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(54) Title: RECOMBINANT PROTEIN TARGETING PD-L1 AND VEGF

(57) Abstract: Provided is a recombinant protein containing 1) an anti-PD-L1 antibody heavy chain and an anti-PD-L1 antibody light chain, which two are linked by a disulfide bond to bind PD-L1, and 2) an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR), linked via a linker to the N-terminus or C-terminus of the heavy chain or the light chain, wherein the recombinant protein is capable of binding PDL1, VEGF and Fc receptor simultaneously. Further provided are a recombinant antibody containing two said recombinant proteins, a polynucleotide encoding the recombinant protein, an expression vector containing the polynucleotide, a method for producing the recombinant protein, a pharmaceutical composition comprising the recombinant protein and a method for treating a disease caused by over expression of VEGF and/or PD-L1 using the recombinant protein.

RECOMBINANT PROTEIN TARGETING PD-L1 AND VEGF

FIELD OF THE INVENTION

[0001] The disclosure relates to a recombinant protein/antibody, and the preparation and use thereof, especially its use in tumor therapies.

BACKGROUND OF THE INVENTION

[0002] Cancer cells have developed several mechanisms to evade a host's immune surveillance. For example, many tumor or cancer cells express on their surfaces a high level of PD-Ll and PD-L2, both of which bind to PD-1 on the surface of T cells, inducing T cell apoptosis.

[0003] In addition, growth of cancer cells depends on sufficient supply of nutrition. Cancer cells themselves can secrete factors that promote blood vessel growth, such as vascular epithelial growth factors (VEGF). Inhibition of the activity of VEGF or its receptors will stop blood supply to a solid tumor, thereby inhibiting its growth.

[0004] *PD-L1 and PD-1*

[0005] PD-L1, also known as programmed death-ligand 1 or CD274, is a transmembrane protein that plays a major role in suppressing the immune system during some particular events such as tissue allografts, autoimmune disease and cancer development.

[0006] In certain cancers, the loss of feedback restriction between transcription factors like STAT3 and NF-κB can lead to increased local PD-L1 expression, which could limit the effectiveness of systemic treatment with agents targeting PD-L1 (Vlahopoulos, SA. Aberrant control of NF-κB in cancer permits transcriptional and phenotypic plasticity to curtail dependence on host tissue: molecular mode. Cancer Biology & Bedicine. 2017, 14: 254–270). An analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5-fold increased risk of death (Thompson RH, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. PNAS. 2004, 101 (49): 17174–9).

[0007] PD-1 is a cell surface receptor of about 268 amino acids. When bound with PD-L1 or PD-L2, it down-regulates the immune system and promotes self-tolerance by suppressing T cell inflammatory activity. The inhibitory effect of PD-1 on immune system prevents autoimmune diseases but also prevents the immune system from killing cancer cells. An anti-PD-1 antibody, BMS-936558, produced objective responses in approximately one in five to one in four patients with non–small-cell lung cancer, melanoma, or renal-cell cancer (Suzanne L. Topalian, et al., Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer, N Engl J Med 2012, 366:2443-2454).

[0008] *VEGF and VEGFR*

[0009] VEGF is a pleiotropic growth factor that is central to control of tissue/wound repair programs, and is classified into VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF. During the tissue healing process, VEGF simultaneously drives formation of new blood vessels (angiogenesis) while down-regulating immunity (Canic M, et al. The role of vascular endothelial growth factor in wound healing. J Surg Res. 2009, 153:347–358; Leung DW, et al.

Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989, 246:1306–1309; Voron T, et al. Control of the immune response by pro-angiogenic factors. Front Oncol. 2014, 4:70). Both of these properties of VEGF are critical to the oncogenic process, as they enable the development of tumor blood vessels and suppress anticancer immunity (Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011, 144:646–674). In particular, VEGF is thought to exert its immune-suppressive effects via three key mechanisms: inhibiting DC maturation, reducing T cell tumor infiltration, and increasing inhibitory cells in tumor microenvironment. Abnormal VEGF expression also contributes to other diseases, including rheumatoid arthritis, diabetic retinopathy, wet form age-related macular degeneration, and glomerular hypertrophy. Avastin, an FDA approved anti-VEGF monoclonal antibody drug, functions to treat cancers (colon cancer, lung cancer) by inhibiting the biological activities of VEGF. Another protein drug targeting VEGF, Aflibercept, was approved in United State and Europe for treatment of wet macular degeneration under tradename Eylea, and for metastatic colorectal cancer as Zaltrap.

[0010] VEGF receptor (VEGFR) has three subtypes, VEGFR-1, VEGFR-2 and VEGFR-3, all having an extracellular portion consisting of 7 immunoglobulin-like domains, a single transmembrane region and an intracellular portion. VEGFR-2 appears to mediate almost all of the known cellular responses to VEGFs, while VEGFR-1 sequesters VEGF from VEGFR-2 binding and modulates VEGFR-2 signaling. VEGF-A, the most dangerous type to human health, binds to both VEGFR-1 and VEGFR-2. VEGFR antagonists are mostly used, or under investigation, for treating cancers. Lenvima, acting as a multiple kinase inhibitor against VEGFR-1, VEGFR-2 and VEGFR-3 kinases, was approved in 2015 for the treatment of differentiated thyroid cancer, and in 2016 for treatment of advanced renal cell carcinoma in combination with Everolimus.

[0011] *Fc and FcR*

[0012] The fragment crystallizable region (Fc region) is the tail region of an antibody and is the domain that determines the effector function of the antibody, that is, how it engages with specific cell receptors or other defense proteins.

[0013] An Fc receptor (FcR) is a protein found on the surface of certain immune effector cells, including B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils, and mast cells. These cells contribute to the protective functions of the immune system.

[0014] An Fc region may interact with Fc receptors and/or proteins of the complement system, activating the immune system. For example, Fc receptors bind to antibodies that are attached to infected cells or invading pathogens, stimulating phagocytic or cytotoxic cells to destroy microbes, or infected cells by antibody-mediated phagocytosis or antibody-dependent cell-mediated cytotoxicity (ADCC).

[0015] *Therapeutic bi-specific or multi-specific fusion proteins/antibodies*

[0016] Protein/antibody therapies have significantly advanced our abilities to treat diseases, including cancers, yet clinical studies have shown that many patients do not adequately respond to monospecific therapy. For example, among patients treated with antibodies targeting PD-L1/PD-1, only a subset experience durable response and/or survival. Two

VEGF targeting proteins mentioned above, Avastin and Aflibercept, inhibit cancer cell growth to certain extent but cannot eliminate the cancer cells.

[0017] To address the above limitations, bi-specific or multi-specific recombinant proteins are developed against two or more separate and different antigens, or different epitopes of the same antigen. For example, some bispecific proteins are engineered to simultaneously bind a cytotoxic cell and a tumor cell to be destroyed. Such proteins are capable of blocking multiple tumor cell growth and survival pathways, and/or activating tumor cell killing pathways, having a potential to better inhibit cancer growth.

[0018] However, bispecific or multi-specific proteins, especially bispecific or multi-specific antibodies, present significant design challenges, because a large number of variables have to be considered, including compatibility of the molecules, whether they can be linked together, and affinity, stability and pharmaceutical properties of the resulting proteins even if they can be linked physically. It is well recognized that simply linking two or more antibodies or proteins together often does not result in synergetic or even advantageous effects. A recombinant antibody disclosed in the Comparative Example below, comprising SIRPαD1, linked by a linker, to an anti-EGFR antibody, has been proved to have inferior anti-tumor activity compared to the anti-EGFR antibody or SIRPαD1-Fc alone in the HT-29 or NCl-H1975 tumor model.

SUMMARY OF THE INVENTION

[0019] Through diligent efforts, the present inventors have successfully designed a recombinant protein and a recombinant antibody that target both PD-L1 and VEGF and at the same time binds to FcR. The recombinant protein/antibody of the present disclosure shows better anti-tumor activity than its mono-specific counterparts, even if they are used in combination.

[0020] Accordingly, in a first aspect, the present disclosure provides a recombinant protein comprising

- an anti-PD-L1 antibody heavy chain and an anti-PD-L1 antibody light chain, which two are linked by a disulfide bond to bind PD-L1, and
- an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR), linked via a linker to the N-terminus or C-terminus of the heavy chain or the light chain,

wherein the recombinant protein is capable of binding PD-L1, VEGF and Fc receptor simultaneously.

[0021] In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the C-terminus of the heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the light chain.

[0022] Also provided is a dimer comprising the recombinant protein described herein. The dimer may be a homodimer comprising two recombinant proteins described above, such as two identical ones.

[0023] In a second aspect, the present disclosure provides a recombinant antibody, comprising an anti-PD-L1 antibody having two heavy chains and two light chains, wherein an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR) is linked via a linker to N-terminus or C-terminus of each heavy chain, or to N-terminus or C-terminus of each light chain, wherein the recombinant antibody is capable of binding PD-L1, VEGF and FcR simultaneously. The recombinant antibody is a homodimer of the recombinant protein described in the first aspect.

[0024] In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of each heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the C-terminus of each heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of each light chain.

[0025] Binding to PD-L1 on target cells such as cancer cells releases the check on T cells by PD-1-mediated inhibitory signals, while binding to VEGF prohibits new blood vessel formation and releases immunity suppression and thus limit target cell growth. Further, binding to FcR on immune cells such as NK cells or macrophages stimulates targeted cell killings by NK cells or macrophages.

[0026] The anti-PD-L1 antibody describe in the first aspect and the second aspect may be an isolated monoclonal antibody such as Atezolizumab, Avelumab, Durvalumab, and an antibody having at least 80%, 85%, 90%, 95%, 98% or 99% amino acid identity to Atezolizumab, Avelumab, or Durvalumab.

