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(56) Related Art
LINGHONG, K. et al, "Expression of fusion IL2-B7.1(IgV+C) and effects on T lymphocytes", Biochemistry and Cell Biology, 2007, vol. 85, no. 6, pages 685-695
CHAN, L. et al, "1131. Generation of Whole Cell Vaccines for Acute Myeloid Leukaemia by Lentivirus Mediated IL-2/CD80 Transduction", Molecular Therapy, 2005, vol. 11, page 436

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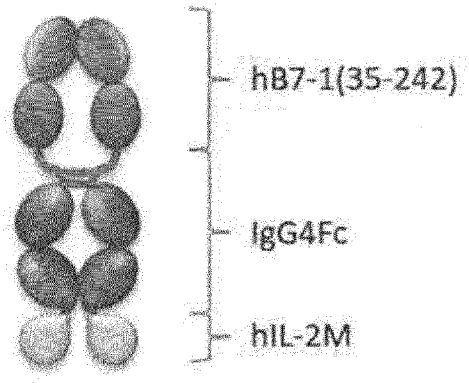
(54) Title: FUSION PROTEIN COMPRISING IL-2 PROTEIN AND CD80 PROTEIN, AND USE THEREOF

(54) 발명의 명칭: IL-2 단백질 및 CD80 단백질을 포함하는 융합단백질 및 이의 용도

[도 1]



(57) Abstract: The present invention provides a fusion protein comprising an IL-2 protein and a CD80 protein. A fusion protein comprising a CD80 fragment, an immunoglobulin Fc, and an IL-2 variant, in one embodiment, can activate immune cells, such as natural killer cells, and, at the same time, can control the immune cell regulatory activity of regulatory T cells. Therefore, a pharmaceutical composition comprising the fusion protein as an active ingredient can increase the immune activity in vivo and can be effectively used for not only cancer but also infectious diseases, and thus is highly industrially applicable.

(57) 요약서: IL-2 단백질 및 CD80 단백질을 포함하는 융합단백질을 제공한다. 일 구체예인 CD80 단편, 면역글로불린 Fc 및 IL-2 변이체를 포함하는 융합단백질은 자연살해세포와 같은 면역세포를 활성화시킬 수 있으며, 동시에 조절 T 세포의 면역세포 조절 활성을 제어할 수 있다. 따라서, 상기 융합단백질을 유효 성분으로 포함하는 약학 조성물은 체내의 면역활성을 증가시켜 암뿐만 아니라 감염성 질환에도 효과적으로 활용할 수 있어 산업적 활용 가능성이 높다.

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Description

Title of Invention

FUSION PROTEIN COMPRISING IL-2 PROTEIN AND CD80 PROTEIN, AND USE THEREOF

Technical Field

The present invention relates to a fusion protein comprising an IL-2 protein and a CD80 protein, and a use thereof. Specifically, the present invention relates to a novel fusion protein having cancer therapeutic and immunopotentiating efficacy.

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).

Furthermore, IL-2 is involved in proliferation and differentiation of B cells, promotes immunoglobulin synthesis by B cells, and stimulates production, proliferation, and activation of natural killer cells (NK cells). Therefore, IL-2 is used as an anticancer agent, because it can increase lymphocyte populations and increase the function of the immune cells in the living body. Currently, therapy with IL-2 has been

approved and used for patients with metastatic renal cell carcinoma and malignant melanoma.

However, IL-2 has a dual function in immune responses in that it is important not only for mediating an increase in number of immune cells and activity thereof, but also for maintaining immune tolerance. In addition, it has been reported that IL-2 may not be optimal for inhibiting tumor growth. The reason is that in the presence of IL-2, activation-induced cell death (AICD) may occur in the resulting cytotoxic T lymphocytes and immune responses may be inhibited by IL-2-dependent regulatory T cells (Treg cells) (Imai *et al.*, *Cancer Sci* 98, 416-423, 2007).

In addition, severe cardiovascular, pulmonary, renal, hepatic, gastrointestinal, neuronal, cutaneous, hematological, and systemic side effects occur in patients who have received immunotherapy with IL-2. Therefore, various IL-2 mutations have been studied to improve therapeutic efficacy of IL-2 and minimize side effects thereof (US 5,229,109 B). However, there are still many problems to be solved in order to utilize IL-2 for pharmacological purposes.

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.

For example, when CD80 and CD86 interact with CD28, costimulatory signals are generated to activate T cells. Eventually, CD80 binds to CTLA4 and stimulates CTLA4 to be upregulated. As a result, CD80 inhibits T cell responses prior to immune response activation caused by CD80/CD28 interaction. This feedback loop allows for fine regulation of immune responses.

In addition, CD80 is known to bind PD-L1, another B7 family member, with

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affinity similar to that with which CD28 binds PD-L1. PD-L1 is known as one of two ligands for programmed death-1 (PD-1) protein, and PD-L1 is known to be involved in T cell regulation. Binding of CD80 to PD-L1 is another mechanism that can block PD-1/PD-L1 interaction, which may prevent inhibition of T cell responses in tumors. At the same time, however, an increase in CD80 levels causes CD80 to bind to CD28 so that CTLA4 is induced, thereby inducing or inhibiting T cell responses.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

By way of clarification and for avoidance of doubt, as used herein and except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additions, components, integers or steps.

Disclosure of Invention

Technical Problem

The present inventors have studied to develop IL-2 which is safe and effective. As a result, the present inventors have discovered that a novel fusion protein comprising, in one molecule, an IL-2 protein and a CD80 protein can activate immune cells and effectively regulate Treg cells, thereby completing the present invention.

Solution to Problem

In an aspect of the present invention, there is provided a fusion protein comprising an IL-2 protein and a CD80 protein.

In another aspect of the present invention, there is provided a fusion protein dimer obtained by attaching the two fusion proteins to each other.

In yet another aspect of the present invention, there is provided a polynucleotide

encoding the fusion protein.

In still yet another aspect of the present invention, there is provided a vector comprising the polynucleotide.

In still yet another aspect of the present invention, there is provided a transformed cell into which the vector has been introduced.

In still yet another aspect of the present invention, there is provided a pharmaceutical composition for preventing or treating cancer or an infectious disease,

comprising, as an active ingredient, the fusion protein or the fusion protein dimer.

In still yet another aspect of the present invention, there is provided a use of the fusion protein for treatment of cancer or an infectious disease.

In still yet another aspect of the present invention, there is provided a use of the fusion protein for manufacture of a medicament for treating cancer or an infectious disease.

Advantageous Effects of Invention

A fusion protein comprising an IL-2 protein and a CD80 protein can not only activate immune cells owing to IL-2, but also effectively regulate Treg cells owing to CD80. Therefore, the fusion protein can attack cancer cells in an efficient manner, and thus can be usefully employed for treatment of cancer or an infectious disease.

Brief Description of Drawings

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

Fig. 2 illustrates a mechanism by which the fusion protein regulates two different types of immune cells; however, it should be understood that the mechanism by which the action of the fusion protein is expressed is not limited thereto.

Fig. 3 illustrates a mechanism by which the fusion protein exhibits an anticancer effect.

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 herein, 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 herein. 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 (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 (GI101) by size exclusion chromatography (SEC).

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

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

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

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

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

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

Fig. 15 illustrates results obtained by identifying the obtained GI102-M72 fusion protein 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.

Figs. 21 and 22 illustrate results obtained by identifying binding ability between GI-101 (hCD80-Fc-hIL-2v) and CTLA-4, and between GI-101 (hCD80-Fc-hIL-2v) and PD-L1. It was identified that GI-101 (hCD80-Fc-hIL-2v) has high binding ability for CTLA-4 and PD-L1.

Fig. 23 illustrates an effect of GI101 on PD-1/PD-L1 binding. GI101 effectively inhibited PD-1/PD-L1 binding.

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

Fig. 25 illustrates results obtained by identifying binding affinity between GI101 and IL-2Rα.

Fig. 26 illustrates results obtained by identifying binding affinity between GI101 and IL-2Rβ.

Fig. 27 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M45.

Fig. 28 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M61.

Fig. 29 illustrates results obtained by identifying binding affinity between IL-2Rα and GI102-M72.

Fig. 30 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M45.

Fig. 31 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M61.

Fig. 32 illustrates results obtained by identifying binding affinity between IL-2Rβ and GI102-M72.

Figs. 33 and 34 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.

Figs. 35 and 36 illustrate results obtained by identifying effects of GI101, GI101C1, GI101C2, and IL-2 (Proleukin) on proliferation of CD8+ T cells.

Fig. 37 illustrates results obtained by identifying effects of GI101 and GI102 on proliferation of CD8+ T cells and CD4+ T cells. Here, Fig. 37A illustrates proportions of CD8+ T cells and CD4+ T cells, Fig. 37B illustrates proliferation capacity of CD8+ T cells, and Fig. 37C illustrates a proportion of CD4+/FoxP3+ Treg cells.

Figs. 38 and 39 illustrate results obtained by identifying effects of GI101 and GI101w on proliferation of CD8+ T cells and NK cells.

Figs. 40 and 41 illustrate results obtained by identifying an effect of GI101 on effector T cells.

Fig. 42 illustrates results obtained by identifying effects of mGI101 and mGI102-M61 on mouse immune cells.

Figs. 43 and 44 illustrate results obtained by identifying an effect of GI101 on cancer cells overexpressing PD-L1.

Figs. 45 and 46 illustrate results obtained by identifying a tumor inhibitory effect of GI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 47 illustrates results obtained by identifying a tumor inhibitory effect of mGI101 in mouse-derived melanoma-transplanted mice.

Fig. 48 illustrates tumor inhibition of mGI101 in mouse-derived melanoma-transplanted mice.

Fig. 49 illustrates results obtained by identifying a tumor inhibitory effect of mGI101, depending on its dose, in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 50 illustrates results obtained by analyzing survival rate of mouse-derived

colorectal cancer cell-transplanted mice having received mGI101.

Fig. 51 illustrates results obtained by identifying a tumor inhibitory effect of GI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 52 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, 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. 53 graphically illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, 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 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, 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 colorectal cancer cell-transplanted mice to treatment with hIgG4, anti-PD-1 antibody, or GI101, and then analyzing, with FACS, macrophages in cancer tissues.

Fig. 56 illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 57 graphically illustrates results obtained by subjecting mouse-derived colorectal cancer cell-transplanted mice to treatment with hIgG4, anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 58 illustrates results obtained by identifying a tumor inhibitory effect of GI101 in mouse-derived lung cancer cell-transplanted mice.

Fig. 59 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, 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. 60 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, anti-PD-1 antibody, or GI101, and then analyzing, with FACS, macrophages in cancer tissues.

Fig. 61 graphically illustrates results obtained by subjecting mouse-derived lung cancer cell-transplanted mice to treatment with hIgG4, anti-PD-1 antibody, or GI101, and then analyzing, with FACS, dendritic cells in cancer tissues.

Fig. 62 illustrates results obtained by identifying a tumor inhibitory effect of mGI102-M61 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 63 illustrates results obtained by analyzing survival rate of mouse-derived colorectal cancer cell-transplanted mice having received mGI102-M61.

Fig. 64 illustrates results obtained by identifying a tumor inhibitory effect of mGI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 65 illustrates tumor inhibition of mGI101 in mouse-derived colorectal cancer cell-transplanted mice.

Fig. 66 illustrates results obtained by making 15-day clinical observations for monkeys having received PBS or GI101.

Figs. 67 and 68 illustrate results obtained by measuring body weights on days -1, 1, 8, and 15 for monkeys having received PBS or GI101.

Fig. 69 illustrates 15-day food consumption for monkeys having received PBS or GI101.

Figs. 70 to 72 illustrate results obtained by analyzing the blood on days -1, 1, 8, and 15 for monkeys having received PBS or GI101.

Figs. 73 to 79 illustrate results obtained by performing clinical and chemical analysis on days -1, 1, 8, and 15 days for monkeys having received PBS or GI101.

Figs. 80 and 81 illustrate results obtained by analyzing cytokines on days -1, 1, 8, and 15 for monkeys having received PBS or GI101.

Figs. 82 to 87 illustrate results obtained by analyzing immune cells on days -1, 1, 8, and 15 for monkeys having received PBS or GI101.

Fig. 88 illustrates results obtained by sacrificing, on day 16, monkeys having received PBS or GI101 to obtain spleen tissues, and pathologically analyzing the spleen tissues.

Fig. 89 illustrates fusion proteins, in each of which CD80 protein and IL-2 protein are bound to a carrier protein. Specifically, Fig. 89A illustrates the fusion protein in which the CD80 protein and the IL-2 protein are bound to N-terminus and C-terminus of the carrier protein, respectively. In addition, Fig. 89B illustrates the fusion protein in which the CD80 protein and the IL-2 protein are bound to C-terminus and N-terminus of the carrier protein, respectively.

