgram
μ L	microliter

TEST ARTICLES

Benchmark (BM)
 CPE hit BA133-04-01
 CPE hit BA133-04-02
 CPE hit BA133-04-03
 CPE hit BA133-04-04

CPE hit BAP133-2-03-03-SL

CPE hit BAP133-2-02-12

B12 isotype control

FORMULATIONS

Test articles were first diluted to 30 µg/mL in pH 6.0 or pH 7.4 FACS buffer, then 3-fold serially diluted in pH 6.0 or pH 7.4 FACS buffer.

CELL CULTURING

293-huCD46 and 293-cynoCD46 cells were maintained in stable cell line culture medium (MEM + 10% FBS + 1mg/mL G418). Colo205 cells that express CD46 (ATCC, Cat#CCL222) were maintained in colo205 culture medium (RPMI1640 + 10%FBS). The cells were routinely sub-cultured twice per week. The cells were harvested during exponential growth phase and counted for plating.

CELL STAINING USING TEST ANTIBODIES

- 1) Seed 3×10^6 cells to T-75 flasks and culture according to the instructions of vendors.
- 2) On the day of FACS analysis, remove and discard culture medium.
- 3) Briefly rinse the cell layer with PBS solution.
- 4) Add 1.5 mL of Detachin solution to each of the T-75 flasks. Wait until cell layer has dispersed.
- 5) Add 4.5 mL of culture media for the corresponding cell lines and resuspend cells by gently pipetting.
- 6) Pool the cells and transfer the cell suspension to a 50-mL conical tube.
- 7) Count the cells with trypan blue staining before centrifugation at 1500 rpm for 5 min at 4°C.
- 8) Wash the cells once with PBS
- 9) Resuspend the cells in pH 6.0 or pH 7.4 FACS buffer to 3.5×10^6 cells/mL.
- 10) Aliquot 3.5×10^5 cells in 100 µL pH 6.0 or pH 7.4 FACS buffer in 96-well U-bottom plates.
- 11) Spin down the cells and discard the buffer.
- 12) Serially dilute test articles in 3-fold dilutions starting at 30 µg/mL in pH 6.0 or pH 7.4 FACS buffer.
- 13) Add 100 µL/well of the diluted test articles to cells, gently mix well and incubate on ice with shaking (200 rpm) for one hour.
- 14) Centrifuge the cells at 1500 rpm for 5 min at 4°C. Wash the cells with 150 µL of pH 6.0 or pH 7.4 wash buffer twice.
- 15) Dilute the goat anti-human IgG AF488 antibody 1:300 in pH 6.0 or pH 7.4 FACS buffers.
- 16) Add 100 µL of the diluted antibody from step above to the cells and incubate on ice with shaking (200 rpm) for 45 minutes, protected from light.
- 17) Pellet the cells and wash with 150 µL of pH 6.0 or pH 7.4 wash buffer three times.
- 18) Fix cells with 4% PFA diluted in 1X PBS for 10 min at R.T., then wash cells with 1X PBS.
- 19) Resuspend the cells in 100 µL of 1X PBS.

- 20) Analyze the cells by NovoCyte Flow Cytometer using Ex488nm/Em530nm. Collect at least 5,000 singlet cells for each data point.

FACS DATA ANALYSIS

The MFI of AF488 in cell singlets was plotted using GraphPad Prism software version 7.03.

Examples 6 and 7

ABBREVIATIONS

°C	degree Celsius
ADC	antibody-drug conjugate
Cyno	cynomolgus
DMEM	Dulbecco's Modified Eagle Medium
FBS	fetal bovine serum
HCl	hydrochloric acid
hr	hour
huCD46	human CD46 extracellular domain
IC50	50% effective inhibitory concentration
log	logarithm
MEM	minimum essential media
min	minute
mL	milliliter
NA	not applicable
NaOH	sodium hydroxide
NEAA	non-essential amino acid
ng	nanogram
PBS	phosphate buffered saline
PE	phycoerythrin
RLU	relative luminescence units
rpm	revolutions per minute
SD	standard deviation
μg	microgram
μL	microliter

TEST ARTICLES ADC

Bench mark
CPE hit BA133-04-04
CPE hit BAP133-2-03-03-SL
B12 isotype control

CELL CULTURING

293-huCD46 cells were maintained in stable cell line culture medium (MEM + 10% FBS + 1mg/mL G418). Colo205 cells that express CD46 (ATCC, Cat#CCL222) were maintained in colo205 culture medium (RPMI1640 + 10%FBS). The cells were routinely sub-cultured twice per week. The cells were harvested during exponential growth phase and counted for plating.