[0027] The anti-PD-L1 antibody may be an isolated monoclonal antibody, comprising two heavy chains each having an amino acid sequence of SEQ ID NO: 2 or 4, and two light chains each having an amino acid sequence of SEQ ID NO: 6, which may be encoded by nucleic acid sequences of SEQ ID NOs: 1, 3 and 5, respectively. The antigen-binding (Fab) portion (or paratope) of the anti-PD-L1 antibody can bind to PD-L1 on the cell surfaces of target cells such as cancer/tumor cells to block the interaction of PD-L1 with PD-1 on the cell surfaces of T cells and thus release the check on T cells by PD-1-mediated inhibitory signals. The Fc portion of the anti-PD-L1 antibody can bind to FcRs on the cell surfaces of immune cells such as NK cells and macrophages to stimulate targeted cell killings by the NK cells or macrophages.

[0028] In some embodiments, the heavy chain of the anti-PD-L1 antibody may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 2, wherein the anti-PD-L1 antibody is able to bind to PD-L1 and block the interaction of PD-L1 with PD-1 on the cell surfaces of T cells, and is also able to bind to FcRs on the cell surfaces of immune cells and thus activate these cells for killing target cells such as cancer cells.

[0029] In some embodiments, the light chain may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 6, wherein the anti-PD-L1 antibody is able to bind to PD-L1 and block the interaction of PD-L1 with PD-1 on the cell surfaces of T cell, and is also able to bind to FcRs on the cell surfaces of immune cells and thus activate these cells for killing target cells such as cancer cells.

[0030] The VEGFR describe in the first aspect and the second aspect may be VEGFR1 and/or VEGFR2, and the extracellular Ig-like domain of the VEGFR may be the second extracellular Ig-like domain of the VEGFR. In one embodiment, the VEGFR is VEGFR1, and the extracellular Ig-like domain of VEGFR1 is the second extracellular Ig-like domain of VEGFR1 (VEGFR1D2). In another embodiment, the VEGFR is VEGFR2, and the extracellular Ig-like domain of VEGFR2 is the second extracellular Ig-like domain of VEGFR2 (VEGFR2D2). The extracellular Ig-like domain of the VEGFR can bind to VEGFs expressed by or around target cells, for instance, cancer/tumor cells, and thus limit growth of target cells.

[0031] In one embodiment, the VEGFR1D2 has a nucleic acid sequence and an amino acid sequence set forth in SEQ ID NOs: 7 and 8, respectively. In some embodiments, the VEGFR1D2 may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 8, wherein the VEGFR1D2 can bind to VEGFs expressed by or around target cells, for instance, cancer/tumor cells and thus limit growth of target cells.

[0032] The linker described in the first aspect and the second aspect may be a peptide of about 5 to 30 amino acid residues. In an embodiment, the linker is a peptide of 10 to 30 amino acid residues. In another embodiment, the linker is a peptide of 10 to 15 amino acid residues. In some embodiments, the linker is -(Gly-Gly-Gly-Gly-Ser)$_2$- (SEQ ID NO: 10) or -(Gly-Gly-Gly-Gly-Ser)$_3$- (SEQ ID NO: 12), which may be encoded by SEQ ID NO: 9 and 11, respectively.

[0033] The VEGFR1D2-Linker-anti-PD-L1 heavy chain, with VEGFR1D2 linked to N-terminus of the anti-PD-L1 heavy chain, comprises an amino acid sequence of SEQ ID NO: 14, which may be encoded by nucleotide of SEQ ID NO: 13. In some embodiments, the VEGFR1D2-Linker-anti-PD-L1 heavy chain comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 14, wherein the VEGFR1D2-Linker-anti-PD-L1 heavy chain together with the light chain of the anti-PD-L1 antibody can bind to VEGF, PD-L1 and FcR, i) blocking the interaction of PD-L1 on target cells with PD-1 on T cells; ii) blocking the interaction of VEGF with VEGFR on target cells' surfaces; and iii) stimulating targeted cell killings by immune cells.

[0034] The anti-PD-L1 heavy chain-Linker-VEGFR1D2, with VEGFR1D2 linked to C-terminus of the anti-PD-L1 heavy chain, comprises an amino acid sequence of SEQ ID NO: 16, which may be encoded by nucleotide of SEQ ID NO: 15. In some embodiments, the anti-PD-L1 heavy chain-Linker-VEGFR1D2 comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 16, wherein the VEGFR1D2-Linker-anti-PD-L1 heavy chain together with the light chain of the anti-PD-L1 antibody can bind to VEGF, PD-L1 and FcR, i) blocking the interaction of PD-L1 on target cells with PD-1 on T cells; ii) blocking the interaction of VEGF with VEGFR on target cells' surfaces; and iii) stimulating targeted cell killings by immune cells..

[0035] The VEGFR1D2-Linker-anti-PD-L1 light chain, with VEGFR1D2 linked to N-terminus of the anti-PD-L1 light chain, comprises an amino acid sequence of SEQ ID NO: 18, which may be encoded by nucleotide of SEQ ID NO: 17. In some embodiments, the VEGFR1D2-Linker-anti-PD-L1 light chain comprises an amino acid sequence having at least

80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 18, wherein the VEGFR1D2-Linker-anti-PD-L1 light chain together with the heavy chain of the anti-PD-L1 antibody can bind to VEGF, PD-L1, and FcR, i) blocking the interaction of PD-L1 on target cells with PD-1 on T cells; ii) blocking the interaction of VEGF with VEGFR on target cells' surfaces; and iii) stimulating targeted cell killings by immune cells.

[0036] In a third aspect, a nucleic acid molecule encoding the recombinant protein/antibody of the present disclosure is also provided, as well as an expression vector comprising the nucleic acid and a host cell comprising the expression vector.

[0037] In a fourth aspect, a method for preparing the recombinant protein/antibody using the host cell comprising the expression vector is also provided, the method comprising the steps of (i) expressing the recombinant protein/antibody in the host cell and (ii) isolating the recombinant protein/antibody from the host cell.

[0038] In a fifth aspect, the present disclosure provides a pharmaceutical composition, comprising the recombinant protein or the recombinant antibody of the present disclosure, and at least one pharmaceutically acceptable carrier.

[0039] In a sixth aspect, the present disclosure provides a method for treating a disease caused by over-expression of VEGF and/or PD-L1, comprising administering to a patient or a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of the present disclosure.

[0040] In one embodiment, the present disclosure provides the use of the recombinant protein or the recombinant antibody in the manufacture of a pharmaceutical composition for the treatment of a disease caused by over-expression of VEGF and/or PD-L1.

[0041] In one embodiment, the method of the present disclosure is for treating a disease selected from the group consisting of acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), bladder cancer, ovarian cancer, prostate cancer, lung cancer, colon cancer, breast cancer, pancreatic cancer, liver cancer, and renal cell carcinoma. In one embodiment, the present disclosure provides a method for treating age-related macular degeneration (AMD), diabetic retinopathy, liver fibrosis or angiosarcoma.

[0042] Other features and advantages of the instant disclosure will be apparent from the following detailed description and examples, which should not be construed as limiting. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

DESCRIPTION OF THE DRAWINGS

[0043] Fig. 1 is a schematic diagram of the structures of recombinant antibodies of the present disclosure.

[0044] Fig. 2 shows SEC-HPLC diagrams of recombinant antibodies of the present disclosure.

[0045] Fig. 3 shows binding activity of recombinant antibodies of the present disclosure to VEGF-165.

[0046] Fig. 4 shows binding activity of recombinant antibodies of the present disclosure to PD-L1.

[0047] Fig. 5 shows simultaneous binding activity of recombinant antibodies of the present disclosure to VEGF-165 and PD-L1.

[0048] Fig. 6 shows IMM2510's ADCC activity against Raji-PD-L1.

[0049] Fig. 7 shows IMM2510's ADCC activity against Raji-PD-L1 in the presence of VEGF-165.

[0050] Fig. 8 shows *in vivo* therapeutic efficacy of IMM2505 in MC38-hPD-L1 xenograft model.

DETAILED DESCRIPTION OF THE INVENTION

[0051] Antibody therapies are approved in various jurisdictions to treat a wide range of diseases, including cancers, and have significantly improved patient outcomes (Komeev KV *et al.*, (2017) TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis. *Cytokine* 89: 127–135). Once bound to a disease antigen, antibodies may induce antibody-dependent cell-mediated cytotoxicity, activate the complement system, and/or prevent a receptor from interacting with its ligand, all of which may lead to cell death. U.S. FDA-approved antibody drugs include Alemtuzumab, Nivolumab, Rituximab and Durvalumab.

[0052] However, clinical studies have shown many patients do not adequately respond to monospecific therapy. For example, the overall response rate of an approved anti-PD-L1 antibody, Avelumab (BAVENCIO), is only 33%. Additionally, acquired antibody resistance frequently occurs following several cycles of treatment.

[0053] Therefore, bispecific or multi-specific antibodies are required against two or more separate and unique antigens, or different epitopes of the same antigen.

[0054] Through diligent experimentation, the present inventor has invented a novel recombinant protein/antibody, which can attack diseases, via three mechanisms of actions, to release the check or inhibition on T cells by PD-1-mediated inhibitory signals, to prohibit new blood vessel formation and release immunity suppression and thus limit target cell growth, and to stimulate targeted cell killings by immune cells such as NK cells and macrophages.

[0055] The recombinant protein of the present disclosure comprises 1) an anti-PD-L1 antibody heavy chain and an anti-PD-L1 antibody light chain, which two are linked by a disulfide bond to bind PD-L1, and 2) an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR), linked via a linker to the N-terminus or C-terminus of the heavy chain or the light chain, wherein the recombinant protein is capable of binding PD-L1, VEGF and Fc receptor simultaneously. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the C-terminus of the heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the light chain. The Fc fragment of the heavy chain will be linked to another Fc fragment by disulfide bonds. For example, two recombinant proteins of the disclosure will be linked together by disulfide bonds to form the recombinant antibody of the present disclosure.