Best Mode for Carrying out the Invention

Fusion protein comprising IL-2 protein and CD80 protein

In an aspect of the present invention, there is provided a fusion protein comprising an IL-2 protein and a CD80 protein.

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 58th, 62nd, 65th, 81st, or 92nd amino acid in the amino acid sequence of SEQ ID NO: 35 with another amino acid. These amino acid residues correspond to the 38th, 42nd, 45th, 61st, and 72nd 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 1st to 34th 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 35th to 288th amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35th to 242nd amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35th to 232nd

amino acids in SEQ ID NO: 11. In addition, an embodiment of the CD80 fragment may be a protein composed of the 35th to 139th 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 (Fig. 89). 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.

Polynucleotide encoding fusion protein

In yet another aspect of the present invention, there is provided a polynucleotide encoding a fusion protein comprising an IL-2 protein and a CD80 protein. Specifically, the polynucleotide may contain the nucleotide sequence of SEQ ID NO: 8, 25, 27, or 29. The fusion protein comprising an IL-2 protein and a CD80 protein is as described above. In the polynucleotide, one or more nucleotides may be altered by substitution, deletion, insertion, or a combination thereof. When a nucleotide sequence is prepared by chemical synthesis, synthetic methods well known in the art may be used, such as those described in Engels and Uhlmann (Angew Chem IntEd Eng., 37: 73-127, 1988). Such methods may include triester, phosphite, phosphoramidite and H-phosphate methods, PCR and other autoprimer methods, oligonucleotide syntheses on solid supports, and the like.

According to an embodiment, the polypeptide may contain a nucleic acid sequence having an identity, to SEQ ID NO: 8, 25, 27, or 29, of at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100%.

The polynucleotide may further contain a nucleic acid encoding a signal sequence or a leader sequence. As used herein, the term "signal sequence" refers to a signal peptide that directs secretion of a target protein. The signal peptide is translated and then cleaved in a host cell. Specifically, the signal sequence is an amino acid sequence that initiates migration of a protein across the endoplasmic reticulum (ER) membrane. In an embodiment, the signal sequence may have the amino acid sequence

of SEQ ID NO: 1.

Signal sequences are well known in the art for their characteristics. Such signal sequences typically contain 16 to 30 amino acid residues, and may contain more or fewer amino acid residues than such amino acid residues. A typical signal peptide is composed of three regions, that is, a basic N-terminal region, a central hydrophobic region, and a more polar C-terminal region. The central hydrophobic region contains 4 to 12 hydrophobic residues that cause the signal sequence to be immobilized during migration of an immature polypeptide through the membrane lipid bilayer.

After initiation, signal sequences are cleaved in the lumen of ER by cellular enzymes, commonly known as signal peptidases. Here, the signal sequence may be a secretory signal sequence of tPa (tissue plasminogen activator), HSV gDs (signal sequence of Herpes simplex virus glycoprotein D), or a growth hormone. Preferably, a secretory signal sequence used in higher eukaryotic cells including mammals and the like may be used. In addition, a signal sequence included in the wild-type IL-2 and/or CD-80 may be used, or a signal sequence that has been substituted with a codon having high expression frequency in a host cell may be used.

Vector with polynucleotide encoding fusion protein

In still yet another aspect of the present invention, there is provided a vector comprising the polynucleotide.

The vector may be introduced into a host cell to be recombined with and inserted into the genome of the host cell. Or, the vector is understood as nucleic acid means containing a polynucleotide sequence which is autonomously replicable as an episome. The vectors include linear nucleic acids, plasmids, phagemids, cosmids, RNA vectors, viral vectors, and analogs thereof. Examples of the viral vector include, but are not limited to, retroviruses, adenoviruses, and adeno-associated viruses.

Specifically, the vector may include plasmid DNA, phage DNA, and the like; and commercially developed plasmids (pUC18, pBAD, pIDTSAMRT-AMP, and the like), E. coli-derived plasmids (pYG601BR322, pBR325, pUC118, pUC119, and the

like), Bacillus subtilis-derived plasmids (pUB110, pTP5, and the like), yeast-derived plasmids (YEp13, YEp24, YCp50, and the like), phage DNA (Charon4A, Charon21A, EMBL3, EMBL4, λ gt10, λ gt11, λ ZAP, and the like), animal viral vectors (retroviruses, adenoviruses, vaccinia viruses, and the like), insect viral vectors (baculoviruses and the like). Since the vector exhibits different expression levels and modification of a protein depending on a host cell, it is preferred to select and use a host cell which is most suitable for the purpose.

As used herein, the term "gene expression" or "expression" of a target protein is understood to mean transcription of DNA sequences, translation of mRNA transcripts, and secretion of fusion protein products or fragments thereof. A useful expression vector may be RcCMV (Invitrogen, Carlsbad) or a variant thereof. Expression vectors may further contain human cytomegalovirus (CMV) promoter for promoting continuous transcription of a target gene in mammalian cells, and a bovine growth hormone polyadenylation signal sequence for increasing the stability level of RNA after transcription.

Transformed cell expressing fusion protein

In still yet another aspect of the present invention, there is provided a transformed cell into which the vector has been introduced.

Host cells for the transformed cell may include, but are not limited to, prokaryotic cells, eukaryotic cells, and cells of mammalian, vegetable, insect, fungal, or bacterial origin. As an example of the prokaryotic cells, E. coli may be used. In addition, as an example of the eukaryotic cells, yeast may be used. In addition, for the mammalian cells, CHO cells, F2N cells, CSO cells, BHK cells, Bowes melanoma cells, HeLa cells, 911 cells, AT1080 cells, A549 cells, HEK 293 cells, HEK293T cells, or the like may be used. However, the mammalian cells are not limited thereto, and any cells which are known to those skilled in the art to be usable as mammalian host cells may be used.

In addition, for the introduction of an expression vector into the host cell, $CaCl_2$ precipitation, Hanahan method whose efficiency has been increased efficiency by using

a reducing agent such as dimethyl sulfoxide (DMSO) in $CaCl_2$ precipitation, electroporation, calcium phosphate precipitation, protoplast fusion, agitation using silicon carbide fiber, Agrobacteria-mediated transformation, transformation using PEG, dextran sulfate-, Lipofectamine-, or dry/inhibition-mediated transformation, or the like may be used.

As described above, for optimization of properties of a fusion protein as a therapeutic agent or for any other purpose, glycosylation pattern of the fusion protein (for example, sialic acids, fucosylations, glycosylations) may be adjusted by manipulating, through methods known to those skilled in the art, glycosylation-related genes possessed by host cells.

Method for producing a fusion protein

In still yet another aspect of the present invention, there is provided a method for producing a fusion protein comprising an IL-2 protein and a CD80 protein, the method comprising culturing the transformed cells. Specifically, the production method may comprise i) culturing the transformed cells to obtain a culture; and ii) collecting the fusion protein from the culture.

Culturing the transformed cells may be carried out using methods well known in the art. Specifically, the culture may be carried out in a batch process, or carried out continuously in a fed batch or repeated fed batch process.

Use of fusion protein or dimer thereof

In still yet another aspect of the present invention, there is provided a pharmaceutical composition for treating or preventing cancer or an infectious disease, and/or for increasing efficacy in treating cancer or an infectious disease, the composition comprising, as an active ingredient, a fusion protein comprising an IL-2 protein and a CD80 protein or a fusion protein dimer where the two fusion proteins are attached.

The fusion protein comprising an IL-2 protein and a CD80 protein, or the fusion protein dimer where the two fusion proteins are attached is as described above.

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. In addition, the infectious disease may be any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

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 or an infectious disease 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 anticancer activity or a therapeutic effect on an infectious disease. 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 or an infectious disease-treating 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 anticancer activity or a therapeutic effect on an infectious disease, so as to boost or reinforce anticancer activity.

In still yet another aspect of the present invention, there is provided a use of a fusion protein comprising an IL-2 protein and a CD80 protein for treating cancer or an infectious disease.

In still yet another aspect of the present invention, there is provided a use of a fusion protein comprising an IL-2 protein and a CD80 protein for enhancing a

therapeutic effect on cancer or an infectious disease.

In still yet another aspect of the present invention, there is provided a use of a fusion protein comprising an IL-2 protein and a CD80 protein for manufacture of a medicament for treating cancer or an infectious disease.

In still yet another aspect of the present invention, there is provided a method for treating cancer or an infectious disease, and/or a method for enhancing a therapeutic effect on cancer or an infectious disease, comprising administering, to a subject, a fusion protein comprising an IL-2 protein and a CD80 protein or a fusion protein dimer where the two fusion proteins are attached.

The subject may be an individual suffering from cancer or an infectious disease. In addition, the subject may be a mammal, preferably a human. The fusion protein comprising an IL-2 protein and a CD80 protein, 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 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. In addition, the fusion protein or fusion protein dimer may be administered in combination with other drugs or physiologically active substances whose therapeutic effect is known with respect to a disease to be treated, or may be formulated in the form of combination preparations with other drugs.

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 and infectious diseases. 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 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™) 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™) 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™) 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™) 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™) 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™) 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 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 and PD-L1

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. On the other hand, Human PD-L1-His Tag (Sino biological, Cat: 10084-H08H) was diluted with 1XNi-NTA kinetic buffer to a concentration of 5 μg/ml, and loaded on the Ni-NTA biosensor chip for 600 seconds and fixed. Then, binding of PD-L1 to GI-101, GI-101C1, hPD-1-Fc (Sino biological, Cat: 10377-H02H), and GI101C2 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 Figs. 21 and 22.

Experimental Example 6. Identification of effect of GI-101 (hCD80-Fc-hIL-2v) on PD-1/PD-L1 binding

A blocking experiment was performed using the Octet RED 384 instrument (ForteBio, Pall Life Science) with agitation at 30°C and 1,000 rpm. Human PD-L1-His Tag (Sino biological, Cat: 10084-H08H) was diluted with 1XNi-NTA kinetic buffer to a concentration of 5 μg/ml, and loaded on the Ni-NTA biosensor chip for 600 seconds and fixed. In order to proceed with the blocking experiment, hPD-L1 fixed on the biosensor chip was allowed to bind to GI-101 at various concentrations (300 nM, 100 nM, 50 nM, 25 nM, 12.5 nM, and 0 nM) for 600 seconds, and then again allowed to bind to the competitor human PD-1 (100 nM) for 600 seconds so as to measure how much more hPD-1 can bind thereto. On the contrary, hPD-L1 was allowed to bind to hPD-1 at various concentrations (300 nM, 100 nM, 50 nM, 25 nM, 12.5 nM, and 0 nM) for 600 seconds, and then again allowed to bind to the competitor GI-101 (100 nM) for 600 seconds so as to measure how much more GI-101 can bind thereto. The blocking experiment was analyzed using the epitope binning menu of Octet Data Analysis HT software ver. 10 provided by Pall Corporation. The results are illustrated in Fig. 23.

Experimental Example 7. 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. 24 to 26.

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 8. 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 8.1. Identification of binding affinity between IL2 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 IL2 alpha receptor and GI101-M45 is illustrated in Fig. 27. In addition, the binding affinity between IL2 alpha receptor and GI101-M61 is illustrated in Fig. 28, and the binding affinity between IL2 alpha receptor and GI101-M72 is illustrated in Fig. 29.

Experimental Example 8.2. 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. 30, and the binding affinity between IL-2Rβ and GI102-M61 was measured as illustrated in Fig. 31. In addition, the binding affinity between IL-2Rβ and GI102-M72 was measured as illustrated in Fig. 32.

III. Identification of immune activity of fusion protein

Experimental Example 9. Identification of IFN-γ production caused by fusion protein

Experimental Example 9.1. 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% 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 plate at $1x10^5$ cells per well. Treatment with 5 μg/ml of PHA (Lactin 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 9.2. FACS analysis

The cell pellets obtained by removing the supernatant were washed with FACS buffer (3% 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 9.3. 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. 33 and 34).

Experimental Example 10. 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% 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 plate at $1x10^5$ cells per well.

Thereafter, treatment with 1 μg/ml of 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. 35 and 36).

Experimental Example 11. 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% 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 plate at $1x10^5$ cells per well.

Thereafter, the CFB-labeled PBMCs were subjected to treatment with 1 μg/ml of 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 anti-human CD4-PE antibody (BioLegend, USA), anti-human CD8-PE/Cy7 antibody (BioLegend, USA), and 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. 37).

Experimental Example 12. 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 (Busan, 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. 38 and 39).

