

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 example, the present disclosure 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 VYDGYWPFAY (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.

[0011a] In one aspect, the present disclosure refers to isolated polypeptides 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 VYDGYWPFAY (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 examples, 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 example, 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 example, the isolated polypeptides of the present disclosure 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. In one aspect, the present disclosure refers to isolated polypeptides comprising a light chain variable region and a heavy chain variable region, each said light chain variable region and each said 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, and SEQ ID NOS: 21 and 13; and said isolated polypeptides specifically bind to human CD46 protein.

[0015] In another example, the present disclosure 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 example, the isolated polypeptides of the present disclosure 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 example, the present disclosure 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 VYDGYWPFAY (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.

[0017a] In one aspect, the present disclosure refers to an isolated antibody or antibody fragment 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 VYDGYWPFAY (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 example, 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 examples 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 example, 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 example, the antibody or antibody fragment of the present disclosure 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 example, the antibody or antibody fragment of the present disclosure 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 example, an antibody or antibody fragment of the present disclosure competes with any of the antibodies or antibody fragments described above for binding to human CD46. In one aspect, the present disclosure refers to an isolated antibody or antibody fragment, which competes with the binding to human CD46 with any of the antibodies or antibody fragments disclosed herein.

[0023] In each of the previous examples, 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 example, the condition is pH. In one example binding activity is measured by binding affinity.

[0024] In each of the previous examples, 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 examples, the antigen binding activity may be measured by an ELISA assay.

[0026] In yet another example, the present disclosure provides an immunoconjugate that includes any of the antibodies or antibody fragments of the disclosure described above. In one aspect, the present disclosure refers to an immunoconjugate comprising the any one of the antibodies or antibody fragments disclosed herein. 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 example, the present disclosure provides a pharmaceutical composition that includes any of the polypeptides, the antibodies or antibody fragments, or the immunoconjugates of the disclosure described above, together with a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the polypeptide disclosed herein, any one of the antibodies or antibody fragments disclosed herein, or any one of the immunoconjugates disclosed herein; and a pharmaceutically acceptable carrier.

[0028] A single dose of the pharmaceutical composition 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. In one aspect, the present disclosure refers to a single dose of any one of the pharmaceutical compositions disclosed herein, comprising 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 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. In one aspect, the present disclosure refers to a single dose of any one of the pharmaceutical compositions disclosed herein, comprising 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.

[0031a] In one aspect, the present disclosure refers to use of any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, any one of the immunoconjugates disclosed herein or any one of the pharmaceutical compositions disclosed herein, in the manufacture of a medicament for treating cancer in a patient with cancer.

[0032] In yet another example, the present disclosure 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 disclosure described above. In one aspect, the present disclosure refers to a kit for diagnosis or treatment, said kit comprising any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, or any one of the immunoconjugates disclosed herein or any one of the pharmaceutical compositions disclosed herein and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.

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.