

FUSION PROTEIN AND USE THEREOF

FIELD OF THE INVENTION

[0001] The present application relates to the field of biomedicine, and particularly to a multispecific fusion protein, and further to a use thereof in the treatment of a tumor and/or an autoimmune disease.

BACKGROUND OF THE INVENTION

[0002] At present, in the field of tumor therapy, there are two major methods of administering targeted drugs and immunotherapy. There may be an interaction between these two therapies, causing a stronger cytotoxic effect, thus alleviating the tumors steadily and sustainably. However, the interaction between targeted drugs and immunotherapy is very complicated, and the overall antitumor effect and the toxicity profile of the combined therapy may be affected by various factors such as species, dosages, order, dosage forms and the like.

[0003] Programmed death 1 (PD-1) antibody is an immunotherapy. PD-1 is expressed in activated T cells, B cells and myeloid cells, which has two ligands, i.e., programmed death ligand 1 (PD-L1) and PD-L2. PD-1 and/or PD-L1 inhibitors can specifically bind to PD-L1 on tumor cells to achieve an anti-tumor effect, but the clinical response rate is low, and there may be some side effects, e.g., causing pneumonia, colitis, hepatitis, etc.

[0004] CD47 protein is a kind of transmembrane glycoprotein, which is a member belonging to the immunoglobulin superfamily. In addition to being expressed by normal tissue cells, CD47 is over-expressed by many tumor cells. CD47 on the surface of tumors cells binds to SIRP α on the surface of macrophages, which prevents the phagocytosis of tumor cells by macrophages, this is considered as

one mechanism by which tumors evade from the surveillance of an immune system. Blocking the interaction between CD47 protein and SIRP α can inhibit the tumor growth.

[0005] However, the current reagents used to block the interaction between CD47 protein and SIRP α have limited recognition activity, their affinities with CD47 protein are always insufficient, so they have limited capacity on inhibiting the tumors. In addition, the current antibody drugs targeting CD47 may cause side effects such as anemia responses or thrombocytopenia. Therefore, it is urgent to obtain an effective therapy which specifically targets both CD47 protein and associated tumor antigens.

SUMMARY OF THE INVENTION

[0006] The present application provides a fusion protein, including a first binding domain that specifically binds PD-L1 and a second binding domain that specifically binds a CD47 protein. The present application also provides an immunoconjugate including the fusion protein; a nucleic acid molecule encoding the fusion protein; a vector, a composition and a cell capable of including and/or expressing the fusion protein; and a method of preparing the fusion protein. The fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition and the cell of the present application have one or more of the following properties: 1) capable of specifically binding the CD47 protein and PD-L1 simultaneously; 2) capable of specifically blocking the interaction between the CD47 protein and SIRP α ; 3) capable of specifically blocking the interaction between PD-1 and PD-L1; 4) capable of effectively inhibiting the growth and/or proliferation of tumors or tumor cells.

[0007] In one ~~aspect~~embodiment, the present application provides a fusion protein, including a first binding domain that specifically binds PD-L1; and a second binding domain that specifically binds a

CD47 protein; wherein, the second binding domain comprises a mutant of a human SIRP α variant 1, the mutant comprises substitution, deletion or addition of an amino acid residue at one or more positions from site 33 to site 149 compared to the sequence as shown in SEQ ID NO: 29.

[0007a] In one aspect, the application provides a fusion protein, comprising: a first binding domain that specifically binds PD-L1; and a second binding domain that specifically binds a CD47 protein; wherein said second binding domain comprises a mutant of a human SIRP α variant 1, said mutant comprises amino acid residue substitutions at one or more positions from site 33 to site 149, compared to the amino acid sequence listed as NCBI RefSeq NP_542970.1, wherein said mutant comprises amino acid substitutions selected from the group consisting of: (1) I61L, V63I, E77I, E84K, V93L, L96S, K98R, N100G and V132L; (2) I61V, E77N, Q82S, K83R and E84H; (3) I61F, V63I, K83R, E84K and V132I; (4) I61L, E77Q, E84D, R107N and V132I; (5) I61L, V63I, E77K, K83R, E84D and N100G; (6) I61V, E77H, Q82R, K83R, E84H and R107S; (7) I61L, E77I, Q82G, E84R, V93L, L96T, N100G, R107S, G109R and V132R; (8) I61L, E77M, Q82G, K83R, E84D and V132L; (9) I61L; (10) I61F, D95H, L96S, G109H and V132S; (11) I61F, D95H, L96S, K98R, G109H and V132S; (12) I61L, E77Q, E84D, V93A, R107N and V132I; (13) E77K, L96S, N100K, G109H and V132L; (14) I61L, V63I, Q82G, E84G, D95R, L96S, N100D and V132I; (15) I61L, E77R, Q82N, K83R, E84G, V93L, D95E, L96T, K98R, N100D and V132L; (16) I61V, E77N, Q82S, K83R, E84H and V93A; (17) I61V, V63I, E77V, K83R, E84D, D95E, L96T, K98R and N100E; (18) I61L, V63I, E77V, K83R, D95E, L96S, K98R, N100D and G109R; (19) I61V, E77L, Q82G, E84G, V93L, D95E, L96T, K98R and N100G; and (20) I61L, V63I, E77N, Q82G and E84G.

[0008] In some embodiments, the mutant comprises amino acid substitutions at one or more amino acid residues selected from the group consisting of: I61, V63, E77, Q82, K83, E84, V93, D95, L96, K98, N100, R107, G109 and V132.

[0009] In some embodiments, the mutant comprises amino acid substitutions at amino acid residues selected from the group consisting of: (1) I61, V63, E77, E84, V93, L96, K98, N100 and V132; (2) I61, E77, Q82, K83 and E84; (3) I61, V63, K83, E84 and V132; (4) I61, E77, E84, R107 and V132; (5) I61, V63, E77, K83, E84 and N100; (6) I61, E77, Q82, K83, E84 and R107; (7) I61, E77, Q82, E84, V93, L96, N100, R107, G109 and V132; (8) I61, E77, Q82, K83, E84 and V132; (9) I61; (10) I61, D95, L96, G109 and V132; (11) I61, D95, L96, K98, G109 and V132; (12) I61, E77, E84, V93, R107 and V132; (13) E77, L96, N100, G109 and V132; (14) I61, V63, Q82, E84, D95, L96, N100 and V132; (15) I61, E77, Q82, K83, E84, V93, D95, L96, K98, N100 and V132; (16) I61, E77, Q82, K83, E84 and V93; (17) I61, V63, E77, K83, E84, D95, L96, K98 and N100; (18) I61, V63, E77, K83, D95, L96, K98, N100 and G109; (19) I61, E77, Q82, E84, V93, D95, L96, K98 and N100; and (20) I61, V63, E77, Q82 and E84.

[0010] In some embodiments, the mutant comprises one or more amino acid substitutions selected from the group consisting of: I61L/V/F, V63I, E77I/N/Q/K/H/M/R/N/V/L, Q82S/R/G/N, K83R, E84K/H/D/R/G, V93L/A, D95H/R/E, L96S/T, K98R, N100G/K/D/E, R107N/S, G109R/H and V132L/R/I/S.

[0011] In some embodiments, the mutant comprises amino acid substitutions selected from the group consisting of: (1) I61L, V63I, E77I, E84K, V93L, L96S, K98R, N100G and V132L; (2) I61V, E77N,

Q82S, K83R and E84H; (3) I61F, V63I, K83R, E84K and V132I; (4) I61L, E77Q, E84D, R107N and V132I; (5) I61L, V63I, E77K, K83R, E84D and N100G; (6) I61V, E77H, Q82R, K83R, E84H and R107S; (7) I61L, E77I, Q82G, E84R, V93L, L96T, N100G, R107S, G109R and V132R; (8) I61L, E77M, Q82G, K83R, E84D and V132L; (9) I61L; (10) I61F, D95H, L96S, G109H and V132S; (11) I61F, D95H, L96S, K98R, G109H and V132S; (12) I61L, E77Q, E84D, V93A, R107N and V132I; (13) E77K, L96S, N100K, G109H and V132L; (14) I61L, V63I, Q82G, E84G, D95R, L96S, N100D and V132I; (15) I61L, E77R, Q82N, K83R, E84G, V93L, D95E, L96T, K98R, N100D and V132L; (16) I61V, E77N, Q82S, K83R, E84H and V93A; (17) I61V, V63I, E77V, K83R, E84D, D95E, L96T, K98R and N100E; (18) I61L, V63I, E77V, K83R, D95E, L96S, K98R, N100D and G109R; (19) I61V, E77L, Q82G, E84G, V93L, D95E, L96T, K98R and N100G; and (20) I61L, V63I, E77N, Q82G and E84G.

[0012] In some embodiments, the mutant comprises an amino acid sequence as shown in any one of SEQ ID NOs: 30-49.

[0013] In some embodiments, the first binding domain comprises an antibody or an antigen-binding fragment or a variant thereof. In some embodiments, the antibody is selected from the group consisting of: a monoclonal antibody, a single-chain antibody, a chimeric antibody, a humanized antibody and a fully human antibody. In some embodiments, the antigen-binding fragment is selected from the group consisting of: Fab, Fab', F(ab')₂, F(ab)₂, dAb, an isolated complementary determining region CDR, Fv and scFv.

[0014] In some embodiments, the PD-L1 is a human PD-L1.

[0015] In some embodiments, the antibody comprises a heavy chain of the antibody or a fragment thereof, the heavy chain of the antibody or the fragment thereof comprises HCDR1-3, and the HCDR1 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 4 and SEQ ID NO: 18. In some embodiments, the HCDR2 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 5 and SEQ ID NO: 19. In some embodiments, the HCDR3 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 6 and SEQ ID NO: 20. In some embodiments, the heavy chain of the antibody or the fragment thereof comprises a heavy chain variable region VH, and the heavy chain variable region VH comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 8 and SEQ ID NO: 22. In some embodiments, the heavy chain of the antibody or the fragment thereof comprises a heavy chain constant region, and the heavy chain constant region comprises IgG. In some embodiments, the IgG is selected from the group consisting of: IgG1 and IgG4. In some embodiments, the heavy chain of the antibody comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 27.

[0016] In some embodiments, the antibody comprises a light chain of the antibody or a fragment thereof, the light chain of the antibody or the fragment thereof comprises LCDR1-3, the LCDR1 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 15. In some embodiments, the LCDR2 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 2 and SEQ ID NO: 16. In some embodiments, the LCDR3 comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID

NO: 3 and SEQ ID NO: 17. In some embodiments, the light chain of the antibody or the fragment thereof comprises a light chain variable region VL, and the light chain variable region VL comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 7 and SEQ ID NO: 21. In some embodiments, the light chain of the antibody or the fragment thereof comprises a light chain constant region, and the light chain constant region comprises Igk. In some embodiments, the light chain of the antibody comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 11 and SEQ ID NO: 25.

[0017] In some embodiments, the first binding domain is located at N-terminal of the second binding domain. In some embodiments, the fusion protein further comprises a linker, the linker is located at C-terminal of the first binding domain and located at N-terminal of the second binding domain. In some embodiments, the linker comprises an amino acid sequence as shown in SEQ ID NO: 52.

[0018] In some embodiments, the fusion protein comprises at least two of the second binding domains. In some embodiments, each of the second binding domains is located at C-terminal of the first binding domain respectively.

[0019] In another aspect, the present application provides an immunoconjugate, which comprises the fusion protein.

[0020] In another aspect, the present application provides one or more isolated nucleic acid molecules, which encode the fusion protein or the immunoconjugate.

[0021] In another aspect, the present application provides one or more vectors, which comprise the nucleic acid molecules.

[0022] In another aspect, the present application provides a composition, which comprises the fusion protein, the immunoconjugate, or the nucleic acid molecule, and optionally, pharmaceutically acceptable excipients.

[0023] In another aspect, the present application provides a cell, which comprises the fusion protein, the immunoconjugate, the nucleic acid molecule, or the vector.

[0024] In another aspect, the present application provides a method of preparing the fusion protein, which comprises culturing the cell under a condition enabling the expression of the fusion protein.

[0025] In another aspect, the present application provides a use of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell in the preparation of a medicament, wherein the medicament is used for treating a tumor.

[0026] In some embodiments, the tumor comprises a solid tumor and a non-solid tumor.

