PHARMACEUTICAL COMPOSITION FOR TREATING CANCER COMPRISING FUSION PROTEIN COMPRISING IL-2 PROTEIN AND CD80 PROTEIN AND IMMUNE CHECKPOINT INHIBITOR

Technical Field

The present invention relates to a pharmaceutical composition for treating cancer comprising, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

Background Art

Interleukin 2 (IL-2), also called T-cell growth factor (TCGF), is a globular glycoprotein that plays a central role in lymphocyte production, survival, and homeostasis. IL-2 has a protein size of 15.5 kDa to 16 kDa and consists of 133 amino acids. IL-2 mediates various immune actions by binding to an IL-2 receptor composed of three distinct subunits.

In addition, IL-2 is synthesized mainly by activated T cells, in particular by CD4+ helper T cells. IL-2 stimulates proliferation and differentiation of T cells, and induces production of cytotoxic T lymphocytes (CTLs) and differentiation of peripheral blood lymphocytes into cytotoxic cells and lymphokine-activated killer cells (LAK cells).

Meanwhile, CD80, also known as B7-1, is a member of the B7 family of membrane-bound proteins that are involved in immune regulation by binding to its ligand by way of delivering costimulatory responses and coinhibitory responses. CD80 is a transmembrane protein expressed on the surface of T cells, B cells,

dendritic cells, and monocytes. CD80 is known to bind CD28, CTLA4 (CD152), and PD-L1. CD80, CD86, CTLA4, and CD28 are involved in a costimulatory-coinhibitory system. For example, they regulate activity of T cells and are involved in proliferation, differentiation, and survival thereof.

In addition, recently, immune checkpoint inhibitors such as Keytruda® are in the spotlight. Immune checkpoint inhibitors are anticancer agents that help to attack cancer cells by activating body's immune system. Cancer treatment to date has focused on killing rapidly dividing cells, which is the characteristic of cancer cells, so that there are side effects of acting on not only cancer cells but also rapidly dividing cells among normal cells. However, it is known that immune anticancer agents use the immune system of a cancer patient to affect cancer cells, so there are few typical side effects of existing anticancer agents. An anti-PD-1 antibody such as Keytruda binds to the specific receptor (PD-1) of T cells and blocks the pathway of cancer cells to avoid surveillance system of active T cells, thereby exhibiting an anticancer effect through immune reactivation which allows T cells in human body to attack cancer cells (Korean Laid-open Patent Publication No. 2018-0030580A).

Detailed Description of the Invention

Technical Problem

Accordingly, as a result of studying to develop a safe and effective anticancer agent, the present inventors found out that a novel fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof in one molecule and an immune checkpoint inhibitor exhibit an excellent anticancer effect, and thereby have completed the present invention.

Solution to Problem

To achieve the above object, an aspect of the present invention provides a pharmaceutical composition for treating cancer containing, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

Effect of the Invention

The fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof can activate immune cells by IL-2. In addition, it was confirmed that the fusion protein dimer exhibits synergistic effects when administered in combination with an immune checkpoint inhibitor. Therefore, the pharmaceutical composition for treating cancer containing, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor may be usefully used for cancer prevention and treatment.

Brief Description of Drawings

Fig. 1 illustrates a schematic embodiment of a fusion protein dimer.

Fig. 2 illustrates a schematic view of a mechanism by which the fusion protein dimer acts in the lymph node.

Fig. 3 illustrates a schematic view of a mechanism by which the fusion protein dimer acts in the tumor microenvironment.

Fig. 4 illustrates a schematic view of the structure of the fusion protein. Here, each of GI101 and mGI101 is an embodiment of the fusion protein, and GI101C1, GI101C2, and mGI101C1 are comparative examples for comparison with activity of

the fusion protein.

Fig. 5 illustrates various embodiments of the fusion protein. Human- and mouse-derived proteins may be combined to prepare a fusion protein. CD80 protein and IL-2 protein may be bound to each other via various linkers other than Fc.

Fig. 6 illustrates a result obtained by identifying the obtained fusion protein dimer (GI101) with SDS-PAGE.

Fig. 7 illustrates amounts of the fusion protein (GI101) depending on absorbance.

Fig. 8 illustrates a result obtained by analyzing the obtained fusion protein dimer (GI101) by size exclusion chromatography (SEC).

Fig. 9 illustrates a result obtained by identifying the obtained mGI101 fusion protein dimer with SDS-PAGE.

Fig. 10 illustrates results obtained by identifying the obtained GI101C1 fusion protein dimer with SDS-PAGE.

Fig. 11 illustrates results obtained by identifying the obtained GI101C2 fusion protein dimer with SDS-PAGE.

Fig. 12 illustrates a result obtained by identifying the obtained mGI101C1 fusion protein dimer with SDS-PAGE.

Fig. 13 illustrates results obtained by identifying the obtained GI102-M45 fusion protein dimer with SDS-PAGE.

Fig. 14 illustrates results obtained by identifying the obtained GI102-M61 fusion protein dimer with SDS-PAGE.

Fig. 15 illustrates results obtained by identifying the obtained GI102-M72 fusion protein dimer with SDS-PAGE.

Fig. 16 illustrates binding affinity between hCTLA4 and GI101.

Fig. 17 illustrates binding affinity between hPD-L1 and GI101.

Fig. 18 illustrates binding affinity between hPD-L1 and hPD-1.

Fig. 19 illustrates binding affinity between mCTLA4 and mGI101.

Fig. 20 illustrates binding affinity between mPD-L1 and mGI101.

Fig. 21 illustrates results obtained by identifying binding ability between GI-101 (hCD80-Fc-hIL-2v) and CTLA-4. It was identified that GI-101 (hCD80-Fc-hIL-2v) has high binding ability for CTLA-4.

Fig. 22 illustrates results obtained by identifying binding affinity between GI101 and IL-2Rα or IL-2Rβ.

Fig. 23 illustrates results obtained by identifying binding affinity between GI101 and IL-2Rα.

Fig. 24 illustrates results obtained by identifying binding affinity between GI101 and IL-2Rβ.

Fig. 25 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M45.

Fig. 26 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M61.

Fig. 27 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M72.

Fig. 28 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M45.

Fig. 29 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M61.

Fig. 30 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M72.

Figs. 31 and 32 illustrate results obtained by measuring amounts of IFN-γ secreted from cells when the cells are subjected to treatment with GI101, GI101C1, GI101C2, or IL-2 at respective concentrations and incubation is performed.

Fig. 33 illustrates results obtained by identifying effects of GI101, GI101C1, GI101C2, and IL-2 (Proleukin) on proliferation of CD8+ T cells.

Fig. 34 illustrates a schematic view of a mechanism by which GI101 acts on effector T cells.

Fig. 35 illustrates results obtained by identifying effects of GI101 and GI102 on proliferation of CD8+ T cells and CD4+ T cells. Here, (A) illustrates proportions of CD8+ T cells and CD4+ T cells, (B) illustrates proliferation capacity of CD8+ T cells, and (C) illustrates a proportion of CD4+/FoxP3+ Treg cells.

Figs. 36 and 37 illustrate results obtained by identifying effects of GI101 and GI101w on proliferation of CD8+ T cells and NK cells.

Figs. 38 and 39 illustrate results obtained by identifying an effect of GI101 on effector T cells.

Fig. 40 illustrates results obtained by identifying effects of mGI101 and mGI102-M61 on mouse immune cells.

Figs. 41 and 42 illustrate results obtained by identifying an effect of GI101 on the inhibition of T cell activity by cancer cells expressing PD-L1 and CTLA-4.

Fig. 43 illustrates results obtained by identifying a tumor inhibitory effect of mGI101, depending on its dose, in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 44 illustrates results obtained by identifying survival rate of mouse-derived colorectal cancer cell-transplanted mice having received mGI101.

Fig. 45 illustrates results obtained by identifying a tumor inhibitory effect of

GI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 46 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, CD8+ T cells, IFN-γ T cells, CD4+ T cells, and Treg cells in cancer tissues.

Fig. 47 graphically illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, CD8+ T cells, IFN-γ T cells, CD4+ T cells, and Treg cells in cancer tissues.

Fig. 48 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, macrophages in cancer tissues.

Fig. 49 graphically illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, macrophages in cancer tissues.

Fig. 50 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 51 graphically illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 52 illustrates results obtained by identifying a tumor inhibitory effect of GI101 in mouse-derived lung cancer cell-transplanted mice.

Fig. 53 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody,

or GI101, and then analyzing, with FACS, CD8+ T cells, IFN-γ T cells, CD4+ T cells, and Treg cells in cancer tissues.

Fig. 54 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, macrophages in cancer tissues.

Fig. 55 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, an anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 56 illustrates results obtained by identifying a tumor inhibitory effect of mGI102-M61 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 57 illustrates results obtained by analyzing survival rate of mouse-derived colorectal cancer cell-transplanted mice having received mGI102-M61.

Fig. 58 illustrates results obtained by identifying a tumor inhibitory effect of mGI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 59 illustrates a tumor inhibition rate of mGI101 in mouse-derived colorectal cancer cell-transplanted mice.

FIG. 60 shows a graph of tumor growth when GI101 and Keytruda are used in combination in human-derived breast cancer cell transplanted mice. Tumor growth was inhibited in the GI101 or Keytruda alone treatment groups compared to the control group (hIgG4). Tumor growth was inhibited in the GI101 and Keytruda combined treatment group compared to the control group. Tumor growth was inhibited in the GI101 and Keytruda combined treatment group compared to the GI101 or Keytruda alone treatment groups.

FIG. 61 shows tumor growth inhibition rate when GI-101 and Keytruda are used in combination in human-derived breast cancer cell transplanted mice. The

IgG4 treatment group had 2 mice having tumor growth inhibition rate of 30% or more, 1 mouse having tumor growth inhibition rate of 50% or more, and 1 mouse having tumor growth inhibition rate of 80%. The GI101 treatment group had 5 mice having tumor growth inhibition rate of 30% or more, 5 mice having tumor growth inhibition rate of 50% or more, and 2 mice having tumor growth inhibition rate of 80%. The Keytruda treatment group had 7 mice having tumor growth inhibition rate of 30% or more, 5 mice having tumor growth inhibition rate of 50% or more, and 3 mice having tumor growth inhibition rate of 80%. The GI101 and Keytruda combined treatment group had 8 mice having tumor growth inhibition rate of 30% or more, 8 mice having tumor growth inhibition rate of 50% or more, and 6 mice having tumor growth inhibition rate of 80%.

FIG. 62 shows the degree of tumor growth of individual experimental animals in each treatment group when GI101 and Keytruda are used in combination in human-derived breast cancer cell transplanted mice.

FIG. 63 shows the degree of tumor growth of individual experimental animals in hIgG4 treatment group in human-derived breast cancer cell transplanted mice.

FIG. 64 shows the degree of tumor growth of individual experimental animals in the GI101 treatment group in human-derived breast cancer cell transplanted mice.

FIG. 65 shows the degree of tumor growth of individual experimental animals in the Keytruda treatment group in human-derived breast cancer cell transplanted mice.

FIG. 66 shows the degree of tumor growth of individual experimental animals in the GI101 and Keytruda combined treatment group in human-derived

breast cancer cell transplanted mice.

FIG. 67 shows the graph of tumor growth when mGI101 and an anti-PD-1 antibody are administered in combination in rodent-derived colorectal cancer cell transplanted mice.

FIG. 68 shows the tumor growth inhibition rate when mGI101 and an anti-PD-1 antibody are administered in combination in rodent-derived colorectal cancer cells transplanted mice.

FIG. 69 shows the degree of tumor growth of individual experimental animals in each treatment group when mGI101 and an anti-PD-1 antibody are administered in combination in rodent-derived colorectal cancer cells transplanted mice.

FIG. 70 shows the degree of tumor growth of individual experimental animals in the hIgG4 treatment group in rodent-derived colorectal cancer cells transplanted mice.

FIG. 71 shows the degree of tumor growth of individual experimental animals in the mGI101 treatment group in rodent-derived colorectal cancer cells transplanted mice.

FIG. 72 shows the degree of tumor growth of individual experimental animals in the anti-PD-1 antibody treatment group in rodent-derived colorectal cancer cells transplanted mice.

FIG. 73 shows the degree of tumor growth of individual experimental animals in the mGI101 and anti-PD-1 antibody combined treatment group is administered in rodent-derived colorectal cancer cells transplanted mice.

FIG. 74 shows the degree of tumor growth of individual experimental animals when rodent-derived colorectal cancer cells were re-injected to the

experimental animals which showed complete remission in the mGI101 and anti-PD-1 antibody combined treatment group in rodent-derived colorectal cancer cells transplanted mice.

FIG. 75 shows a graph of tumor growth when mGI101 and an anti-PD-L1 antibody are administered in combination in rodent-derived colorectal cancer cells transplanted mice.