[0056] The recombinant antibody of the disclosure comprises an anti-PD-L1 antibody having two heavy chains and two light chains, wherein an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR) is linked via a linker to N-terminus or C-

terminus of each heavy chain, or to N-terminus or C-terminus of each light chain, wherein the recombinant antibody is capable of binding PD-L1, VEGF and FcR simultaneously. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of each heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the C-terminus of each heavy chain. In some embodiments, the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of each light chain.

[0057] The inventors of the present application surprisingly found that the Fc portion of an antibody remains FcR binding capacity even when a polypeptide is linked to the C-terminus of the Fc fragment. In particular, the inventors, in an unpublished study, designed and prepared a recombinant antibody comprising a normal Ig-like antibody and two VEGFR1D2 fragments, wherein the two VEGFR1D2 fragments are linked to two Fc fragments of the antibody, respectively. The recombinant antibody actively lysed target cells in an ADCC assay with a bit lower activity than the normal antibody, the maximum lysis% and lysis EC_{50} were about 45.0% and 68.71 ng/ml for the recombinant antibody, and the maximum lysis% and lysis EC_{50} for the normal antibody were about 58.0% and 49.55 ng/ml. In a later *in vivo* anti-tumor test, the recombinant antibody showed a better anti-tumor effect than the combined use of the normal antibody and VEGFR1D2-Fc.

[0058] The three main components contained in the recombinant protein/antibody of the present disclosure are the extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR), the linker, and the anti-PD-L1 antibody. A person of ordinary skills in the art will recognize that there are many design choices for selecting the above three components. Preferably, human-derived sequence is used in human disease therapies, as the strong immunogenicity of proteins or peptides from non-human animals may lead to allergy and other adverse effects. However, other animal proteins or peptides, humanized if appropriate, may also be used in the present disclosure based on different application purposes.

[0059] Any anti-PD-L1 antibody may be used in the formation of the recombinant protein or antibody of the present disclosure. The anti-PD-L1 antibody may be an isolated monoclonal antibody selected from the group consisting of Atezolizumab, Avelumab, and Durvalumab.

[0060] In some embodiments, the anti-PD-L1 antibody is an isolated monoclonal antibody comprising two heavy chains each having an amino acid sequence of SEQ ID NO: 2 or 4, and two light chains each having an amino acid sequence of SEQ ID NO: 6, which two may be encoded by nucleic acid sequences of SEQ ID NOs: 1, 3 and 5, respectively. The Fab portion (or paratope) of the anti-PD-L1 antibody can bind to PD-L1 on the cell surfaces of target cells such as tumor/cancer cells to block the interaction of PD-L1 with PD-1 on the cell surfaces of T cells and thus release the check on T cells by PD-1-mediated inhibitory signals, while the Fc portion of the anti-PD-L1 antibody can bind to FcRs on the cell surfaces of immune cells such as NK cells and macrophages to stimulate targeted cell killings by the NK cells or macrophages. In some embodiments, the heavy chain of the anti-PD-L1 antibody may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 2, wherein the anti-PD-L1 antibody is able to bind to PD-L1 and block the interaction of PD-L1 with PD-1 on the cell surfaces of T cells, and is also able to bind to FcRs

on the cell surfaces of immune cells and thus activate these cells for killing target cells such as cancer cells. In some embodiments, the light chain may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 6, wherein the anti-PD-L1 antibody is able to bind to PD-L1 and block the interaction of PD-L1 with PD-1 on the cell surfaces of T cells, and is also able to bind to FcRs on the cell surfaces of immune cells and thus activate these cells for killing target cells such as cancer cells.

[0061] Any extracellular Ig-like domain of any VEGFR (VEGFR1, VEGFR2, and VEGFR3) capable of binding with VEGF, especially VEGF-A, may be selected for construction of the recombinant protein. In one embodiment, the VEGFR in the recombinant protein is VEGFR1, and the extracellular Ig-like domain of the VEGFR is the second extracellular Ig-like domain of VEGFR1 (VEGFR1D2).

[0062] In one embodiment, the VEGFR1D2 has a nucleic acid sequence and an amino acid sequence set forth in SEQ ID Nos: 7 and 8, respectively. In another embodiment, the VEGFR1D2 may comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 8, wherein the VEGFR1D2 can bind to VEGFs expressed by or around target cells, for instance, cancer/tumor cells and thus limit growth of target cells.

[0063] Linkers serve primarily as a spacer between the extracellular Ig-like domain of VEGFR and the N-terminus or C-terminus of the heavy chain or light chain of an anti-PD-L1 antibody. The linker may be made up of amino acids linked together by peptide bonds, preferably from 5 to 30 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. One or more of these amino acids may be glycosylated, as is understood by those of skill in the art. In one embodiment, the 5 to 30 amino acids may be selected from glycine, alanine, proline, asparagine, glutamine, serine and lysine. In one embodiment, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines (particularly (Glys, $(Gly)_8$, poly(Gly-Ala), and polyalanines. One exemplary suitable linker as shown in the Examples below is (Gly-Ser), such as $-(Gly-Gly-Gly-Gly-Ser)_3-$ and $-(Gly-Gly-Gly-Gly-Ser)_2-$.

[0064] Linkers may also be non-peptide linkers. For example, alkyl linkers such as -NH-, $-(CH_2)s-C(O)-$, wherein s=2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_{1-4}) lower acyl, halogen (e.g., CI, Br), CN, NH_2, phenyl, etc.

[0065] Also, the present disclosure provides a polynucleotide molecule encoding the recombinant protein or antibody and an expression vector expressing the recombinant protein or antibody. Examples of vectors include but are not limited to plasmids, viral vectors, yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), transformation-competent artificial chromosomes (TACs), mammalian artificial chromosomes (MACs) and human artificial episomal chromosomes (HAECs).

[0066] The present disclosure provides host cells comprising the above expression vectors. The host cells may be transformed or transfected with the expression vectors. Suitable host cells include *Escherichia coli*, yeasts and other eukaryotes. Preferably, *Escherichia coli*, yeast or mammalian cell lines (such as COS or CHO) are used.

[0067] In another aspect, the present disclosure provides a pharmaceutical composition comprising the recombinant protein or antibody of the present disclosure formulated together with a pharmaceutically acceptable carrier. The composition may optionally contain one or more additional pharmaceutically active ingredients, such as another antibody or a drug. The pharmaceutical compositions of the disclosure also can be administered in a combination therapy with, for example, another immune-stimulatory agent, anti-cancer agent, an anti-viral agent, or a vaccine.

[0068] The pharmaceutical composition can comprise any number of excipients. Excipients that can be used include carriers, surface active agents, thickening or emulsifying agents, binders, dispersion or suspension aids, solubilizers, colorants, flavoring agents, coatings, disintegrating agents, lubricants, sweeteners, preservatives, isotonic agents, and combinations thereof. The selection and use of suitable excipients are taught in Gennaro, ed., Remington: The Science and Practice of Pharmacy, 20th Ed. (Lippincott Williams & Wilkins 2003), the disclosure of which is incorporated herein by reference.

[0069] The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in injection. For example, the vehicle or carrier may be neutral buffered saline or saline mixed with serum albumin. Other exemplary pharmaceutical compositions comprise Tris buffers, or acetate buffers, which may further include sorbitol or a suitable substitute thereof. In one embodiment of the present disclosure, compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, supra) in the form of a lyophilized cake or an aqueous solution. Further, the therapeutic composition may be formulated as a lyophilizate using appropriate excipients such as sucrose.

[0070] Preferably, the pharmaceutical composition is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active molecule can be coated in a material to protect it from the action of acids or enzymes and other natural conditions that may inactivate it. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, an antibody of the disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, e.g., intranasally, orally, vaginally, rectally, sublingually or topically.

[0071] Pharmaceutical compositions can be in the form of sterile aqueous solutions or dispersions. They can also be formulated in a microemulsion, liposome, or other ordered structure suitable to high drug concentration.

[0072] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration and will generally be that amount of the composition which

produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01% to about 99% of active ingredient, preferably from about 0.1% to about 70%, most preferably from about 1% to about 30% of active ingredient in combination with a pharmaceutically acceptable carrier.

[0073] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active molecule calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Alternatively, the recombinant protein can be administered as a sustained release formulation, in which case less frequent administration is required.

[0074] For administration of the recombinant protein, the dosage may range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 10 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration twice per week, once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for the recombinant protein of the disclosure include 3 mg/kg body weight or 6 mg/kg body weight via intraperitoneal administration, with the protein being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks; (vi) 6 mg/kg body weight, one dosage per week. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg/ml and in some methods about 25-300 μg/ml.

[0075] A "therapeutically effective dosage" of a recombinant protein of the disclosure preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumor-bearing subjects, a "therapeutically effective dosage" preferably inhibits tumor growth by at least about 40%, more preferably by at least about 60%, even more preferably by at least about 80%, and still more preferably by at least about 99% relative to untreated subjects. A therapeutically effective amount of a fusion protein of the present disclosure can decrease tumor size, or otherwise ameliorate symptoms in a subject, which is typically a human or can be another mammal.

[0076] The pharmaceutical composition can be a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. See, e.g., Sustained and

Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0077] Therapeutic compositions can be administered via medical devices such as (1) needleless hypodermic injection devices (e.g., U.S. Pat. Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; and 4,596,556); (2) micro-infusion pumps (U.S. Pat. No. 4,487,603); (3) transdermal devices (U.S. Pat.No. 4,486,194); (4) infusion apparatuses (U.S. Pat.Nos. 4,447,233 and 4,447,224); and (5) osmotic devices (U.S. Pat. Nos. 4,439,196 and 4,475,196); the disclosures of which are incorporated herein by reference.