[0027] In some embodiments, the solid tumor and the non-solid tumor comprise multiple myeloma, leukemia, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, neuroglioma, germinoma, sarcoma, mesothelioma, placentoma, cerebral cancer, bone cancer, skin cancer, nasopharynx cancer, lung cancer, oral cancer, esophagus cancer, gastric cancer, liver cancer, pancreatic cancer, prostate cancer, intestinal cancer, breast cancer, cervical cancer, ovarian cancer and testicular cancer, frontal sinus tumor, hypopharyngeal cancer, olfactory neuroblastoma, tongue cancer, gingival carcinoma, ampulla

carcinoma, colon cancer, rectal cancer, kidney cancer, ureteral carcinoma, bladder cancer, penile cancer, fallopian tube carcinoma, eyelid cancer, retinoblastoma.

[0028] In another embodiment~~aspect~~, the present application provides the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell, which are used for treating a tumor.

[0029] In another embodiment~~aspect~~, the present application provides a method of blocking the interaction between the PD-L1 protein and PD-1, comprising administering to a subject in need thereof an effective amount of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell.

[0029a] In another aspect, the present application provides a use of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell in the preparation of a medicament, wherein the medicament is to be used for blocking the interaction between PD-L1 protein and PD-1

[0030] In another ~~aspect~~embodiment, the present application provides a method of blocking the interaction between the CD47 protein and SIRP α , comprising administering to a subject in need thereof an effective amount of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell.

[0030a] In another aspect, the present application provides a use of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell in the preparation

of a medicament, wherein said medicament is to be used for blocking the interaction between CD47 protein and SIRP α .

[0031] In another aspect embodiment, the present application provides a method of inhibiting the growth and/or proliferation of tumors o tumor cells, comprising administering to a subject in need thereof an effective amount of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell.

[0032] In another aspect embodiment, the present application provides a method of preventing or treating tumors in a subject, comprising administering to the subject in need thereof an effective amount of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell.

[0033] In another aspect embodiment, the present application provides the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell, which are used for preventing or treating tumors in a subject.

[0034] Other aspects and advantages of the present application can be conceived easily by those skilled in the art from the following detailed description. The following detailed description only shows and describes exemplary embodiments of the present application. As will be recognized by those skilled in the art, the content of the present application enables those skilled in the art to make changes to the disclosed specific embodiments without departing from the spirit and scope of the

invention to which the present application is related. Correspondingly, the attached drawings of the present application and the description of the specification are only exemplary, but not restrictive.

BRIEF DESCRIPTION OF THE DRAWING

[0035] The specific features related to the present application are shown in the accompanying claims.

The characteristics and advantages related to the present application will be better understood with reference to the exemplary embodiments and the attached drawings described in detail below. The attached drawings are illustrated briefly as below:

[0036] FIG. 1 shows an exemplary structure of the fusion protein according to the present application.

[0037] FIGs. 2-3 show the binding ability of the fusion protein to PD-L1 according to the present application.

[0038] FIG. 4 shows the binding ability of the fusion protein to CD47 according to the present application.

[0039] FIG. 5 shows the binding ability of the fusion protein to PD-L1 and CD47 simultaneously according to the present application.

[0040] FIG. 6 shows that the fusion protein of the present application competitively blocks the binding between CD47 and its ligand SIRPα.

[0041] FIGs. 7-8 show that the fusion protein of the present application competitively blocks the binding between PD-1 and PD-L1.

[0042] FIGs. 9-10 show that the growth of tumor volume in a mouse tumor model of human lymphoma is inhibited by use of the fusion protein of the present application.

DETAILED DESCRIPTION

[0043] The implementation of the present application will be illustrated in the following specific embodiments, and other advantages and effects of the present application will be easily known by persons familiar with the technology from the disclosures in the specification.

[0044] In the present application, the term “fusion protein” generally refers to a protein obtained from the fusion of two or more proteins or polypeptides. The fusion protein can be prepared artificially through a recombinant DNA technology. For example, the genes or nucleic acid molecules encoding the two or more proteins or polypeptides can be linked with each other to form a fusion gene or a fused nucleic acid molecule, which can encode the fusion protein. The translation of the fusion genes can produce a single polypeptide, which can possess the properties of at least one, or even each of the two or more proteins or polypeptides before fusion.

[0045] In the present invention, the term “specifically binds” generally refers to the non-random binding reaction between two molecules, such as the reaction between an antibody and an antigen producing the antibody. One antibody specific to a certain antigen means binding to the antigen with an affinity (KD) $\leq 10^{-5}$ M (e.g., 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, etc.), wherein KD refers to the ratio of dissociation rate to binding rate (k_{off}/k_{on}), which can be determined by a method familiar to technicians in the field.

[0046] In the present application, the term “binding domain” generally means a domain that can specifically bind and/or recognize a specific epitope on a target (e.g., an antigen). In the present application, the term “domain” generally refers to a closely spherical structural region that is clearly separated in the subunit structure of a protein. For example, a polypeptide chain firstly can be a regular secondary structure formed from adjacent amino acid residues in some regions, then adjacent secondary structural fragments can be assembled together to form a super-secondary structure; on such a basis, the polypeptide chain can be folded into a tertiary structure that is almost spherical. For larger protein molecules or subunits, the polypeptide chain often may be a tertiary structure that is formed by the association of two or more relatively independent regional structures which can be clearly distinguished in space, and such a relatively independent regional structure can be referred as a domain.

[0047] In the present application, “the first”, “the second” as used in the terms “the first binding domain” and “the second binding domain” are only used for distinction in description.

[0048] In the present application, the term “CD47 protein” generally refers to an integrin-associated protein (IAP), which is a multiple transmembrane receptor belonging to the immunoglobulin superfamily. For example, CD47 protein can bind to membrane integrins, and also bind to their ligands thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRP α). CD47 protein is widely expressed on the surface of cell membrane. In the present application, the CD47 protein may comprise any variants, isotypes and species homologues of a human CD47. The amino acid sequence of the human CD47 protein is listed as CEJ95640.1 in the GenBank. The CD47 protein can be expressed naturally by cells or expressed on cells transfected with CD47 genes.

[0049] In the present application, the term “SIRP α ” generally refers to a regulatory membrane glycoprotein from the SIRP family, which can be used as the ligand of CD47 protein. In the present application, the SIRP α may comprise human SIRP α , for example, SIRP α variant 1 and SIRP α variant 2. The SIRP α variant 2 is different from the SIRP α variant 1 in 13 amino acids, and its amino acid sequence is listed as CAA71403.1 in GenBank. In the present application, the term “SIRP α variant 1” generally refers to a SIRP α protein of which the amino acid sequence is listed as NCBI RefSeq NP_542970.1 (residues 31-504 constitute a mature type), then the amino acid sequence of the SIRP α variant 1 is shown in SEQ ID NO: 29.

[0050] The term “antibody” generally refers to a protein comprising one or more polypeptides essentially encoded by immunoglobulin genes or immunoglobulin gene fragments. For example, immunoglobulin genes may comprise κ , λ , α , γ , δ , ϵ and μ constant region genes, as well as numerous variable region genes of immunoglobulin. For example, the light chain can be classified into κ or λ , which can define the types of immunoglobulin respectively: Ig κ and Ig λ . The heavy chain can be classified into γ , μ , α , δ or ϵ , which, in turn, define the types of immunoglobulin respectively: IgG, IgM, IgA, IgD and IgE. For example, the antibody may have structural units comprising tetramers, each of the tetramers can be composed of two pairs of the same polypeptide chains, and each pair has a “light chain” (about 25 kD) and a “heavy chain” (about 50-70 kD), the N-terminal of each member can define a variable region of about 100 to 110 or more amino acids, which is mainly responsible for the recognition of antigens. For example, the terms “light chain variable region (VL)” and “heavy chain variable region (VH)” generally refer to the variable region of a light chain and a heavy chain

respectively. The antibody may exist as a complete immunoglobulin or as many fully characterized fragments produced by digestion with various peptidases or de novo expression.

[0051] In the present invention, the term “antigen-binding fragment” generally refers to one or more parts of a full-length antibody, in which the parts substantially maintain the capacity of binding the same antigen (e.g., PD-L1) that the antibody binds, and they can compete with the full-length antibody for specifically binding to the antigen. In general, see *Fundamental Immunology*, Ch. 7 (Paul, W., ed., Edition 2, Raven Press, N.Y. (1989), the entire contents of which are incorporated herein by reference. Antigen-binding fragments can be produced by a recombinant DNA technology or through the enzymatic or chemical cleavage of a complete antibody. In some cases, the antigen-binding fragments comprise Fab, Fab', F(ab')₂, (Fab)₂, Fd, Fv, dAb and complementary determining region (CDR) fragments, a single-chain antibody (e.g., scFv), a chimeric antibody, a diabody and a polypeptide that comprises at least a portion of an antibody sufficient to confer specific antigen binding capacity to the polypeptide. Conventional technologies known to technicians in the field (e.g., a recombinant DNA technology or an enzymatic or chemical cleavage process) can be used to obtain the antigen-binding fragments of an antibody from a given antibody, and screen the antigen-binding fragments of the antibody in terms of specificity in the same way as for complete antibodies. For example, pepsin can digest the antibodies in the hinge region below the disulfide bond so as to produce F(ab')₂.

[0052] In the present application, the term “Fab” generally refers to antibody fragments composed of VL, VH, CL and CH1 domains.

[0053] In the present application, the term “Fab” generally refers to antibody fragments with several additional residues at the carboxyl terminal of CH1 domain compared to Fab fragments. For example, Fab' may comprise one or more cysteine coming from the hinge region of the antibody.

[0054] In the present application, the term “F(ab)₂” generally refers to antigen-binding fragments obtained from paired Fab fragments linked with cysteine.

[0055] In the present application, the term “dAb fragments” generally refers to antibody fragments composed of VH domains (Ward et al, Nature 341:544-546 (1989)).

[0056] In the present application, the term “complementary determining regions CDR” generally refers to the 3 hypervariable regions (HVRs) of the light chain variable region (VL) and the heavy chain variable region (VH), and the hypervariable regions are also known as complementary determining regions because these regions may form precise spatial complementarity with antigenic determinants.

[0057] In the present application, the term “Fv fragments ” generally refers to antibody fragments composed of single-armed VL and VH domains of the antibody.

[0058] In the present application, the term “scFv” generally refers to a molecule composed of the heavy chain variable region and the light chain variable region of the antibody linked by a short peptide linker, which is also known as a single-chain antibody.

[0059] In the present application, the term “monoclonal antibody” generally refers to a group of antibodies which are substantially homologous, and various antibodies contained in this group may be

identical except for the possible presence of naturally occurring mutations in trace amounts. The monoclonal antibodies are highly specific, directly with respect to a single antigenic site. In addition, as opposed to a polyclonal antibody preparation that comprises different antibodies with respect to different determinants (epitopes), the modifier “monoclonal” of each monoclonal antibody with respect to the single determinant on the antigen is not interpreted as requiring the production of antibodies by any particular methods. For example, monoclonal antibodies may be prepared by a hybridoma technique or monoclonal antibodies may be produced in bacterial, eukaryotic animal or plant cells by a recombinant DNA process, and can also be obtained from a phage antibody library, for example, using the technology described in Clackson et al., *Nature*, 352:624-628(1991) and Marks et al., *Mol. Biol.*, 222:581-597 (1991).

[0060] In the present application, the term “chimeric antibody” generally refers to such an antibody, wherein a part of each heavy chain or light chain amino acid sequence is homologous to a corresponding amino acid sequence in an antibody from a particular species, or belongs to a particular category, and the remaining segments of the chain are homologous to the corresponding sequences of another species. For example, the variable regions of the light chain and the heavy chain are derived from the variable regions of an antibody in one animal species (e.g., mice, rats, etc.), while the constant part is homologous to the sequence of an antibody from another species (e.g., human). For example, to obtain chimeric antibodies, non-human B cells or hybridoma cells can be utilized to produce variable regions, and the constant regions combined with them are derived from human. The variable regions have the advantage of easy preparation, and their specificity is not affected by the source of

the constant regions combined with them. Meanwhile, because the constant regions of chimeric antibodies may be derived from human, so chimeric antibodies are less likely to elicit an immune response at the time of injection than using antibodies with non-human derived constant regions.