FIG. 76 shows a graph of tumor growth when mGI101 and an anti-TIGIT antibody are administered in combination in rodent-derived colorectal cancer cells transplanted mice.

Best Mode for Carrying Out the Invention

An aspect of the present invention provides a pharmaceutical composition for treating cancer containing, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

Immune checkpoint inhibitor

As used herein, the term "immune checkpoint" refers to an intracellular signaling system that maintains self-tolerance and protects tissues from excessive immune responses that cause damage. The immune checkpoint proteins is a cell membrane protein that regulates immune checkpoint and can inhibit differentiation, proliferation, and activity of immune cells. Specifically, the immune checkpoint protein is expressed in activated T cells so as to function to reduce T cell proliferation, cytokine secretion, and cytotoxicity, and inhibit excessive activity of T cells. Some immune checkpoints are known to be one of the main mechanisms of tumor cells resulting in immune evasion. Therefore, an "immune checkpoint

inhibitor" targets immune checkpoint proteins to inhibit or block the immune checkpoints and increase activation of T cells, thereby enhancing antitumor immunity, and thus exhibiting an anticancer effect. In addition to advantages of having fewer side effects such as vomiting and hair loss than conventional cytotoxic anticancer agents, and a greater therapeutic effect, immune checkpoint inhibitors are known to have a long-lasting therapeutic effect even after drug administration is discontinued because they take advantage of an immune response system with excellent memory capacity.

Specifically, an immune checkpoint inhibitor may target CTLA-4, PD-1, PD-L1, PD-L2, B7-H4, HVEM(Herpesvirus entry mediator), BTLA, TIM3, GAL9, LAG3, VISTA, KIR or TIGIT.

In particular, the immune checkpoint inhibitor may include, but not be limited to, an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-TIM3 antibody, an anti-GAL9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody and an anti-TIGIT antibody.

As used herein, the term "cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)" is referred to as CD152, and is expressed on the membrane surface of activated T cells. CTLA-4 binds to CD80 (B7-1) and CD86 (B7-2) of antigen presenting cells and inhibits T cell activity. CTLA-4 inhibitors may be ipilimumab(YERVOY®) and tremelimumab.

As used herein, the term "programmed cell death protein 1 (PD-1)" is referred to as CD279, and is expressed on the surface of activated T cells. PD-1 reacts with a protein on the surface of cancer cells, PD-L1 (B7-H1) and PD-L2 (B7-DC), and

inhibits T-cell activity, growth factor, and cytokine production which are mediated by TCR (T cell receptor) and CD28 to induce negative signaling. The PD-1 inhibitor may be, for example, pembrolizumab (Keytruda®), MK-3475, nivolumab (Opdivo®), cemiplimab (Libtayo®), JTX-4014, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, INCMGA00012, AMP-224, and AMP-514.

As used herein, the term "programmed death-ligand 1 (PD-L1)" is referred to as CD274 or B7-H1, and is a protein presented on the surface of cancer cells or on hematopoietic cells. PD-L1 presented on the cancer surface can bind to PD-1 presented on the surface of T cells. The PD-L1 inhibitor may be, for example, atezolizumab, avelumab(Bavencio®), durvalumab(Imfinzi®), KN035, CK-301, AUNP12, CA-170, and BMS-986189.

As used herein, the term "B7-H4" is referred to as V-set domain-containing T-cell activation inhibitor 1 (VTCN1), and is expressed on the membrane surface of antigen-presenting cells. B7-H4 binds to CD28 protein of T cells and inhibits activity, growth, and cytokine production of T cells to negatively regulate T-cell mediated immune responses.

As used herein, the term "herpesvirus entry mediator (HVEM)" is referred to as CD270, and is also known as tumor necrosis factor receptor superfamily member 14 (TNFRSF14). HVEM is expressed on the membrane surface of various immune cells including T cells, and binds to various partner proteins to regulate inflammation and immune responses. When HVEM binds to B and T lymphocyte attenuator (BTLA, CD272) or CD160 of T cells, immune activity of T cells is inhibited. On the other hand, when HVEM binds to TNFSF14 (LIGHT), HVEM induces dendritic cell maturation, T cell proliferation and cytokine production to activate inflammation and immune responses.

As used herein, the term "T cell membrane protein 3 (TIM3)" is also referred to as hepatitis A virus cellular receptor 2 (HAVCR2), and is expressed in various immune cells. When TIM3 is activated by binding to a water-soluble protein GAL9 (galectin 9), intracellular calcium influx is increased to induce apoptosis of T cells, which in turn causes immune tolerance. In addition, with GAL9, TIM3 binds to a cell adhesion molecule 1 (CEACAM1) which is a cell surface protein to inhibit immune activity of T cells, and binds to high mobility group protein 1 (HMGB1) or phospatidyl serine (PTdSer) which is a water-soluble protein to inhibit immune activity. TIM3 inhibitors may be LY3321367, MBG453, and TSR-022.

As used herein, the term "lymphocyte activation gene 3 (LAG3)" is referred to as CD223, and binds to a major histocompatibility complex (MHC) class II to inhibit proliferation and activity of T cells. LAG3 inhibitors may be IMP321, relatlimab, and GSK2831781.

As used herein, the term "V-domain Ig suppressor of T cell activation (VISTA)" belongs to B7 family (B7-H5), and is expressed in various immune cells to inhibit proliferation, activity, and cytokine production of T cells. VISTA inhibitors may be JNJ-63723283.

As used herein, the term "killer cell immunoglobulin-like receptor (KIR)" is a membrane-bound protein expressed in NK cells and T cells, and is a family protein having genetic diversity and homology. Among them, KIR2DL1, KIR2DL2/L3, KIR3DL1, and KIR3DL2 may bind to MHC class I to inhibit cell immune activity of NK cells.

As used herein, the term “T cell immunoglobulin and ITIM domain (TIGIT)” is a membrane-bound protein expressed on the surface of NK cells and T cells and may bind to CD155, CD112 and CD113 to inhibit immune activity.

A fusion protein comprising an IL-2 protein and a CD80 protein, and a dimer thereof

As used herein, the term "IL-2" or "interleukin-2", unless otherwise stated, refers to any wild-type IL-2 obtained from any vertebrate source, including mammals, for example, primates (such as humans) and rodents (such as mice and rats). IL-2 may be obtained from animal cells, and also includes one obtained from recombinant cells capable of producing IL-2. In addition, IL-2 may be wild-type IL-2 or a variant thereof.

In the present specification, IL-2 or a variant thereof may be collectively expressed by the term "IL-2 protein" or "IL-2 polypeptide." IL-2, an IL-2 protein, an IL-2 polypeptide, and an IL-2 variant specifically bind to, for example, an IL-2 receptor. This specific binding may be identified by methods known to those skilled in the art.

An embodiment of IL-2 may have the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 36. Here, IL-2 may also be in a mature form. Specifically, the mature IL-2 may not contain a signal sequence, and may have the amino acid sequence of SEQ ID NO: 10. Here, IL-2 may be used under a concept encompassing a fragment of wild-type IL-2 in which a portion of N-terminus or C-terminus of the wild-type IL-2 is truncated.

In addition, the fragment of IL-2 may be in a form in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous amino acids are truncated from N-terminus of a protein having the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 36. In addition, the fragment of IL-2 may be in a form in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

23, 24, or 25 contiguous amino acids are truncated from C-terminus of a protein having the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 36.

As used herein, the term “IL-2 variant” refers to a form in which a portion of amino acids in the full-length IL-2 or the above-described fragment of IL-2 is substituted. That is, an IL-2 variant may have an amino acid sequence different from wild-type IL-2 or a fragment thereof. However, an IL-2 variant may have activity equivalent or similar to the wild-type IL-2. Here, "IL-2 activity" may, for example, refer to specific binding to an IL-2 receptor, which specific binding can be measured by methods known to those skilled in the art.

Specifically, an IL-2 variant may be obtained by substitution of a portion of amino acids in the wild-type IL-2. An embodiment of the IL-2 variant obtained by amino acid substitution may be obtained by substitution of at least one of the 38^{th}, 42^{nd}, 45^{th}, 61^{st}, and 72^{nd} amino acids in the amino acid sequence of SEQ ID NO: 10.

Specifically, the IL-2 variant may be obtained by substitution of at least one of the 38^{th}, 42^{nd}, 45^{th}, 61^{st}, or 72^{nd} amino acid in the amino acid sequence of SEQ ID NO: 10 with another amino acid. In addition, when IL-2 is in a form in which a portion of N-terminus in the amino acid sequence of SEQ ID NO: 35 is truncated, the amino acid at a position complementarily corresponding to that in the amino acid sequence of SEQ ID NO: 10 may be substituted with another amino acid. For example, when IL-2 has the amino acid sequence of SEQ ID NO: 35, its IL-2 variant may be obtained by substitution of at least one of 58^{th}, 62^{nd}, 65^{th}, 81^{st}, or 92^{nd} amino acid in the amino acid sequence of SEQ ID NO: 35 with another amino acid. These amino acid residues correspond to the 38^{th}, 42^{nd}, 45^{th}, 61^{st}, and 72^{nd} amino acid residues in the amino acid sequence of SEQ ID NO: 10, respectively. According to an embodiment, one, two, three, four, five, six, seven, eight, nine, or ten amino acids

may be substituted as long as such IL-2 variant maintains IL-2 activity. According to another embodiment, one to five amino acids may be substituted.

In an embodiment, an IL-2 variant may be in a form in which two amino acids are substituted. Specifically, the IL-2 variant may be obtained by substitution of the 38th and 42nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th and 45th amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 42nd and 45th amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 42nd and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 42nd and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 45th and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 45th and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 61st and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10.

Furthermore, an IL-2 variant may be in a form in which three amino acids are substituted. Specifically, the IL-2 variant may be obtained by substitution of the 38th,

42nd, and 45th amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 42nd, and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 42nd, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 45th, and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 45th, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 42nd, 45th, and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 42nd, 45th, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 45th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10.

In addition, an IL-2 variant may be in a form in which four amino acids are substituted. Specifically, the IL-2 variant may be obtained by substitution of the 38th, 42nd, 45th, and 61st amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 42nd, 45th, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of the 38th, 45th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution

of the 38th, 42nd, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10. In addition, in an embodiment, the IL-2 variant may be obtained by substitution of 42nd, 45th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10.

Furthermore, an IL-2 variant may be in a form in which five amino acids are substituted. Specifically, the IL-2 variant may be obtained by substitution of each of the 38th, 42nd, 45th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10 with another amino acid.

Here, the "another amino acid" introduced by the substitution may be any one selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. However, regarding amino acid substitution for the IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 38th amino acid cannot be substituted with arginine, the 42nd amino acid cannot be substituted with phenylalanine, the 45th amino acid cannot be substituted with tyrosine, the 61st amino acid cannot be substituted with glutamic acid, and the 72nd amino acid cannot be substituted with leucine.

Regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 38th amino acid, arginine, may be substituted with an amino acid other than arginine. Preferably, regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 38th amino acid, arginine, may be substituted with alanine (R38A).

Regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 42nd amino acid, phenylalanine, may be substituted with an amino acid other than phenylalanine. Preferably, regarding amino acid

substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 42nd amino acid, phenylalanine, may be substituted with alanine (F42A).

Regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 45th amino acid, tyrosine, may be substituted with an amino acid other than tyrosine. Preferably, regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 45th amino acid, tyrosine, may be substituted with alanine (Y45A).

Regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 61st amino acid, glutamic acid, may be substituted with an amino acid other than glutamic acid. Preferably, regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 61st amino acid, glutamic acid, may be substituted with arginine (E61R).

Regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 72nd amino acid, leucine, may be substituted with an amino acid other than leucine. Preferably, regarding amino acid substitution for an IL-2 variant, in the amino acid sequence of SEQ ID NO: 10, the 72nd amino acid, leucine, may be substituted with glycine (L72G).

Specifically, an IL-2 variant may be obtained by at least one substitution selected from the group consisting of R38A, F42A, Y45A, E61R, and L72G, in the amino acid sequence of SEQ ID NO: 10.

Specifically, an IL-2 variant may be obtained by amino acid substitutions at two, three, four, or five positions among the positions selected from the group consisting of R38A, F42A, Y45A, E61R, and L72G.

In addition, an IL-2 variant may be in a form in which two amino acids are substituted. Specifically, an IL-2 variant may be obtained by the substitutions, R38A

and F42A. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A and Y45A. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A and Y45A. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, E61R and L72G.

Furthermore, an IL-2 variant may be in a form in which three amino acids are substituted. Specifically, an IL-2 variant may be obtained by the substitutions, R38A, F42A, and Y45A. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, F42A, and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, F42A, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, Y45A, and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, Y45A, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A, Y45A, and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A, Y45A, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A, E61R, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, Y45A, E61R, and L72G.