[0078] In certain embodiments, the recombinant protein of the disclosure can be formulated to ensure proper distribution *in vivo*. For example, to ensure that the therapeutic proteins of the disclosure cross the blood-brain barrier, they can be formulated in liposomes, which may additionally comprise targeting moieties to enhance selective transport to specific cells or organs. See, e.g. U.S. Pat. Nos. 4,522,811; 5,374,548; 5,416,016; and 5,399,331.

[0079] A gene therapy *in vivo* is also envisioned wherein a nucleic acid molecule encoding the recombinant protein of the present disclosure, or a derivative thereof is introduced directly into the subject. For example, a nucleic acid sequence encoding a recombinant protein of the present disclosure is introduced into target cells via local injection of a nucleic acid construct with or without an appropriate delivery vector, such as an adeno-associated virus vector. Alternative viral vectors include, but are not limited to, retroviruses, adenovirus, herpes simplex vims and papilloma virus vectors. Physical transfer of the virus vector may be achieved *in vivo* by local injection of the desired nucleic acid construct or other appropriate delivery vector containing the desired nucleic acid sequence, liposome- mediated transfer, direct injection (naked DNA), or microparticle bombardment (gene-gun).

[0080] The compositions of the present disclosure may be used alone or in combination with other therapeutic agents to enhance their therapeutic effects or decrease potential side effects.

[0081] Another object of the present disclosure is to provide a method for preparing the above recombinant protein or recombinant antibody and the pharmaceutical composition comprising the same. In one embodiment, the method comprises (1) providing a recombinant protein-encoding polynucleotide molecule; (2) constructing an expression vector comprising the polynucleotide molecule of (1); (3) transfecting or transforming suitable host cells with the expression vector of (2) and cultivating the host cells to express the protein; and (4) purifying the protein or antibody. The preparation may be carried out with well-known technologies by an ordinarily skilled artisan.

[0082] Another object of the present disclosure is to provide a method of treating diseases using the pharmaceutical composition of the present disclosure, comprising administrating an effective amount of the aforementioned pharmaceutical composition to the patients or subjects in need thereof.

[0083] In one embodiment, the pharmaceutical composition is used to treat VEGF and/or PD-L1-overexpressing tumors or cancers, including but not limited to acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), non-hodgkins lymphoma (NHL), multiple myeloma (MM), bladder cancer, ovarian cancer,

prostate cancer, lung cancer, colon cancer, breast cancer, pancreatic cancer, liver cancer and renal cancer.

[0084] In one embodiment, the present disclosure provides a method for treating diseases related to over-expressions of VEGF, which include, but not limited to, age-related macular degeneration (AMD), diabetic retinopathy, liver fibrosis and angiosarcoma.

[0085] The present disclosure is now further described with the non-limiting examples below.

[0086] EXAMPLES

[0087] In the descriptions below, IMM25 refers to a monoclonal anti-PD-L1 antibody that targets PD-L1. This antibody has two heavy chains each having an amino acid sequence of SEQ ID NO: 2, and two light chains each having an amino acid sequence of SEQ ID NO: 6.

[0088] IMM2510 refers to a recombinant antibody, containing two VEGFR1D2s each linked via a GS-linker, to the N-terminus of each heavy chain of an anti-PD-L1 antibody. The VEGFR1D2-linker-anti-PD-L1 heavy chain is encoded by a nucleic acid sequence of SEQ ID NO: 13, and has an amino acid sequence of SEQ ID NO: 14, respectively. The light chain of the anti-PD-L1 antibody has an amino acid sequence of SEQ ID NO: 6.

[0089] IMM25011 refers to a recombinant antibody, containing two VEGFR1D2s each linked via a GS-linker, to the C-terminus of each heavy chain of an anti-PD-L1 antibody. The anti-PD-L1 heavy chain-Linker-VEGFR1D2 is encoded by a nucleic acid sequence of SEQ ID NO: 15, and has an amino acid sequence and SEQ ID NO: 16. The light chain of the anti-PD-L1 antibody has an amino acid sequence of SEQ ID NO: 6.

[0090] IMM25031 refers to a recombinant antibody, containing two VEGFR1D2s each linked via a GS-linker, to the N-terminus of each light chain of an anti-PD-L1 antibody. The VEGFR1D2-linker-anti-PD-L1 light chain is encoded by a nucleic acid sequence of SEQ ID NO: 17, and has an amino acid sequence of SEQ ID NO: 18. The heavy chain of the anti-PD-L1 antibody had an amino acid sequence of SEQ ID NO: 2.

[0091] VEGFR1-Fc is a recombinant protein, containing two VEGFR1D2s linked to an antibody Fc portion. Each VEGFR1D2-Fc fragment has an amino acid sequence of SEQ ID NO:22, which may be encoded by a nucleic acid sequence of SEQ ID NO:21.

[0092] The basic structures of these four recombinant antibodies are shown in Fig. 1.

[0093] **Example 1.** Construction of vectors expressing IMM25, IMM25011, IMM25031 and IMM2510

[0094] 1.1 IMM25

[0095] Full-length coding sequence of IMM25 was designed artificially. Specifically, 57 nucleotides encoding the signal peptide of mouse IgG1 heavy chain (SEQ ID NO.: 19) were added to the 5' end of the anti-PD-L1 heavy chain-coding sequence (SEQ ID NO.: 1), and a Kozak sequence (SEQ ID NO: 20) was added to the 5' end of the signal peptide sequence. Lastly, HindIII and NheI restriction sites were added to the 5' and 3' ends of the resulting sequence, respectively. For the light chain, the same signal sequence as well as the Kozac sequence was used, but HindIII and the XbaI restriction sites were added to the resulting sequence, respectively. The two resulting sequences were synthesized by Genscript (ID#: C0015BJ110-1 (heavy chain); C0015BJ110-2 (light chain)) and subcloned, respectively, into the pMac-H and pMac-L vectors.

[0096] 1.2 IMM2510

[0097] Full-length coding sequence of IMM2510 was designed artificially. Specifically, for the heavy chain, the coding sequence of the second extracellular domain of VEGFR1 (VEGFR1D2) (SEQ ID NO.: 7) was linked through the GS-linker (SEQ ID NO.: 11) to the 5' end of the anti-PD-L1 heavy chain coding sequence (SEQ ID NO.:3). 57 nucleotides encoding the signal peptide of mouse IgG1 heavy chain (SEQ ID NO.: 19) were added to the 5' end of VEGFR1D2-coding sequence, and a Kozak sequence (SEQ ID NO.: 20) was added to the 5' end of the signal peptide sequence. Lastly, HindIII and NheI restriction sites were added to the 5' and 3' ends of the resulting sequence, respectively. The resulting sequence was synthesized by Genscript (ID#: C9143DA150-1) and subcloned into the pMac-H vector. The expression vector for the light chain of IMM2510 is identical to that of IMM25.

[0098] 1.3 IMM25011

[0099] Full-length coding sequence of IMM25011 was designed artificially. Specifically, for the heavy chain, the coding sequence of anti-PD-L1 heavy chain coding sequence (SEQ ID NO.:3) was linked through the GS-linker (SEQ ID NO.: 9) to the 3' end of the coding sequence of the second extracellular domain of VEGFR1 (VEGFR1D2) (SEQ ID NO.: 7), and NheI and SalI restriction sites were added to the 5' and 3' ends of the resulting sequence, respectively. The resulting sequence was synthesized by Genscript (ID#: C8379CJ170-1) and subcloned into the IMM25 heavy chain expression vector. The expression vector for the light chain of IMM2510 is identical to that of IMM25.

[00100] 1.4 IMM25031

[00101] Full-length coding sequence of IMM25011 was designed artificially. Specifically, for the light chain, the coding sequence of the second extracellular domain of VEGFR1 (VEGFR1D2) (SEQ ID NO.: 7) was linked through the GS-linker (SEQ ID NO.: 11) to the 5' end terminal of the anti-PD-L1 light chain coding sequence (SEQ ID NO.:5). 57 nucleotides encoding the signal peptide of mouse IgG1 heavy chain (SEQ ID NO.: 19) were added to the 5' end of VEGFR1D2-coding sequence, and a Kozak sequence (SEQ ID NO.: 20) was added to the 5' end of the signal peptide sequence. Lastly, HindIII and XbaI restriction sites were added to the 5' and 3' ends of the resulting sequence, respectively. The resulting sequence was synthesized by Genscript (ID#: C4608CE150-1) and subcloned into the pMac-L vector. The expression vector for the heavy chain of IMM25031 is identical to that of IMM25.

[00102] **Example 2.** Manufacture and quality analysis of recombinant fusion antibodies

[00103] To manufacture the recombinant proteins, the expression vectors were transfected into Free StyleTM CHO-S cells (Thermo Fisher Scientific,Cat#R80007) using Polyetherimide (PEI) (polysciences,Cat#24765-1) as the tranfectant. Cells were cultured for about 7-10 days before harvesting cell culture supernatant for protein purification by affinity chromatography. The purity of the recombinant proteins was analyzed by SEC-HPLC.

[00104] The SEC-HPLC diagram in Fig. 2 showed a big difference among the three recombinant antibodies of the present disclosure. IMM2510 had the highest purity (90.67%), followed by IMM25011 (85.58%) and IMM25031 (71.97%). IMM25011 had high percentage of aggregates (15.42%), while IMM25031 had more degradation (27.11%).