[0061] In the present application, the term “humanized antibody” generally refers to a modified antibody which reduces the immunogenicity of an antibody, an immunoglobulin binding protein and a polypeptide derived from non-human species (e.g., mice or rats) to humans, and still retain the antigen-binding properties of the original antibody. For example, the humanized antibody can be prepared by the genetic engineering technology, and non-human binding domains can be humanized using CDR grafting (Jones et al., *Nature* 321:522 (1986)) and the variant thereof by technical means including reshaping (Verhoeyen, et al., 1988 *Science* 239:1534-1536; Riechmann, et al., 1988 *Nature* 332:323-337; Tempest, et al., *Bio/Technol* 1991 9:266-271), hyperchimerization (Queen, et al., 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, et al., 1991 *Proc Natl Acad Sci USA* 88:2869-2873; Co, et al., 1992 *J Immunol* 148:1149-1154) and veneering (Mark, et al., “Derivation of therapeutically active humanized and veneered anti-CD18 antibodies.” In: Metcalf B W, Dalton B J, eds. *Cellular adhesion: molecular definition to therapeutic potential*. New York: Plenum Press, 1994: 291-312). If other regions, for example, the hinge region and the constant region domains, are also derived from non-human sources, these regions may also be humanized.

[0062] In the present application, the term “fully human antibody” generally refers to an antibody obtained by expressing genes encoding human antibody in genetically engineered animal with antibody gene deletion. For example, by means of a transgenic or trans-chromosomal technology, all

the genes that encode human antibody can be transferred into genetically engineered animal with antibody gene deletion so that the animal can express the human antibody.

[0063] The protein, polypeptide and/or amino acid sequence referred in the present application should also be further understood to comprise the following range: variants or homologs having the same or similar functions as the protein or polypeptide.

[0064] In the present application, the variant may be a protein or a polypeptide with substitution, deletion or addition of one or more amino acids in the amino acid sequence of the protein and/or the polypeptide (e.g., an antibody or an fragment thereof that specifically binds the PD-L1 protein). For example, the functional variant may comprise a protein or a polypeptide with amino acid modifications by at least one, for example 1-30, 1-20 or 1-10, further for example 1, 2, 3, 4 or 5 amino acid substitution, deletion and/or insertion. The functional variant can essentially maintain the biological characteristics of the protein or the polypeptide before modification (e.g., substitution, deletion or addition). For example, the functional variant can maintain at least 60%, 70%, 80%, 90%, or 100% of the biological characteristics of the protein or the polypeptide before modification (e.g., antigen binding capacity). For example, the substitution may be conservative substitution.

[0065] In the present application, the homolog may be a protein or a polypeptide that has at least about 85% (e.g., having at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or higher) of sequence homology with the amino acid sequence of the protein and/or the polypeptide (e.g., the antibody or a fragment thereof that specifically binds the PD-L1 protein).

[0066] In the present application, the homology generally refers to the comparability, similarity, or relevance between two or more sequences. The “sequence homology percentage” can be calculated by the following means: two sequences to be compared are compared in a comparison window to determine the number of positions of the same nucleic acid bases (e.g., A, T, C, G, I) or the same amino acid residues (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) contained in the two sequences so as to get the number of matched positions, which is divided by the total number of positions in the comparison window (i.e., the window size), and then multiplied by 100 to get the sequence homology percentage. The comparison for determining the sequence homology percentage can be achieved through various ways known in the art, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Technicians in this field can determine parameters appropriate for aligning sequences, including any algorithms required for realizing the maximum alignment in the range of the full-length sequence being compared or in the target sequence region. The homology can also be determined by the following methods: FASTA and BLAST. The description of FASTA algorithm can be seen in Improved Tool for Comparison of Biological Sequences to W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci.*, 85: 2444-2448, 1988; and Fast and Sensitive Protein Comparability Search to D. J. Lipman and W. R. Pearson, *Science*, 227: 1435-1441, 1989. The description of BLAST algorithm can be seen in A Basic Search Tool for Local Alignment to S. Altschul, W. Gish, W. Miller, E. W. Myers and D. Lipman, *Journal of Molecular Biology*, 215: 403-410, 1990.

[0067] In the present application, the term “PD-L1” generally refers to the ligand of programmed death-1 (PD-1) protein, which is also known as CD274, B7-H or B7H1. PD-1 negatively modulates the signaling of a T cell antigen receptor by interacting with the specific ligand (PD-L). The PD-L1 may be the prognostic indicator of various tumors. In the present application, the PD-L1 may be a human PD-L1. The Gene ID of the human PD-L1 in GenBank is 29126.

[0068] In the present application, the term “PD-1” generally refers to a member in the synuclein family, which can also be known as NACP, PARK1 or PARK4. In the present application, the PD1 may be a human PD1. The Gene ID of the human PD-L1 in GenBank is 6622.

[0069] Generally, in a polypeptide chain, an amino group is linked with another carboxyl group in the polypeptide chain so as to become one chain, but at the two terminals of the protein, there remain amino acid residues that do not form peptide bonds respectively, which are a polypeptide chain terminal carrying free amino groups and a polypeptide chain terminal carrying carboxyl groups respectively. In the present application, the term “N-terminal” generally refers to the polypeptide chain terminal with an amino acid residue carrying free amino groups. In the present application, the term “C-terminal” generally refers to the polypeptide chain terminal with an amino acid residue carrying free carboxyl groups.

[0070] In the present application, the term “nucleic acid molecule” generally refers to nucleotide, deoxyribonucleotide or ribonucleotide or an analogue thereof in isolated forms of any length which is isolated from natural environment or synthesized artificially.

[0071] In the present application, the term “immunoconjugate” generally refers to a polypeptide molecule with immune function in which one or more heterogenous molecules (including, but not limited to, cytotoxin) are conjugated. In the present application, “conjugate” and “link”, “fusion” can be used interchangeably in the present application, and generally refers to that two or more chemical elements, sequences or components are linked together, for example by means including chemical conjugation or recombination. The heterogenous molecule may be a cytotoxin, a chemotherapeutic agent, etc. For example, the fusion protein of the present application can be conjugated with one or more heterogenous molecules (e.g., cytotoxin) to get the immunoconjugate.

[0072] In the present invention, the term “vector” refers to a nucleic acid delivery vehicle into which a polynucleotide encoding a certain protein can be inserted so that the protein can be expressed. The vector makes the genetic material element it carries be expressed in a host cell through transforming, transducing or transfecting the host cell. For example, the vector comprises: plasmid; phagemid; cosmid; artificial chromosomes, such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1-derived artificial chromosome (PAC); phages, such as λ phage or M13 phage; and animal viruses and the like. The varieties of animal viruses used as the vector comprise retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpes virus (e.g., herpes simplex virus), poxvirus, baculovirus, papilloma virus, papovavirus (e.g., SV40). A vector may contain various elements for controlling the expression, including a promoter sequence, a transcription initiation sequence, an enhancer sequence, a selection element and a reporter gene. In addition, the vector may

also contain replication origins. The vector could also comprise a component that help it get into the cell, such as a virion, a liposome or a protein coat, but not just these substances.

[0073] In the present application, the term “tumor” generally refers to neoplasms formed from the proliferation of local histocytes in the organisms of mammals (e.g., cells or parts thereof) under the action of various tumorigenic factors. In the present application, the tumor may comprise a solid tumor and a non-solid tumor. The solid tumor may comprise neuroglioma, germinoma, sarcoma, mesothelioma, placentoma, cerebral cancer, bone cancer, skin cancer, nasopharynx cancer, lung cancer, oral cancer, esophagus cancer, gastric cancer, liver cancer, pancreatic cancer, prostate cancer, intestinal cancer, breast cancer, cervical cancer, ovarian cancer and testicular cancer. In the present application, the non-solid tumor may comprise multiple myeloma, leukemia, Non-Hodgkin lymphoma, Hodgkin’s lymphoma.

[0074] In the present application, the term “comprise” generally means including definitely specified features, but not excluding other factors.

[0075] In the present application, the term “about” generally refers to variations in a range of 0.5%-10% above or below a specified value, for example, variations in a range of 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10% above or below a specified value.

[0076] Fusion protein

[0077] In one aspect, the present application provides a fusion protein, the fusion protein may comprise a first binding domain and a second binding domain. The first binding domain can specifically bind PD-L1; the second binding domain can specifically bind a CD47 protein, the second binding domain may comprise a mutant of a human SIRP α variant 1, and the mutant comprises substitution, deletion or addition of amino acid residues at one or more (e.g., 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10 or more) positions from site 33 to site 149 compared to the sequence as shown in SEQ ID NO: 29. The fusion protein of the present application can specifically bind both tumor-associated antigen and CD47 protein, thereby playing a role in the treatment of tumors and/or autoimmune diseases.

[0078] In the present application, the term “the first binding domain” generally refers to a domain that can specifically bind PD-L1. The term “the second binding domain” generally refers to a domain that can specifically bind a CD47 protein.

[0079] The second binding domain that specifically binds CD47

[0080] In the present application, the mutant (e.g., a mutant of a human SIRP α variant 1 that specifically binds a CD47 protein) comprises amino acid substitutions at one or more (e.g., 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10 or more) amino acid residues selected from the group consisting of: I61, V63, E77, Q82, K83, E84, V93, D95, L96, K98, N100, R107, G109 and V132.

[0081] In the present application, the positions of amino acid residues in the amino acid substitution mean the numbers of the residues determined based on the amino acid sequence as shown in SEQ ID NO: 29.

[0082] In the present application, “amino acid substitution X_n” means that an amino acid substitution occurs at the residue X of site n in the corresponding amino acid sequence as shown in SEQ ID NO: 29, in which n is a positive integer, X is the abbreviation of any one amino acid residue. For example, “amino acid substitution I61” indicates that an amino acid substitution occurs at the residue I of site 61 in the corresponding amino acid sequence as shown in SEQ ID NO: 29.

[0083] In the present application, the amino acid substitutions may be nonconservative substitutions. The nonconservative substitutions may comprise changing amino acid residues in target proteins or polypeptides in a nonconservative form, for example, changing an amino acid residue with a certain side chain size or a certain property (e.g., hydrophilic) into another amino acid residue with a different side chain size or a different property (e.g., hydrophobic).

[0084] In the present application, the amino acid substitutions may also be conservative substitutions. The conservative substitutions may comprise changing amino acid residues in target proteins or polypeptides in a conservative form, for example, changing an amino acid residue with a certain side chain size or a certain property (e.g., hydrophilic) into another amino acid residue with the same or similar side chain size or the same or similar property (e.g., still hydrophilic). Such conservative substitutions generally do not greatly affect the structure or function of the resulting protein. In the present application, the amino acid sequence variant of the fusion protein or a fragment thereof may

comprise conservative amino acid substitutions which do not significantly change the structure or function of the protein (e.g., a mutant of a human SIRP α variant 1 that blocks CD47 and specifically binds a CD47 protein).

[0085] As an example, the mutual substitutions of various amino acids in each group of the following groups can be considered as conservative substitutions in the present application: a group of amino acids with nonpolar side chains: alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine; a group of uncharged amino acids with polar side chains: glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine; a group of negatively charged amino acids with polar side chains: aspartic acid and glutamic acid; positively charged basic amino acids: lysine, arginine and histidine; and amino acids carrying phenyl groups: phenylalanine, tryptophan and tyrosine.

[0086] In the present application, the mutant may comprise amino acid substitutions at amino acid residues selected from the group consisting of: (1) I61, V63, E77, E84, V93, L96, K98, N100 and V132; (2) I61, E77, Q82, K83 and E84; (3) I61, V63, K83, E84 and V132; (4) I61, E77, E84, R107 and V132; (5) I61, V63, E77, K83, E84 and N100; (6) I61, E77, Q82, K83, E84 and R107; (7) I61, E77, Q82, E84, V93, L96, N100, R107, G109 and V132; (8) I61, E77, Q82, K83, E84 and V132; (9) I61; (10) I61, D95, L96, G109 and V132; (11) I61, D95, L96, K98, G109 and V132; (12) I61, E77, E84, V93, R107 and V132; (13) E77, L96, N100, G109 and V132; (14) I61, V63, Q82, E84, D95, L96, N100 and V132; (15) I61, E77, Q82, K83, E84, V93, D95, L96, K98, N100 and V132; (16) I61, E77, Q82, K83, E84 and V93; (17) I61, V63, E77, K83, E84, D95, L96, K98 and N100; (18) I61, V63,

E77, K83, D95, L96, K98, N100 and G109; (19) I61, E77, Q82, E84, V93, D95, L96, K98 and N100; and (20) I61, V63, E77, Q82 and E84.

[0087] In the present application, the mutant may comprise one or more (e.g., 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10 or more) amino acid substitutions selected from the group consisting of: I61L/V/F, V63I, E77I/N/Q/K/H/M/R/N/V/L, Q82S/R/G/N, K83R, E84K/H/D/R/G, V93L/A, D95H/R/E, L96S/T, K98R, N100G/K/D/E, R107N/S, G109R/H and V132L/R/I/S.