Experimental Example 13. 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% FBS). 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 white plat 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. 40 and 41).

Experimental Example 14. 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. 42).

IV. Identification of anticancer effect of fusion protein

Experimental Example 15. Identification of effect of GI101 on cancer cells overexpressing PD-L1

NCl-H292 cancer cell line overexpressing PD-L1 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 plate. 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. 43 and 44).

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

5×10^6 cells/0.05 ml of mouse-derived CT-26 cancer cell line was mixed with 0.05 ml Matrigel matrix phenol red-free (BD), and transplantation of 0.1 ml of the mixture was performed by subcutaneous administration in the right dorsal region of 6-week-old female BALB/c mice (Orient Bio). A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 80 mm^3 to 120 mm^3 were separated. Then, the subjects were intravenously administered with 0.1 ml of GI101. A total of three administrations were given once every three days after the first administration, and PBS was given to a negative control group. The tumor size was measured daily to identify an anticancer effect.

As a result, it was observed that the CT-26 cancer cell line-transplanted mice treated with GI101 exhibited a remarkable decrease in tumor size as compared with the

negative control group (Figs. 45 and 46).

Experimental Example 17. Identification of anticancer effect of mGI101 in mouse-derived melanoma-transplanted mice

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 B16F10 cancer cell line (ATCC, USA) were mixed with 0.05 ml of Matrigel matrix phenol red-free (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 50 mm^3 to 120 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, and an anti-PD-1 antibody was administered at a dose of 5 mg/kg to a positive control group. For experimental groups, mGI101 at a dose of 1 mg/kg or 4 mg/kg was administered intravenously thereto. Additionally, groups having received mGI101 at a dose of 4 mg/kg and an anti-PD-1 antibody at a dose of 5 mg/kg were also set as experimental groups. A total of three administrations were given once every three days after the first administration. The tumor size was measured daily.

As a result, the initial tumor volume of all groups was 90 mm^3, and standard error (S.E.) of each group was 5 mm^3 to 6 mm^3. In the negative control group, a change in tumor volume was observed during the experimental period, in which the tumor volume increased from 90 mm^3 to 1,434 mm^3 up to 15 days after the administration.

In the group having received mGI101 at a dose of 1 mg/kg, the tumor volume was observed to increase from 90 mm^3 to 885 mm^3 during the experimental period which is the same period as the negative control group, and a statistically significant inhibition of tumor growth was observed at some measurement time points (p-value: 0.5 on day 11, p-value < 0.01 on day 7, p-value < 0.001 on day 3). In the group having

received mGI101 at a dose of 4 mg/kg, the tumor volume was observed to increase from 90 mm^3 to 748 mm^3 during the experimental period which is the same period as the negative control group, and a statistically significant inhibition of tumor growth was observed at some measurement time points (p-value: 0.5 on day 9, p-value < 0.01 on days 7 and 11).

In addition, tumor growth inhibition rate was analyzed by using, as a reference, the group having received mIgG at a dose of 4 mg/kg and comparing this group with each of the other groups. In the group having received mGI101 at a dose of 1 mg/kg, growth inhibition rate of 36.5% was observed as compared with the negative control group, and no statistically significant difference (p-value: 0.5) was observed. In the group having received mGI101 at a dose of 4 mg/kg, a statistically significant (p-value: 0.5) tumor growth inhibition rate was observed as compared with the negative control group. A total of two administrations were given once every three days after the first administration. The tumor size was measured daily.

Through this, it was found that in tumor growth inhibitory efficacy test for B16F10, a melanoma allotransplanted into C57BL/6 mice, mGI101 had an effect of inhibiting tumor growth in a dose-dependent manner (Figs. 47 and 48).

Experimental Example 18. Identification of anticancer effect of mGI101 in mouse-derived colorectal cancer cell-transplanted mice

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 Matrigel matrix phenol red-free (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, it was found that the experimental group having received mGI101 at a dose of 6 mg/kg or 12 mg/kg mGI101 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. 49). In addition, as a result of measuring a survival rate, it was found that the experimental group having received mGI101 at a dose of 6 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. 50).

Experimental Example 19. Identification of anticancer effect of GI101 in mice transplanted with mouse-derived colorectal cancer cells

Experimental Example 19.1. 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 anti-PD-1 antibody, anti-PD-1 antibody and 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. 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. 51).

Experimental Example 19.2. Immune cell analysis in cancer tissue

The mice of each group in Experimental Example 19.1 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: 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).

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 anti-PD-1 antibody alone at a dose of 5 mg/kg (* $p < 0.05$, Figs. 52 and 53). 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. 52 and 53). 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 anti-PD-1 antibody alone (Figs. 54 and 55). In addition, all experimental groups having received GI101 exhibited an increased level of CD86 expression in macrophages and dendritic cells (* $p < 0.05$, Figs. 54 to 57).

Experimental Example 20. Identification of anticancer effect of GI101 in mice transplanted with mouse-derived lung cancer cells Experimental Example 20.1. 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. 58).

Experimental Example 20.2. Immune cell analysis in cancer tissue

The mice of each group in Experimental Example 20.1 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 19.2 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 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. 59 to 61).

Experimental Example 21. 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 Matrigel matrix phenol red-free (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. 62). 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. 63).

Experimental Example 22. 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 Matrigel matrix phenol red-free (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. 64 and 65).

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 excellent anticancer efficacy as compared with the group having received a combination of mCD80 and IL-2v (Figs. 64 and 65). 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. Toxicity evaluation of fusion protein

Experimental Example 23. Toxicity evaluation of GI101 using monkeys

Experimental Example 23.1. Monkey breeding and drug administration

In the present experiment, nine male Philippine monkeys (Cynomolgus monkeys) aged 2 to 3 years were used. The experiment was carried out in accordance with the "Act on Welfare and Management of Animals" in Japan and the "Guidance for Animal Care and Use" of Ina Research Inc. The experimental protocol was reviewed by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc, and then approved by AAALAC International (Accredited Unit No. 001107).

The experiment was conducted from one day before drug administration up to 15 days after drug administration. Each monkey was observed around the cage, and the stool status was additionally checked. Body weights were measured using a digital scale (LDS-150H, Shimadzu Corporation) one day before drug administration, and on days 1, 8, and 15 after drug administration. In addition, the remaining amount of food was measured from one day before drug administration up to sacrifice of the monkeys.

Here, a disposable syringe (24G) was filled with the drug GI101, and a total of two administrations were given via an intravenous route, each administration being made at a rate of 0.17 ml/sec. GI101 was given twice, at a week's interval, at a dose of 5 mg/kg/day or 10 mg/kg/day. A control group was administered PBS (pH 7.4) in the same manner.

Experimental Example 23.2. Clinical observation, identification of changes in body weight and food intake

Clinical observation, and measurement of changes in body weight and food intake were performed from one day before drug administration up to days 1, 8, and 15 after drug administration. As a result, no toxicity was caused by GI101 (Figs. 66 to 69).

Experimental Example 23.3. Blood analysis

Blood was collected from the monkeys in Experimental Example 23.1 one day before drug administration, and on days 1, 8, and 15 after drug administration. Here, the blood was collected via the femoral vein with a disposable syringe (22G). The

collected blood was subjected to blood analysis using the Automated Hematology System XN-2000 (Sysmex Corporation) and the Automated Blood Coagulation Analyzer CA-510 (Sysmex Corporation) for the items listed in Table 2 below.

[Table 2]

Parameter	Abbr.	Unit	Method	Equipment
Complete blood count				
Red blood cell count	RBC	$10^6/\mu L$	DC sheath-flow detection	XN-2000
Hemoglobin concentration	HGB	g/dL	SLS-hemoglobin	XN-2000
Hematocrit	HCT	%	RBC pulse height detection	XN-2000
Mean corpuscular volume	MCV	fL	HCT/RBC ($X10^4/\mu L$) X 1000	XN-2000
Mean corpuscular hemoglobin	MCH	pg	HGB/RBC ($X10^4/\mu L$) X 1000	XN-2000
Mean corpuscular hemoglobin concentration	MCHC	g/dL	HGB/HCT X 100	XN-2000
Reticulocytes Ratio Count	RET % RET #	% $10^9/L$	Flow cytometry	XN-2000
Platelet count	PLT	$10^3/\mu L$	Flow cytometry	XN-2000
White blood cell count	WBC	$10^3/\mu L$	Flow cytometry	XN-2000
Differential white blood cells [a)]Ratio Count	Diff WBC % Diff WBC #	% $10^3/\mu L$	Flow cytometry	XN-2000
Coagulation tests				
Prothrombin time	PT	s	Light scattering detection	CA-510
Activated partial thromboplastin time	APTT	s	Light scattering detection	CA-510

[a)] Neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO) and basophils (BASO)

As a result, the group having received GI101 at a dose of 5 mg/kg/day or 10 mg/kg/day exhibited an increase in numbers of reticulocytes, leukocytes, and lymphocytes on day 15 (Figs. 70 to 72).

Experimental Example 23.4. Clinical and chemical analysis

Blood was collected from the monkeys in Experimental Example 23.1 one day before drug administration, and on days 1, 8, and 15 after drug administration. Here,

the blood was collected in the same manner as in Experimental Example 23.3. The collected blood was subjected to clinical and chemical analysis using the Clinical Analyzer Model 7180 (Hitachi High-Technologies Corporation) for the items listed in Table 3 below.

[Table 3]

Parameter	**Abbr.**	**Unit**	**Method**
Aspartate aminotransferase	AST	U/L	JSCC traceable method
Alanine aminotransferase	ALT	U/L	JSCC traceable method
Alkaline phosphatase	ALP	U/L	JSCC traceable method
Lactate dehydrogenase	LD	U/L	JSCC traceable method
Creatine kinase	CK	U/L	JSCC traceable method
Glucose	GLU	mg/dL	Enzymatic (Gluc-DH)
Total bilirubin	BIL	mg/dL	Enzymatic (BOD)
Urea nitrogen	UN	mg/dL	Enzymatic (urease-LEDH)
Creatinine	CRE	mg/dL	Enzymatic
Total cholesterol	CHO	mg/dL	Enzymatic (cholesterol oxidase)
Triglycerides	TG	mg/dL	Enzymatic (GK-GPO with free glycerol elimination)
Phospholipids	PL	mg/dL	Enzymatic (choline oxidase)
Inorganic phosphorus	IP	mg/dL	Enzymatic (maltose phosphorylase)
Calcium	CA	mg/dL	OCPC
Sodium	NA	mEq/L	Ion-selective electrode
Potassium	K	mEq/L	Ion-selective electrode
Chloride	CL	mEq/L	Ion-selective electrode
Total protein	TP	g/dL	Biuret
Albumin	ALB	g/dL	BCG
Albumin-globulin ratio	A/G	-	Calculated
JSCC: Japan Society of Clinical Chemistry			

As a result, no toxicity caused by GI101 was detected in the clinical and chemical analysis (Figs. 73 to 79).

Experimental Example 21.5. Cytokine analysis

Blood was collected from the monkeys in Experimental Example 23.1 one day before drug administration, and on days 1, 8, and 15 after drug administration. Here, the blood was collected in the same manner as in Experimental Example 23.3. Using

the Bio-Plex 200 (Bio-Rad Laboratories, Inc.) instrument and the Non-Human Primate Cytokine Magnetic Bead Panel (EMD Millipore) Assay Kit, the collected blood was analyzed for TNF-α, IFN-γ IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12. As a result, no toxicity caused by GI101 was detected with respect to the cytokine analysis (Figs. 80 and 81).

Experimental Example 23.6. Immune cell analysis

Blood was collected from the monkeys in Experimental Example 23.1 one day before drug administration, and on days 1, 8, and 15 after drug administration. Here, the blood was collected in the same manner as in Experimental Example 23.3. Using a flow cytometer (LSRFortessa X-20, Becton, Dickinson and Company), the collected blood was analyzed for the following items:

1) Ki67 + CD4: CD45+/CD3+/CD4+/Ki67+

2) Ki67 + CD8: CD45+/CD3+/CD8+/Ki67+

3) Ki67 + Treg: CD45+/CD3+/FoxP3+/Ki67+

4) Ki67 + ICOS + Treg: CD45+/CD3+/FoxP3+/Ki67+/CD278+

5) ICOS + Treg: CD45+/CD3+/FoxP3+/CD278+

6) Ki67 + NK cell: CD45+/CD16+ and CD56+/Ki67+.

As a result, in the immune cell analysis, all groups having received GI101 exhibited, on day 15, an increase in numbers of T cells, CD4+ T cells, CD8+ T cells, regulatory T cells, NK cells and Ki67+ T cells, Ki67+ CD4+ T cells, Ki67+ CD8+ T cells, Ki67+ regulatory T cells, Ki67+ ICOS+ regulatory T cells, Ki67+ NK cells, ICOS+ regulatory T cells.