[00105] **Example 3.** Recombinant antibodies bound to VEGF-165

[00106] For VEGF binding, recombinant human VEGF-165 (Cat# 11066-HNAH，Sino Biologicals) was prepared in coating buffer (Product code: 1001329288 C3041-100CAP, Sigma-Aldrich Co.) and transferred to the ELISA plate (Cat#442404, Nunc™) at 50 ng/well, and the plate was placed in 4°C refrigerator overnight. Then, the plate was washed for three times with PBS containing 0.05% of Tween-20 (PBS-T) before serially diluted recombinant antibodies and control antibody (Avastin) were added, and the plate was incubated at room temperature for 1 hour and then washed again for 5 times with PBS-T. HRP-Rabbit Anti-Human IgG Fc (Cat#:309-036-008, Jackson ImmunoResearch Lab) was added to the plate and the plate was incubated at room for one hour. After washing the plate for 5 times with PBS-T, substrate was added to the plate which was read in a plate reader after the color changing was stopped by 1N H_2S0_4.

[00107] As shown in Fig. 3, the three recombinant antibodies of the present disclosure had similar binding activities to VAGF-165 but relatively 2 to 4 folds lower than that of Avastin.

[00108] **Example 4.** Recombinant antibodies bound to PD-L1

[00109] For PD-L1 binding analysis, 100 μl of 1×10^6/ml CHO-PD-L1 cells (ImmuneOnco, Cat#YMAK-C006) were incubated with 100 μl of serially titrated recombinant antibodies as well as control antibodies (Atezolizumab and Herceptin) at 4°C for 40 minutes. After washed with cold PBS, cells were incubated with FITC-conjugated anti-human IgG-Fc antibody (Sigma, Cat#F9512). Then, the cells were washed for two times and subjected to FACS analysis.

[00110] The results as shown in Fig. 4 revealed that IMM25011, as expected due to its structure, had identical binding activity to that of Atezolizumab (EC_{50} = 0.22 nM for Atezolizumab, EC_{50} = 0.23 nM for IMM25011), while IMM25031 (EC50 = 0.96 nM) and IMM2510 (0.85 nM) had similar binding activities but relatively 4 folds lower than that of Atezolizumab.

[00111] **Example 5.** Recombinant antibodies bound to VEGF-165 and PD-L1 simultaneously

[00112] For analysis of simultaneous binding activity of the recombinant antibodies to two targets, 100 μl of 1×10^6/ml CHO-PD-L1 cells (ImmuneOnco, Cat#YMAK-C006) were incubated with 100 μl of serially diluted IMM2510 for 45 minutes at 4℃. Cells were washed with cold PBS for two times, then incubated with 100 μl 40 nM biotin-conjugated VEGF-165 (Cat# 11066-HNAH，Sino Biologicals) for 45 minutes at 4℃. After being washed for two times, cells were stained with FITC-conjugated Streptavidin (BD Pharmingen, Cat#554060, Lot#6169673) for another 45 minutes at 4℃. After washes, cells were subject to FACS analysis.

[00113] As shown in Fig. 5, with the increase of IMM2510 concentration, a dose-dependent increase in fluorescence signal was observed, suggesting a simultaneous binding of the two targets. The binding affinity of IMM2510 (EC_{50} = 0.63 nM) was similar to that seen in single target-binding assay with PD-L1 (EC_{50} = 0.85 nM).

[00114] **Example 6.** Recombinant antibodies induced antibody-dependent cell-mediated cytotoxicity (ADCC)

[00115] CFSE-labeled Raji-PD-L1 cells (ImmuneOnco, Cat#YMAK-C007) as target cells, 6×10^5/ml, 50 μl, were mixed with 100 μl of 6×10^5/ml NK92MI cells (ATCC, Cat#CRL-2408)

as effector cells stably expressing FcγRIIIa at a 1:2 ratio, and the mixed cells were cultured for 4 hours at 37℃ under 5% CO_2 in the presence of 50 μl serially diluted IMM2510 or IMM25. Then propidium iodide (PI) (Sigma, Cat#P4170) was added to the cell culture at a concentration of 5 μg/ml, and the cell culture was subjected to FACS analysis for PI signals.

[00116] To confirm that ADCC activity would not be impacted by VEGF binding, VEGF-165 protein (Cat# 11066-HNAH，Sino Biologicals) at a low (25 ng/ml) or high concentration (250 ng/ml) was added respectively together with IMM2510 or control antibodies to the cell culture, ADCC was analyzed following the procedure as described above.

[00117] Percentage of cell lysis caused by ADCC was calculated based on the following formula: % Lysis (or %ADCC) = (% PI Positive Cells with IMM2510 or IMM25 - % PI Positive Cells with negative control protein)/(100 - % PI Positive Cells with negative control protein)*100.

[00118] The ADCC activity of IMM2510 showed a six folds decrease when compared to that of IMM25, as shown in Fig. 6, which might be due to the relatively lower PD-L1-binding affinity (EC_{50} = 0.85 nM vs. EC_{50} = 0.22 nM for IMM25). ADCC activity of IMM2510 was not impacted when VEGF-165 was added to the assay, as shown in Fig. 7, further suggesting a simultaneous but respective binding of the two targets.

[00119] **Example 7.** IMM2510 had good anti-tumor effect

[00120] *In vivo* efficacy was evaluated using B-hPD-1 humanized mice (BIOCYTOGEN). Mouse colon cancer cells (MC-38) stably expressing human PD-L1 (MC38-PD-L1, BIOCYTOGEN) were prepared in serum-free medium and subcutaneously injected into the right flank of the B-hPD-1 humanized mice at the age of 6-8 weeks. When the tumor volume reached 100-200 mm^3, mice were randomly assigned into five groups with 6 mice in each group. Mice were treated respectively with PBS, IMM25 (5.0 mg/kg), VEGFR1-Fc (2.0 mg/kg), IMM2510 (6.0 mg/kg), and combination of IMM25 (5.0 mg/kg) and VEGFR1-Fc (2.0 mg/kg). Treatment was conducted intraperitoneally, twice a week for four weeks. Tumor volume and body weight were measured twice a week.

[00121] The tumor volume (V) was calculated as (length × $width^2$)/2. Tumor growth inhibition rate (TGI) was calculated by the formula: Tumor growth inhibition rate = (1-tumor volume change in administration group/tumor volume change in control group) ×100%.

[00122] Results were expressed as mean ± S.E.M. Comparisons between two groups were made by Dunnett's multi-comparison test, wherein $P < 0.05$ was considered significant.

Table 1. Anti-tumor effect of IMM2510 and other agents

Group	Drug	Animal#	Dose (mg/kg)	Treatment	TGI*
1	PBS	6	N/A	i.p, b.i.w. ×4	
2	IMM25	6	5.0	i.p, b.i.w. ×4	27.15%
3	VEGFR1-Fc	6	2.0	i.p, b.i.w. ×4	30.02%
4	IMM2510	6	6.0	i.p, b.i.w. ×4	79.01%
5	IMM25+VEGFR1-Fc	6	5.0+2.0	i.p, b.i.w. ×4	48.99%

[00123] Tumor volume and TGI can be found in Table 1 and Fig. 8. Data showed that the tumor volume of the single agent-treated mice grew relatively slowly (mean tumor volume at day 28: IMM25 = 2168.0 mm^3, VEGFR1-Fc = 2088.0 mm^3) compared to PBS (Mean value =

2934.0 mm^3), while the tumor volume of mice treated with combination of the two single agents grew significantly more slowly (Mean value = 1552.0 mm^3) than that treated with single agent. The recombinant antibody IMM2510 inhibited tumor growth with much stronger efficacy (Mean value = 706.0 mm^3), even significantly better than the combination treatment.

[00124] **Comparative Example 1.** IMM0404's anti-tumor activity in HT-29 or NCl-H1975 xenograft model

[00125] IMM0404 in this Example is a recombinant antibody, containing two SIRPαD1s each linked via a GS-linker, to an anti-EGFR antibody, Eribitux, at the N-terminus of each heavy chain. The SIRPαD1-GS-linker-anti-EGFR heavy chain has an amino acid sequence of SEQ ID NO: 23. The light chain of the anti-EGFR antibody has an amino acid sequence of SEQ ID NO: 24.

[00126] SIRPαD1-Fc is a fusion protein consisting of SIRPαD1 linked to an Fc fragment, which was described in WO2016169261. The amino acid sequence of this fusion protein is set forth in SEQ ID NO: 25.

[00127] *HT-29 xenograft model*

[00128] HT-29 human colon cancer cells were cultured in the McCoy's 5A medium containing 10% FBS at 37℃ and 5% CO_2. Cells at the logarithmic phase were collected and re-suspended in 1×PBS. The suspension was added with and mixed with Matrige at a volume ratio of 1:1, and the mixture contained 3×10^7cells per mL.

[00129] Forty mice were injected subcutaneously with HT-29 cells, $3x10^6$ cells per mouse, at the right flank. When tumor volume reached 100-200 mm^3, these animals were randomly allocated into 5 groups with 8 mice in each group. Mice were respectively treated, once per week, through intraperitoneal injection with PBS, SIRPαD1-Fc (1.2 mg/kg), Erbitux (2.0 mg/kg), IMM0404 (2.7 mg/kg), and SIRPαD1-Fc+Erbitux (1.2 mg/kg + 2.0 mg/kg), for 4 weeks. Totally four treatments were given. The day upon first dosing was defined as Day 0. Tumor volume and body weight were measured twice a week.

[00130] The tumor volume (V) was calculated as (length × $width^2$)/2. Tumor growth inhibition rate (TGI) was calculated by the formula: Tumor growth inhibition rate = (1-tumor volume change in administration group/tumor volume change in control group) ×100%. The student test was used to calculate group differences.

Table 2. Anti-tumor effect of IMM0404 and other antibodies

Group	Drug	Animal#	Dose (mg/kg)	Treatment	TGI*
1	PBS	8	N/A	i.p, q.w. × 4	
2	SIRPαD1-Fc	8	1.2	i.p, q.w. × 4	32.79%
3	Eribitux	8	2.0	i.p, q.w. × 4	40.00%
4	IMM0404	8	2.7	i.p, q.w. × 4	18.48%
5	SIRPαD1-Fc + Eribitux	8	1.2+2.0	i.p, q.w. × 4	33.04%

[00131] It can be seen from Table 2 that IMM0404 did not show better anti-tumor activity than other proteins in this xenograft model.