[0088] In the present application, the amino acid substitution “X_nY/Z” means that the residue X of site n in the corresponding amino acid sequence as shown in SEQ ID NO: 29 is substituted by an amino acid residue Y or an amino acid residue Z, in which n is a positive integer, X, Y and Z are the abbreviations of any amino acid residues independently, and X is different from Y or Z. For example, the amino acid substitution “I61L/V/F” means that the residue I of site 61 in the corresponding amino acid sequence as shown in SEQ ID NO: 29 is substituted by an amino acid residue L, V or F.

[0089] In the present application, the mutant may comprise amino acid substitutions selected from the group consisting of: (1) I61L, V63I, E77I, E84K, V93L, L96S, K98R, N100G and V132L; (2) I61V, E77N, Q82S, K83R and E84H; (3) I61F, V63I, K83R, E84K and V132I; (4) I61L, E77Q, E84D, R107N and V132I; (5) I61L, V63I, E77K, K83R, E84D and N100G; (6) I61V, E77H, Q82R, K83R, E84H and R107S; (7) I61L, E77I, Q82G, E84R, V93L, L96T, N100G, R107S, G109R and V132R; (8) I61L, E77M, Q82G, K83R, E84D and V132L; (9) I61L; (10) I61F, D95H, L96S, G109H and V132S; (11) I61F, D95H, L96S, K98R, G109H and V132S; (12) I61L, E77Q, E84D, V93A, R107N and V132I; (13) E77K, L96S, N100K, G109H and V132L; (14) I61L, V63I, Q82G, E84G, D95R,

L96S, N100D and V132I; (15) I61L, E77R, Q82N, K83R, E84G, V93L, D95E, L96T, K98R, N100D and V132L; (16) I61V, E77N, Q82S, K83R, E84H and V93A; (17) I61V, V63I, E77V, K83R, E84D, D95E, L96T, K98R and N100E; (18) I61L, V63I, E77V, K83R, D95E, L96S, K98R, N100D and G109R; (19) I61V, E77L, Q82G, E84G, V93L, D95E, L96T, K98R and N100G; and (20) I61L, V63I, E77N, Q82G and E84G.

[0090] In the present application, based on the human SIRP α variant 1 (the amino acid sequence as shown in SEQ ID NO: 29, i.e., residues at site 33 to site 149 in the amino acid sequence of a human SIRP α), the mutants of the SIRP α variant 1 which comprise the amino acid substitutions of the above groups (1)-(20) respectively are named as M1, M5, M12, M35, M37, M41, M57, M67, M81, M82, M84, M91, M99, M102, M111, M122, M126, M130, M135 and M145 successively. The mutants of the SIRP α variant 1 can successively comprise the amino acid sequences as shown in any one of SEQ ID NOs: 30-49.

[0091] In some embodiments, the mutant of the SIRP α variant 1 is M91, and the mutant of the SIRP α variant 1 comprises the amino acid sequence as shown in SEQ ID NO: 41.

[0092] The first binding domain that specifically binds PD-L1

[0093] In the present application, the first binding domain may comprise an antibody or an antigen-binding fragment or a variant thereof. For example, the antibody may be selected from the group consisting of: a monoclonal antibody, a single-chain antibody, a chimeric antibody, a humanized antibody and a fully human antibody. For example, the antigen-binding fragment is selected from the

group consisting of: Fab, Fab', (Fab')₂, F(ab)₂, dAb, an isolated complementary determining region CDR, Fv and scFv.

[0094] The antibody or the antigen-binding fragment thereof of the present application may kill tumor cells and/or inhibit the tumor growth by specifically binding the PD-L1 protein. For example, the tumor may comprise a PD-L1 positive tumor. For example, the PD-L1 positive tumor may be selected from the group consisting of: gastric cancer, breast cancer, cervical cancer, lung cancer, head and neck tumor, melanoma, glioma, lymphoepithelioma, esophagus cancer or colorectal cancer. In the present application, the antibody and the antigen-binding fragment thereof can kill gastric cancer, breast cancer, cervical cancer, lung cancer, head and neck tumor, melanoma, glioma, lymphoepithelioma, esophagus cancer or colorectal cancer cells or inhibit the growth of gastric cancer, breast cancer, cervical cancer, lung cancer, head and neck tumor, melanoma, glioma, lymphoepithelioma, esophagus cancer or colorectal cancer cells.

[0095] The PD-L1 protein of the present application may be a human PD-L1 protein or a functional fragment thereof. For example, the PD-L1 protein may not be a mouse PD-L1 protein, or may not be a rat PD-L1 protein. In some embodiments, the antibody or the antigen-binding fragment thereof in the present application substantially does not bind the mouse PD-L1 protein or the rat PD-L1 protein.

[0096] The antibody or the antigen-binding fragment thereof in the present application can compete with the reference antibody to bind the PD-L1 protein. The reference antibody may comprise a light chain variable region and a heavy chain variable region. For example, the light chain variable region of the reference antibody may comprise LCDR1-3, the LCDR1 may comprise an amino acid sequence

as shown in any one of the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 15; the LCDR2 may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 2 and SEQ ID NO: 16; and the LCDR3 may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 3 and SEQ ID NO: 17. The heavy chain variable region of the reference antibody may comprise HCDR1-3, the HCDR1 may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 4 and SEQ ID NO: 18; the HCDR2 may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 5 and SEQ ID NO: 19; and the HCDR3 may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 6 and SEQ ID NO: 20.

[0097] For example, the amino acid sequence of the light chain variable region of the reference antibody may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 7 and SEQ ID NO: 21, and the amino acid sequence of the heavy chain variable region of the reference antibody may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 8 and SEQ ID NO: 22. Further for example, the light chain of the reference antibody may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 11 and SEQ ID NO: 25; and the heavy chain of the reference antibody may comprise an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 27. For example, the light chain of the reference antibody may comprise an amino acid sequence as shown in SEQ ID NO: 11, and the heavy chain of the reference antibody may comprise an amino acid sequence as shown in SEQ ID NO: 13. For example, the light chain of the reference antibody may comprise an

amino acid sequence as shown in SEQ ID NO: 25, and the heavy chain of the reference antibody may comprise an amino acid sequence as shown in SEQ ID NO: 27.

[0098] The antibody or the antigen-binding fragment thereof in the present application may comprise a light chain of the antibody or a fragment thereof. For example, the light chain of the antibody or the fragment thereof may comprise a Igk constant region, e.g., it may comprise a human Igk constant region.

[0099] For example, the light chain of the antibody or the fragment thereof may comprise LCDR1, and the LCDR1 may comprise an amino acid sequence as below: SEQ ID NO: 1. The light chain of the antibody or the fragment thereof may comprise LCDR2, and the LCDR2 may comprise an amino acid sequence as below: SEQ ID NO: 2. The light chain of the antibody or the fragment thereof may comprise LCDR3, and the LCDR3 may comprise an amino acid sequence as below: SEQ ID NO: 3. Further for example, the light chain of the antibody or the fragment thereof may comprise LCDR1, and the LCDR1 may comprise an amino acid sequence as below: SEQ ID NO: 15. The light chain of the antibody or the fragment thereof may comprise LCDR2, and the LCDR2 may comprise an amino acid sequence as below: SEQ ID NO: 16. The light chain of the antibody or the fragment thereof may comprise LCDR3, and the LCDR3 may comprise an amino acid sequence as below: SEQ ID NO: 17.

[0100] The light chain of the antibody or the fragment thereof in the present application may comprise a light chain variable region VL, and the amino acid sequence of the light chain variable region VL may be: SEQ ID NO: 7. In some embodiments, the amino acid sequence of the light chain of the antibody or the fragment thereof may be: SEQ ID NO: 11. Further for example, the amino acid

sequence of the light chain variable region VL may be: SEQ ID NO: 21. In some embodiments, the amino acid sequence of the light chain of the antibody or the fragment thereof may be: SEQ ID NO: 25.

[0101] The antibody or the antigen-binding fragment thereof in the present application may comprise a heavy chain of the antibody or a fragment thereof. For example, the heavy chain of the antibody or the fragment thereof further comprises a human constant region. Wherein, the human constant region may comprise a human IgG constant region. Wherein, the IgG constant region may comprise a human IgG1 constant region or IgG4.

[0102] For example, the heavy chain of the antibody or the fragment thereof may comprise HCDR1, and the HCDR1 may comprise an amino acid sequence as below: SEQ ID NO: 4. The heavy chain of the antibody or the fragment thereof may comprise HCDR2, and the HCDR2 may comprise an amino acid sequence as below: SEQ ID NO 5. The heavy chain of the antibody or the fragment thereof may comprise HCDR3, and the HCDR3 may comprise an amino acid sequence as below: SEQ ID NO: 6. Further for example, the heavy chain of the antibody or the fragment thereof may comprise HCDR1, and the HCDR1 may comprise an amino acid sequence as below: SEQ ID NO: 18. The heavy chain of the antibody or the fragment thereof may comprise HCDR2, and the HCDR2 may comprise an amino acid sequence as below: SEQ ID NO 19. The heavy chain of the antibody or the fragment thereof may comprise HCDR3, and the HCDR3 may comprise an amino acid sequence as below: SEQ ID NO: 20.

[0103] The heavy chain of the antibody or the fragment thereof may comprise a heavy chain variable region VH, and the heavy chain variable region VH may comprise an amino acid sequence as below: SEQ ID NO: 8. In some embodiments, the heavy chain of the antibody may comprise an amino acid sequence as below: SEQ ID NO: 13. Further for example, the heavy chain variable region VH may comprise an amino acid sequence as below: SEQ ID NO: 22. In some embodiments, the heavy chain of the antibody may comprise an amino acid sequence as below: SEQ ID NO: 27.

[0104] In some embodiments, the amino acid sequence of the light chain of the antibody or the antigen-binding fragment thereof in the present application comprises SEQ ID NO: 11; and the amino acid sequence of its heavy chain comprises SEQ ID NO: 13; or the amino acid sequence of the light chain of the antibody or the antigen-binding fragment thereof in the present application comprises SEQ ID NO: 25; and the amino acid sequence of its heavy chain comprises SEQ ID NO: 27.

[0105] In some embodiments, the amino acid sequence of LCDR1 in the antibody or the antigen-binding fragment thereof of the present application may comprise SEQ ID NO: 1; the amino acid sequence of LCDR2 may comprise SEQ ID NO: 2; the amino acid sequence of LCDR3 may comprise SEQ ID NO: 3; and the amino acid sequence of HCDR1 may comprise SEQ ID NO: 4; or the amino acid sequence of HCDR2 may comprise SEQ ID NO: 5; the amino acid sequence of HCDR3 may comprise SEQ ID NO: 6. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1201 or an antibody having the same LCDR1-3 and HCDR1-3 as those in the antibody SG1201. In some embodiments, the light chain of the antibody or the antigen-binding fragment thereof in the present application may comprise a light chain variable region, and the amino

acid sequence of the light chain variable region may comprise SEQ ID NO: 7; and its heavy chain may comprise a heavy chain variable region, and the amino acid sequence of the heavy chain variable region may comprise SEQ ID NO: 8. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1201 or an antibody having the same light chain variable region and heavy chain variable region as those in the antibody SG1201. In some embodiments, the antibody or the antigen-binding fragment thereof of the present application may comprise a light chain and a heavy chain, the amino acid sequence of the light chain is shown in SEQ ID NO: 11 and the amino acid sequence of the heavy chain is shown in SEQ ID NO: 13. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1201 or have the same light chain and heavy chain amino acid sequences as those in the antibody SG1201.

[0106] In some embodiments, the antibody of the present application may be SG1201. The amino acid sequences of LCDR1-3 in the antibody SG1201 are shown in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3 respectively; the amino acid sequence of VL is shown in SEQ ID NO: 7; the amino acid sequence of the light chain is shown in SEQ ID NO: 11; the amino acid sequences of HCDR1-3 are shown in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 respectively; the amino acid sequence of VH is shown in SEQ ID NO: 8; and the amino acid sequence of the heavy chain is shown in SEQ ID NO: 13.