Specifically, in lymphocytes, proportions of T cells, CD4+ T cells, regulatory T cells increased and a proportion of NK cells decreased, while a proportion of CD8+ T cells did not change. A proportion of regulatory T cells increased on day 3 and decreased on days 8 and 15. However, the proportion was still higher than the control group.

In addition, regarding proportions of immune cells, which are Ki67+, in the respective immune cells, proportions of Ki67+ T cells, Ki67+ CD4+ T cells, Ki67+ CD8+ T cells, Ki67+ regulatory T cells, Ki67+ ICOS+ regulatory T cells, Ki67+ NK cells, and ICOS+ regulatory T cells increased.

Furthermore, proportions of Ki67+ T cells, Ki67+ CD8+ T cells, and Ki67+ NK cells increased on days 3, 8, and 15; proportions of Ki67+ CD4+ T cells and Ki67+ regulatory T cells increased on days 3 and 8; and proportions of Ki67+ ICOS+ regulatory T cells and ICOS+ regulatory T cells increased only on day 8 (Figs. 82 to 87).

Experimental Example 23.7. Pathological analysis

On day 16, the monkeys in Experimental Example 23.1 were sacrificed and all organs and tissues were fixed using 10% formalin. However, the testes were fixed using a formalin-sucrose-acetic acid (FSA) solution, and the eyes and optic nerve were fixed using 1% formaldehyde-2.5% glutaraldehyde in phosphate buffer. Hematoxylin-eosin staining was performed on the organs and tissues in the items listed in Table 4 below, and observations were made under an optical microscope.

[Table 4]

Organ/tissue	Fixation	Organ weight	Specimen preparation	
			HE-stained	Note
Heart	O	O	-	Left ventricular papillary muscle, right ventricular wall and areas including the coronary artery and aortic valve
Aorta (thoracic)	O	-		
Sternum	O	-		Decalcified
Sternal bone marrow		-		
Femurs	O (R&L)	-		Distal articular cartilage and shaft; decalcified
Femoral bone marrow	O (R)	-		Decalcified
Thymus	O	O	O	
Spleen	O	O	O	
Submandibular lymph nodes	O	-	O	
Mesenteric lymph nodes	O	-	O	

Organ/tissue	Fixation	Organ weight	Specimen preparation	
			HE-stained	Note
Trachea	O	-		Decalcified
Bronchi Lungs	O (R&L)	O (R&L separated)	-	Left anterior and right posterior lobes
Tongue	O	-		
Submandibular glands	O (R&L)	O (R&L combined)		
Parotid glands	O (R&L)	-		
Esophagus	O	-		
Stomach	O	-		Cardia, body and pylorus
Duodenum	O	-		
Jejunum	O	-		
Ileum Peyer's patches	O	-		
Cecum	O	-		
Colon	O	-		
Rectum	O	-		
Liver Gallbladder	O	O (with bile-drained gallbladder)	O O	Left lateral lobe and right medial lobe including the gallbladder
Pancreas	O	O	-	
Kidneys	O (R&L)	O (R&L separated)	O (R&L)	
Urinary bladder	O	-		
Pituitary	O	O		
Thyroids Parathyroids	O (R&L)	O (R&L separated)		
Adrenals	O (R&L)	O (R&L separated)		
Testes	O (R&L)	O (R&L separated)		
Epididymides	O (R&L)	O (R&L separated)		
Prostate	O	O		
Seminal vesicles	O	O	-	
Brain	O	O	-	Cerebrum (frontal, parietal (including basal ganglia and hippocampus) and occipital lobes); cerebellum; pons; and medulla oblongata
Spinal cord (thoracic)	O	-		
Sciatic nerve	O (L)	-		

Organ/tissue	Fixation	Organ weight	Specimen preparation	
			HE-stained	Note
Eyes	O (R&L)	-		
Optic nerves	O (R&L)	-		
Lacrimal glands	O (R&L)	-		
Skeletal muscle (biceps femoris)	O (L)	-		
Skin (thoracic)	O	-		
Injection site (tail vein)	O	-		Decalcified
Skin of the thoracic or medial femoral region with ID No.	O	-	-	

O: conducted -: Not conducted

R&L: Both the right and left organs/tissues were conducted.

L: Either the right or left organ/tissue (usually the left) was conducted.

R: Either the right or left organ/tissue (usually the right) was conducted

As a result, the group treated with GI101 at a dose of 5 mg/kg/day or 10 mg/kg/day exhibited an increase in spleen weight (Fig. 88). No significant changes were observed in the other tissues. In conclusion, in the groups having received GI101, some changes were observed but no toxicity was observed.

VI. Experimental Example 24 for identifying anticancer effect of GI102. Identification of anticancer effect of GI102-M45

Experimental Example 24.1. Identification of anticancer effect of GI102-M45 in mice transplanted with mouse-derived colorectal cancer cells 5×10^6 cells/0.05 ml of mouse-derived CT-26 cancer cell line were mixed with 0.05 ml Matrigel matrix phenol red-free (BD), and transplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of 6-week-old female BALB/c mice (Orient Bio). A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 80 mm^3 to 120 mm3 were separated. Then, the subjects were intravenously administered 0.1 ml of GI102-M45. A total of three administrations were given once every three days after the first administration, and PBS was given for a negative control. The tumor size was measured daily to identify an anticancer effect. Activity of GI102-M45 was identified in the same manner as in Experimental Example 16.

Experimental Example 24.2. Identification of anticancer effect of GI102-M45 in mice transplanted with mouse-derived lung cells

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, GI102-M45 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. Activity of GI102-M45 was identified in the same manner as in Experimental Example 20.1.

Experimental Example 25. Identification of anticancer effect of GI102-M61

Experimental Example 25.1. Identification of anticancer effect of GI102-M61 in mice transplanted with mouse-derived colorectal cancer cells

5×10^6 cells/0.05 ml of mouse-derived CT-26 cancer cell line were mixed with 0.05 ml Matrigel matrix phenol red-free (BD), and transplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of 6-week-old female BALB/c mice (Orient Bio). A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 80 mm^3 to 120 mm^3 were separated. Then, the subjects were intravenously administered 0.1 ml of GI102-M61. A total of three administrations were given once every three days after the first administration, and PBS was given to a negative control. The tumor size was measured daily to identify an anticancer effect. Activity of GI102-M61 was

identified in the same manner as in Experimental Example 16.

Experimental Example 25.2. Identification of antitumor effect of GI102-M61 in mice transplanted with mouse-derived lung cancer cells

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, GI102-M61 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. Activity of GI102-M61 was identified in the same manner as in Experimental Example 20.1.

Experimental Example 26. Identification of anticancer effect of GI102-M72

Experimental Example 26.1. Identification of antitumor effect of GI102-M72 in mice transplanted with mouse-derived colorectal cancer cells

5×10^6 cells/0.05 ml of mouse-derived CT-26 cancer cell line were mixed with 0.05 ml Matrigel matrix phenol red-free (BD), and transplantation of the mixture was performed by subcutaneous administration at 0.1 ml in the right dorsal region of 6-week-old female BALB/c mice (Orient Bio). A certain period of time after the cancer cell transplantation, the tumor volume was measured and subjects that reached about 80 mm^3 to 120 mm^3 were separated. Then, the subjects were intravenously administered 0.1 ml of GI102-M72. A total of three administrations were given once every three days after the first administration, and PBS was given to a negative control. The tumor

size was measured daily to identify an anticancer effect. Activity of GI102-M72 was identified in the same manner as in Experimental Example 16.

Experimental Example 26.2. Identification of anticancer effect of GI102-M72 in mice transplanted with mouse-lung cancer cells

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, GI102-M72 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. Activity of GI102-M72 was identified in the same manner as in Experimental Example 20.1.

Claims

1. A fusion protein comprising an IL-2 variant protein and a CD80 fragment, wherein the fusion protein consists of the following structural formula (I):

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

in the structural formula (I),

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

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

X is the CD80 fragment,

wherein the CD80 fragment consists of the 35th amino acid to 242nd amino acid in the amino acid sequence of SEQ ID NO: 11,

Y is the IL-2 variant protein,

wherein the IL-2 variant is obtained by substitution of at least one selected from the 38th, 42nd, 45th, 61st, and 72nd amino acids in the amino acid sequence of SEQ ID NO: 10,

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

n and m are each independently 0 or 1.

2. The fusion protein of claim 1, wherein the IL-2 variant is 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.

3. The fusion protein of claim 1, wherein the IL-2 variant contains 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.

4. The fusion protein of claim 1, wherein the IL-2 variant has the amino acid sequence of SEQ ID NO: 6, 22, 23, or 24.

5. The fusion protein of claim 1, wherein the Fc domain is a wild type or variant.

6. The fusion protein of claim 1, wherein the Fc domain has the amino acid sequence of SEQ ID NO: 4.

7. The fusion protein of claim 5, wherein the variant of the Fc domain has the amino acid sequence of SEQ ID NO: 12.

8. The fusion protein of claim 1, wherein the linker (1) consists of 5 to 80 contiguous amino acids and the linker (2) consists of 1 to 50 contiguous amino acids.

9. The fusion protein of claim 1, wherein the linker (1) is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 3.

10. The fusion protein of claim 1, wherein the linker (2) is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 5.

11. The fusion protein of claim 1, wherein the fusion protein has the amino acid sequence of SEQ ID NO: 9, 26, 28, or 30.

12. A fusion protein dimer wherein two fusion proteins of any one of claims 1 to 11

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are attached to each other.

13. The fusion protein dimer of claim 12, wherein the fusion protein dimer is a homodimer.

14. A polynucleotide encoding the fusion protein of any one of claims 1 to 11.

15. The polynucleotide of claim 14, wherein the polynucleotide has the nucleotide sequence of SEQ ID NO: 8, 25, 27 or 29.

16. A vector comprising the polynucleotide of claim 15.

17. A transformed cell into which the vector of claim 16 has been introduced.

18. A pharmaceutical composition when used for preventing or treating cancer or an infectious disease, comprising as an active ingredient:

the fusion protein of any one of claims 1 to 11; or

the fusion protein dimer of claim 12 or 13.

19. The pharmaceutical composition of claim 18, further comprising a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 18, 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, laryngeal cancer, acute myeloid leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

21. The pharmaceutical composition of claim 18, wherein the infectious disease is any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus infection, cytomegalovirus infection, viral respiratory disease, and influenza.

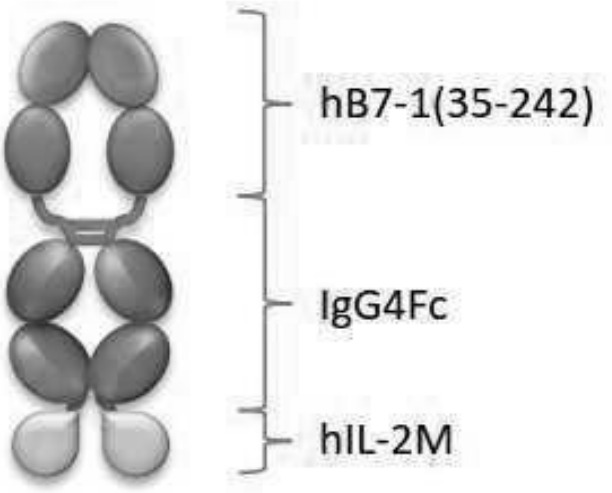
22. A use of the fusion protein of any one of claims 1 to 11, the fusion protein dimer of claim 12 or 13, or the pharmaceutical composition of any one of claims 18-21 for treatment of cancer or an infectious disease.

23. A use of the fusion protein of any one of claims 1 to 11, the fusion protein dimer of claim 12 or 13, or the pharmaceutical composition of any one of claims 18-21 in the manufacture of a medicament for treating cancer or an infectious disease.