[00132] *NCI-H1975 xenograft model*

[00133] NCI-H1975 non-small cell lung cancer cells were cultured in the RPMI-1640 medium containing 10% FBS (GIBCO, US) at 37℃ and 5% CO_2. Cells at the logarithmic phase were collected and re-suspended in 1×PBS, 1×10^7cells per mL.

[00134] Forty SCID mice were injected subcutaneously with NCI-H1975 cells, $1x10^6$ cells per mouse, at the right flank. When tumor volume reached 100-200 mm^3, these animals were randomly allocated into 5 groups with 8 mice in each group. Mice were respectively treated, once per week, through intraperitoneal injection with PBS, SIRPαD1-Fc (2.7 mg/kg), Erbitux (5.0 mg/kg), IMM0404 (6.0 mg/kg), and SIRPαD1-Fc+Erbitux (2.7 mg/kg + 5.0 mg/kg), for 3 weeks. Totally three treatments were given. The day upon first dosing was defined as Day 0. Tumor volume and body weight were measured twice a week.

[00135] The tumor volume (V) was calculated as (length × $width^2$)/2. Tumor growth inhibition rate (TGI) was calculated by the formula: Tumor growth inhibition rate = (1-tumor volume change in administration group/tumor volume change in control group) ×100%. The student test was used to calculate group differences.

Table 3. Anti-tumor effect of IMM0404 and other antibodies

Group	Drug	Animal#	Dose (mg/kg)	Treatment	TGI*
1	PBS	8	N/A	i.p, q.w. × 3	
2	SIRPαD1-Fc	8	2.7	i.p, q.w. × 3	49.49%
3	Eribitux	8	5.0	i.p, q.w. × 3	85.69%
4	IMM0404	8	6.0	i.p, q.w. × 3	68.77%
5	SIRPαD1-Fc + Eribitux	8	2.7+5.0	i.p, q.w. × 3	76.03%

[00136] It can be seen from Table 3 that IMM0404's anti-tumor activity was better than SIRPαD1-Fc, but interior to Eribitux and SIRPαD1-Fc +Eribitux.

[00137] While the disclosure has been described above in connection with one or more embodiments, it should be understood that the disclosure is not limited to those embodiments, and the description is intended to cover all alternatives, modifications, and equivalents, as may be included within the spirit and scope of the appended claims. All referenced cited herein are further incorporated by reference in their entirety.

[00138] Several important amino acid sequences of the disclosure are listed below.

SEQ ID NO./ Description/ Sequence
SEQ ID NO.: 14 VEGFR1D2-linker-anti-PD-L1 heavy chain (IMM2510) SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISAATYKEIG LLTCEATVNGHLYKTNYLTHRQTNTGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTF SDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCA RRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PG
SEQ ID NO.: 16

anti-PD-L1 heavy chain-Linker-VEGFR1D2 (IMM25011) EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSSDTGRPFVEMYSEIPEIIHMTEGRELVIPC RVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISAATYKEIGLLTCEATVNGHLYKTNYLTHRQTNT
SEQ ID NO.: 6 Light chain of IMM2510, IMM25011 and IMM25 DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
SEQ ID NO.: 18 VEGFR1D2-linker-anti-PD-L1 light chain (IMM25031) SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISAATYKEIG LLTCEATVNGHLYKTNYLTHRQTNTGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDV STAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO.: 2 Heavy chain of IMM25031 and IMM25 EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

We Claim:

1. A recombinant protein, comprising

- an anti-PD-L1 antibody heavy chain and an anti-PD-L1 antibody light chain, which two are linked by a disulfide bond to bind PD-L1, and

- an extracellular Ig-like domain of a vascular epithelial growth factor receptor (VEGFR), linked via a linker to N-terminus or C-terminus of the heavy chain or the light chain,

wherein the recombinant protein is capable of binding PD-L1, VEGF and Fc receptor simultaneously.

2. The recombinant protein according to claim 1, wherein the extracellular Ig-like domain of VEGFR is a second extracellular Ig-like domain of VEGFR1 (VEGFR1D2).

3. The recombinant protein according to claim 2, wherein the VEGFR1D2 comprises an amino acid sequence of SEQ ID NO.: 8.

4. The recombinant protein according to claim 1, wherein the anti-PD-L1 antibody is Atezolizumab, Avelumab or Durvalumab.

5. The recombinant protein according to claim 1, wherein the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the heavy chain.

6. The recombinant protein according to claim 5, wherein the heavy chain with the extracellular Ig-like domain of VEGFR comprises an amino acid sequence of SEQ ID NO.:14, and the light chain comprises an amino acid sequence of SEQ ID NO.: 6.

7. The recombinant protein according to claim 1, wherein the extracellular Ig-like domain of VEGFR is linked via a linker to the C-terminus of the heavy chain.

8. The recombinant protein according to claim 7, wherein the heavy chain with the extracellular Ig-like domain of VEGFR comprises an amino acid sequence of SEQ ID NO.:16, and the light chain comprises an amino acid sequence of SEQ ID NO.: 6.

9. The recombinant protein according to claim 1, wherein the extracellular Ig-like domain of VEGFR is linked via a linker to the N-terminus of the light chain.

10. The recombinant protein according to claim 9, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO.: 2, and the light chain with the extracellular Ig-like domain of VEGFR comprises an amino acid sequence of SEQ ID NO.:18.

11. An recombinant antibody, comprising two identical recombinant proteins according to any one of claims 1 to 10.

12. A polynucleotide encoding the recombinant protein according to any one of claims 1 to 10.

13. A pharmaceutical composition, comprising the recombinant protein according to any one of claims 1 to 10, the recombinant antibody according to claim 11, or the polynucleotide according to claim 12, and a pharmaceutically acceptable carrier.

14. Use of the pharmaceutical composition according to claim 13 in preparation of a medicament for treating a disease caused by over-expression of VEGF or PD-L1, or both.

15. The use according to claim 14, wherein the disease is selected from the group consisting of acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), bladder cancer, ovarian cancer, prostate cancer, lung cancer, colon cancer, breast cancer, pancreatic cancer, liver cancer, renal cell carcinoma, age-related macular degeneration, diabetic retinopathy, liver fibrosis and angiosarcoma.

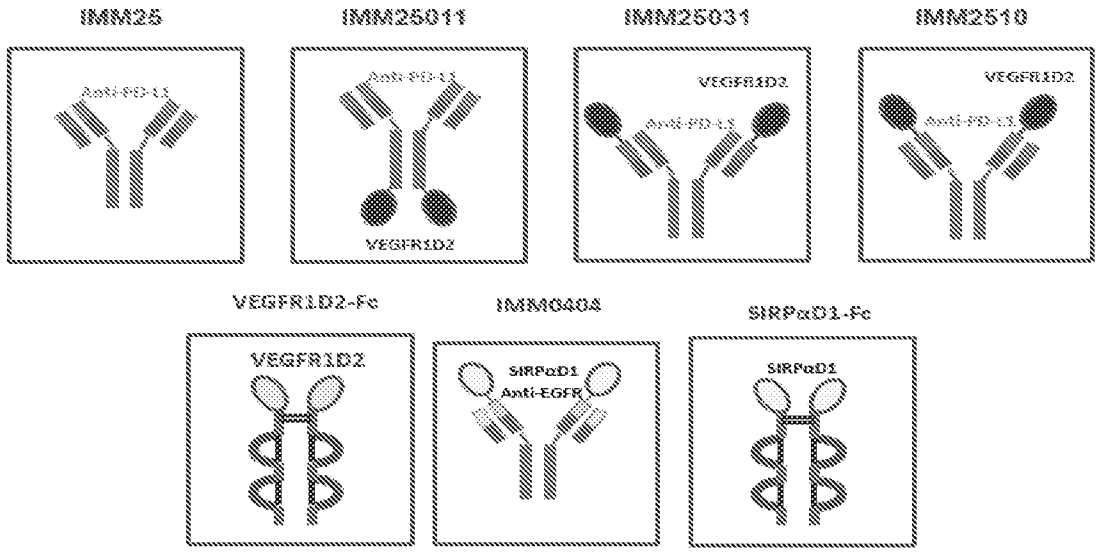
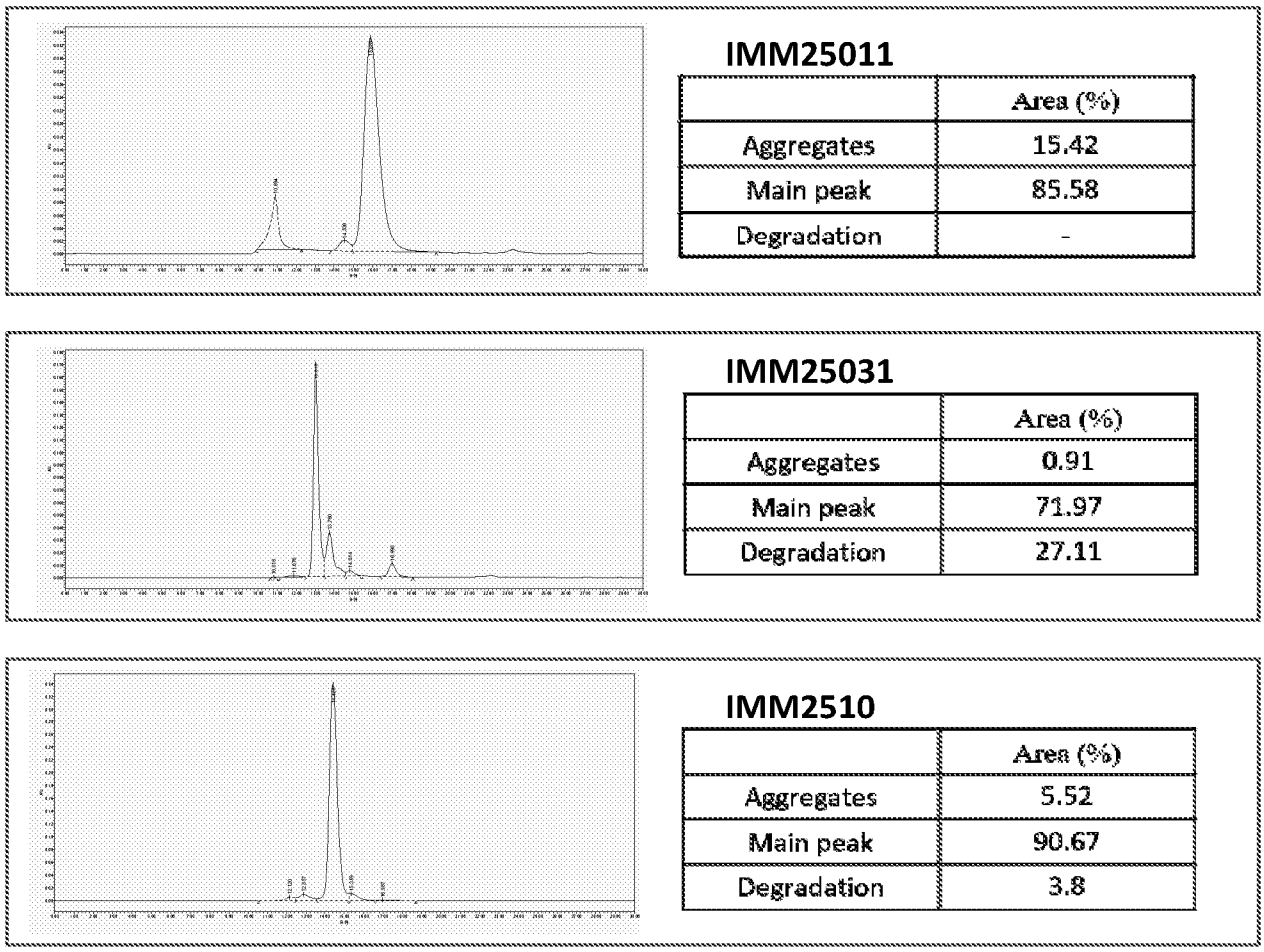