[0107] In some embodiments, the amino acid sequence of LCDR1 in the antibody or the antigen-binding fragment thereof of the present application may comprise SEQ ID NO: 15; the amino acid sequence of LCDR2 may comprise SEQ ID NO: 16; the amino acid sequence of LCDR3 may comprise

SEQ ID NO: 17; and the amino acid sequence of HCDR1 may comprise SEQ ID NO: 18; the amino acid sequence of HCDR2 may comprise SEQ ID NO: 19; the amino acid sequence of HCDR3 may comprise SEQ ID NO: 20. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1202 or an antibody having the same LCDR1-3 and HCDR1-3 as those in the antibody SG1202. In some embodiments, the light chain of the antibody or the antigen-binding fragment thereof in the present application may comprise a light chain variable region, and the amino acid sequence of the light chain variable region may comprise SEQ ID NO: 21; and its heavy chain may comprise a heavy chain variable region, the amino acid sequence of the heavy chain variable region may comprise SEQ ID NO: 22. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1202 or an antibody having the same light chain variable region and heavy chain variable region as those in the antibody SG1202. In some embodiments, the antibody or the antigen-binding fragment thereof in the present application may comprise a light chain and a heavy chain, the amino acid sequence of the light chain is shown in SEQ ID NO: 25 and the amino acid sequence of the heavy chain is shown in SEQ ID NO: 27. For example, the antibody or the antigen-binding fragment thereof may comprise an antibody SG1202 or have the same light chain and heavy chain amino acid sequences as those in the antibody SG1202.

[0108] In some embodiments, the antibody of the present application may be SG1202. The amino acid sequences of LCDR1-3 of the antibody SG1202 are shown in SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17, respectively; the amino acid sequence of VL is shown in SEQ ID NO: 21; the amino acid sequences of HCDR1-3 are shown in SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20,

respectively; the amino acid sequence of VH is shown in SEQ ID NO: 22; the amino acid sequence of the light chain is shown in SEQ ID NO: 25; and the amino acid sequence of the heavy chain is shown in SEQ ID NO: 27.

[0109] The antibody or the antigen-binding fragment thereof in the present application may also comprise one or more random mutations (e.g., one or more, e.g. one or several amino acid substitutions) in the amino acid sequence of the light chain and/or the heavy chain of SG1201 and/or SG1202. For example, the antibody or the antigen-binding fragment thereof may comprise one or more random mutations (e.g., one or more, e.g. one or several amino acid substitutions) at one or more sites of framework regions L-FR1-4 in the light chain variable region of SG1201 and/or SG1202, and/or comprise one or more random mutations (e.g., one or more, e.g. one or several amino acid substitutions) at one or more sites of framework regions H-FR1-4 in the heavy chain variable region of SG1201 and/or SG1202.

[0110] The linkage between the first binding domain and the second binding domain

[0111] In the present application, the first binding domain may be located at the N-terminal of the second binding domain. For example, the C-terminal of the first binding domain may be linked to the N-terminal of the second binding domain indirectly through a linker. In some instances, the C-terminal of the first binding domain may also be linked to the N-terminal of the second binding domain directly (e.g., in-frame).

[0112] In the present application, the fusion protein may also comprise a linker, which may be located at the C-terminal of the first binding domain and located at the N-terminal of the second binding domain. For example, in the fusion protein, the C-terminal of the first binding domain may be linked to the N-terminal of the linker, and the C-terminal of the linker may be linked to the N-terminal of the second binding domain. For example, the first binding domain, the linker and the second binding domain can be comprised in the fusion protein successively from N-terminal to C-terminal.

[0113] In the present application, the linker may comprise an amino acid sequence as shown in SEQ ID NO:52.

[0114] In some instances, the fusion protein may comprise at least 2 (e.g., at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or more) of the second binding domain. In the present application, each of the second binding domains can be located at the C-terminal of the first binding domain respectively. In the present application, the more than two second binding domains can be linked to the C-terminal of the first binding domain directly or indirectly.

[0115] In the present application, the fusion protein may comprise a first binding domain that specifically binds PD-L1, and a second binding domain that specifically binds a CD47 protein, in which the second binding domain may comprise a mutant of a human SIRP α variant 1, the C-terminal of the antibody that specifically binds PD-L1 or the antigen-binding fragment or the variant thereof can be directly or indirectly linked to the N-terminal of the mutant of the human SIRP α variant 1. For example, the second binding domain may comprise at least two mutants of the human SIRP α variant 1, and the N-terminals of the two mutants of the human SIRP α variant 1 are linked to the C-terminals

of the antibody that specifically binds PD-L1 or the antigen-binding fragment or the variant thereof, respectively.

[0116] For example, as shown in FIG. 1, the first binding domain of the fusion protein (SG12473) may comprise SG1201, and the second binding domain thereof may comprise two mutants M91 of the SIRP α variant 1, the sequence of the used linker 1 is shown in SEQ ID NO: 52, the N-terminals of the two M91 are linked to the C-terminals of two heavy chains of SG1201 through the linker 1 respectively. In the fusion protein, M91 is linked to the C-terminal of the heavy chain of SG1201 to get the second polypeptide chain, and the light chain of SG1201 may be named as the first polypeptide chain. The amino acid sequences of the second polypeptide chain and the first polypeptide chain of SG12473 are shown in SEQ ID NO: 53 and SEQ ID NO: 11 respectively.

[0117] For example, as shown in FIG. 1, the first binding domain of the fusion protein (SG12474) may comprise SG1202, the second binding domain thereof may comprise two mutants M91 of the SIRP α variant 1, the sequence of the used linker 1 is shown in SEQ ID NO: 52, the N-terminals of the two M91 are linked to the C-terminals of two heavy chains of SG1202 through the linker 1 respectively. In the fusion protein, M91 is linked to the C-terminal of the heavy chain of SG1202 to get the second polypeptide chain, and the light chain of SG1202 may be named as the first polypeptide chain. The amino acid sequences of the second polypeptide chain and the first polypeptide chain of SG12474 are shown in SEQ ID NO: 54 and SEQ ID NO: 25 respectively.

[0118] Nucleic acid molecule, vector and cell as well as preparation method

[0119] In another aspect, the present application provides one or more isolated nucleic acid molecules, which encode the fusion protein or the immunoconjugate. For example, each nucleic acid molecule of the one or more nucleic acid molecules may encode the whole antibody or the antigen-binding fragment thereof, and may also encode a part thereof (e.g., one or more of HCDR1-3, LCDR1-3, VL, VH, light chains or heavy chains).

[0120] The nucleic acid molecule of the present application may be isolated. For example, they can be produced or synthesized by the processes below: (i) amplification in vitro, for example being produced by amplification through polymerase chain reaction (PCR), (ii) being produced by cloning recombination, (iii) being purified, for example fractioned by enzymatic digestion and gel electrophoresis, or, (iv) being synthesized, for example through chemical synthesis. In some embodiments, the isolated nucleic acid is a nucleic acid molecule prepared by a recombinant DNA technology.

[0121] Recombinant DNA and molecular cloning techniques comprise those described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, (1989) (Maniatis) and by T. J. Silhavy, M. L. Bannan and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) as well as by Ausubel, F. M. et al, *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987). In brief, the nucleic acids can be prepared from genomic DNA fragments, cDNA and RNA, and all of these nucleic acids can be extracted from cells

directly or produced by recombination through various amplification methods (including, but not limited to, PCR and RT-PCR).

[0122] The direct chemical synthesis of nucleic acids generally involves sequentially adding 3'-capped and 5'-capped nucleotide monomers into the terminal 5'-hydroxyl of the growing nucleotide polymer chain, in which each addition is realized by nucleophilic attacking the terminal 5'-hydroxyl of the growth chain at 3'-position of the added monomers, and the monomers are generally phosphorus derivatives, such as phosphotriester, phosphoramidite, etc. See, for example, Matteuci et al, Tet. Lett. 521:719 (1980); US Patent No. 4,500,707 to Caruthers et al; and US Patent Nos. 5,436,327 and 5,700,637 to Southern et al. In another aspect, the present application provides a vector comprising the isolated polynucleotide of the present application. The vector may be any linear nucleic acid, plasmid, phagemid, cosmid, RNA vector, viral vector, and the like. Non-limiting examples of the viral vector may comprise a retrovirus, an adenovirus and an adeno-associated virus. In some embodiments, the vector is an expression vector, for example, a phage display vector.

[0123] In another aspect, the present application provides one or more vectors including the nucleic acid molecules. For example, the vector may comprise the one or more nucleic acid molecules of the present application. Each vector may comprise one or more of the nucleic acid molecules. In addition, the vector may further comprise other genes, such as marker genes which allow to select the vector in appropriate host cells and under appropriate conditions. In addition, the vector may further comprise an expression control element allowing the correct expression of the coding regions in an appropriate host. Such a control element is well known to those of ordinary skills in the art. For example, it may

comprise promoters, ribosome bind sites, enhancers and other control elements for regulating gene transcription or mRNA translation, and the like. In some embodiments, the expression control sequences are tunable elements. The specific structures of the expression control sequences may change depending on the species or the functions of cell types, but generally comprise 5'-non-transcribed sequences and 5' and 3'-non-translated sequences which participate in the initiation of transcription and translation respectively, e.g., TATA cassettes, capped sequences, CAAT sequences, etc. For example, 5'-non-transcribed expression control sequence may comprise a promoter region, which may comprise a promoter sequence for transcribing and controlling the functionally linked nucleic acids. The expression control sequence may further include an enhancer sequence or an upstream activator sequence. In the present application, suitable promoters may comprise, for example, promoters for SP6, T3 and T7 polymerases, a human U6RNA promoter, a CMV promoter and an artificial hybrid promoter (e.g., CMV), in which a certain part of the promoter may be fused with a certain part of the gene promoter of other cell proteins (e.g., human GAPDH, glyceraldehyde-3-phosphate dehydrogenase), and it may comprise or not comprise additional introns. The one or more nucleic acid molecules of the present application can be linked with the expression control elements operably.

[0124] The vector may comprise, for example, a plasmid, a cosmid, a virus, a phage or other vectors commonly used in genetic engineering for example. In some embodiments, the vector may be an expression vector.

[0125] The vector can also contain one or more selective marker genes, which, after the expression, can confer one or more phenotypic traits that can be used to select or identify host cells carrying the vector in other ways. Non-limiting examples of suitable selective markers for eukaryocytes comprise dihydrofolate reductase and neomycin resistance.

[0126] In another aspect, the present application provides a cell, which comprises the fusion protein, the immunoconjugate, the nucleic acid molecule, or the vector. The cell may be a host cell. For example, the cell may comprise various cell types as below: prokaryotic cells such as *Escherichia coli* or *Bacillus subtilis*, fungal cells such as yeast cells or *Aspergillus*, insect cells such as S2 *Drosophila* cells or Sf9, or animal cells such as fibroblasts, CHO cells, COS cells, NSO cells, HeLa cells, BHK cells, HEK 293 cells or human cells.

[0127] For example, the vector can be introduced into the host cell stably or transiently through a variety of established technologies. For example, one method involves calcium chloride treatment, in which the vector is introduced through calcium precipitation. Other salts can also be used following similar methods, for example calcium phosphate. In addition, electroporation (i. e., applying electric current to increase the permeability of cells to nucleic acids) can be used. Other examples of transformation methods comprise microinjection, DEAE dextran-mediated transformation and heat shock in the presence of lithium acetate. Lipid complexes, liposomes and dendrimers can also be used to transfect host cells.

[0128] When heterogenous sequences are introduced into a host cell, a variety of methods can be applied to identify host cells into which the vector has been introduced. One exemplary selection

method comprises subculturing a single cell to form a single colony, then testing the expression of the desired protein product. Another method requires the selection of the host cell containing a heterogenous sequence based on the phenotypic traits conferred by the expression of selective marker genes included within the vector.

[0129] For example, the introduction of various heterogenous sequences of the present application into the host cell can be confirmed by the following methods such as PCR, Southern blotting or Northern blotting hybridization. For example, nucleic acids can be prepared from the obtained host cell, and specific target sequences can be amplified by PCR using primers that are specific to the target sequences. The amplified products are subjected to agarose gel electrophoresis, polyacrylamide gel electrophoresis or capillary electrophoresis, then stained with ethidium bromide, SYBR Green solution or the like, or detected for DNA by means of UV detection. Alternatively, nucleic acid probes that are specific to the target sequences can be used in hybridization reactions. The expression of specific gene sequences can be determined by hybridization with PCR or Northern blotting or by the reverse transcription detection of corresponding mRNA using immunoassays of antibodies that react with the encoded gene products. Exemplary immunoassays comprise, but not limited to, ELISA, radioimmunoassay and sandwich immunoassay.

[0130] In addition, the introduction of various heterogenous sequences of the present application into the host cell can be confirmed by the enzymatic activity of enzymes (e.g., enzymatic markers) that are encoded with heterogenous sequences. Enzymes can be determined by various methods known in the art. Generally, enzymatic activity can be determined by the formation of the products or by the

transformation of the substrate of the enzymatic reaction under investigation. The reaction can be performed in vitro or in vivo.

[0131] In another aspect, the present application provides a method of preparing the fusion protein, which may comprise culturing the cell under a condition enabling the expression of the fusion protein. For example, suitable culture media, suitable temperature and culture time could be used, and all these methods are known to those of ordinary skills in the art.