In addition, an IL-2 variant may be in a form in which four amino acids are substituted. Specifically, an IL-2 variant may be obtained by the substitutions,

R38A, F42A, Y45A, and E61R. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, F42A, Y45A, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, F42A, E61R, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, R38A, Y45A, E61R, and L72G. In addition, in an embodiment, an IL-2 variant may be obtained by the substitutions, F42A, Y45A, E61R, and L72G.

Furthermore, an IL-2 variant may be obtained by the substitutions, R38A, F42A, Y45A, E61R, and L72G.

Preferably, an embodiment of the IL-2 variant may contain which are any one selected from the following substitution combinations (a) to (d) in the amino acid sequence of SEQ ID NO: 10:

(a) R38A/F42A

(b) R38A/F42A/Y45A

(c) R38A/F42A/E61R

(d) R38A/F42A/L72G

Here, when IL-2 has the amino acid sequence of SEQ ID NO: 35, an amino acid substitution may be present at a position complementarily corresponding to that in the amino acid sequence of SEQ ID NO: 10. In addition, even when IL-2 is a fragment of the amino acid sequence of SEQ ID NO: 35, an amino acid substitution may be present at a position complementarily corresponding to that in the amino acid sequence of SEQ ID NO: 10.

Specifically, an IL-2 variant may have the amino acid sequence of SEQ ID NO: 6, 22, 23, or 24.

In addition, an IL-2 variant may be characterized by having low *in vivo* toxicity. Here, the low *in vivo* toxicity may be a side effect caused by binding of IL-

2 to the IL-2 receptor alpha chain (IL-2Rα). Various IL-2 variants have been developed to ameliorate the side effect caused by binding of IL-2 to IL-2Rα, and such IL-2 variants may be those disclosed in US Patent No. 5,229,109 and Korean Patent No. 1667096. In particular, IL-2 variants described in the present application have low binding ability for the IL-2 receptor alpha chain (IL-2Rα) and thus have lower *in vivo* toxicity than the wild-type IL-2.

As used herein, the term "CD80", also called "B7-1", is a membrane protein present in dendritic cells, activated B cells, and monocytes. CD80 provides co-stimulatory signals essential for activation and survival of T cells. CD80 is known as a ligand for the two different proteins, CD28 and CTLA-4, present on the surface of T cells. CD80 is composed of 288 amino acids, and may specifically have the amino acid sequence of SEQ ID NO: 11. In addition, as used herein, the term "CD80 protein" refers to the full-length CD80 or a CD80 fragment.

As used herein, the term "CD80 fragment" refers to a cleaved form of CD80. In addition, the CD80 fragment may be an extracellular domain of CD80. An embodiment of the CD80 fragment may be obtained by elimination of the 1^{st} to 34^{th} amino acids from N-terminus which are a signal sequence of CD80. Specifically, an embodiment of the CD80 fragment may be a protein composed of the 35^{th} to 288^{th} amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35^{th} to 242^{nd} amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35^{th} to 232^{nd} amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35^{th} to 139^{th} amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein

composed of the 142nd to 242nd amino acids in SEQ ID NO: 11. In an embodiment, a CD80 fragment may have the amino acid sequence of SEQ ID NO: 2.

In addition, the IL-2 protein and the CD80 protein may be attached to each other via a linker or a carrier. Specifically, the IL-2 or a variant thereof and the CD80 (B7-1) or a fragment thereof may be attached to each other via a linker or a carrier. In the present description, the linker and the carrier may be used interchangeably.

The linker links two proteins. An embodiment of the linker may include 1 to 50 amino acids, albumin or a fragment thereof, an Fc domain of an immunoglobulin, or the like. Here, the Fc domain of immunoglobulin refers to a protein that contains heavy chain constant region 2 (CH2) and heavy chain constant region 3 (CH3) of an immunoglobulin, and does not contain heavy and light chain variable regions and light chain constant region 1 (CH1) of an immunoglobulin. The immunoglobulin may be IgG, IgA, IgE, IgD, or IgM, and may preferably be IgG4. Here, Fc domain of wild-type immunoglobulin G4 may have the amino acid sequence of SEQ ID NO: 4.

In addition, the Fc domain of an immunoglobulin may be an Fc domain variant as well as wild-type Fc domain. In addition, as used herein, the term "Fc domain variant" may refer to a form which is different from the wild-type Fc domain in terms of glycosylation pattern, has a high glycosylation as compared with the wild-type Fc domain, or has a low glycosylation as compared with the wild-type Fc domain, or a deglycosylated form. In addition, an aglycosylated Fc domain is included therein. The Fc domain or a variant thereof may be adapted to have an adjusted number of sialic acids, fucosylations, or glycosylations, through culture conditions or genetic manipulation of a host.

In addition, glycosylation of the Fc domain of an immunoglobulin may be modified by conventional methods such as chemical methods, enzymatic methods, and genetic engineering methods using microorganisms. In addition, the Fc domain variant may be in a mixed form of respective Fc regions of immunoglobulins, IgG, IgA, IgE, IgD, and IgM. In addition, the Fc domain variant may be in a form in which some amino acids of the Fc domain are substituted with other amino acids. An embodiment of the Fc domain variant may have the amino acid sequence of SEQ ID NO: 12.

The fusion protein may have a structure in which, using an Fc domain as a linker (or carrier), a CD80 protein and an IL-2 protein, or an IL-2 protein and a CD80 protein are linked to N-terminus and C-terminus of the linker or carrier, respectively. Linkage between N-terminus or C-terminus of the Fc domain and CD-80 or IL-2 may optionally be achieved by a linker peptide.

Specifically, a fusion protein may consist of the following structural formula (I) or (II):

N'-X-[linker (1)]$_n$-Fc domain-[linker (2)]$_m$-Y-C' (I)

N'-Y-[linker (1)]$_n$-Fc domain-[linker (2)]$_m$-X-C' (II)

Here, in the structural formulas (I) and (II),

N′ is the N-terminus of the fusion protein,

C′ is the C-terminus of the fusion protein,

X is a CD80 protein,

Y is an IL-2 protein,

the linkers (1) and (2) are peptide linkers, and

n and m are each independently 0 or 1.

Preferably, the fusion protein may consist of the structural formula (I). The IL-2 protein is as described above. In addition, the CD80 protein is as described above. According to an embodiment, the IL-2 protein may be an IL-2 variant with one to five amino acid substitutions as compared with the wild-type IL-2. The CD80 protein may be a fragment obtained by truncation of up to about 34 contiguous amino acid residues from the N-terminus or C-terminus of the wild-type CD80. Alternatively, the CD protein may be an extracellular immunoglobulin-like domain having the activity of binding to the T cell surface receptors CTLA-4 and CD28.

Specifically, the fusion protein may have the amino acid sequence of SEQ ID NO: 9, 26, 28, or 30. According to another embodiment, the fusion protein includes a polypeptide having a sequence identity of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to the amino acid sequence of SEQ ID NO: 9, 26, 28, or 30. Here, the identity is, for example, percent homology, and may be determined through homology comparison software such as BlastN software of the National Center of Biotechnology Information (NCBI).

The peptide linker (1) may be included between the CD80 protein and the Fc domain. The peptide linker (1) may consist of 5 to 80 contiguous amino acids, 20 to 60 contiguous amino acids, 25 to 50 contiguous amino acids, or 30 to 40 contiguous amino acids. In an embodiment, the peptide linker (1) may consist of 30 amino acids. In addition, the peptide linker (1) may contain at least one cysteine. Specifically, the peptide linker (1) may contain one, two, or three cysteines. In addition, the peptide linker (1) may be derived from the hinge of an immunoglobulin. In an embodiment, the peptide linker (1) may be a peptide linker consisting of the amino acid sequence of SEQ ID NO: 3.

The peptide linker (2) may consist of 1 to 50 contiguous amino acids, 3 to 30 contiguous amino acids, or 5 to 15 contiguous amino acids. In an embodiment, the peptide linker (2) may be $(G4S)_n$ (where n is an integer of 1 to 10). Here, in $(G4S)_n$, n may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In an embodiment, the peptide linker (2) may be a peptide linker consisting of the amino acid sequence of SEQ ID NO: 5.

In another aspect of the present invention, there is provided a dimer obtained by binding of two fusion proteins, each of which comprises an IL-2 protein and a CD80 protein. The fusion protein comprising IL-2 or a variant thereof and CD80 or a fragment thereof is as described above.

Here, the binding between the fusion proteins constituting the dimer may be achieved by, but is not limited to, a disulfide bond formed by cysteines present in the linker. The fusion proteins constituting the dimer may be the same or different fusion proteins from each other. Preferably, the dimer may be a homodimer. An embodiment of the fusion protein constituting the dimer may be a protein having the amino acid sequence of SEQ ID NO: 9.

A pharmaceutical composition of the present invention containing, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor show an efficacy for preventing or treating cancer.

The cancer may be selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

A preferred dose of the pharmaceutical composition varies depending on the

patient's condition and body weight, severity of disease, form of drug, route and duration of administration and may be appropriately selected by those skilled in the art. In the pharmaceutical composition for treating or preventing cancer of the present invention, the active ingredient may be contained in any amount (effective amount) depending on application, dosage form, blending purpose, and the like, as long as the active ingredient can exhibit an anticancer activity. A conventional effective amount thereof will be determined within a range of 0.001% to 20.0% by weight, based on the total weight of the composition. Here, the term "effective amount" refers to an amount of an active ingredient capable of inducing an anticancer effect. Such an effective amount can be experimentally determined within the scope of common knowledge of those skilled in the art.

As used herein, the term "treatment" may be used to mean both therapeutic and prophylactic treatment. Here, prophylaxis may be used to mean that a pathological condition or disease of an individual is alleviated or mitigated. In an embodiment, the term "treatment" includes both application or any form of administration for treating a disease in a mammal, including a human. In addition, the term includes inhibiting or slowing down a disease or disease progression; and includes meanings of restoring or repairing impaired or lost function so that a disease is partially or completely alleviated; stimulating inefficient processes; or alleviating a serious disease.

As used herein, the term "efficacy" refers to capacity that can be determined by one or parameters, for example, survival or disease-free survival over a certain period of time such as one year, five years, or ten years. In addition, the parameter may include inhibition of size of at least one tumor in an individual.

Pharmacokinetic parameters such as bioavailability and underlying

parameters such as clearance rate may also affect efficacy. Thus, “enhanced efficacy” (for example, improvement in efficacy) may be due to enhanced pharmacokinetic parameters and improved efficacy, which may be measured by comparing clearance rate and tumor growth in test animals or human subjects, or by comparing parameters such as survival, recurrence, or disease-free survival.

As used herein, the term "therapeutically effective amount" or "pharmaceutically effective amount" refers to an amount of a compound or composition effective to prevent or treat the disease in question, which is sufficient to treat the disease at a reasonable benefit/risk ratio applicable to medical treatment and does not cause adverse effects. A level of the effective amount may be determined depending on factors including the patient's health condition, type and severity of disease, activity of drug, the patient's sensitivity to drug, mode of administration, time of administration, route of administration and excretion rate, duration of treatment, formulation or simultaneously used drugs, and other factors well known in the medical field. In an embodiment, the therapeutically effective amount means an amount of drug effective to treat cancer.

Here, the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may be any carrier as long as the carrier is a non-toxic substance suitable for delivery to a patient. Distilled water, alcohol, fat, wax, and inert solid may be contained as the carrier. A pharmaceutically acceptable adjuvant (buffer, dispersant) may also be contained in the pharmaceutical composition.

Specifically, by including a pharmaceutically acceptable carrier in addition to the active ingredient, the pharmaceutical composition may be prepared into a parenteral formulation depending on its route of administration using conventional

methods known in the art. Here, the term "pharmaceutically acceptable" means that the carrier does not have more toxicity than the subject to be applied (prescribed) can adapt while not inhibiting activity of the active ingredient.

When the pharmaceutical composition is prepared into a parenteral formulation, it may be made into preparations in the form of injections, transdermal patches, nasal inhalants, or suppositories with suitable carriers according to methods known in the art. In a case of being made into injections, sterile water, ethanol, polyol such as glycerol or propylene glycol, or a mixture thereof may be used as a suitable carrier; and an isotonic solution, such as Ringer's solution, phosphate buffered saline (PBS) containing triethanol amine or sterile water for injection, and 5% dextrose, or the like may preferably be used. Formulation of pharmaceutical compositions is known in the art, and reference may specifically be made to Remington's Pharmaceutical Sciences (19th ed., 1995) and the like. This document is considered part of the present description.

A preferred dose of the pharmaceutical composition may range from 0.01 μg/kg to 10 g/kg, or 0.01 mg/kg to 1 g/kg, per day, depending on the patient's condition, body weight, sex, age, severity of the patient, and route of administration. The dose may be administered once a day or may be divided into several times a day. Such a dose should not be construed as limiting the scope of the present invention in any aspect.