FIG. 1



IMM25011

	Area (%)
Aggregates	15.42
Main peak	85.58
Degradation	-

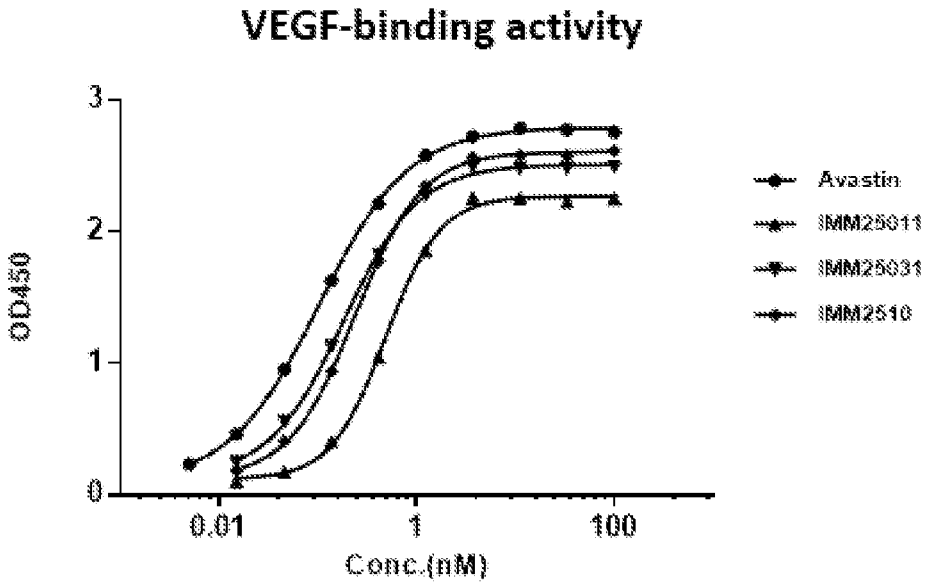
IMM25031

	Area (%)
Aggregates	0.91
Main peak	71.97
Degradation	27.11

IMM2510

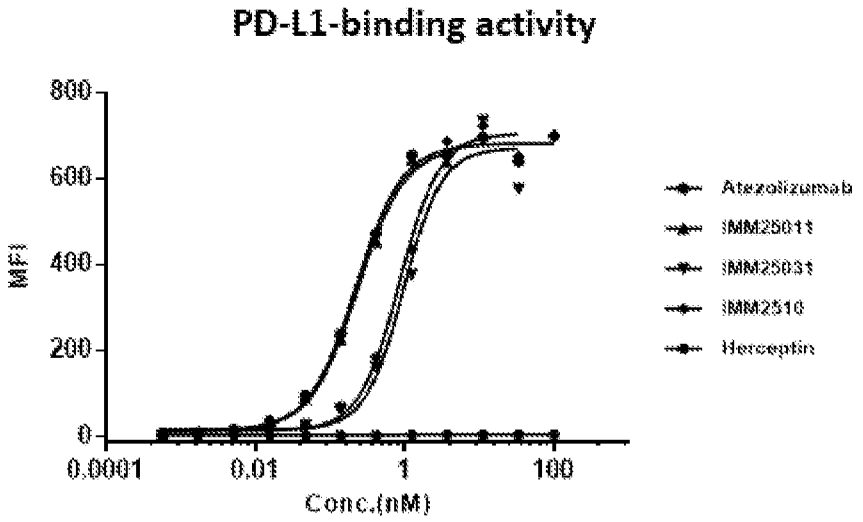
	Area (%)
Aggregates	5.52
Main peak	90.67
Degradation	3.8

FIG. 2



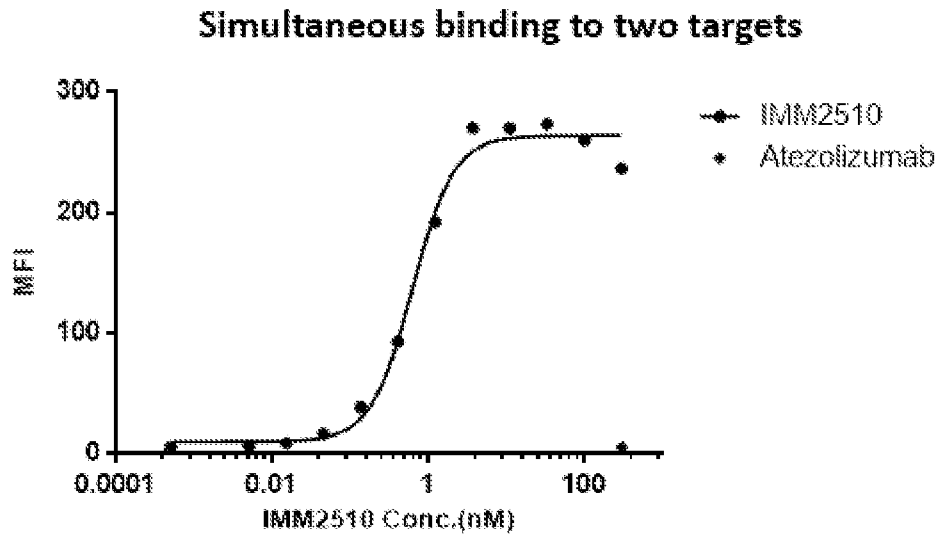
	Avastin	IMM25011	IMM25031	IMM2510
EC50	0.1027	0.4829	0.1826	0.2398

FIG. 3



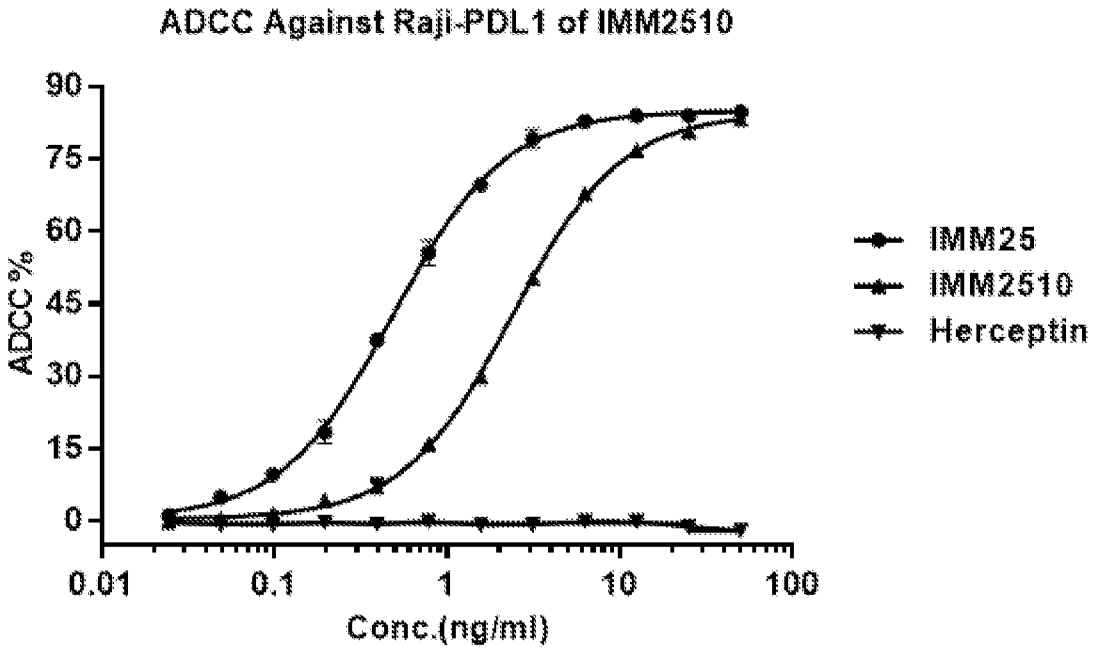
	Atezolizumab	IMM25011	IMM25031	IMM2510
EC50	0.2190	0.2334	0.9623	0.8482