[0132] In some instances, the method may further comprise steps of separating and/or purifying the fusion protein. For example, the fusion protein of the present application can be purified and isolated by affinity chromatography using protein G-agarose or protein A-agarose, or by gel electrophoresis and/or high performance liquid chromatography.

[0133] Immunoconjugate, composition and application

[0134] In another aspect, the present application provides an immunoconjugate comprising the fusion protein. For example, the immunoconjugate may be fusion protein-drug conjugate (ADC), in which the fusion protein of the present application is conjugated with one or more therapeutic agents, and the therapeutic agents comprise, but not limited to, cytotoxic agents, radiotoxic agents (e.g., radioisotopes) and/or immune inhibitors (e.g., any agents that kill cells by means of inhibiting immune responses) and the like. In some embodiments, the therapeutic agents may be those capable of treating tumor-associated diseases or disorders.

[0135] The conjugation can be performed by a peptide linker (e.g., a cleavable linker) or through other ways. For example, the linker may be an acid labile linker, a peptidase sensitive linker, a photolabile linker, and the like.

[0136] In another aspect, the present application provides a composition comprising the fusion protein, the immunoconjugate, or the nucleic acid molecule, and optionally, pharmaceutically acceptable excipients.

[0137] For example, the pharmaceutically acceptable excipients may comprise buffering agents, antioxidants, preservatives, low molecular weight polypeptides, proteins, hydrophilic polymers, amino acids, sugar, chelating agents, counter ions, metal complexes and/or nonionic surfactants and the like.

[0138] In the present application, the composition can be formulated with pharmaceutically acceptable carriers or diluents and any other known auxiliary agents and excipients by conventional technical means in this field, for example following the technology disclosed in Remington: The Science and Practice of Pharmacy, Edition 19, Gennaro ed., Mack Publishing Co., Easton, PA, 1995.

[0139] In the present application, the composition can be formulated for oral administration, intravenous administration, intramuscular administration, in situ administration at tumor sites, inhalation, rectal administration, vaginal administration, transdermal administration or administration by subcutaneous reservoir.

[0140] For example, the composition can be used to inhibit the tumor growth. For example, the composition of the present application can inhibit or delay the development or progress of diseases,

reduce the size of tumors (even essentially eliminating tumors), and/or relieve and/or stabilize the status of diseases.

[0141] For example, the composition of the present application may be suitable forms for oral administration, such as tablets, capsules, pills, powders, sustained release preparations, solutions, suspensions; or for parenteral injection, such as sterile solutions, suspensions or emulsions; or for local administration as ointment or cream; or for rectal administration as suppositories. The composition may be unit dose forms suitable for single dose at precise dosages. The composition may further comprise conventional drug carriers or excipients. In addition, the composition may comprise other drugs or agents, carriers, adjuvants, etc.

[0142] The composition of the present application may comprise a therapeutically effective amount of the fusion protein. The therapeutically effective amount is a dosage required for preventing and/or treating (at least partially treating) diseases or disorders (e.g., tumors) and/or any complications thereof in a subject suffering from or being at risk of these diseases or disorders. The specific amount/concentration of the dosage may change depending on the administration method and the demand of the patient, and can be determined, for example, based on the size of the patient, the viscosity and/or the body weight. For example, a suitable dosage may be about 0.1 mg or 1 mg/kg/day to about 50 mg/kg/day; sometimes, the dosage may be higher. It should be understood that these specific dosages can be adjusted conveniently by persons skilled in the art (e.g., doctors or pharmacists) based on the specific patient, preparations and/or the status of disease.

[0143] In the present application, the terms “treating” or “curing” or “relieving” or “improving” can be used interchangeably in the present application, and refer to those methods capable of obtaining beneficial or desired results (including, but not limited to, therapeutic benefits and/or preventive benefits). In the present application, the therapeutic benefits generally refer to eradicating or reducing the severity of the underlying conditions being treated. In addition, therapeutic benefits are realized by eradicating or reducing the severity of the underlying conditions or reducing the incidence of one or more physical symptoms associated with the underlying conditions so as to observe improvements in the subject (although the subject may still suffer from the underlying conditions). With regard to the preventive benefits, the composition can be administered to the subject at risk of developing a specific disease or the subject with one or more physical symptoms of the reported disease, even if the disease may not have been diagnosed.

[0144] In another aspect, the present application provides a use of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell in the preparation of a medicament, in which the medicament may be used for treating a tumor.

[0145] In another aspect, the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition or the cell of the present application may be used for treating the tumor.

[0146] In another aspect, the present application provides a method of treating a tumor, including administering to the subject the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition or the cell of the present application.

[0147] In another aspect, the present application provides a method of blocking the interaction between CD47 protein and SIRP α , including administering (for example, administering to a subject in need thereof or cells or biological samples) the fusion protein or the composition of the present application.

[0148] In another aspect, the present application provides a method of blocking the interaction between PD-L1 and PD1, including administering (for example, administering to a subject in need thereof or cells or biological samples) the fusion protein or the composition of the present application.

[0149] In another aspect, the present application provides a method of inhibiting a tumor or the growth and/or proliferation of tumor cells, including contacting the fusion protein or the composition of the present application with the tumor or the tumor cells. For example, the contact may be in vitro.

[0150] In another aspect, the present application provides a method capable of inhibiting a tumor or the growth and/or proliferation of tumor cells, including administering to a subject in need thereof an effective amount of the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell.

[0151] In some embodiments, the tumor may comprise a solid tumor and a non-solid tumor.

[0152] In some embodiments, the solid tumor and the non-solid tumor may comprise multiple myeloma, leukemia, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, neuroglioma, germinoma, sarcoma, mesothelioma, placentoma, cerebral cancer, bone cancer, skin cancer, nasopharynx cancer, lung cancer, oral cancer, esophagus cancer, gastric cancer, liver cancer, pancreatic cancer, prostate

cancer, intestinal cancer, breast cancer, cervical cancer, ovarian cancer and testicular cancer, frontal sinus tumor, hypopharyngeal cancer, olfactory neuroblastoma, tongue cancer, gingival carcinoma, ampulla carcinoma, colon cancer, rectal cancer, kidney cancer, ureteral carcinoma, bladder cancer, penile cancer, fallopian tube carcinoma, eyelid cancer, retinoblastoma.

[0153] In another aspect, the present application provides the fusion protein, the immunoconjugate, the nucleic acid molecule, the vector, the composition, or the cell, which can be used for treating a tumor or an autoimmune disease

[0154] In the present application, the term “subject” generally refers to human or non-human animals, including, but not limited to, cat, dog, horse, pig, cow, sheep, goat, rabbit, mouse, rat or monkey.

[0155] Without intending to be bound by any theory, the embodiments below are only for interpreting the fusion protein, the preparation method and the use of the present application, rather than limiting the inventive scope of the present application.

[0156] Examples

[0157] Example 1 Construction of the fusion protein

[0158] Referring to the fusion protein structure as shown in FIG. 1, taking Roche's PD-L1 antibody Atezolizumab (SG1201) and the mutant M91 (SEQ ID: NO 41) of the SIRP α variant 1 for example, the selected linker 1 (SEQ ID: NO 52) is used from N terminal to C terminal to successively link SG1201, the linker and two M91, in which the N-terminals of the two M91 are linked to the C-terminal of the heavy chain of SG1201 respectively, so as to get the fusion protein SG12473.

[0159] The amino acid sequences of LCDR1-3 of the antibody SG1201 are shown in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively; the amino acid sequence of VL is shown in SEQ ID NO: 7; the nucleotide sequence encoding VL is shown in SEQ ID NO: 9; the amino acid sequences of HCDR1-3 of the antibody SG1201 are shown in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively; the amino acid sequence of VH is shown in SEQ ID NO: 8; the nucleotide sequence encoding VH is shown in SEQ ID NO: 10. The amino acid sequence of the light chain of the antibody SG1201 is shown in SEQ ID NO: 11; and the nucleotide sequence encoding its light chain is shown in SEQ ID NO: 12. The amino acid sequence of the heavy chain of the antibody SG1201 is shown in SEQ ID NO: 13; and the nucleotide sequence encoding its heavy chain is shown in SEQ ID NO: 14.

[0160] The fusion protein SG12473 is composed of a first polypeptide chain and a second polypeptide chain, in which the first polypeptide chain is the light chain of SG1201, the amino acid sequence of which is shown in SEQ ID NO: 11; the second polypeptide chain is the polypeptide chain obtained from the linkage between the heavy chain of SG1201 with a mutation in Fc region and M91 through the linker 1, the amino acid sequence of which is shown in SEQ ID NO: 53.

[0161] Referring to the fusion protein structure as shown in FIG. 1, taking AstraZeneca PD-L1 antibody Durvalumab (SG1202) and the mutant M91 (SEQ ID: NO 41) of the SIRP α variant 1 for example, the selected linker 1 (SEQ ID: NO 52) is used from N terminal to C terminal to successively link SG1202, the linker and two M91, in which the N-terminals of the two M91 are linked to the C-terminal of the heavy chain of SG1202 respectively, so as to get the fusion protein SG12474.

[0162] The amino acid sequences of LCDR1-3 of the antibody SG1202 are shown in SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17, respectively; the amino acid sequence of VL is shown in SEQ ID NO: 21; the nucleotide sequence encoding VL is shown in SEQ ID NO: 23; the amino acid sequences of HCDR1-3 of the antibody SG1202 are shown in SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, respectively; the amino acid sequence of VH is shown in SEQ ID NO: 22; the nucleotide sequence encoding VH is shown in SEQ ID NO: 24. The amino acid sequence of the light chain of the antibody SG1202 is shown in SEQ ID NO: 25; the nucleotide sequence encoding its light chain is shown in SEQ ID NO: 26. The amino acid sequence of the heavy chain of the antibody SG1202 is shown in SEQ ID NO: 27; and the nucleotide sequence encoding its heavy chain is shown in SEQ ID NO: 28.

[0163] The fusion protein SG12474 is composed of a first polypeptide chain and a second polypeptide chain, in which the first polypeptide chain is the light chain of SG1202, the amino acid sequence of which is shown in SEQ ID NO: 25; the second polypeptide chain is the polypeptide chain obtained from the linkage between the heavy chain of SG1202 with a mutation in Fc region and M91 through the linker 1, the amino acid sequence of which is shown in SEQ ID NO: 54.

[0164] Wherein, the amino acid sequence of IgG1-Fc is shown in SEQ ID NO: 50; and the amino acid sequence of Fc with a mutation is shown in SEQ ID NO: 51.

[0165] The fusion protein SS002M91 between the mutant M91 (SEQ ID: NO 41) of the SIRP α variant 1 and IgG1-Fc is a homodimer, and the amino acid sequence of its monomer is shown in SEQ ID NO: 55.

[0166] Example 2 Detection on the activity of binding two antigens

[0167] (1) With a humanized antibody against different antigens as the control, the binding activity between a corresponding bifunctional protein and a related antigen was evaluated by ELISA.

[0168] PD-L1 (human-derived, PD-L1/B7-H1/CD274 Protein (His Tag) purchased from Sino Biological Co.) was coated with ELISA strips at 4°C overnight; after washing with PBST, 10% of fetal calf serum was added and blocked at 37°C for 1 hour; different concentrations of the antibody SG1201 and the fusion protein SG12473, or different concentrations of the antibody SG1202 and the fusion protein SG12474 were added and reacted at 37°C for 1 hour; after washing with PBST, a horseradish peroxidase-labeled goat anti-human IgG secondary antibody (Goat Anti human IgG HRP, Thermo Fisher Scientific) was added and reacted at 37°C for 30 minutes, and washed with PBST for 5 times; each well was added with 100 µl TMB (eBioscience), placed in dark at room temperature (20±5°C) for 1-2 min; then each well was added with 100 µl 2N H₂SO₄ stop solution to terminate the reaction of the substrate, OD values were read at 450 nm on the microplate reader, and the capacity of the bifunctional protein to bind the related target antigen was analyzed.

[0169] The results were shown in FIGs. 2-3. FIGs. 2-3 showed that although the antibody types of PD-L1 in the fusion protein SG12473 and the fusion protein SG12474 were different, the capacities of the fusion protein SG12473 and the fusion protein SG12474 to bind PD-L1 were not affected.

[0170] (2) With the mutant M91 of the CD47 receptor as the control, the binding activity between a corresponding bifunctional protein and CD47 was evaluated by ELISA.