Subjects to which the pharmaceutical composition can be applied (prescribed) are mammals and humans, with humans being particularly preferred. In addition to the active ingredient, the pharmaceutical composition of the present application may further contain any compound or natural extract, which has already been validated for safety and is known to have an anticancer activity, so as to boost or reinforce an

anticancer activity.

In still yet another aspect of the present invention, there is provided a kit for treating cancer containing a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

In still yet another aspect of the present invention, there is provided a use of a composition for combined administration containing a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor for preventing or treating cancer.

In still yet another aspect of the present invention, there is provided a use of a composition for combined administration containing a fusion protein comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor for enhancing a therapeutic effect on cancer.

In still yet another aspect of the present invention, there is provided a use of a composition for combined administration containing a fusion protein comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor for manufacture of a medicament for treating cancer.

In still yet another aspect of the present invention, there is provided a method for preventing or treating cancer and/or a method for enhancing a therapeutic effect on cancer, comprising administering, to a subject, a composition for combined administration containing a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, or a fusion protein dimer where the two fusion proteins are attached, and an immune checkpoint inhibitor.

The subject may be an individual suffering from cancer. In addition, the subject may be a mammal, preferably a human. The fusion protein comprising an

IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, or the fusion protein dimer where the two fusion proteins are attached is as described above.

Route of administration, dose, and frequency of administration of the fusion protein or fusion protein dimer and NK cells may vary depending on the patient's condition and the presence or absence of side effects, and thus the fusion protein or fusion protein dimer may be administered to a subject in various ways and amounts. The optimal administration method, dose, and frequency of administration can be selected in an appropriate range by those skilled in the art.

Due to IL-2 activity, the fusion protein in an embodiment of the present invention can activate immune cells such as natural killer cells. Thus, the fusion protein can be effectively used for cancer. In particular, it was identified that as compared with the wild type, an IL-2 variant with two to five amino acid substitutions, in particular, an IL-2 variant that contains amino acid substitutions at two, three, four, or five positions among the positions selected from the group consisting of R38A, F42A, Y45A, E61R, and L72G, has low binding ability for the IL-2 receptor alpha chain and thus exhibits improved characteristics with respect to pharmacological side effects of conventional IL-2. Thus, such an IL-2 variant, when used alone or in the form of a fusion protein, can decrease incidence of vascular (or capillary) leakage syndrome (VLS), a problem with IL-2 conventionally known.

Mode for Carrying out the Invention

Hereinafter, the present invention will be described in more detail by way of the following examples. However, the following examples are only for illustrating the present invention, and the scope of the present invention is not limited thereto.

I. Preparation of fusion protein

Preparation Example 1. Preparation of hCD80-Fc-IL-2 variant (2M): GI101

In order to produce a fusion protein comprising a human CD80 fragment, an Fc domain, and an IL-2 variant, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 8) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (2M) (R38A, F42A) (SEQ ID NO: 6) having two amino acid substitutions, in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 9. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI101".

Purification was carried out using chromatography containing MabSelect SuRe protein A resin. The fusion protein was bound thereto under a condition of 25 mM Tris, 25 mM NaCl, pH 7.4. Then, elution was performed with 100 mM NaCl, 100 mM acetic acid, pH 3. 20% 1 M Tris-HCl at pH 9 was placed in a collection tube, and then the fusion protein was collected. For the collected fusion protein, the buffer was exchanged through dialysis with PBS buffer for 16 hours.

Thereafter, absorbance at 280 nm wavelength was measured, over time, with size exclusion chromatography using a TSKgel G3000SWXL column (TOSOH Bioscience), to obtain a highly concentrated fusion protein. Here, the isolated and

purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition, and stained with Coomassie Blue to check its purity (Fig. 6). It was identified that the fusion protein was contained at a concentration of 2.78 mg/ml when detected with NanoDrop (Fig. 7). In addition, the results obtained by analysis using size exclusion chromatography are provided in Fig. 8.

Preparation Example 2. Preparation of mCD80-Fc-IL-2 variant (2M): mGI101

In order to produce a fusion protein comprising a mouse CD80, an Fc domain, and an IL-2 variant, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 14) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a mCD80 (SEQ ID NO: 13), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (2M) (R38A, F42A) (SEQ ID NO: 6) with two amino acid substitutions, in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 15. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "mGI101".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 9). It was found that the fusion

protein was contained at a concentration of 1.95 mg/ml when detected by absorbance at 280 nm using NanoDrop.

Preparation Example 3. Preparation of hCD80-Fc: GI101C1

In order to produce a fusion protein comprising a human CD80 fragment and an Fc domain, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 16) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), and an Fc domain (SEQ ID NO: 4). The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 17. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI101C1".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 10). It was observed that the fusion protein was contained at a concentration of 3.61 mg/ml when detected by absorbance at 280 nm using NanoDrop.

Preparation Example 4. Preparation of Fc-IL-2 variant (2M): GI101C2

In order to produce a fusion protein comprising an Fc domain and an IL-2 variant, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide

contains a nucleotide sequence (SEQ ID NO: 18) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (2M) (R38A, F42A) (SEQ ID NO: 6) with two amino acid substitutions, in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 19. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI101C2".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 11). It was found that the fusion protein was contained at a concentration of 4.79 mg/ml when detected by absorbance at 280 nm using NanoDrop.

Preparation Example 5. Preparation of mCD80-Fc: mGI101C1

In order to produce a fusion protein comprising a mouse CD80 and an Fc domain, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 20) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a mCD80 (SEQ ID NO: 13), an Ig hinge (SEQ ID NO: 3), and an Fc domain (SEQ ID NO: 4), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of

SEQ ID NO: 21. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "mGI101C1".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 12). It was observed that the fusion protein was contained at a concentration of 2.49 mg/ml when detected by absorbance at 280 nm using NanoDrop.

The fusion proteins prepared in Preparation Examples 1 to 5 are summarized in Table 1 below.

[Table 1]

Item	N-terminus	Linker	C-terminus
Preparation Example 1 (GI101)	hCD80 fragment	Fc domain	hIL-2m
Preparation Example 2 (mGI101)	mCD80 fragment	Fc domain	hIL-2m
Preparation Example 3 (GI101C1)	CD80 fragment	Fc domain	-
Preparation Example 4 (GI101C2)	-	Fc domain	IL-2m
Preparation Example 5 (mGI101C1)	mCD80 fragment	Fc domain	-

Preparation Example 6. Preparation of CD80-Fc-IL-2: GI101w

In order to produce a fusion protein comprising a human CD80 fragment, an Fc domain, and a human IL-2, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically,

the polynucleotide contais a nucleotide sequence (SEQ ID NO: 31) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and mature human IL-2 (SEQ ID NO: 10), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO™) to express the fusion protein of SEQ ID NO: 32. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI101w". The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1.

Preparation Example 7. Preparation of hCD80-Fc-IL-2 variant (3M): GI102-M45

In order to produce a fusion protein comprising a human CD80 fragment, an Fc domain, and an IL-2 variant (3M) (R38A, F42A, Y45A) (GI102-M45) with three amino acid substitutions, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 25) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (SEQ ID NO: 22), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO™) to express the fusion protein of SEQ ID NO: 26. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture

was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI102-M45". The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 13).

Preparation Example 8. Preparation of hCD80-Fc-IL-2 variant (3M): GI102-M61

In order to produce a fusion protein comprising a human CD80 fragment, an Fc domain, and an IL-2 variant (3M) (R38A, F42A, E61R) (GI102-M61) with three amino acid substitutions, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 27) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (SEQ ID NO: 23), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 28. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI102-M61".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein

was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 14).

Preparation Example 9. Preparation of hCD80-Fc-IL-3M: GI102-M72

In order to produce a fusion protein comprising a human CD80 fragment, an Fc domain, and an IL-2 variant (3M) (R38A, F42A, L72G) (GI102-M72) with three amino acid substitutions, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 29) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a CD80 fragment (SEQ ID NO: 2), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (SEQ ID NO: 24), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO^{TM}) to express the fusion protein of SEQ ID NO: 30. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "GI102-M72".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1. The isolated and purified fusion protein was subjected to SDS-PAGE under reduced (R) or non-reduced (NR) condition and stained with Coomassie Blue to check its purity (Fig. 15).

Preparation Example 10. Preparation of mCD80-Fc-IL-3M: mGI102-M61

In order to produce a fusion protein comprising a mouse CD80 fragment, an Fc domain, and an IL-2 variant (3M) (R38A, F42A, E61R) (GI102-M61) with three

amino acid substitutions, a polynucleotide was synthesized through the Invitrogen GeneArt Gene Synthesis service of ThermoFisher Scientific. Specifically, the polynucleotide contains a nucleotide sequence (SEQ ID NO: 33) which encodes a fusion protein that contains a signal peptide (SEQ ID NO: 1), a mCD80 fragment (SEQ ID NO: 13), an Ig hinge (SEQ ID NO: 3), an Fc domain (SEQ ID NO: 4), a linker (SEQ ID NO: 5), and an IL-2 variant (SEQ ID NO: 23), in this order, from the N-terminus. The polynucleotide was inserted into pcDNA3_4 vector. In addition, the vector was introduced into CHO cells (Expi-CHO™) to express the fusion protein of SEQ ID NO: 34. After the vector was introduced, culture was performed for 7 days in an environment of 37°C, 125 rpm, and 8% CO_2 concentration. Then, the culture was harvested and the fusion protein was purified therefrom. The purified fusion protein was designated "mGI102-M61".

The purification and collection of the fusion protein were carried out in the same manner as in Preparation Example 1.

II. Identification of binding affinity between fusion protein and its ligand

In order to identify the binding affinity between the fusion protein and its ligand, the binding affinity was measured using Octet RED 384.

Experimental Example 1. Identification of binding affinity between hCTLA-4 and GI101

AR2G biosensor (Amine Reactive 2nd gen, ForteBio, Cat: 18-5092) was previously hydrated with 200 μl of distilled water in a 96-well microplate (GreinerBio-one, Cat: 655209). A ligand (CTLA-4, Human CTLA-4/CD152, His tag, Sino Biological, Cat: 11159-H08H) to be attached to the AR2G biosensor was diluted with 10 mM acetate buffer (pH 5, AR2G reagent Kit, ForteBio, Cat: 18-5095) to a concentration of 5 μg/ml. In addition, GI101 to be attached to the ligand was

diluted with 1X AR2G kinetic buffer (AR2G reagent Kit, ForteBio, Cat: 18-5095) to a concentration of 1,000 nM, 500 nM, 250 nM, 125 nM, or 62.5 nM. Activation buffer was prepared by mixing 20 mM EDC and 10 mM s-NHS (AR2G reagent Kit, ForteBio, Cat: 18-5095) in distilled water. 80 μl of each reagent was placed in a 384-well microplate (Greiner Bio-one, Cat: 781209) and the program was set up.

As a result, the binding affinity between hCTLA-4 and GI101 was measured as illustrated in Fig. 16.

Experimental Example 2. Identification of binding affinity between hPD-L1/GI101 and hPD-L1/PD-1

Ni-NTA (Nickel charged Tris-NTA, Ni-NTA Biosensors, ForteBio, 18-5101) was previously hydrated with 200 μl of 1X Ni-NTA kinetic buffer (10X Kinetics buffer, ForteBio, 18-1042) in a 96-well microplate (GreinerBio-one, Cat: 655209). A ligand (Human PD-L1/B7-H1 protein, His-tag, Sino biological, Cat: 10084-H08H) to be attached to the Ni-NTA Biosensors was diluted with 1X Ni-NTA kinetic buffer to a concentration of 5 μg/ml. GI101 to be attached to the ligand was diluted with 1X Ni-NTA kinetic buffer at 1,000 nM, 500 nM, 250 nM, 125 nM, or 62.5 nM. In addition, human PD-1/PDCD1 (Human PD-1/PDCD1, Fc Tag, Sino Biological, Cat: 10377-H02H) to be attached to the ligand was diluted with 1X Ni-NTA kinetic buffer to a concentration of 2,000 nM, 1,000 nM, 500 nM, 250 nM, or 125 nM. Then, 80 μl of each reagent was placed in a 384-well microplate and the program was set up.

As a result, the binding affinity between hPD-L1 and GI101 was measured as illustrated in Fig. 17. In addition, the binding affinity between hPD-L1 and hPD-1 was measured as illustrated in Fig. 18.

Experimental Example 3. Identification of binding affinity between mCTLA-4 and mGI101

The binding affinity between mCTLA-4 and mGI101 was examined in the same manner as in Experimental Example 1. Here, the equipment used is as follows: Biosensor: AR2G, Ligand: mCTLA-4 (Recombinant Mouse CTLA-4 Fc chimera, R&D Systems, Cat: 434-CT-200), Analyte: mGI101 (500 nM, 250 nM, 125 nM, 62.5 nM, 31.3 nM).