FIG. 4



EC50	0.6318

FIG. 5



	IMM25	IMM2510
EC50	0.4855	2.364

FIG. 6

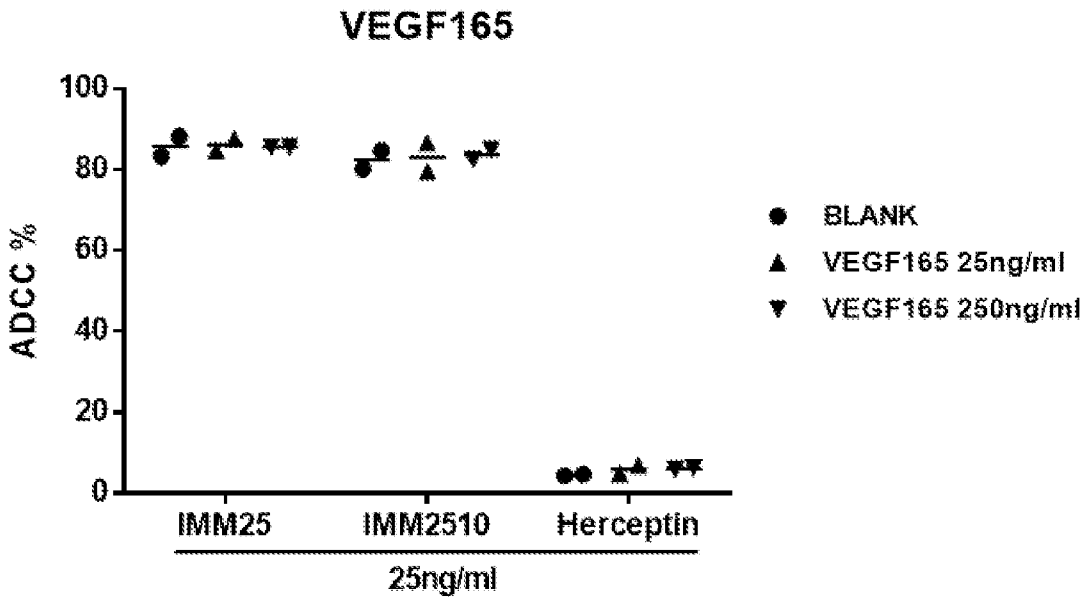

VEGF165
ADCC %
100
80
60
40
20
0
IMM25
IMM2510
Herceptin
25ng/ml
BLANK
VEGF165 25ng/ml
VEGF165 250ng/ml

FIG. 7

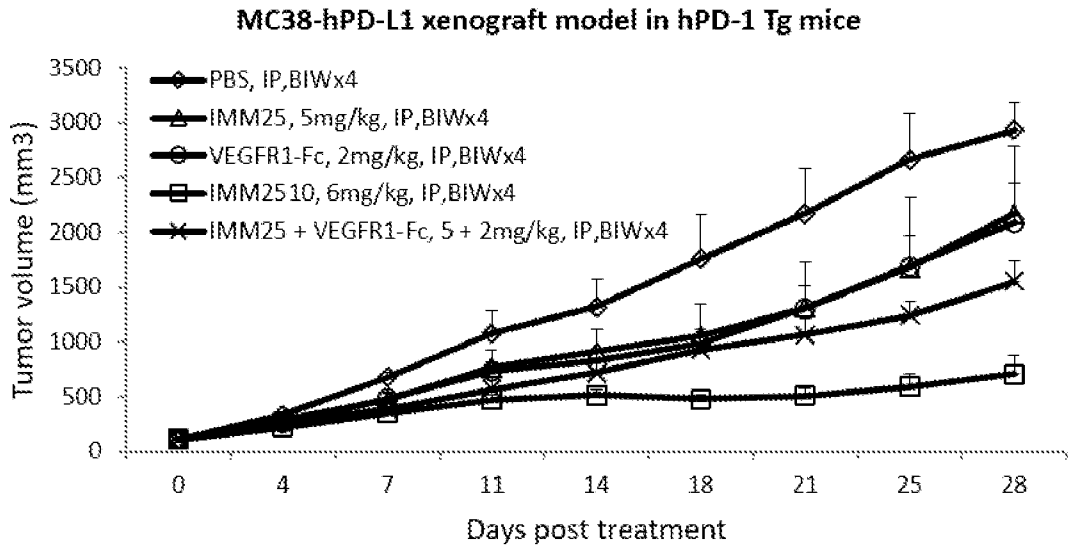

MC38-hPD-L1 xenograft model in hPD-1 Tg mice
Tumor volume (mm3)
3500
3000
2500
2000
1500
1000
500
0
PBS, IP,BIWx4
IMM25, 5mg/kg, IP,BIWx4
VEGFR1-Fc, 2mg/kg, IP,BIWx4
IMM2510, 6mg/kg, IP,BIWx4
IMM25 + VEGFR1-Fc, 5 + 2mg/kg, IP,BIWx4
0
4
7
11
14
18
21
25
28
Days post treatment

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/122446

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C07K 16/30(2006.01)i; C07K 19/00(2006.01)i; C12N 15/13(2006.01)i; C12N 15/62(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K,C12N,A61K,A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS,SIPOABS,DWPI,CNKI,WANFANG DATA,DUXIU,PUBMED,ISI web of knowledge,Elsevier Science Direct, Springerlink,Genbank,Uniport,STN,BAIDU:IMMUNEONCO BIOPHARMACEUTICALS,TIAN WENZHI,LI SONG,PD-L1,PD-1,programmed death-ligand 1,CD274,VEGF,VEGFR1,VEGFR1D2,recombinant,antibody, bi-specific, bispecific, disulfide,Atezolizumab, Avelumab,Durvalumab,SEQ ID NOs:2,6,8,14,16,18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2019062642 A1 (BEIJING BEYOND BIOTECHNOLOGY CO., LTD) 04 April 2019 (2019-04-04) claims 1-16	1-15
PX	WO 2019168947 A1 (AP BIOSCIENCES, INC.) 06 September 2019 (2019-09-06) claims 1-22	1-15
Y	WO 2015200905 A2 (OLIGASIS, LLC) 30 December 2015 (2015-12-30) claims 1, 63-65, description paragraphs 16, 27, 28, 107, 111, 191, 233, figure 39	1-15
Y	WO 2017136562 A2 (KADMON CORPORAIION, LLC) 10 August 2017 (2017-08-10) claims 1, 15-17, 22, description page 2 line 29 to page 3 line 6	1-15
A	CN 108671229 A (HUABO BIOPHARM SHANGHAI CO., LTD) 19 October 2018 (2018-10-19) SEQ ID NO:1	1-15
A	WO 2015148416 A1 (JING, D.) 01 October 2015 (2015-10-01) the whole document	1-15

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2020	**04 March 2020**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **LI,Nan** Telephone No. **86-(10)-53962096**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 103319610 A (HUABO BIOMEDICAL TECHNOLOGY SHANGHAI CO) 25 September 2013 (2013-09-25) the whole document	1-15
A	LU, D. et al. "Abstract 572: A novel anti-PDL1 x anti-VEGFR2 bispecific antibody for enhanced antitumor immunity" *Cancer Research*, Vol. 14 supplement, No. 76, 31 July 2016 (2016-07-31), the whole document	1-15
A	WANG, Q. "Expression, purification, and preliminary functional study of PD-1-VEGF multi-trageting Fc fusion protein PVP" *Chinese Master's Theses Full-text Database Medicine and Health Sciences*, No. 12, 15 December 2013 (2013-12-15), E072-9	1-15
A	LEE, H.T. et al. "Molecular mechanism of PD-1/PD-L1 blockade via anti-PD-L1 antibodies atezolizumab and durvalumab" *Scientific Reports*, Vol. 7, No. 1, 17 July 2017 (2017-07-17), e5532	1-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/122446

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2019062642	A1	04 April 2019	CN	109575140	A	05 April 2019
WO	2019168947	A1	06 September 2019	WO	2019168947	A8	03 October 2019
				TW	201945023	A	01 December 2019
WO	2015200905	A2	30 December 2015	JP	2017526635	A	14 September 2017
				EP	3161000	A2	03 May 2017
				CA	2953698	A1	30 December 2015
				EP	3161000	A4	02 May 2018
				CN	107428824	A	01 December 2017
				AU	2015279560	A1	19 January 2017
				MX	2016017290	A	24 August 2017
				WO	2015200905	A2	30 December 2015
WO	2017136562	A2	10 August 2017	EA	201891732	A1	28 February 2019
				WO	2017136562	A3	28 September 2017
				EP	3411068	A2	12 December 2018
				JP	2019506863	A	14 March 2019
				CN	109310755	A	05 February 2019
CN	108671229	A	19 October 2018	WO	2019214551	A1	14 November 2019
WO	2015148416	A1	01 October 2015	JP	6355032	B2	11 July 2018
				EP	3122783	A4	22 November 2017
				JP	2017510249	A	13 April 2017
				KR	20160132117	A	16 November 2016
				EP	3122783	A1	01 February 2017
				US	9527901	B2	27 December 2016
				US	2015266942	A1	24 September 2015
				EP	3122783	B1	04 September 2019
				CN	106459218	A	22 February 2017
CN	103319610	A	25 September 2013	CN	103319610	B	27 January 2016