[0171] CD47 (human-derived, CD47 Protein (His Tag) purchased from Sino Biological Co.) was coated with ELISA strips at 4°C overnight; after washing with PBST, 10% of fetal calf serum was added and blocked at 37°C for 1 hour; different concentrations of the fusion protein SS002M91, the fusion protein SG12473 and the fusion protein SG12474 were added and reacted at 37°C for 1 hour; after washing with PBST, a horseradish peroxidase-labeled goat anti-human IgG secondary antibody (Goat Anti human IgG HRP, Thermo Fisher Scientific) was added and reacted at 37°C for 30 minutes, and washed with PBST for 5 times; each well was added with 100 µl TMB (eBioscience), placed in dark at room temperature (20±5°C) for 1-2 min; then each well was added with 100 µl 2N H₂SO₄ stop solution to terminate the reaction of the substrate, OD values were read at 450 nm on the microplate reader, and the capacity of the fusion protein SG12473 and the fusion protein SG12474 to bind CD47 was analyzed.

[0172] The results were shown in FIG. 4. FIG. 4 showed that although the antibody types of PD-L1 in the fusion protein SG12473 and the fusion protein SG12474 were different, the capacities of the fusion protein SG12473 and the fusion protein SG12474 to bind CD47 were not affected.

[0173] Example 3 Detection on the activity of simultaneously binding two antigens

[0174] With a humanized antibody against different antigens as the control, the biological activity of the fusion protein SG12473 and the fusion protein SG12474 to simultaneously bind two antigens was evaluated by ELISA.

[0175] PD-L1 was coated with ELISA strips at 4°C overnight; after washing with PBST, 10% of fetal calf serum was added and blocked at 37°C for 1 hour; different concentrations of the antibody SG1201, the fusion protein SG12473, the antibody SG1202 and the fusion protein SG12474 were added and reacted at 37°C for 1 hour; after washing with PBST, biotin-labeled CD47 (Biotin-Fc-CD47) was added and reacted at 37°C for 30 minutes, and washed with PBST for 5 times; horseradish peroxidase-labeled avidin (Streptavidin-HRP, Jiaxuan Bio.) was added and reacted at 37°C for 30 minutes, and washed with PBST for 5 times; each well was added with 100 µl TMB (eBioscience), placed in dark at room temperature (20±5°C) for 1-2 min; then each well was added with 100 µl 2N H₂SO₄ stop solution to terminate the reaction of the substrate, OD values were read at 450 nm on the microplate reader, and the capacities of the fusion protein SG12473 and the fusion protein SG12474 to simultaneously bind PD-L1 and CD47 were analyzed.

[0176] The results were shown in FIG. 5. FIG. 5 showed that although the antibody types of PD-L1 in the fusion protein SG12473 and the fusion protein SG12474 were different, the capacities of the fusion protein SG12473 and the fusion protein SG12474 to simultaneously bind PD-L1 and CD47 were not affected.

[0177] Example 4 Analysis on the activity of blocking the CD47/SIRPα interaction

[0178] With the fusion protein SS002M91 as the control, the biological activities of the fusion proteins SG12473 and SG12474 to block the CD47/SIRPα interaction were evaluated.

[0179] SIRP α -His was coated on the assay plate at 1 ug/ml overnight at 4°C; after washing with PBST, 10% of fetal calf serum was added and blocked at 37°C for 1 hour; SS002M91, SG12473, SG12474 were diluted gradiently with 10% of fetal bovine blood respectively, and Biotin-Fc-CD47 was added into the samples until a final concentration of 2 μ g/ml, and pre-incubated at 37°C for 30 min, as the primary antibody; after the assay plate was washed with PBST, the primary antibody was added and incubated at 37°C for 1 hour; after washing with PBST for 5 times, horseradish peroxidase-labeled avidin (Streptavidin-HRP, Jiaxuan Bio.) was added and incubated at 37°C for 30 minutes; after washing with PBST for 5 times, each well was added with 100 μ l TMB (eBioscience), and placed in dark at room temperature (20 $\pm$ 5°C) for 1-5 min; then each well was added with 100 μ l 2N H₂SO₄ stop solution to terminate the reaction of the substrate, OD values were read at 450 nm on the microplate reader, and the blocking effects of SS002M91, SG12473, SG12474 on CD47/SIRP α were analyzed.

[0180] The results were shown in FIG. 6. FIG. 6 showed that the same as the fusion protein SS002M91, the fusion proteins SG12473, SG12474 could competitively block the binding between CD47 and its ligand SIRP α . Wherein, IC₅₀ value of SG12473 was 1.26 nM, IC₅₀ value of SG12474 was 0.77 nM, and IC₅₀ value of SS002M91 was 1.16 nM.

[0181] Example 5 Analysis on the activity of blocking the PD-1/PD-L1 interaction

[0182] With SG1201 and SG1202 as the control, the biological activities of the fusion proteins SG12473 and SG12474 to block the PD-1/PD-L1 interaction were evaluated.

[0183] PD-L1-Fc was coated on the assay plate at 2 ug/ml overnight at 4°C; after washing with PBST, 10% of fetal calf serum was added and blocked at 37°C for 1 hour; SG1201, SG12473, SG1202 and SG12474 were diluted gradiently with 10% of fetal bovine blood respectively, and Biotin-Fc-PD1 was added into the samples until a final concentration of 1 ug/ml, and pre-incubated at 37°C for 30 min, as the primary antibody; after the assay plate was washed with PBST, the primary antibody was added and incubated at 37°C for 1 hour; after washing with PBST for 5 times, horseradish peroxidase-labeled avidin (Streptavidin-HRP, Jiaxuan Bio.) was added and incubated at 37°C for 30 minutes; after washing with PBST for 5 times, each well was added with 100 µl TMB (eBioscience), and placed in dark at room temperature (20±5°C) for 1-5 min; then each well was added with 100 µl 2N H₂SO₄ stop solution to terminate the reaction of the substrate, OD values were read at 450 nm on the microplate reader, and the blocking effects of SG1201, SG12473, SG1202 and SG12474 on PD-1/PD-L1 were analyzed.

[0184] As can be seen from FIGs. 7-8, the same as SG1201 and SG1202, the fusion proteins SG12473 and SG12474 could competitively block the binding between PD-1 and PD-L1. Wherein, IC₅₀ value of SG1201 is 11.23 nM, IC₅₀ value of SG12473 is 13.22 nM, IC₅₀ value of SG1202 is 10.89 nM, and IC₅₀ value of SG12474 is 9.12 nM.

[0185] Example 6 Fusion protein inhibits the tumor activity in vivo

[0186] A female NCG mouse MiXeno animal model was transplanted heterogeneously with a human-derived lymphoma KARPAS-299 cell line subcutaneously so as to evaluate the inhibitory effect of the fusion protein SG12473 on the tumor activity.

[0187] Female NCG mice of 6-8 weeks (purchased from Jiangsu Jicui Yaokang Biotechnology Co. Ltd.) were chosen for test. The mice were inoculated with KARPAS-299 cells subcutaneously and the growth profiles of tumors were observed periodically. When the tumors grew to an average size of 35 mm³, the mice were randomly grouped according to the tumor size and the body weight of the mice for administration. At the day of grouping (about at Day 4), a fresh human PBMC (deriving from a donor) was inoculated through the tail vein so as to establish a KARPAS-299 humanized mouse model.

[0188] The tests were divided into a solvent control group, a SG1201 group as well as low, medium and high dosage groups of SG12473, in which there were 12 mice in each group and every 6 mice utilized PBMC from the same source. Drugs were given by intraperitoneal injection twice a week, for a period of totally three weeks. In particular,

[0189] Group 1A and Group 1B: solvent control groups

[0190] Group 2A and Group 2B: SG1201 groups, SG1201 at 5 mg/Kg

[0191] Group 3A and Group 3B: low dosage groups of SG12473, SG12473 at 5 mg/Kg

[0192] Group 4A and Group 4B: medium dosage groups of SG12473, SG12473 at 10 mg/Kg

[0193] Group 5A and Group 5B: high dosage groups of SG12473, SG12473 at 20 mg/Kg;

[0194] The therapeutic efficacy was evaluated according to the relative tumor growth inhibition (TGI), and safety was evaluated based on the changes in animal weight and the death rate.

[0195] The results showed that, in mice using PBMC derived from the donor A, significant tumor inhibitory effects were displayed in all the low, medium and high dosage groups of SG12473. As shown from the results in FIG. 9, TGI in the low, medium and high dosage groups of SG12473 at the completion of administration were 44.71%, 47.16% and 96.49% respectively; and 4 days after the completion of administration, TGI were 43.71%, 47.92% and 95.94% respectively, which had statistically significant difference relative to the solvent control groups (p values were all <0.01). No significant tumor inhibitory effect was displayed at the test dosage of SG1201.

[0196] In mice using PBMC derived from the donor B, significant tumor inhibitory effects were displayed in all the low, medium and high dosage groups of SG12473. As shown from the results in FIG. 10, TGI in the low, medium and high dosage groups of SG12473 at the completion of administration were 36.96%, 62.06% and 98.90% respectively; and 4 days after the completion of administration, TGI were 33.44%, 57.57% and 98.83% respectively, which had statistically significant difference relative to the solvent control groups (p values were all <0.01). No significant tumor inhibitory effect was displayed at the test dosage of SG1201.

[0197] There were no animal deaths in each treatment group, no obvious drug toxicity was displayed, and the tolerance was good during the treatment.

[0198] The foregoing detailed description is provided by means of explanations and examples, but not intending to limit the scope of the attached claims. The various variations of the embodiments currently listed in the present application are apparent to those with ordinary skills in the art, and reserved within the scope of the attached claims and its equivalent schemes.

WHAT IS CLAIMED IS:

1. A fusion protein, comprising:

a first binding domain that specifically binds PD-L1; and

a second binding domain that specifically binds a CD47 protein;

wherein, said second binding domain comprises a mutant of a human SIRP α variant 1, said mutant comprises ~~substitution, deletion or addition of the~~ amino acid residue substitutions at one or more positions from site 33 to site 149, compared to the amino acid sequence listed as NCBI RefSeq NP_542970.1 as shown in SEQ ID NO: 29, wherein said mutant comprises amino acid substitutions selected from the group consisting of:

(1) I61L, V63I, E77I, E84K, V93L, L96S, K98R, N100G and V132L;

(2) I61V, E77N, Q82S, K83R and E84H;

(3) I61F, V63I, K83R, E84K and V132I;

(4) I61L, E77Q, E84D, R107N and V132I;

(5) I61L, V63I, E77K, K83R, E84D and N100G;

(6) I61V, E77H, Q82R, K83R, E84H and R107S;

(7) I61L, E77I, Q82G, E84R, V93L, L96T, N100G, R107S, G109R and V132R;

(8) I61L, E77M, Q82G, K83R, E84D and V132L;

(9) I61L;

(10) I61F, D95H, L96S, G109H and V132S;

(11) I61F, D95H, L96S, K98R, G109H and V132S;

(12) I61L, E77Q, E84D, V93A, R107N and V132I;

(13) E77K, L96S, N100K, G109H and V132L;

(14) I61L, V63I, Q82G, E84G, D95R, L96S, N100D and V132I;

(15) I61L, E77R, Q82N, K83R, E84G, V93L, D95E, L96T, K98R, N100D and V132L;

(16) I61V, E77N, Q82S, K83R, E84H and V93A;

(17) I61V, V63I, E77V, K83R, E84D, D95E, L96T, K98R and N100E;

(18) I61L, V63I, E77V, K83R, D95E, L96S, K98R, N100D and G109R;

(19) I61V, E77L, Q82G, E84G, V93L, D95E, L96T, K98R and N100G; and

(20) I61L, V63I, E77N, Q82G and E84G.