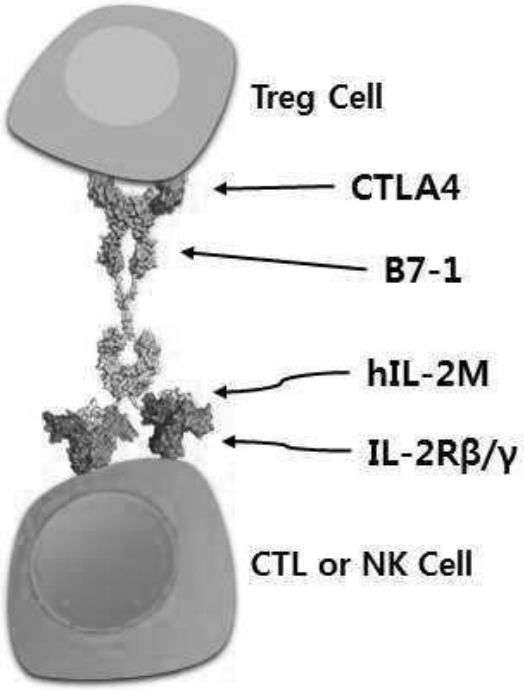
24. A method for treating cancer or an infectious disease, comprising:

administering, to a subject, the fusion protein of any one of claims 1 to 11, the fusion protein dimer of claim 12 or 13, or the pharmaceutical composition of any one of claims 18-21.

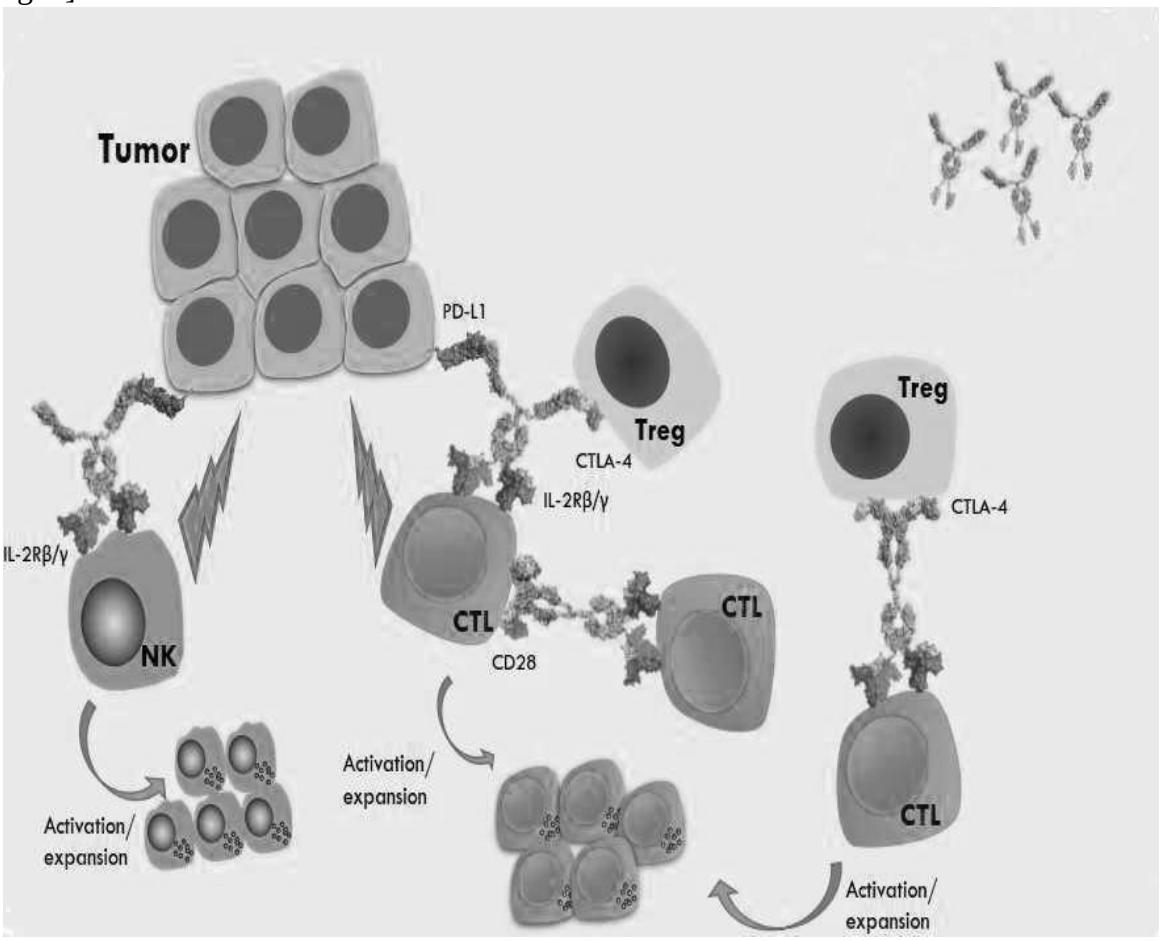
[Fig. 1]


hB7-1(35-242)
IgG4Fc
hIL-2M

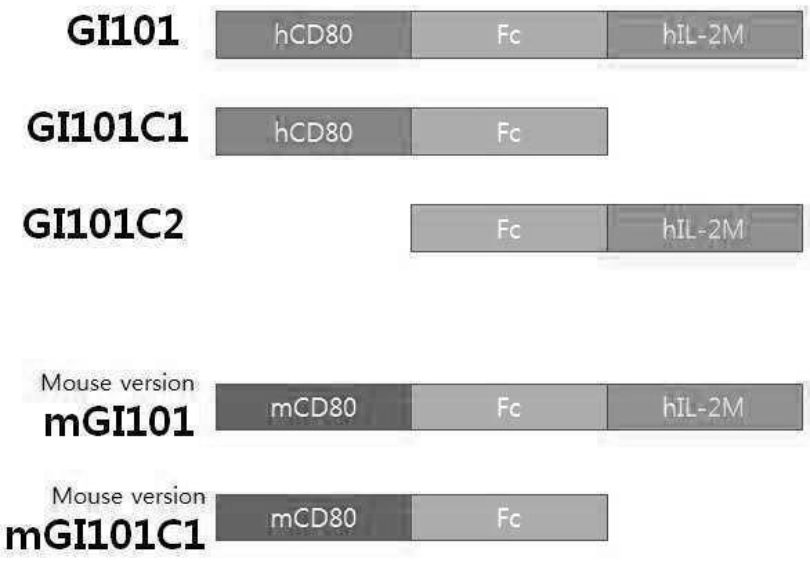
[Fig. 2]


Treg Cell
CTLA4
B7-1
hIL-2M
IL-2Rβ/γ
CTL or NK Cell

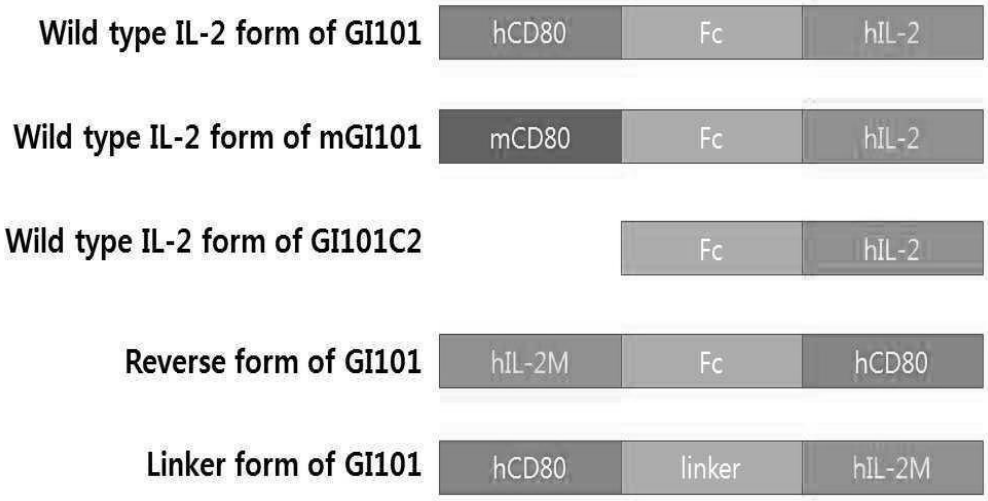
[Fig. 3]


Tumor
PD-L1
Treg
CTLA-4
IL-2Rβ/γ
Treg
CTLA-4
IL-2Rβ/γ
NK
CTL
CD28
CTL
Activation/
expansion
Activation/
expansion
CTL
Activation/
expansion

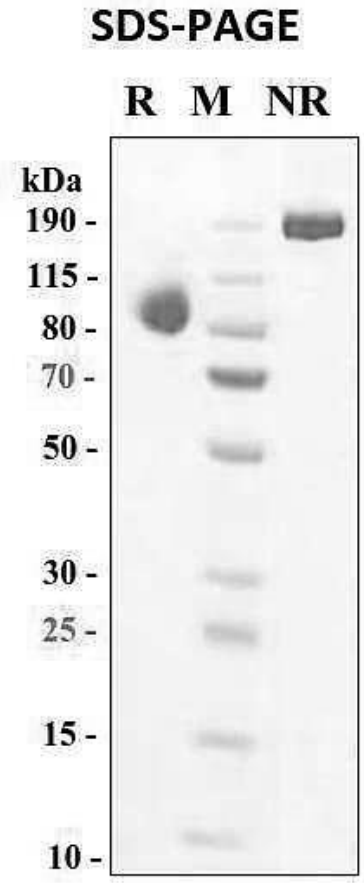
[Fig. 4]


GI101
hCD80
Fc
hIL-2M
GI101C1
hCD80
Fc
GI101C2
Fc
hIL-2M
Mouse version
mGI101
mCD80
Fc
hIL-2M
Mouse version
mGI101C1
mCD80
Fc

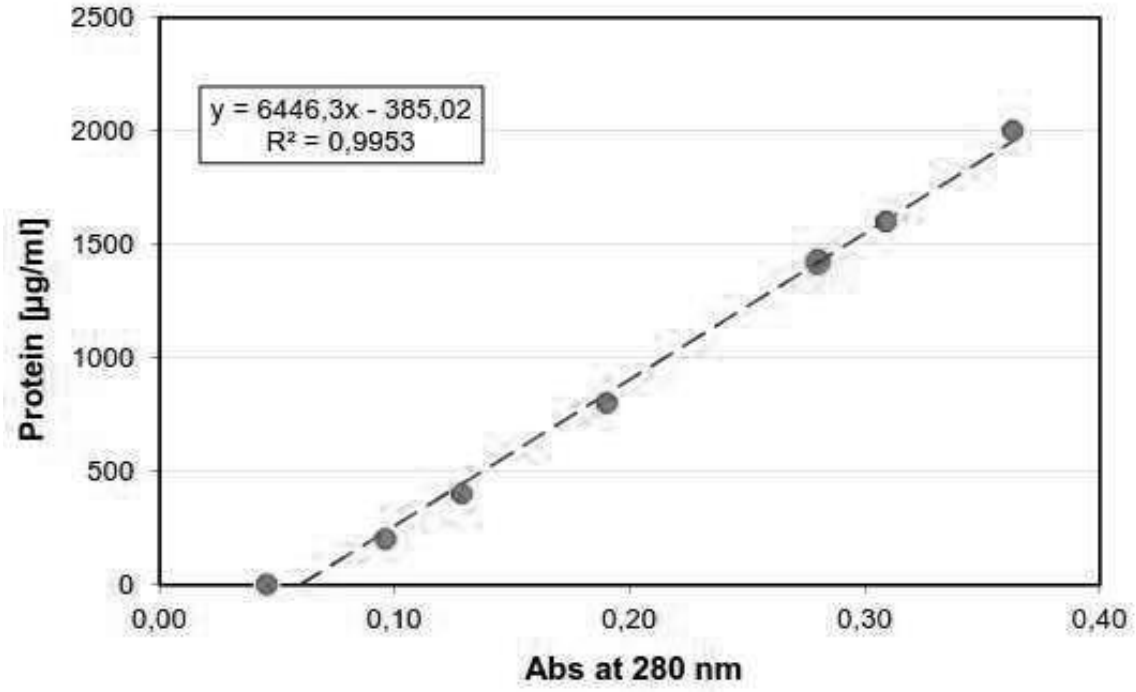
[Fig. 5]


Wild type IL-2 form of GI101
hCD80
Fc
hIL-2
Wild type IL-2 form of mGI101
mCD80
Fc
hIL-2
Wild type IL-2 form of GI101C2
Fc
hIL-2
Reverse form of GI101
hIL-2M
Fc
hCD80
Linker form of GI101
hCD80
linker
hIL-2M