As a result, the binding affinity between mCTLA-4 and mGI101 was measured as illustrated in Fig. 19.

Experimental Example 4. Identification of binding affinity between mPD-L1 and mGI101

The binding affinity between mPD-L1 and mGI101 was identified in the same manner as in Experimental Example 1. Here, the equipment used is as follows. Biosensor: AR2G, Ligand: mPD-L1 (Recombinant Mouse mGI101 B7-H1/PD-L1 Fc chimera, R&D Systems, Cat: 434-CT-200), Analyte: mGI101 (500 nM, 250 nM, 125 nM, 62.5 nM, 31.3 nM).

As a result, the binding affinity between mPD-L1 and mGI101 was measured as illustrated in Fig. 20.

Experimental Example 5. Identification of binding ability of GI-101 (hCD80-Fc-hIL-2v) to CTLA-4

Binding kinetics measurements were performed using the Octet RED 384 instrument (ForteBio, Pall Life Science) with agitation at 30°C and 1,000 rpm. The binding ability for CTLA-4 was measured using the Amine Reactive 2 generation (AR2G) biosensor chip, and the binding ability for PD-L1 was measured using the Nickel charged Tris-NTA (Ni-NTA) biosensor chip. The AR2G biosensor chip was

activated with a combination of 400 mM EDC and 100 mM sulfo-NHS. Then, Human CTLA-4-His Tag (Sino Biological, Cat: 11159-H08H) was diluted with 10 mM acetate buffer (pH 5) to 5 μg/ml, and loaded on the AR2G biosensor chip for 300 seconds and fixed.

Then, binding of CTLA-4 to GI-101 (hCD80-Fc-hIL-2v), GI-101C1 (hCD80-Fc), Ipilimumab (Bristol-Myers Squibb), and GI-101C2 (Fc-hIL-2v) at various concentrations was measured for 300 seconds and dissociation thereof was also measured for 300 seconds. Binding kinetics analysis was performed using Octet Data Analysis HT software ver. 10 provided by Pall Corporation. The results are illustrated in Fig. 21.

Experimental Example 6. Identification of binding affinity between IL-2Rα or IL-2Rβ and GI101

The binding ability for IL-2Rα was measured using the AR2G biosensor, and the binding ability for IL-2Rβ was measured using the Ni-NTA biosensors (Nickel charged Tris-NTA, Ni-NTA Biosensors, ForteBio, 18-5101).

A ligand (IL-2Rα-His Tag, Acro, Cat: ILA-H52H9) to be attached to the AR2G biosensor was diluted with 10 mM acetate buffer (pH 5, AR2G reagent Kit, ForteBio, Cat: 18-5095) to a concentration of 5 μg/ml. The AR2G biosensor was activated with a buffer prepared by mixing 400 mM EDC and 100 mM sulfo-NHS, and then the diluted ligand was loaded on the AR2G biosensor for 300 seconds and fixed.

Meanwhile, a ligand (IL-2Rβ-His Tag, Acro, Cat: CD2-H5221) to be attached to the Ni-NTA biosensor was diluted with 1X Ni-NTA kinetic buffer to a concentration of 5 μg/ml. The diluted ligand was loaded on the Ni-NTA biosensor for 600 seconds and fixed.

Thereafter, GI101, GI101w, or Proleukin (Novartis, hIL-2), at various concentrations, to be attached to the ligand was loaded thereon for 300 seconds. Then, binding thereof was measured and dissociation thereof was also measured for 300 seconds. Binding kinetics analysis was performed using Octet Data Analysis HT software ver. 10 provided by Pall Corporation. The results are illustrated in Figs. 22 to 24.

As a result, it was identified that GI101 has low binding ability for the IL-2 receptor alpha chain, IL-2Rα, and high binding ability for IL-2Rβ, as compared with GI101w and Proleukin.

Experimental Example 7. Measurement of binding affinity between fusion protein and ligand

In order to identify binding affinity between the fusion protein and its ligand, binding affinity was measured using Octet RED 384.

Experimental Example 7.1. Identification of binding affinity between IL-2 alpha receptor and GI101-M45, GI101-M61, or GI101-M72

AR2G biosensor (Amine Reactive 2nd gen, ForteBio, Cat: 18-5092) was previously hydrated with 200 μl of distilled water (DW) in a 96-well microplate (GreinerBio-one, Cat: 655209). A ligand (Human IL-2 R alpha protein, His Tag, Acro, ILA-H52H9) to be attached to the biosensor was diluted with 10 mM acetate buffer (pH 5) (AR2G reagent Kit, ForteBio, Cat: 18-5095) to a concentration of 5 μg/ml. An analyte (GI101-M45, GI101-M61, GI101-M72) to be attached to the ligand was diluted with 1X AR2G kinetic buffer (AR2G reagent Kit, ForteBio, Cat: 18-5095) to 500 nM, 250 nM, 125 nM, and 62.5 nM, respectively. Activation buffer was prepared by mixing 20 mM EDC and 10 mM s-NHS (AR2G reagent Kit,

ForteBio, Cat: 18-5095) in DW. 80 μl of each reagent was placed in a 384-well microplate (Greiner Bio-one, Cat: 781209) and the program was set up.

As a result, the binding affinity between IL-2 alpha receptor and GI101-M45 is illustrated in Fig. 25. In addition, the binding affinity between IL-2 alpha receptor and GI101-M61 is illustrated in Fig. 26, and the binding affinity between IL-2 alpha receptor and GI101-M72 is illustrated in Fig. 27.

Experimental Example 7.2. Identification of binding affinity of GI102-M45, GI102-M61, and GI102-M72 to IL-2Rβ

Ni-NTA Biosensors were previously hydrated with 200 μl of 1X Ni-NTA kinetic buffer (10X Kinetics buffer, ForteBio, 18-1042) in a 96-well microplate. A ligand (Human IL-2 R beta protein, His-Tag, Acro, CD2-H5221) to be attached to the biosensor was diluted with 1X Ni-NTA kinetic buffer to a concentration of 2 μg/ml. GI102-M45, GI102-M61, or GI102-M72 to be attached to the ligand was diluted with 1X Ni-NTA kinetic buffer to a concentration of 500 nM, 250 nM, 125 nM, or 62.5 nM. 80 μl of each reagent was placed in a 384-well microplate and the program was set up.

As a result, the binding affinity between IL-2Rβ and GI102-M45 was measured as illustrated in Fig. 28, and the binding affinity between IL-2Rβ and GI102-M61 was measured as illustrated in Fig. 29. In addition, the binding affinity between IL-2Rβ and GI102-M72 was measured as illustrated in Fig. 30.

III. Identification of immune activity of fusion protein

Experimental Example 8. Identification of IFN-γ production caused by fusion protein

Experimental Example 8.1. Culture of CFSE-labeled PBMCs

Peripheral blood mononuclear cells (PBMCs) isolated from a human were labeled with carboxyfluorescein succinimidyl ester (CFSE) by being reacted with 1 μM CellTrace CFSE dye at 37°C for 20 minutes. CFSE not bound to the cells was removed by being reacted for 5 minutes with a culture medium having a 5-fold volume of the staining reaction solution and then by being centrifuged at 1,300 rpm for 5 minutes. The CFB-labeled PBMCs were resuspended in the culture medium (RPMI1640 medium containing 10% fetal bovine serum (FBS), 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol, 1 mM non-essential amino acid, and 2 mM L-glutamine), and then added to a 96-well microplate at $1x10^5$ cells per well. Treatment with 5 μg/ml of PHA (Lectin from Phaseolus Vulgaris, red kidney bean, Sigma-Aldrich, St. Louis, MO, USA, Cat. No. L1668-5MG), and GI101, GI101C1, GI101C2, or IL-2 (Aldesleukin; human recombinant IL-2, Novartis) was performed and incubation was performed in a 5% CO_2 incubator at 37°C for 6 days.

Here, the treatment with GI101, GI101C1, GI101C2, and IL-2 was performed at a concentration of 1 nM, 10 nM, or 100 nM. The cells were analyzed by FACS, and human IFN-γ present in the culture medium was measured using an ELISA kit (Biolegend, San Diego, CA, USA, Cat. No. 430103).

Experimental Example 8.2. FACS analysis

The cell pellets obtained by removing the supernatant were washed with FACS buffer (3% fetal bovine serum (FBS), 10 mM EDTA, 1M HEPES, 100 unit/ml Penicillin, Streptomycin, 10 μg/ml, 1 mM sodium pyruvate), and then reacted with Fc blocker (Biolegend, Cat. No. 422302) at 4°C for 5 minutes. Then, treatment with APC anti-CD3 Ab (Biolegend, Cat. No. 300412) and PE anti-CD8a Ab (Biolegend, Cat. No. 300908) was performed and reaction was allowed to proceed at 4°C for 20

minutes. Then, the resultant was washed with FACS buffer. The cell pellets were resuspended in FACS buffer and then analyzed using BD LSR Fortessa (BD Biosciences, San Diego, CA, USA) and FlowJo software.

Experimental Example 8.3. Human IFN-γ ELISA

The amount of human IFN-γ secreted into the supernatant of each sample in which the cells had been cultured was measured using a human IFN-γ ELISA kit (Biolegend, Cat. No. 430103). Briefly, anti-human-IFN-γ antibodies were added to an ELISA plate, and reaction was allowed to proceed overnight at 4°C so that these antibodies were coated thereon. Then, blocking was performed at room temperature for 1 hour with a PBS solution to which 1% BSA had been added. Washing with a washing buffer (0.05% Tween-20 in PBS) was performed, and then a standard solution and each sample were properly diluted and added thereto. Then, reaction was allowed to proceed at room temperature for 2 hours.

After the reaction was completed, the plate was washed and secondary antibodies (detection antibodies) were added thereto. Reaction was allowed to proceed at room temperature for 1 hour. Washing with a washing buffer was performed, and then an Avidin-HRP solution was added thereto. Reaction was allowed to proceed at room temperature for 30 minutes. A substrate solution was added thereto and color development reaction was induced in the dark at room temperature for 20 minutes. Finally, H_2SO_4 was added thereto to stop the color development reaction, and the absorbance at 450 nm was measured with Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

As a result, it was found that cells treated with GI101 exhibited a remarkable increase in IFN-γ secretion, as compared with cells treated with GI101C1, GI101C2, or IL-2 (Figs. 31 and 32).

Experimental Example 9. Identification of effect of GI101 on proliferation of CD8+ T cells

Peripheral blood mononuclear cells (PBMCs) isolated from a human were labeled with CFSE by being reacted with 1 μM CellTrace CFSE dye at 37°C for 20 minutes. CFSE not bound to the cells was removed by being reacted for 5 minutes with a culture medium having a 5-fold volume of the staining reaction solution and then by being centrifuged at 1,300 rpm for 5 minutes. The CFB-labeled PBMCs were resuspended in the culture medium (RPMI1640 medium containing 10% fetal bovine serum (FBS), 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol, 1 mM non-essential amino acid, and 2 mM L-glutamine), and then added to a 96-well microplate at $1x10^5$ cells per well.

Thereafter, treatment with 1 μg/ml of an anti-CD3ε antibody (Biolegend Cat. No. L1668-5MG), and GI101, GI101C1, GI101C2, or Proleukin (Novartis) was performed and incubation was performed in a 5% CO_2 incubator at 37°C for 6 days. Here, the cells were treated with GI101, GI101C1, GI101C2, and IL-2 at a concentration of 100 nM. The incubated cells were examined for their degree of proliferation by measuring, with FACS analysis using APC-TCRαβ and PE-CD8α antibodies, a proportion of CD8+ T cells that had not been labeled with CFSE.

As a result, it was found that GI101 activated proliferation of CD8+ T cells *in vitro* to a similar extent to the wild-type IL-2 Proleukin (Figs. 33 and 34).

Experimental Example 10. Identification of effect of GI101 and GI102 on proliferation of CD8+ T cells

Human PBMCs were purchased from Allcells (Lot # 3014928, USA). 1M CellTrace CFSE dye was used, which was reacted with the human PBMCs under a light-blocking condition at room temperature for 20 minutes. The cells were labeled

with CFSE by being reacted with 1 μM CellTrace CFSE dye at 37°C for 20 minutes. CFSE not bound to the cells was removed by being reacted for 5 minutes with culture medium having a 5-fold volume of the staining reaction solution and then by being centrifuged at 1,300 rpm for 5 minutes. The CFB-labeled PBMCs were resuspended in the culture medium (RPMI1640 medium containing 10% fetal bovine serum (FBS), 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol, 1 mM non-essential amino acid, and 2 mM L-glutamine), and then added to a 96-well microplate at $1x10^5$ cells per well.

Thereafter, the CFB-labeled PBMCs were subjected to treatment with 1 μg/ml of an anti-CD3ε antibody (OKT3, eBioscience, USA), and GI101, GI101C1, GI101C2, or Proleukin (Novartis), and incubation was performed in a 5% CO_2 incubator at 37°C for 7 days. Here, the cells were subjected to treatment with GI101, GI101C1, GI101C2, and IL-2 at a concentration of 10 μM.