~~2. The fusion protein according to claim 1, wherein said mutant comprises amino acid substitutions at one or more amino acid residues selected from the group consisting of: I61, V63, E77, Q82, K83, E84, V93, D95, L96, K98, N100, R107, G109 and V132.~~

~~3. The fusion protein according to claim 2, wherein said mutant comprises amino acid substitutions at amino acid residues selected from the group consisting of:~~

~~(1) I61, V63, E77, E84, V93, L96, K98, N100 and V132;~~

~~(2) I61, E77, Q82, K83 and E84;~~

~~(3) I61, V63, K83, E84 and V132;~~

~~(4) I61, E77, E84, R107 and V132;~~

~~(5) I61, V63, E77, K83, E84 and N100;~~

~~(6) I61, E77, Q82, K83, E84 and R107;~~

~~(7) I61, E77, Q82, E84, V93, L96, N100, R107, G109 and V132;~~

~~(8) I61, E77, Q82, K83, E84 and V132;~~

~~(9) I61;~~

~~(10) I61, D95, L96, G109 and V132;~~

~~(11) I61, D95, L96, K98, G109 and V132;~~

~~(12) I61, E77, E84, V93, R107 and V132;~~

~~(13) E77, L96, N100, G109 and V132;~~

~~(14) I61, V63, Q82, E84, D95, L96, N100 and V132;~~

~~(15) I61, E77, Q82, K83, E84, V93, D95, L96, K98, N100 and V132;~~

~~(16) I61, E77, Q82, K83, E84 and V93;~~

~~(17) I61, V63, E77, K83, E84, D95, L96, K98 and N100;~~

~~(18) I61, V63, E77, K83, D95, L96, K98, N100 and G109;~~

~~(19) I61, E77, Q82, E84, V93, D95, L96, K98 and N100; and~~

~~(20) I61, V63, E77, Q82 and E84.~~

~~4. The fusion protein according to any one of claims 1-3, wherein said mutant comprises one or more amino acid substitutions selected from the group consisting of: I61L/V/F, V63I, E77I/N/Q/K/H/M/R/N/V/L, Q82S/R/G/N, K83R, E84K/H/D/R/G, V93L/A, D95H/R/E, L96S/T, K98R, N100G/K/D/E, R107N/S, G109R/H and V132L/R/I/S.~~

~~5. The fusion protein according to claim 4, wherein said mutant comprises amino acid substitutions selected from the group consisting of:—~~

~~(1) I61L, V63I, E77I, E84K, V93L, L96S, K98R, N100G and V132L;~~

~~(2) I61V, E77N, Q82S, K83R and E84H;~~

~~(3) I61F, V63I, K83R, E84K and V132I;~~

- (4) ~~I61L, E77Q, E84D, R107N and V132I;~~
- (5) ~~I61L, V63I, E77K, K83R, E84D and N100G;~~
- (6) ~~I61V, E77H, Q82R, K83R, E84H and R107S;~~
- (7) ~~I61L, E77I, Q82G, E84R, V93L, L96T, N100G, R107S, G109R and V132R;~~
- (8) ~~I61L, E77M, Q82G, K83R, E84D and V132L;~~
- (9) ~~I61L;~~
- (10) ~~I61F, D95H, L96S, G109H and V132S;~~
- (11) ~~I61F, D95H, L96S, K98R, G109H and V132S;~~
- (12) ~~I61L, E77Q, E84D, V93A, R107N and V132I;~~
- (13) ~~E77K, L96S, N100K, G109H and V132L;~~
- (14) ~~I61L, V63I, Q82G, E84G, D95R, L96S, N100D and V132I;~~
- (15) ~~I61L, E77R, Q82N, K83R, E84G, V93L, D95E, L96T, K98R, N100D and V132L;~~
- (16) ~~I61V, E77N, Q82S, K83R, E84H and V93A;~~
- (17) ~~I61V, V63I, E77V, K83R, E84D, D95E, L96T, K98R and N100E;~~
- (18) ~~I61L, V63I, E77V, K83R, D95E, L96S, K98R, N100D and G109R;~~
- (19) ~~I61V, E77L, Q82G, E84G, V93L, D95E, L96T, K98R and N100G; and~~
- (20) ~~I61L, V63I, E77N, Q82G and E84G.~~

6.2. The fusion protein according to ~~any one of~~ claims 1-5, wherein said mutant comprises an amino acid sequence as ~~shown~~ set forth in any one of SEQ ID NOs: 30-49.

7.3. The fusion protein according to any one of claims 1-62, wherein said first binding domain comprises an antibody or an antigen-binding fragment thereof.

~~8.4.~~The fusion protein according to claim ~~7~~3, wherein said antibody is selected from the group consisting of: a monoclonal antibody, a single-chain antibody, a chimeric antibody, a humanized antibody and a fully human antibody.

~~9.5.~~The fusion protein according to any one of claims ~~7-8~~3-4, wherein said antigen-binding fragment is selected from the group consisting of: Fab, Fab', F(ab)2, dAb, an isolated complementary determining region CDR, Fv and scFv.

~~10.6.~~____The fusion protein according to any one of claims 1-~~9~~5, wherein said PD-L1 is a human PD-L1.

~~11.7.~~____The fusion protein according to any one of claims ~~7-10~~3-6, wherein said antibody comprises a heavy chain of the antibody or a fragment thereof and a light chain of the antibody or a fragment thereof, said heavy chain of the antibody or the fragment thereof comprises HCDR1-3 and said light chain of the antibody or the fragment thereof comprises LCDR1-3, said HCDR1-3 and LCDR1-3 comprises an the amino acid sequences as shown in any one of the group consisting of: SEQ ID NO: 4 and SEQ ID NO: 18selected from the group consisting of:

(1) said HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, said HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, said HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6, said LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, said LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2, said LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 3; and

~~(1)(2)~~ said HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 18,
said HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 19, said
HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 20, said LCDR1
comprises an amino acid sequence as set forth in SEQ ID NO: 15, said LCDR2 comprises
an amino acid sequence as set forth in SEQ ID NO: 16, said LCDR3 comprises an amino
acid sequence as set forth in SEQ ID NO: 17.

~~12. The fusion protein according to claim 11, wherein said HCDR2 comprises an amino acid~~
~~sequence as shown in any one of the group consisting of: SEQ ID NO: 5 and SEQ ID NO:~~
~~19.~~

~~13. The fusion protein according to any one of claims 11-12, wherein said HCDR3 comprises an~~
~~amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 6 and SEQ~~
~~ID NO: 20.~~

~~14.8.~~ The fusion protein according to any one of claims 11-13, wherein said heavy chain of
the antibody or the fragment thereof comprises a heavy chain variable region VH, and said
heavy chain variable region VH comprises an amino acid sequence as ~~shown~~ set forth in any
~~one of the group consisting of: SEQ ID NO: 8 and or~~ SEQ ID NO: 22.

~~15.9.~~ The fusion protein according to any one of claims 11-14, wherein said heavy chain
of the antibody or the fragment thereof comprises a heavy chain constant region, and said
heavy chain constant region comprises IgG.

~~16.10.~~ The fusion protein according to claim 9, wherein said IgG is selected from the group
consisting of: IgG1 and IgG4.

~~17.11.~~ The fusion protein according to any one of claims ~~11-16~~7-10, wherein said heavy chain of the antibody comprises an amino acid sequence as ~~shown~~ set forth in ~~any one of the group consisting of:~~ SEQ ID NO: 13 ~~and or~~ SEQ ID NO: 27.

~~18.~~ The fusion protein according to any one of claims ~~7-17~~, wherein said antibody comprises a light chain of the antibody or a fragment thereof, said light chain of the antibody or the fragment thereof comprises ~~LCDR1-3~~, said ~~LCDR1~~ comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 15.

~~19.~~ The fusion protein according to claim ~~18~~, wherein said ~~LCDR2~~ comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 2 and SEQ ID NO: ~~16~~.

~~20.~~ The fusion protein according to any one of claims ~~18-19~~, wherein said ~~LCDR3~~ comprises an amino acid sequence as shown in any one of the group consisting of: SEQ ID NO: 3 and SEQ ID NO: 17.

~~21.12.~~ The fusion protein according to any one of claims ~~18-20~~7-11, wherein said light chain of the antibody or the fragment thereof comprises a light chain variable region VL, and said light chain variable region VL comprises an amino acid sequence as ~~shown~~ set forth in ~~any one of the group consisting of:~~ SEQ ID NO: 7 ~~and or~~ SEQ ID NO: 21.

~~22.13.~~ The fusion protein according to any one of claims ~~18-21~~7-12, wherein said light chain of the antibody or the fragment thereof comprises a light chain constant region, and the light chain constant region comprises Igk.

~~23.14.~~ The fusion protein according to any one of claims ~~18-22~~7-13, wherein said light chain

of the antibody comprises an amino acid sequence as ~~shown set forth in any one of the group~~
~~consisting of:~~ SEQ ID NO: 11 ~~and or~~ SEQ ID NO: 25.

~~24.15.~~ The fusion protein according to any one of claims 1-~~23~~14, wherein said first binding domain is located at N-terminal of the second binding domain.

~~25.16.~~ The fusion protein according to any one of claims 1-~~24~~15, wherein said fusion protein further comprises a linker, the linker is located at C-terminal of the first binding domain and located at N-terminal of the second binding domain.

~~26.17.~~ The fusion protein according to claim ~~25~~16, wherein said linker comprises an amino acid sequence as shown in SEQ ID NO: 52.

~~27.18.~~ The fusion protein according to any one of claims 1-~~26~~17, comprising at least two of the second binding domain.

~~28.19.~~ The fusion protein according to claim ~~27~~18, wherein each of said second binding domains is located at C-terminal of the first binding domain respectively.

~~29.20.~~ An immunoconjugate, comprising the fusion protein according to any one of claims 1-~~28~~19.

~~30.21.~~ One or more isolated nucleic acid molecules, encoding the fusion protein according to any one of claims 1-~~28~~19 or the immunoconjugate according to claim ~~29~~20.

~~31.22.~~ One or more vectors, comprising the nucleic acid molecules according to claim ~~30~~21.

~~32.23.~~ A composition, comprising the fusion protein according to any one of claims 1-~~28~~19, the immunoconjugate according to claim ~~29~~20, or the nucleic acid molecule according to claim ~~30~~21, and ~~optionally~~, a pharmaceutically acceptable excipient.

~~33.24.~~ A cell, comprising the fusion protein according to any one of claims 1-~~28~~19, the immunoconjugate according to claim ~~29~~20, the nucleic acid molecule according to claim ~~30~~21, or the vector according to claim ~~31~~22.

~~34.25.~~ A method of preparing the fusion protein according to any one of claims 1-~~28~~19, comprising culturing the cell according to claim ~~33~~-24 under a condition enabling the expression of said fusion protein.

~~35.26.~~ A use of the fusion protein according to any one of claims 1-~~28~~19, the immunoconjugate according to claim ~~29~~20, the nucleic acid molecule according to claim ~~30~~21, the vector according to claim ~~31~~22, the composition according to claim ~~32~~23, or the cell according to claim ~~33~~-24 in the preparation of a medicament, wherein said medicament is to be used for treating a tumor.

~~36.27.~~ The use according to claim ~~35~~26, wherein said tumor comprises a solid tumor and a non-solid tumor.

~~37.28.~~ The use according to claim ~~36~~27, wherein said solid tumor and non-solid tumor comprise multiple myeloma, leukemia, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, neuroglioma, germinoma, sarcoma, mesothelioma, placentoma, cerebral cancer, bone cancer, skin cancer, nasopharynx cancer, lung cancer, oral cancer, esophagus cancer, gastric cancer, liver cancer, pancreatic cancer, prostate cancer, intestinal cancer, breast cancer, cervical cancer, ovarian cancer and testicular cancer, frontal sinus tumor, hypopharyngeal cancer, olfactory neuroblastoma, tongue cancer, gingival carcinoma, ampulla carcinoma, colon cancer, rectal cancer, kidney cancer, ureteral carcinoma, bladder cancer, penile cancer,

fallopian tube carcinoma, eyelid cancer, retinoblastoma.

~~38.~~29. ~~A method of blocking the interaction between PD-L1 protein and PD-1, comprising~~
~~administering to a subject in need thereof an effective amount~~use of the fusion protein
according to any one of claims 1-~~19~~28, the immunoconjugate according to claim ~~29~~20, the
nucleic acid molecule according to claim ~~30~~21, the vector according to claim ~~31~~22, the
composition according to claim ~~32~~23, or the cell according to claim ~~33~~24 in the preparation
of a medicament, wherein the medicament is to be used for blocking the interaction between
PD-L1 protein and PD-1.

~~39.~~30. ~~A method of blocking the interaction between CD47 protein and SIRP α , comprising~~
~~administering to a subject in need thereof an effective amount~~use of the fusion protein
according to any one of claims 1-~~28~~19, the immunoconjugate according to claim ~~29~~20, the
nucleic acid molecule according to claim ~~30~~21, the vector according to claim ~~31~~22, the
composition according to claim ~~32~~23, or the cell according to claim ~~33~~24 in the preparation
of a medicament, wherein said medicament is to be used for blocking the interaction between
CD47 protein and SIRP α .

~~40.~~ ~~A method of inhibiting the growth and/or proliferation of tumors or tumor cells,~~
~~comprising administering to a subject in need thereof an effective amount of the fusion~~
~~protein according to any one of claims 1-28, the immunoconjugate according to claim 29, the~~
~~nucleic acid molecule according to claim 30, the vector according to claim 31, the~~
~~composition according to claim 32, or the cell according to claim 33.~~