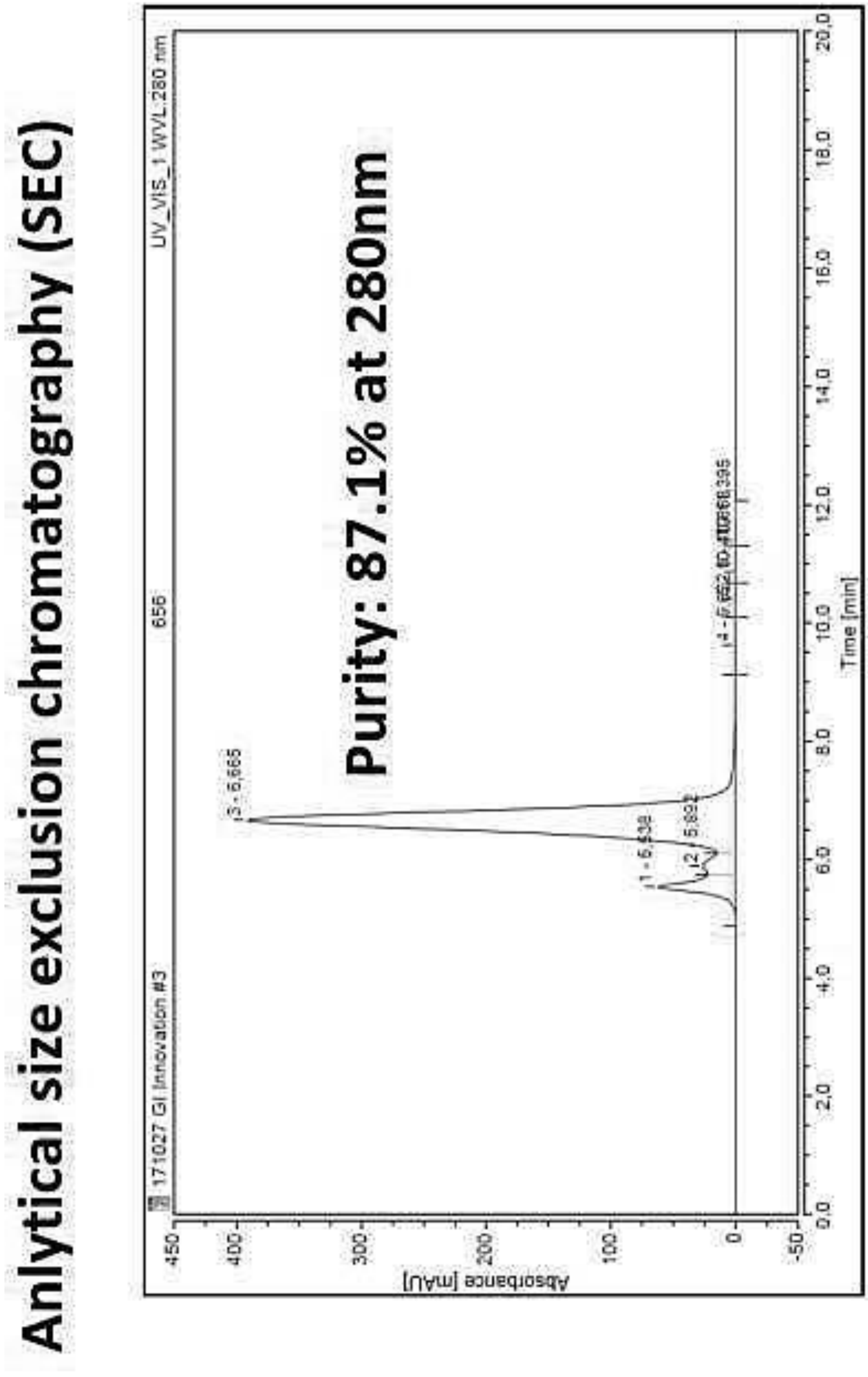
[Fig. 6]


SDS-PAGE
R
M
NR
kDa
190 -
115 -
80 -
70 -
50 -
30 -
25 -
15 -
10 -

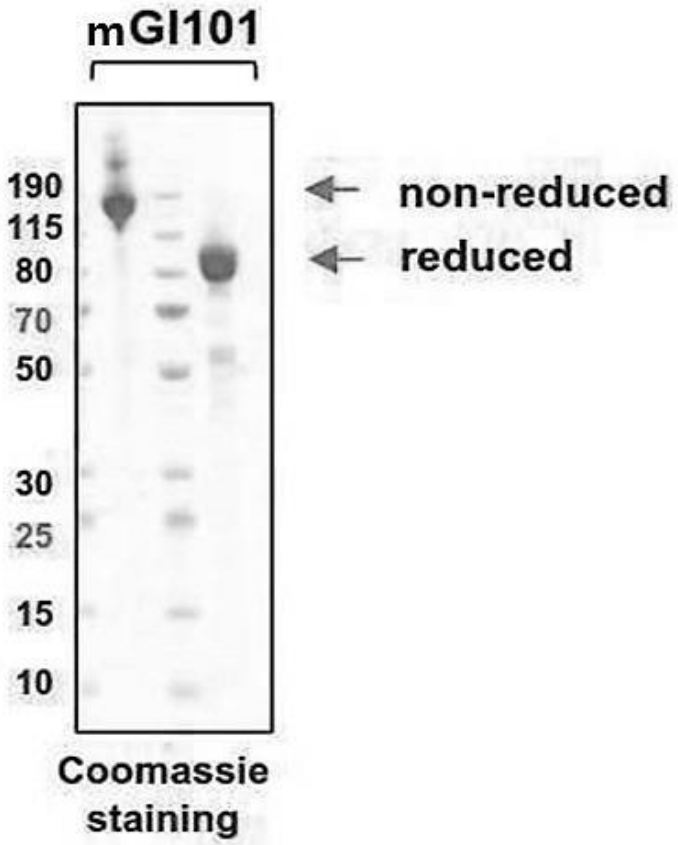
[Fig. 7]


y = 6446,3x - 385,02
R² = 0,9953
Protein [µg/ml]
2500
2000
1500
1000
500
0
0,00
0,10
0,20
0,30
0,40
Abs at 280 nm

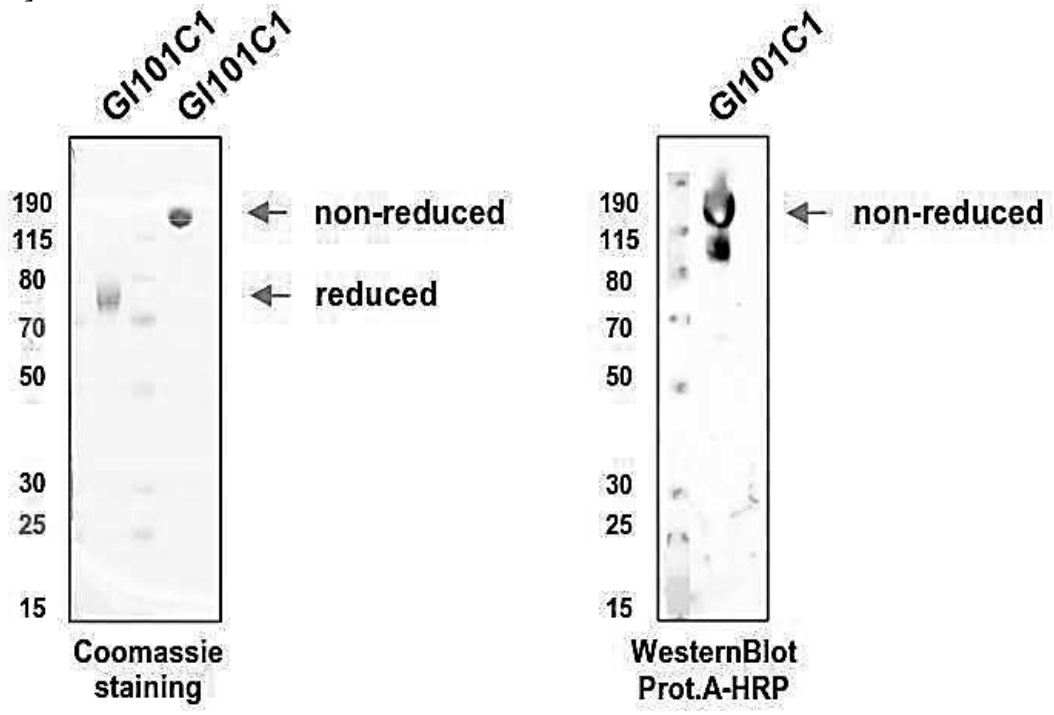
[Fig. 8]


Anlytical size exclusion chromatography (SEC)
Purity: 87.1% at 280nm
171027 GI Innovation #3
656
UV_VIS_1 WVL:280 nm
3 - 6,665
1 - 5,538
2 - 5,892
Absorbance [mAU]
Time [min]

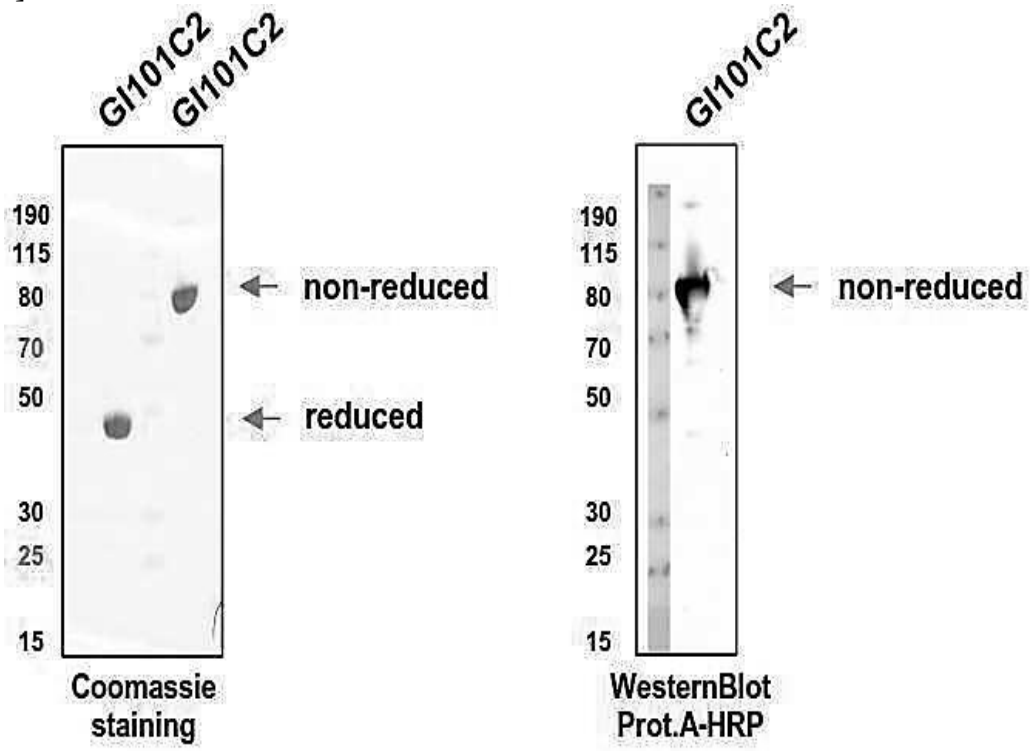
[Fig. 9]


mGI101
190
115
80
70
50
30
25
15
10
non-reduced
reduced
Coomassie
staining

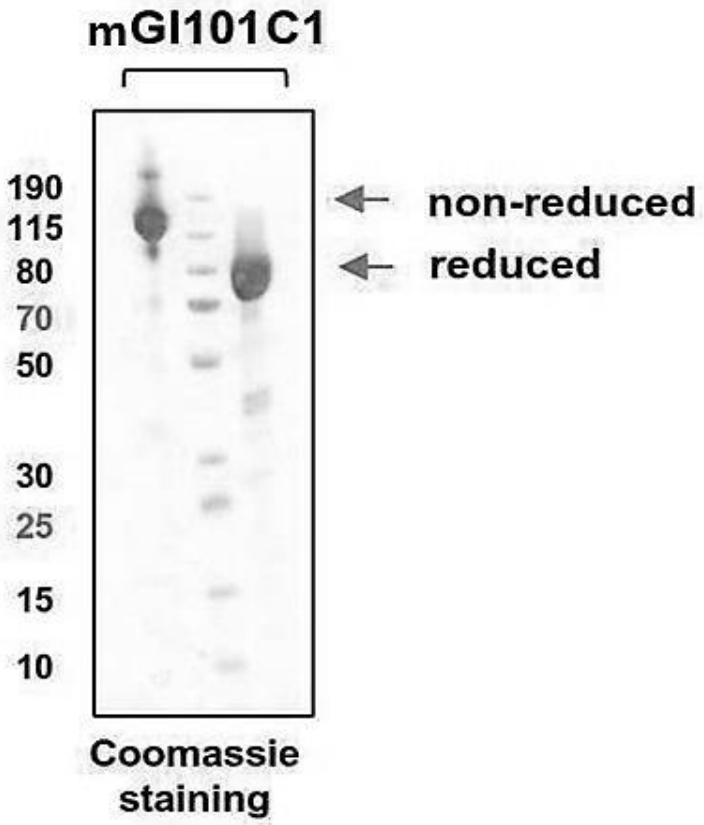
[Fig. 10]