The incubated cells were examined for their degree of proliferation by measuring, with FACS analysis using an anti-human CD4-PE antibody (BioLegend, USA), an anti-human CD8-PE/Cy7 antibody (BioLegend, USA), and an anti-human FoxP3-APC antibody (BioLegend, USA), a proportion of CD8+ T cells that had not been labeled with CFSE.

As a result, the GI101, GI102_M61, GI101C2, and Proleukin treatment groups exhibited a significant increase in proportion of CD8+ T cells, as compared with the control group (no stimulus), the anti-CD3 antibody alone treatment group, and the GI101C1 treatment group. In addition, as compared with the negative control group (no stimulus) and the anti-CD3 alone treatment group, the GI101, GI101C2, and Proleukin treatment groups exhibited a significant increase in proliferation of CD4+/FoxP3+ Treg cells, whereas the GI102 and GI101C1 treatment

groups did not exhibit a significant increase in proliferation of CD4+/FoxP3+ Treg cells (Fig. 35).

Experimental Example 11. Identification of effect of GI101 or GI101w on proliferation of CD8+ T cells and NK cells

7-week-old C57BL/6 mice purchased from Orient Bio (Korea) were divided into 3 groups, each group containing 3 mice, and PBS, GI101, or GI101w was injected intraperitoneally thereinto. Here, GI101 and GI101w were respectively prepared to be at 40.5 μg in 200 μl of PBS, and injected intraperitoneally thereinto. Five days after the injection, the spleens were removed from the mice of each group. The cells were isolated therefrom, and the total number of cells was measured using a hematocytometer. Splenocytes were examined for proportions of CD8+ T cells and NK cells therein, with FACS analysis using staining with APC-CD3ε antibody (Biolegend; 145-2C11), PE-NK1.1 antibody (Biolegend; PK136), and Pacific blue-CD8α antibody (BD; 53-6.7). As such, the numbers of CD8+ T cells and NK cells present in the spleen were calculated.

As a result, it was identified that GI101 activated proliferation of CD8+ T cells and NK cells *in vivo* as compared with GI101w (Figs. 36 and 37).

Experimental Example 12. Identification of effect of GI101 on function of T cells

An experiment was performed using a CTLA-4 blockade bioassay kit (Promega Cat. No. JA4005). The experiment is briefly described as follows. CTLA-4 effector cells kept in liquid nitrogen were thawed in a 37°C constant temperature water bath for 3 minutes, and 0.8 ml of CTLA-4 effector cells were mixed well with 3.2 ml of pre-warmed assay buffer (90% RPMI + 10% fetal bovine serum). Then, the mixture was added to a 96-well white cell culture plate (SPL, Cat. No. 30196) at

25 μl per well. Then, 25 μl of GI101 at various concentrations was added thereto. For a negative control, 25 μl of assay buffer was added thereto. Then, the 96-well-white cell culture plate was covered and placed at room temperature until aAPC/Raji cells were prepared.

aAPC/Raji cells kept in liquid nitrogen were thawed in a 37°C constant temperature water bath for 3 minutes, and 0.8 ml of aAPC/Raji cells were mixed well with 3.2 ml of pre-warmed assay buffer. Then, 25 μl of the mixture was added to the plate at per well, and reaction was allowed to proceed in a 5% CO_2 incubator at 37°C for 16 hours. After the reaction was completed, the resultant was allowed to stand at room temperature for 15 minutes, and then the Bio-Glo reagent was added thereto while taking care to avoid bubbles. The Bio-Glo reagent was also added to three of the outermost wells and the wells were used as blanks to correct the background signal. Reaction was allowed to proceed at room temperature for 10 minutes, and then luminescence was measured with Cytation 3 (BioTek Instruments, Inc., Winooski, VT, USA). Final data analysis was performed by calculating RLU (GI101-background)/RLU (no treatment-background).

As a result, it was found that GI101 attached to CTLA-4 expressed on effector T cells, and activated the function of T cells rather than inhibiting the same (Figs. 38 and 39).

Experimental Example 13. Identification of effect of mGI101 and mGI102 on immune cells

7-week-old C57BL/6 mice purchased from Orient Bio (Korea) were divided into 3 groups, each group containing 3 mice, and PBS, 3 mg/kg, 6 mg/kg, or 12 mg/kg of GI101, or 3 mg/kg, 6 mg/kg, or 12 mg/kg of mGI102 (mGI102-M61) was administered intravenously thereinto. On days 1, 3, 5, 7, and 14 after the injection,

the spleens were removed from the mice of each group. Thereafter, for the spleen tissue, the numbers of effector CD8+ T cells, NK cells, and Treg cells were calculated with FACS analysis using respective antibodies, and proportions of effector CD8+ T cells and NK cells with respect to Treg cells were respectively calculated. The information on the antibodies used in each cell assay is as follows:

Effector CD8+ T cells: PB anti-mouse CD3ε antibody (Biolegend, # 155612; KT3.1.1), FITC anti-mouse CD8α antibody (BD, # 553031, 53-6.7), PE/Cy7 anti-mouse CD44 antibody (Biolegend, # 103030; IM7), APC anti-mouse CD122 antibody (Biolegend, # 123214; TM-β1)

NK cells: PB anti-mouse CD3ε antibody (Biolegend, # 155612; KT3.1.1), PE anti-mouse NK-1.1 (Biolegend, # 108708; PK136)

Treg cells: FITC anti-mouse CD3 antibody (Biolegend, # 100204; 17A2), PB anti-mouse CD4 antibody (Biolegend, # 100531; RM4-5), PE anti-mouse CD25 antibody (Biolegend, # 102008; PC61), APC anti-mouse Foxp3 antibody (Invitrogen, # FJK-16s, 17-5773-82).

As a result, the group having received mGI101 or mGI102 (mGI102-M61) exhibited a significant increase in numbers of CD8+ T cells and NK cells at the time points from 3 days to 14 days after administration, as compared with the PBS administration group. In addition, it was found that the group having received mGI102 exhibited a significant increase in proportions of activated CD8+ T cells/Treg cells and NK cells/Treg cells at the time points from 3 days to 14 days after administration, as compared with the PBS administration group (Fig. 40).

IV. Identification of anticancer effect of fusion protein

Experimental Example 14. Identification of effect of GI101 on inhibition of T cell activity by cancer cells expressing PD-L1 and CTLA-4

NCl-H292 cancer cell line expressing PD-L1 and CTLA-4 was cultured for 3 hours in a culture medium containing 10 μg/ml Mitomycin C (Sigma), and then Mitomycin C was removed by washing with the culture medium. Thereafter, 5×10^4 cells of the Mitomycin C-treated NCl-H292 cancer cell line were incubated with 1×10^5 cells of human PBMCs in a 96-well microplate. Here, treatment with 5 μg/ml of PHA (Sigma) was performed for T cell activity. In addition, GI101C1 and GI101 at a concentration of 50 nM were reacted with IgG1-Fc (Biolegend) or abatacept (= Orencia; Bristol-Myers Squibb) at a concentration of 50 nM for 30 minutes at 4°C, and then the resultant was used to treat the NCl-H292 cancer cells. After 3 days, the supernatant of the cell incubate was collected and the amount of IFN-γ was quantified using an ELISA kit (Biolegend).

As a positive control group, human PBMCs stimulated with PHA in the absence of the Mitomycin C-treated NCl-H292 cancer cell line were used; and as a negative control group, human PBMCs stimulated with PHA in the presence of the Mitomycin C-treated NCl-H292 cancer cell line was used. An experimental method using the IFN-γ ELISA kit was carried out in the same manner as in Experimental Example 9.3.

As a result, GI101 effectively activated the immune response that had been inhibited by the cancer cell line overexpressing PD-L1. In addition, it was discovered that GI101 inhibited signaling of CTLA-4 expressed on effector T cells (Figs. 41 and 42).

Experimental Example 15. Identification of anticancer effect of mGI101 in mice transplanted with mouse-derived colorectal cancer cells

BALB/c mice (female, 7-week-old) acquired from Orient Bio were subjected to an acclimation period of 7 days. Then, $5x10^6$ cells of CT-26 cancer cell line

(ATCC, USA) were mixed with 0.05 ml of phenol red-free matrigel matrix (BD), and allotransplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of the mice. A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 28 mm^3 were selected, and then the selected mice were grouped evenly based on tumor size and body weight, each group containing 10 mice. Thereafter, using a disposable syringe (31G, 1 mL), hIgG4 was administered at a dose of 6 mg/kg to a negative control group. For experimental groups, mGI101 at a dose of 3 mg/kg, 6 mg/kg or 12 mg/kg was administered intravenously thereto. A total of three administrations were given once every three days after the first administration. The tumor size was measured daily.

As a result, in the groups having received mGI101 at a dose of 6 mg/kg and 12 mg/kg, respectively, the tumor size was significantly inhibited as compared with the negative control group at some measurement time points and at the end of the test (Fig. 43). Further, as a result of measuring the survival rate, in the group having received mGI101 at a dose of 6 mg/kg, significant improvement was observed as compared with the negative control group at some measurement time points and at the end of the test (Fig. 44).

Experimental Example 16. Identification of anticancer effect of GI101 in mice transplanted with mouse-derived colorectal cancer cells

Experimental Example 16.1. Identification of tumor inhibitory effect

BALB/c mice (female, 7-week-old) acquired from Orient Bio were subjected to an acclimation period of 7 days. Then, $5x10^6$ cells of CT-26 cancer cell line (ATCC, USA) were suspended in 0.1 ml PBS, and allotransplantation of the suspension was performed by subcutaneous administration at 0.1 ml in the right

dorsal region of the mice. A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 50 mm^3 to 200 mm^3 were selected, and then the selected mice were grouped evenly based on tumor size and body weight, each group containing 10 mice. Thereafter, using a disposable syringe (31G, 1 mL), no drug was administered to a negative control group, and an anti-PD-1 antibody at a dose of 5 mg/kg, or an anti-PD-1 antibody at a dose of 5 mg/kg and an anti-CTLA-4 antibody at a dose of 5 mg/kg were administered intravenously to positive control groups. For experimental groups, GI101 at a dose of 0.1 mg/kg or 1 mg/kg was administered intravenously thereto. A total of three administrations were given once every three days after the first administration. The tumor size was measured daily.

As a result, in the CT-26 cancer cell line-transplanted mice, all groups having received an anti-PD-1 antibody, an anti-PD-1 antibody and an anti-CTLA-4 antibody, or GI101 at a dose of 0.1 mg/kg or 1 mg/kg exhibited significant inhibition of tumor growth, as compared with the negative control group. In particular, the experimental group having received GI101 at a dose of 0.1 mg/kg exhibited a significant tumor inhibitory effect, as compared with the anti-PD-1 antibody treatment group (* $p < 0.05$) (Fig. 45).

Experimental Example 16.2. Immune cell analysis in cancer tissues

The mice of each group in Experimental Example 16.1 were sacrificed when the tumor volume reached an average of 200 mm^3, and cancer tissues were collected. Thereafter, the cancer tissues were separated to a single-cell level to analyze immune cells therein, and then FACS analysis was performed on immune cells in the cancer tissues using the following antibodies. Specifically, Anti-mouse-CD3 (Biolegend, Cat. No. 100320), Anti-mouse-CD4 (Biolegend, Cat. No. 100526), Anti-mouse-CD8

(Biolegend, Cat. No. 100750), Anti-mouse-FoxP3 (eBioscience, Cat. No. 12-5773-82), Anti-mouse-CD25 (Biolegend, Cat. No. 102049), Anti-mouse-CD44 (eBioscience, Cat. No. 61-0441-82), Anti-mouse-PD-1 (Biolegend, Cat. No. 135218), Anti-mouse-IFN-gamma (Biolegend, Cat. No. 505832), Anti-mouse-CD49b (Biolegend, Cat. No. 108906), Anti-mouse-H2 (Invitrogen, Cat. No. A15443), Anti-mouse-CD11c (Biolegend, Cat. No. 117343), Anti-mouse-CD80 (eBioscience, Cat. No. 47-4801-82), Anti-mouse-CD86 (Biolegend, Cat. No. 104729), Anti-mouse-F4/80 (eBioscience, Cat. No. 47-4801-82), and Anti-mouse-CD206 (eBioscience, Cat. No. 17-2061-80) were used as the antibodies.