FORMULATIONS

- 1) Make 5-fold serial dilution of 10 x test article ADC or B12 isotype ADC stocks in pH 6.0 or pH 7.4 assay media in duplicates starting at 50 µg/mL
- 2) Centrifuge the plates and gently remove the culture medium then add 90 µL of pH assay media before adding ADCs
- 3) Add 10 µL of serially diluted 10 x ADCs or B12 sample stocks to the wells containing 3000 cells (Final starting concentration is 5 µg/mL)
- 4) Incubate the treated cells at 37°C in a 5% CO₂ incubator for 72 hours

CELLTITER-GLO LUMINESCENT CELL VIABILITY ASSAY

- 1) Thaw the CellTiter-Glo buffer and equilibrate to room temperature prior to use
- 2) Equilibrate the lyophilized CellTiter-Glo substrate to room temperature prior to use
- 3) Transfer the entire liquid volume of CellTiter-Glo buffer into the amber bottle containing CellTiter-Glo substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms the CellTiter-Glo reagent
- 4) Mix by gently vortex to obtain a homogeneous solution
- 5) Equilibrate the plate and its contents to room temperature
- 6) Add 70 µL CellTiter-Glo reagent in each well. Mix contents for 2 minutes on an orbital shaker at 100 rpm to induce cell lysis
- 5) Allow the plate to incubate at room temperature for 10 minutes to stabilize luminescent signal
- 6) Record luminescence on the SpectraMax i3X plate reader

DATA ANALYSIS

The inhibitions of different dose of test antibodies were plotted in concentration-response luminescence signals and IC₅₀ was calculated. The data were interpreted by GraphPad Prism software.

[0280] It is to be understood, however, that even though numerous characteristics and advantages of the present invention have been set forth in the foregoing description, together with details of the structure and function of the invention, the disclosure is illustrative only, and changes may be made in detail, especially in matters of shape, size and arrangement of parts within the principles of the invention to the full extent indicated by the broad general meanings of the terms in which the appended claims are expressed.

[0281] All documents mentioned herein are hereby incorporated by reference in their entirety or alternatively to provide the disclosure for which they were specifically relied upon. The applicant(s) do not intend to dedicate any disclosed embodiments to the public, and to the

extent any disclosed modifications or alterations may not literally fall within the scope of the claims, they are considered to be part hereof under the doctrine of equivalents.

POLYPEPTIDE (INCLUDING ANTIBODY) SEQUENCES OF THE INVENTION

SEQ ID NO: 1

RAX₁QX₂IX₃NYLN

SEQ ID NO: 2

YTSSLX₄X₅

SEQ ID NO: 3

QQYIKLPWT

SEQ ID NO: 4

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLNWYQQKP GKAPKLLIYY TSSLHSGVPS RFSGSGSGTD
FTLTSSLQP EDFATYYCQQ YIKLPWTFGG GTKLEIKR

SEQ ID NO: 5

RASQGISNYLN

SEQ ID NO: 6

YTSSLHS

SEQ ID NO: 7

QVQLQESGPG LVKPSETLSL TCTVSGGSVS SYDISWIRQP PGKGLEWIGV IWTDDGGTNYN SAFMSRVTIS
VDTSKNQFSL KLSSVTAADT AVYYCARVYD GYPWFAYWGQ GTLVTVSS

SEQ ID NO: 8

GGSVSSYDIS

SEQ ID NO: 9

VIWTDGGTNYNSAFMS

SEQ ID NO: 10

VYDGYPFAY

SEQ ID NO: 11

AIQLTQSPSS LSASVGDRVT ITCRASQWIS NYLNWYLQKP GQSPQLLIYY TSSLHSGVPS RFSGSGSGTD
FTLKISRVEA EDVGVYYCQQ YIKLPWTFGG GTKVEIK

SEQ ID NO: 12
RASQWIS NYLN

SEQ ID NO: 13
EVQLVESGGG LVQPGGSLRL SCAASGGSVS SYDISWIRQS PSRGLEWLGV IWTGDDGTNYN SAFMSRFTIS
RDDSKNTAYL QMNSLKTEDT AVYYCERVYD GYPWFAYWGQ GTLVTVSS

SEQ ID NO: 14
AIQLTQSPSS LSASVGDRVT ITCRASQGIA NYLNWYLQKP GQSPQLLIYY TSSLHSGVPD RFSGSGSGTD
FTLKISRVEA EDVGVYYCQQ YIKLPWTFGQ GTKVEIK

SEQ ID NO: 15
RASQGIANYLN

SEQ ID NO: 16
AIQLTQSPSS LSASVGDRVT ITCRASQGIS NYLNWYLQKP GQSPQLLIYY TSSLFSGVPD RFSGSGSGTD
FTLKISRVEA EDVGVYYCQQ YIKLPWTFGQ GTKVEIK

SEQ ID NO: 17
YTSSLFS

SEQ ID NO: 18
AIQLTQSPSS LSASVGDRVT ITCRASQGIS NYLNWYLQKP GQSPQLLIYY TSSLHEGVPD RFSGSGSGTD
FTLKISRVEA EDVGVYYCQQ YIKLPWTFGQ GTKVEIK

SEQ ID NO: 19
YTSSLHE

SEQ ID NO: 20
AIQLTQSPSS LSASVGDRVT ITCRASQGIS NYLNWYLQKP GQSPQLLIYY TSSLHSGVPD RFSGSGSGTD
FTLKISRVEA EDVGVYYCQQ YIKLPWTFGQ GTKVEIK

SEQ ID NO: 21
AIQLTQSPSS LSASVGDRVT ITCRALQGIS NYLNWYQQKP GKAPKLLIYY TSSLHSGVPS RFSGSGSGTE
FTLTSSLQP DDFATYYCQQ YIKLPWTFGQ GTKVEIK

SEQ ID NO: 22
RALQGISNYLN