GI101C1
GI101C1
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie
staining
GI101C1
190
115
80
70
50
30
25
15
non-reduced
WesternBlot
Prot.A-HRP

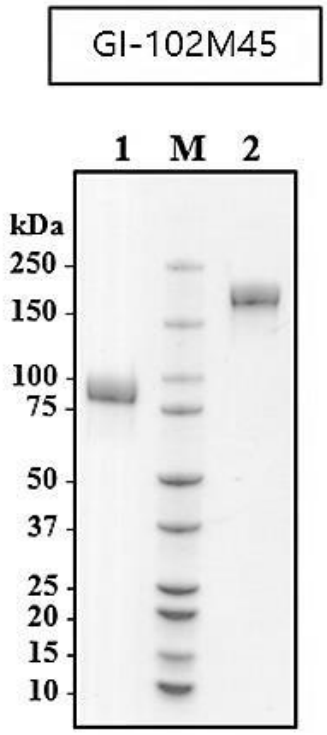
[Fig. 11]


GI101C2
GI101C2
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie staining
GI101C2
190
115
80
70
50
30
25
15
non-reduced
WesternBlot Prot.A-HRP

[Fig. 12]


mGI101C1
190
115
80
70
50
30
25
15
10
non-reduced
reduced
Coomassie staining

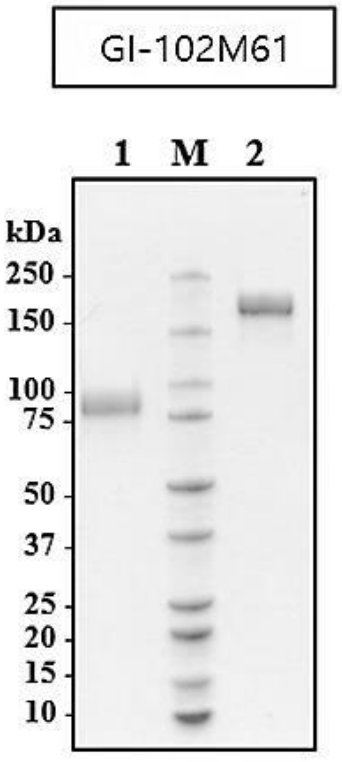
[Fig. 13]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

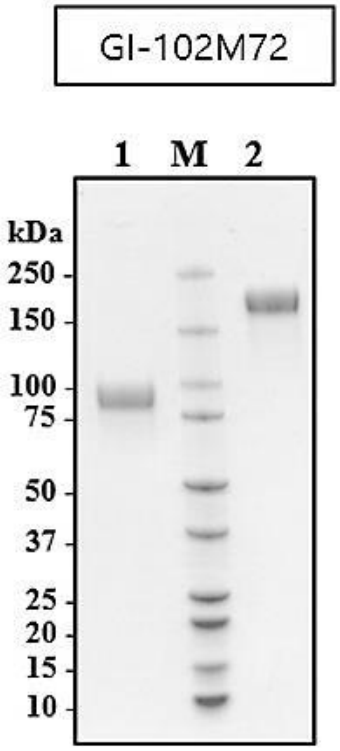
[Fig. 14]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

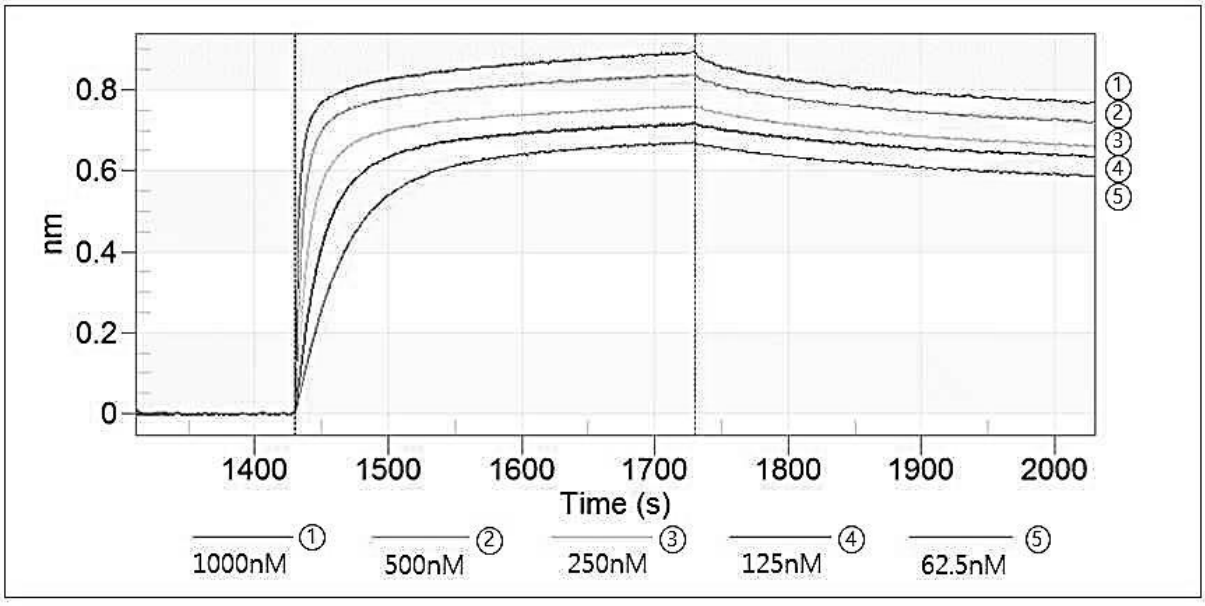
[Fig. 15]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

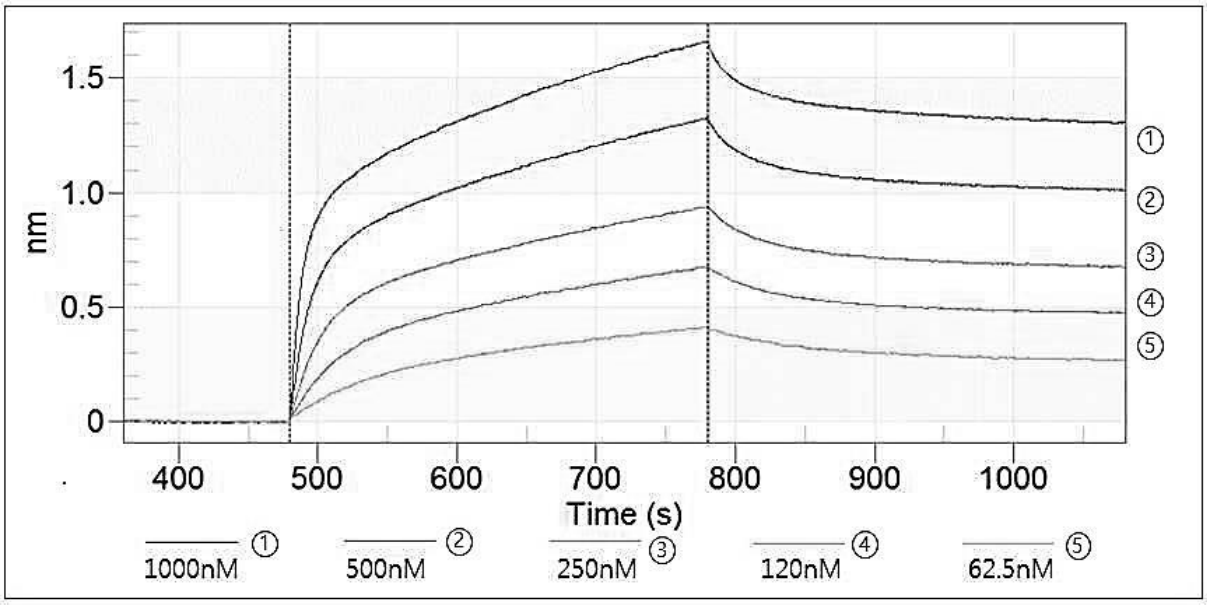
BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

[Fig. 16]



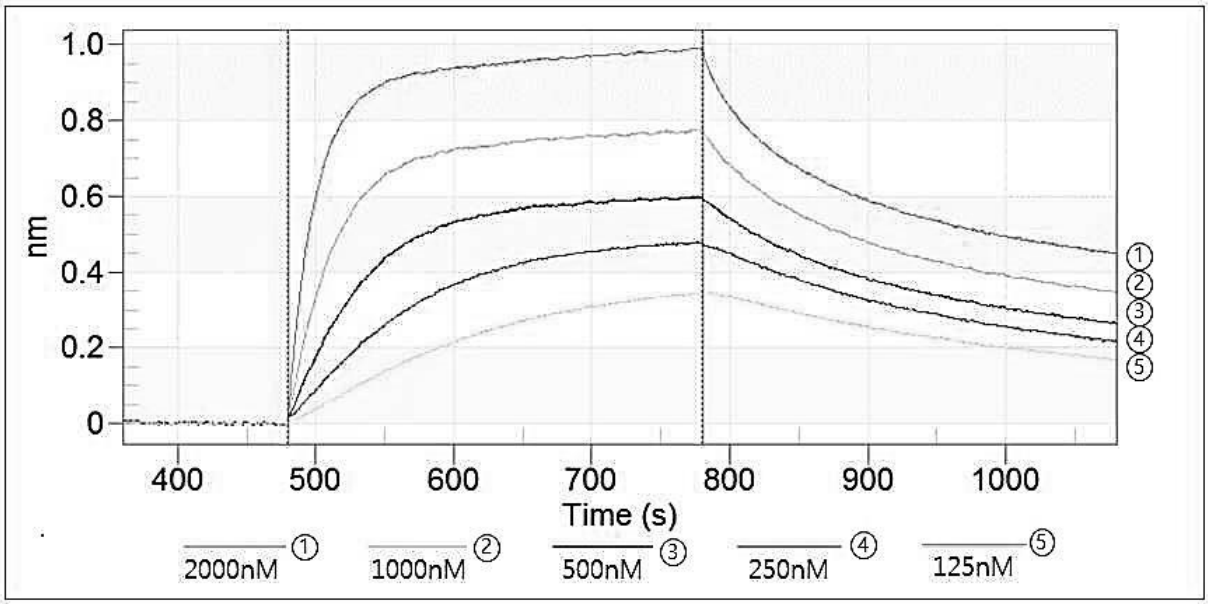
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	1.07E-09	3.86E+05	4.14E-04

[Fig. 17]



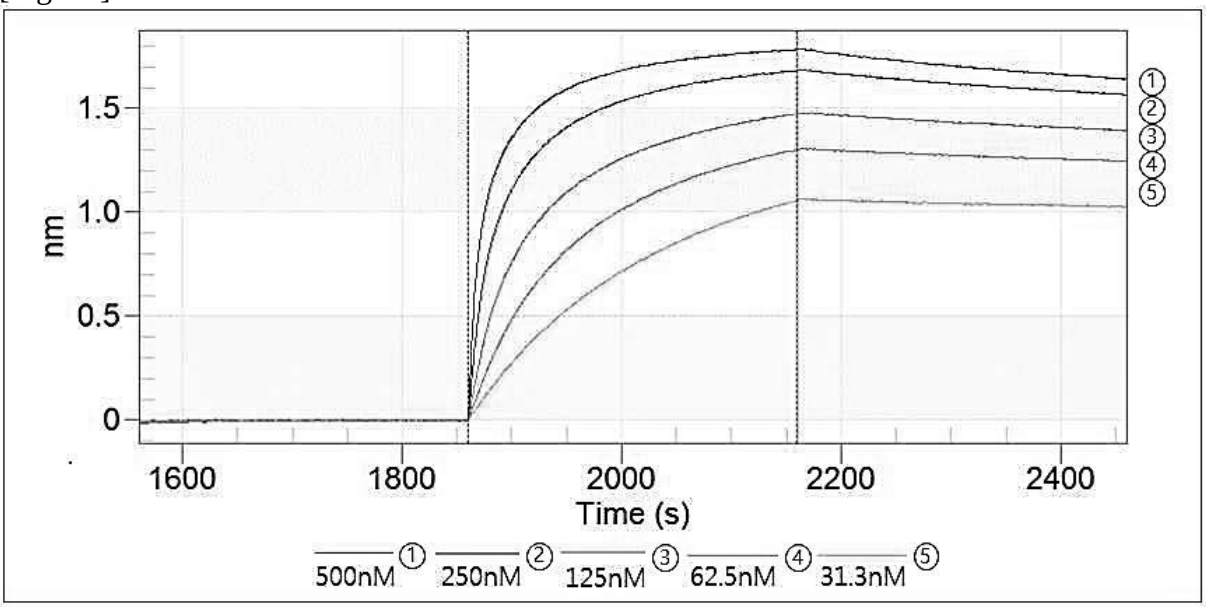
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	3.46E-08	7.0E+04	2.42E-03