As a result, the experimental group having received GI101 at a dose of 0.1 mg/kg exhibited a significant increase in CD8+ T cells, as compared with the positive control group having received an anti-PD-1 antibody alone at a dose of 5 mg/kg (* $p < 0.05$, Figs. 46 and 47). Furthermore, all experimental groups having received GI101 exhibited a significantly increased level of expression of IFN-γ in T cells, as compared with the negative control group (* $p < 0.05$, Figs. 46 and 47). In addition, the experimental group having received GI101 at a dose of 0.1 mg/kg exhibited an increase in M1 macrophages as compared with the negative control group and the positive control group having received an anti-PD-1 antibody alone (Figs. 48 and 49). In addition, all experimental groups having received GI101 exhibited an increased level of CD86 expression in macrophages and dendritic cells (* $p < 0.05$, Figs. 48 to 51).

Experimental Example 17. Identification of anticancer effect of GI101 in mice transplanted with mouse-derived lung cancer cells Experimental Example 17.1. Identification of tumor inhibitory effect

C57BL/6 mice (female, 7-week-old) acquired from Orient Bio were subjected to an acclimation period of 7 days. Then, $5x10^6$ cells of LLC2 cancer cell line (ATCC, USA) were suspended in 0.1 ml PBS, and allotransplantation of the suspension was performed by subcutaneous administration at 0.1 ml in the right dorsal region of the mice. A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 50 mm^3 to 200 mm^3 were selected, and then the selected mice were grouped evenly based on tumor size and body weight, each group containing 10 mice. Thereafter, using a disposable syringe (31G, 1 mL), no drug was administered to a negative control group, and an anti-PD-1 antibody at a dose of 5 mg/kg, or an anti-PD-1 antibody at a dose of 5 mg/kg and an anti-CTLA-4 antibody at a dose of 5 mg/kg were administered intravenously to positive control groups. For experimental groups, GI101 at a dose of 0.1 mg/kg or 1 mg/kg was administered intravenously thereto. A total of three administrations were given once every three days after the first administration. The tumor size was measured daily.

As a result, all experimental groups exhibited a significant tumor inhibitory effect, as compared with the negative control group (* $p < 0.05$) (Fig. 52).

Experimental Example 17.2. Immune cell analysis in cancer tissues

The mice of each group in Experimental Example 17.1 were sacrificed when the tumor volume reached an average of 200 mm^3, and cancer tissues were collected. Thereafter, FACS analysis was performed in the same manner as Experimental Example 16.2 to analyze immune cells in the cancer tissues.

As a result, the experimental group having received GI101 at a dose of 0.1 mg/kg exhibited a significant increase in CD8+ T cells, as compared with the positive control group having received an anti-PD-1 antibody alone (* $p < 0.05$, Fig.

59). Furthermore, all experimental groups having received GI101 exhibited a significantly increased level of expression of IFN-γ, as compared with the negative control group (* $p < 0.05$, Fig. 59). In addition, all experimental groups having received GI101 exhibited an increased level of CD86 expression in macrophages and dendritic cells (* $p < 0.05$, Figs. 53 to 55).

Experimental Example 18. Identification of anticancer effect of mGI102-M61 in mice transplanted with mouse-derived colorectal cancer cells

BALB/c mice (female, 7-week-old) acquired from Orient Bio were subjected to an acclimation period of 7 days. Then, $5x10^6$ cells of CT-26 cancer cell line (ATCC, USA) were mixed with 0.05 ml of phenol red-free matrigel matrix (BD), and allotransplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of the mice. A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 28 mm^3 were selected, and then the selected mice were grouped evenly based on tumor size and body weight, each group containing 10 mice. Thereafter, using a disposable syringe (31G, 1 mL), hIgG4 was administered at a dose of 6 mg/kg to a negative control group. For experimental groups, mGI102-M61 at a dose of 3 mg/kg, 6 mg/kg, or 12 mg/kg was administered intravenously thereto. A total of three administrations were given once every three days after the first administration. The tumor size was measured daily.

As a result, it was identified that the experimental group having received mGI102-M61 at a dose of 12 mg/kg exhibited significant inhibition of tumor growth at some measurement time points and at the end of the test, as compared with the negative control group (Fig. 56). In addition, as a result of measuring a survival rate, it was identified that the experimental group having received mGI102-M61 at a dose

of 12 mg/kg exhibited significant improvement at some measurement time points and at the end of the test, as compared with the negative control group (Fig. 57).

Experimental Example 19. Identification of anticancer effect of mGI101 in mice transplanted with mouse-derived colorectal cancer cells

BALB/c mice (female, 7-week-old) acquired from Orient Bio were subjected to an acclimation period of 7 days. Then, $5x10^6$ cells of CT-26 cancer cell line (ATCC, USA) were mixed with 0.05 ml of phenol red-free matrigel matrix (BD), and allotransplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of the mice. A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 200 mm^3 to 250 mm^3 were selected, and then the selected mice were grouped evenly based on tumor size and body weight, each group containing 10 mice.

Thereafter, using a disposable syringe (31G, 1 mL), hIgG4 was administered at a dose of 4 mg/kg to a negative control group. For experimental groups, mGI101 at a dose of 1 mg/kg, 4 mg/kg, or 6 mg/kg was administered intravenously thereto. Additionally, groups having received mCD80 at 4.9 mg/kg or Fc-IL-2v (GI101C2) at 2.8 mg/kg were set as control groups. In addition, a group having simultaneously received mCD80 at 4.9 mg/kg and Fc-IL-2v (GI101C2) at 2.8 mg/kg was set as a control group.

In tumor volume measurement, it was identified that the group having received mGI101 at a dose of 6 mg/kg exhibited significant inhibition at some measurement time points and at the end of the test, as compared with the negative control. An excellent tumor growth inhibition rate was observed as compared with

the group having received a combination of mCD80 and Fc-IL-2v (GI101C2) (Figs. 58 and 59).

In conclusion, in the tumor growth-inhibitory efficacy test on BALB/c mice allotransplanted with CT-26, a BALB/c mouse-derived colorectal cancer cell line, it was demonstrated that the test substance mGI101 had tumor inhibitory efficacy under this test condition as compared with mCD80 and IL-2v single preparations; and it was identified that mGI101 exhibited an excellent anticancer efficacy as compared with the group having received a combination of mCD80 and IL-2v (Figs. 58 and 59). In particular, the group having received mGI101 at a dose of 6 mg/kg exhibited significant inhibition of tumor size, as compared with the negative control group and the group having received a combination of mCD80 and Fc-IL2v (GI101C2).

V. Determination of anticancer effect of combined administration of fusion protein dimer and immune checkpoint inhibitor

Experimental example 20. Determination of anticancer effect by combined administration of GI101 and anti-PD-1 antibody in human-derived breast cancer cell transplanted mice

This test evaluated tumor growth inhibitory effect in a tumor model transplanted with xenogeneic MDA-MB-231 cells which are human-derived breast cancer cells, using a humanized mouse model prepared by xenogeneic transplant of human PBMC into a NSGb2m mouse, after intraperitoneal administration of GI101 as a test material, and Keytruda (Pembrolizumab, MSD), an anti-PD-1 antibody as a positive control material, alone or in combination.

Stock solutions of the test material, negative control material, and positive control material described in Table 2 were diluted by adding an excipient according

to each dose.

[Table 2]

-	Test material	Positive control material	Negative control material	Excipient
Name of material	GI101	Keytruda	hIgG4	PBS
Description	clear liquid	clear liquid	clear liquid	clear liquid
Component	Fc fusion protein	anti-PD-1 antibody	-	-
pH	7.5	-	-	-
Storage condition	stored under refrigeration (4℃)	stored under refrigeration (4℃)	stored under refrigeration (4℃)	stored under refrigeration (4℃)
Handling precautions	stored under refrigeration until administration and used by reconstitution on the administration day	stored under refrigeration until administration and used by reconstitution on the administration day	stored under refrigeration until administration and used by reconstitution on the administration day	-

Human-derived breast cancer cells, MDA-MB-231 (Homo sapiens, human mammary gland/breast; derived from metastatic site: pleural effusion) were purchased from Korea cell line bank (Korea) and used in the test. A cell culture medium was used by mixing fetal bovine serum (FBS,16000-044, Thermofisher scientific, U.S.A.), penicillin-streptomycin; 10,000 units/ml penicillin and 10,000 μg/ml streptomycin (15140122, Thermofisher scientific, U.S.A.), and RPMI1640 (A1049101, Thermofisher scientific, U.S.A.) per 100 ml as the composition shown in the table below.

[Table 3]

Name	Composition (㎖)
FBS	10

Penicillin-Streptomycin	1
RPMI1640	89
Total volume	100

Cells to be used in the test were thawed, placed in a flask for cell culture, and cultured at 37°C, in a 5% CO_2 incubator (MCO-170M, Panasonic, Japan). The cultured cells were suspended using trypsin-EDTA (Cat. 25200-072, Thermofisher scientific, U.S.A.). The suspended cells were collected by centrifugation (125xg, 5 minutes) by using a centrifuge, transferred to a new medium and a new flask, and subcultured. On the day of cell line transplantation, the cultured cells were placed in a centrifuge tube, recovered, and then centrifuged (125xg, 5 minutes) to discard the supernatant. Then, a cell suspension ($5x10^6$ cells/0.05 ml) was made with PBS (Cat. LB 001-04, Welgene Inc., KOREA), and stored on ice until inoculation.

For the test, 8-week-old female NSGb2m (NOD.Cg-$B2m^{tm1Unc}Prkdc^{scid}Il2rg^{tm1Wjl}$/SzJ) mice were purchased from JoongABio (Korea) and used. The body weight was measured the next day after the end of quarantine and acclimatization period, and then a human-derived PBMC cell suspension ($5x10^6$ cells/0.2 ml) prepared for healthy animals was filled in a disposable syringe, and administered to the caudal vein of the animals. General symptoms were observed once a day after cell transplantation.

A solution prepared by adding phenol red-free matrigel matrix (0.05 ml, 356237, BD, U.S.A.) to the prepared MDA-MB-231 cell suspension ($5x10^6$ cells/0.05 ml) was filled in a disposable syringe, and transplanted by administering subcutaneously (0.1 ml/head) to the right back of the animal transplanted with human PBMC. General symptoms were observed once a day during the engraftment and growth period following cell line transplantation.

After a certain period following cell transplantation, the tumor volume was measured for mice showing no abnormal health status, and 32 individuals were selected so that the average of each group reached 40 to 80 mm^3. The selected animals were divided into 4 groups (8 animals per group) as evenly as possible, based on the tumor volume and body weight.

The test groups were organized as shown in Table 4. The test material was administered to animals using a disposable syringe (31G, 1 ml), and the frequency of administration was twice a week with a total of 4 administrations.

[Table 4]

Group		Dosage (mg/kg)	Volume administered (㎖/kg)	Number of animal
G1	hIgG4	6	10	8
G2	GI101	6	10	8
G3	Keytruda	5	10	8
G7	GI101 + Keytruda	6 + 5	10	8

During the observation period, general symptoms such as appearance, behavior, excretion, or the like were observed once a day, and deceased animals were identified. Body weight was measured on the day of cell line transplantation, twice a week, and on sacrifice day of the animal.

During the observation period, maximum length (L) and perpendicular width (W) of the tumor were measured three times a week using a digital caliper (mitutoyo, Japan), and applied to the following equation to calculate the tumor volume (TV).

<Equation 1>

TV (㎣) = $(W^2XL)/2$

<Equation 2>

%TGI(Tumor Growth Inhibition)= (1-(Ti-T0)/(Vi-V0))X100

The tumor volume of each individual before administration was set as the value measured at the time of grouping.

On day 21, day 25, day 28, and day 31 after tumor transplantation, the drugs shown in Table 4 were respectively administered, and as a result, tumor growth was inhibited in the GI101 or Keytruda alone treatment groups compared to the control group (hIgG4). Tumor growth was inhibited in the GI101 and Keytruda combined treatment groups compared to the control group. Tumor growth was inhibited in the GI101 and Keytruda combined treatment group compared to the GI101 or Keytruda alone treatment groups (FIG. 60).

Tumor growth inhibition rate was calculated at the end of the experiment (day 42 after tumor transplantation), as compared to the drug treatment day 1 (day 17 after tumor transplantation). As a result, the hIgG4 treatment group had 2 mice having tumor growth inhibition rate of 30% or more, 1 mouse having tumor growth inhibition rate of 50% or more, and 1 mouse having tumor growth inhibition rate of 80% or more; the GI101 treatment group had 5 mice having tumor growth inhibition rate of 30% or more, 5 mice having tumor growth inhibition rate of 50% or more, and 2 mice having tumor growth inhibition rate of 80% or more; the Keytruda treatment group had 7 mice having tumor growth inhibition rate of 30% or more, 5 mice having tumor growth inhibition rate of 50% or more, and 3 mice having tumor growth inhibition rate of 80% or more; and the GI101 and Keytruda combined treatment group had 8 mice having tumor growth inhibition rate of 30% or more, 8 mice having tumor growth inhibition rate of 50% or more, and 6 mice having tumor growth inhibition rate of 80% or more (FIG. 61).

In addition, the degree of tumor growth of individual experimental animals in each treatment group when GI101 and Keytruda were used in combination in mice transplanted with human-derived breast cancer cells are shown in FIGS. 62 to 66.

Experimental example 21. Determination of anticancer effect by

combined administration of mGI101 and anti-PD-1 antibody in mouse-derived colorectal cancer cell transplanted mice

This test evaluated tumor growth inhibitory effect in a tumor model in which allogeneic murine colon adenocarcinoma cells (MC38) were transplanted to C57BL/6 mice, after intraperitoneal administration of mGI101 as a test material, and an anti-PD-1 antibody as a positive control material, alone or in combination.

Murine colon adenocarcinoma cells (MC38), which are rodent-derived colorectal cancer cells, were purchased from Kerafast Inc. (USA) and used in the test. MC38 cells were cultured in a RPMI1640 medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% antibiotic/antifungal agent (Gibco). The cultured cells were harvested using trypsin and suspended in PBS. $1x10^6$ MC38 cells were injected s.c. into the right flank of C57BL/6 female mice (7-week-old) to establish an allograft tumor model.

Mice were randomly assigned (5 per group) based on the tumor volume (30 mm^3). Tumor grafts were identified about 2 days after cell inoculation. The test groups were organized as shown in Table 5, and test materials were administered.

[Table 5]

Test group		**Administration route, administration interval**	**Dosage**	**Number of animal**
G1	Vehicle control(hIgG4)	*i.p.* BIW x 16 days	10 mg/kg	5
G2	mGI101	*i.p.* day 1, 5, 9	6 mg/kg	6
G3	Anti-PD-1 antibody (cloneRMP1-14, InVivoMab)	*i.p.* BIW x 16 days	5 mg/kg	5
G4	mGI101 + anti-PD-1 antibody	*i.p.* day 1, 5, 9 (mGI101)	0.6 mg/kg	5
		i.p. BIW x 16 days (anti-PD-1 antibody)	5 mg/kg	

During the test period, clinical symptoms such as disease and behavior changes, or the like were observed once a day, and deceased animals were identified. The animals were sacrificed after the test period. The size of MC38 solid cancer was measured using a tumor 3D scanner (TM900, Peria, belgium). The average weight

loss and percentage change, and average tumor growth inhibition were calculated for each experimental group. Antitumor efficacy was evaluated compared to the vehicle control group.

All statistical calculations were performed using Prism 8.0 (Graph Pad Software Inc., USA). Tumor volume measurements were compared through one-way ANOVA (end time) followed by Bonferroni's multiple comparison test. A "p" value <0.05 was considered significant.

All test animals remained healthy without showing signs of pathological abnormalities after administration of mGI101 and co-administration of mGI101 and an anti-PD-1 antibody. The results of combination therapy using mGI101 and/or an anti-PD-1 antibody against MC38 tumors are shown in FIGS. 67 to 73. An anticancer effect was observed in the drug treatment group compared to the control group, and the difference in tumor size was noticeable during 16 days of test period. MC38 tumor was known as a model responsive to anti-PD-1 antibodies in previous literature, and an anticancer effect was also observed in the anti-PD-1 antibody administration group of this test ($p>0.01$). The anticancer effect was also found in the mGI101 (6 mpk) alone administration group as much as in the anti-PD-1 antibody administration group ($p>0.01$). mGI101 (0.6 mpk) + anti-PD-1 (5 mpk) combined administration group showed a remarkably excellent anticancer effect ($p>0.0001$).

Individual tumor sizes for each test group are shown in FIGS. 69 to 73. According to the results of individual tumor size, slight tumor regression was observed in some animals among the anti-PD-1 antibody administration group. The mGI101 (6 mpk) alone administration group showed better tumor growth inhibitory effect than the anti-PD-1 antibody administration group. The tumor size remained

the same until 5 to 7 days, but regrew after 7 days. The tumor size remained the same until 5 to 7 days, but regrew after 7 days. The combined administration group (GI101 (0.6 mpk) + anti-PD-1 antibody (5 mpk)) showed remarkably excellent tumor growth inhibition. In particular, two of the combined administration group showed complete response (no tumor).

MC38 cells were re-injected into the left flank (opposite to the initial injection site of cancer cells) of the two mice of the combined administration group showing complete remission. These mice continued anti-PD-1 antibody administration (5 mpk, BIW) until day 32 (FIG. 74). A small tumor (>30 mm^3) was found in one of the two mice, but the tumor size did not grow any more until day 35 (FIG. 69). Tumor was not found in another mouse after tumor was re-injected (FIGS. 69 and 74).

In conclusion, the anti-tumor efficacy of mGI101 alone and in combination with an anti-PD-1 antibody was tested in the MC38 allogeneic tumor model, and as a result, the combined administration group (GI101 (0.6 mpk) + anti-PD-1 (5 mpk)) showed the best anti-tumor efficacy. Two experimental animals of the combined administration group showed a complete response, and the mice showing a complete response showed an anticancer effect when MC38 was re-injected (Table 6).

[Table 6]

Days After treatment	CR mouse	
	No.1 Tumor Vol.(mm^3)	No.2 Tumor Vol.(mm^3)
D1	78.2	23.6
D2	84.5	13.5
D3	36.3	0
D4	0	0
D5	0	0
D6	0	0
D7	0	0
D8	0	0
D9	0	0

D10	0	0
D11	0	0
D12	0	0
D13	0	0
D14	0	0
D15	0	0
D16	0	0
D17	0	0
D18	0	0
D19	0	0
D20	0	0
D21	0	0
D22	0	0
D23	0	0
D24	0	0
*D25	0	0
D26	0	0
D27	0	0
D28	0	0
D29	0	0
D30	0	0
D31	0	0
D32	12.8	0

Experimental example 22. Determination of anticancer effect by combined administration of mGI101 and anti-PD-L1 antibody in mouse-derived colorectal cancer cell transplanted mice

This test evaluated tumor growth inhibitory effect in a tumor model in which allogeneic murine colon carcinoma cells (CT26) were transplanted to BALB/c mice, after intraperitoneal administration of mGI101 as a test material, and an anti-PD-L1 antibody (BioXcell, Cat# BE0101) as a positive control material, alone or in combination.

CT26 cells were cultured in a RPMI1640 medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% antibiotic/antifungal agent (Gibco). The cultured cells were harvested using trypsin and suspended in PBS. $5x10^5$ CT26 cells were subcutaneously injected into the right flank of BALB/c female mice (7-week-old) to establish an allograft tumor model.

Mice were randomly assigned (4 per group) based on the tumor volume (50 ~ 120 mm^3). Tumor grafts were identified about 2 days after cell inoculation. The test groups were organized as shown in Table 7, and test materials were administered.

[Table 7]

Test group		**Administration route, administration interval**	**Dosage**	**Number of animal**
G1	Vehicle control(PBS)	*i.p.* BIW x 9 days	-	4
G2	mGI101	*i.v.* QW x 9 days	3 mg/kg	4
G3	Anti-PD-L1 antibody (BioXcell, Cat# BE0101)	*i.p.* BIW x 9 days	10 mg/kg	4
G4	mGI101 + anti-PD-L1 antibody	*i.v.* QW x 9 days (mGI101)	3 mg/kg	4
G4	mGI101 + anti-PD-L1 antibody	*i.p.* BIW x 9 days (anti-PD-L1 antibody)	10 mg/kg	4

During the test period, clinical symptoms such as disease and behavior changes, or the like were observed once a day, and deceased animals were identified. The animals were sacrificed after the test period. The size of CT26 solid cancer was measured using a tumor 3D scanner (TM900, Peria, belgium). The average weight loss and percentage change, and average tumor growth inhibition were calculated for each experimental group. An antitumor efficacy was evaluated compared to the vehicle control group.

All statistical calculations were performed using Prism 8.0 (Graph Pad Software Inc., USA). Tumor volume measurements were compared through one-way ANOVA (end time) followed by Bonferroni's multiple comparison test. A "p" value <0.05 was considered significant.

The anti-tumor efficacy of mGI101 alone and in combination with an anti-PD-L1 antibody was tested in the CT26 allogeneic tumor model, and as a result, the

combined administration group (mGI101 (3 mpk) + anti-PD-L1 (10 mpk)) showed the best anti-tumor efficacy (FIG. 75).

Experimental example 23. Determination of anticancer effect by combined administration of mGI101 and anti-TIGIT antibody in mouse-derived colorectal cancer cell transplanted mice

This test evaluated tumor growth inhibitory effect in a tumor model in which allogeneic murine colon carcinoma cells (CT26) were transplanted to BALB/c mice, after intraperitoneal administration of mGI101 as a test material, and an anti-TIGIT antibody specifically binding to the extracellular domain (ECD) of TIGIT having the amino acid sequence of SEQ ID NO: 39 as a positive control material, alone or in combination.

CT26 cells were cultured in a RPMI1640 medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% antibiotic/antifungal agent (Gibco). The cultured cells were harvested using trypsin and suspended in PBS. $5x10^5$ CT26 cells were subcutaneously injected into the right flank of BALB/c female mice (7-week-old) to establish an allograft tumor model.

Mice were randomly assigned (5 per group) based on the tumor volume (50 ~ 120 mm^3). Tumor grafts were identified about 2 days after cell inoculation. The test groups were organized as shown in Table 8, and test materials were administered.

[Table 8]

Test group		**Administration route, administration interval**	**Dosage**	**Number of animal**
G1	Vehicle control(PBS)	*i.p.* BIW x 9 days	-	5
G2	mGI101	*i.v.* QW x 9 days	3 mg/kg	5
G3	Anti-TIGIT antibody (Merck, MK-7684)	*i.p.* BIW x 9 days	20 mg/kg	5

G4	mGI101 + anti-TIGIT antibody	*i.v.* QW x 9 days (mGI101)	3 mg/kg	5
		i.p. BIW x 9 days (anti-TIGIT antibody)	20 mg/kg	

During the test period, clinical symptoms such as disease and behavior changes, or the like were observed once a day, and deceased animals were identified. The animals were sacrificed after the test period. The size of CT26 solid cancer was measured using a tumor 3D scanner (TM900, Peria, belgium). The average weight loss and percentage change, and average tumor growth inhibition were calculated for each experimental group. An antitumor efficacy was evaluated compared to the vehicle control group.

All statistical calculations were performed using Prism 8.0 (Graph Pad Software Inc., USA). Tumor volume measurements were compared through one-way ANOVA (end time) followed by Bonferroni's multiple comparison test. A "p" value <0.05 was considered significant.

The anti-tumor efficacy of mGI101 alone and in combination with an anti-TIGIT antibody was tested in the CT26 allogeneic tumor model, and as a result, the combined administration group (mGI101 (3 mpk) + anti-TIGIT (20 mpk)) showed the best anti-tumor efficacy (FIG. 76). An anti-tumor effect was not observed in the anti-TIGIT antibody alone administration group, as compared with the control group. However, the combined administration of an anti-TIGIT antibody and mGI101 showed a remarkably superior anti-tumor effect, as compared with the mGI101 alone administration group.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for preventing or treating cancer comprising, as active ingredients, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

2. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the IL-2 protein or a variant thereof and the CD80 protein or a fragment thereof are attached via a linker.

3. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the IL-2 protein has the amino acid sequence of SEQ ID NO: 10.

4. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the CD80 has the amino acid sequence of SEQ ID NO: 11.

5. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the fusion protein has the amino acid sequence of SEQ ID NO: 9.

6. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the immune checkpoint inhibitor is any one selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-TIM3 antibody, an anti-GAL9 antibody, an anti-LAG3 antibody, an anti-

VISTA antibody, an anti-KIR antibody, an anti-BTLA antibody and an anti-TIGIT antibody.

7. The pharmaceutical composition for preventing or treating cancer according to claim 6, wherein the anti-CTLA-4 antibody is any one selected from the group consisting of ipilimumab and tremelimumab;

the anti-PD-1 antibody is any one selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, JTX-4014, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, INCMGA00012, AMP-224, and AMP-514;

the anti-PD-L1 antibody is any one selected from the group consisting of atezolizumab, avelumab, durvalumab, KN035, CK-301, AUNP12, CA-170, and BMS-986189;

the anti-TIM3 antibody is any one selected from the group consisting of LY3321367, MBG453, and TSR-022;

the anti-LAG3 antibody is any one selected from the group consisting of IMP321, relatlimab, and GSK2831781; or

the anti-VISTA antibody is JNJ-63723283.

8. The pharmaceutical composition for preventing or treating cancer according to claim 1, wherein the cancer is any one selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, larynx cancer, acute myeloid leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary cancer, and lymphoma.

9. A method for preventing or treating cancer, comprising:

administering, to a subject having cancer, a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor.

10. A use of a fusion protein dimer comprising an IL-2 protein or a variant thereof and a CD80 protein or a fragment thereof, and an immune checkpoint inhibitor for preventing or treating cancer.