[Fig. 18]



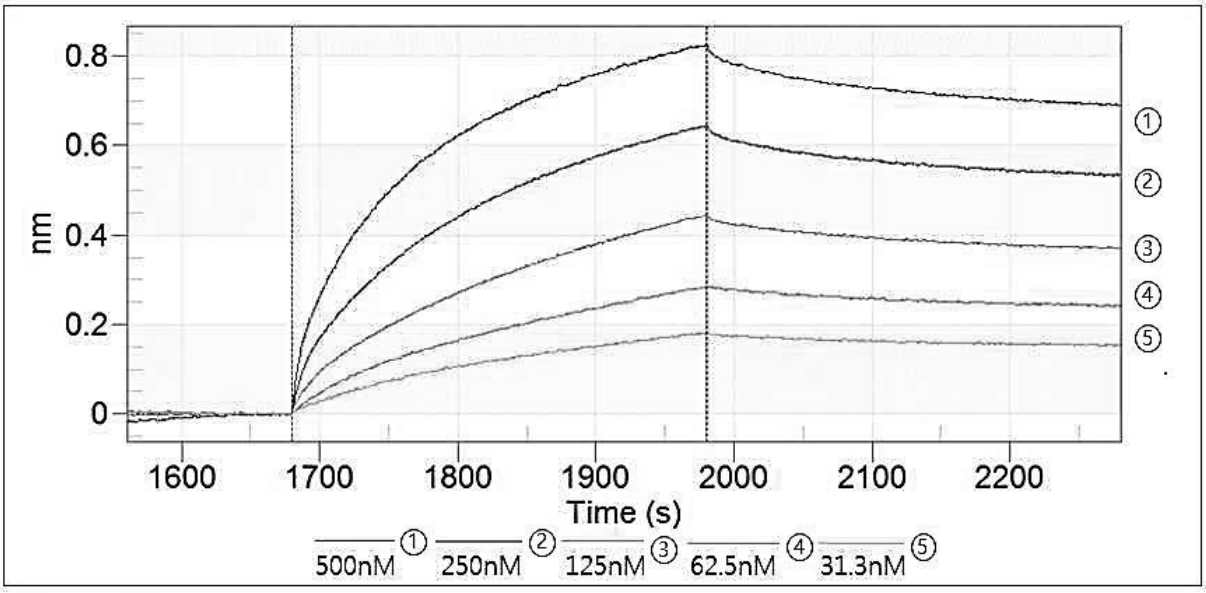
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
PD-1	1.13E-07	2.72E+04	3.07E-03

[Fig. 19]



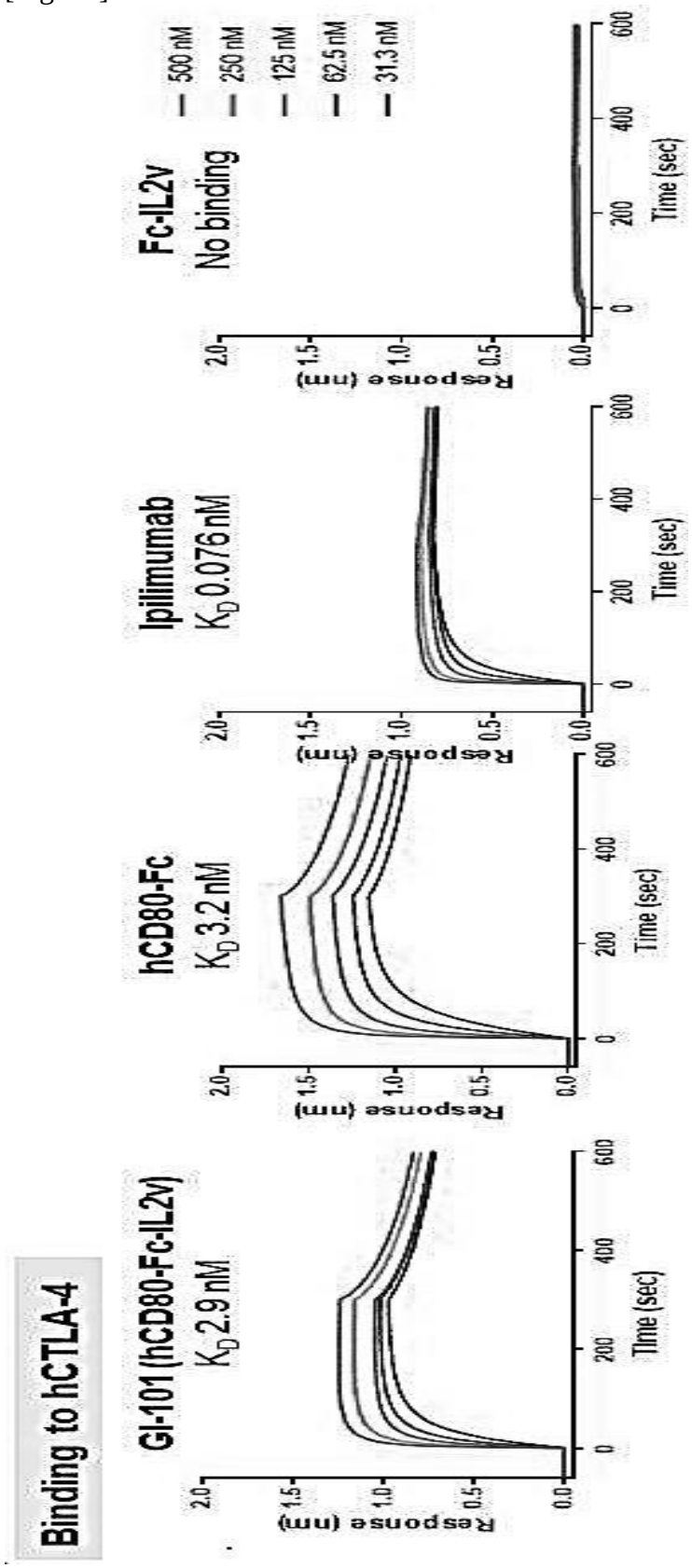
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	2.58E-08	3.05E+05	7.88E-04

[Fig. 20]

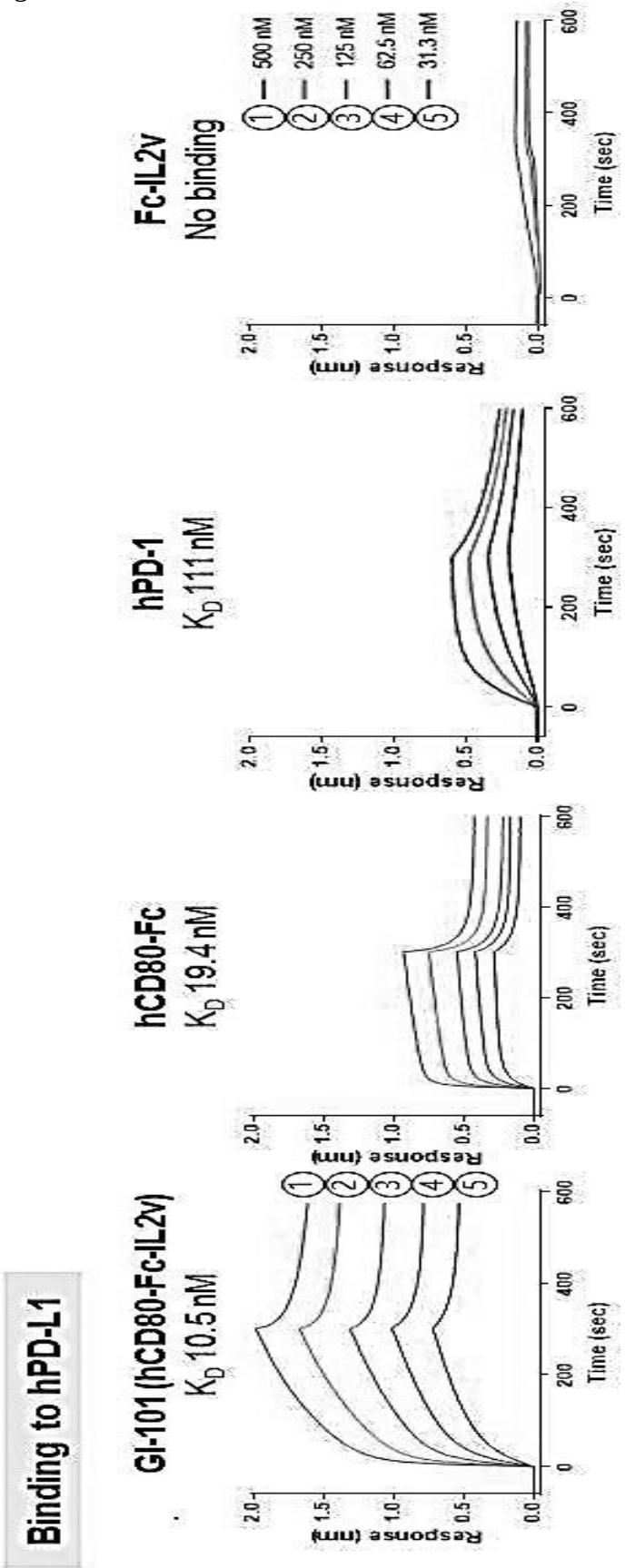


Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	9.91E-09	8.12E+05	8.04E-03

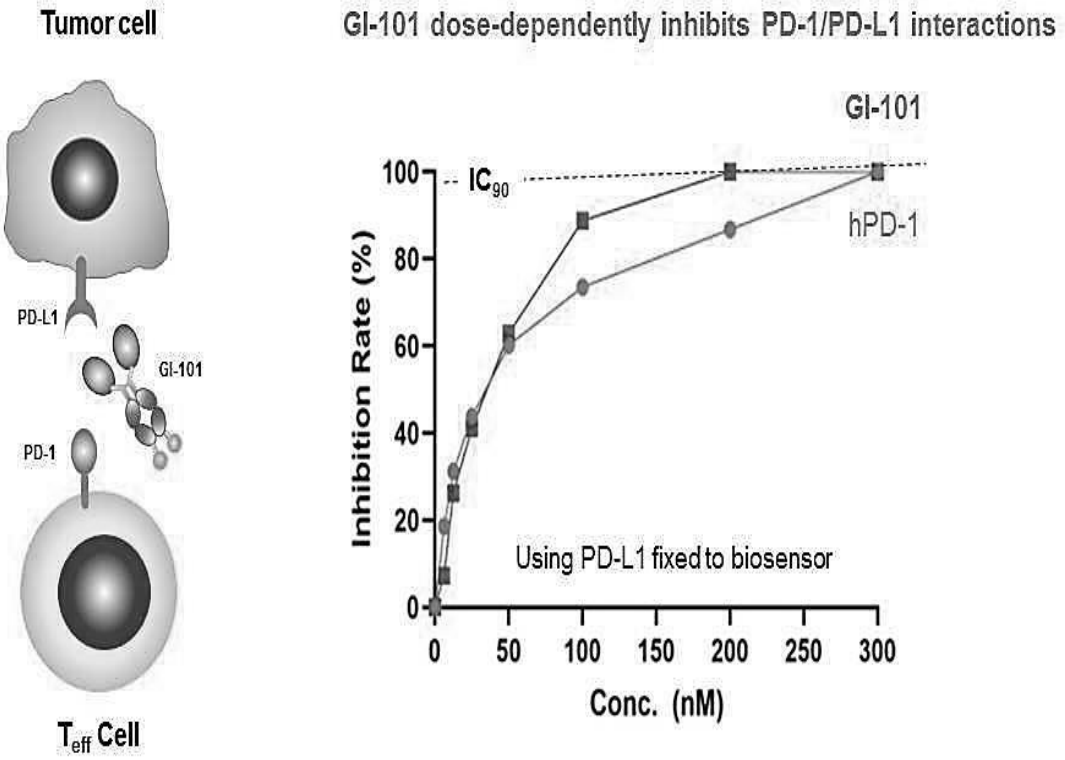
[Fig. 21]


Binding to hCTLA-4
GI-101 (hCD80-Fc-IL2v)
K_D 2.9 nM
hCD80-Fc
K_D 3.2 nM
Ipilimumab
K_D 0.076 nM
Fc-IL2v
No binding
500 nM
250 nM
125 nM
62.5 nM
31.3 nM
Response (nm)
Time (sec)

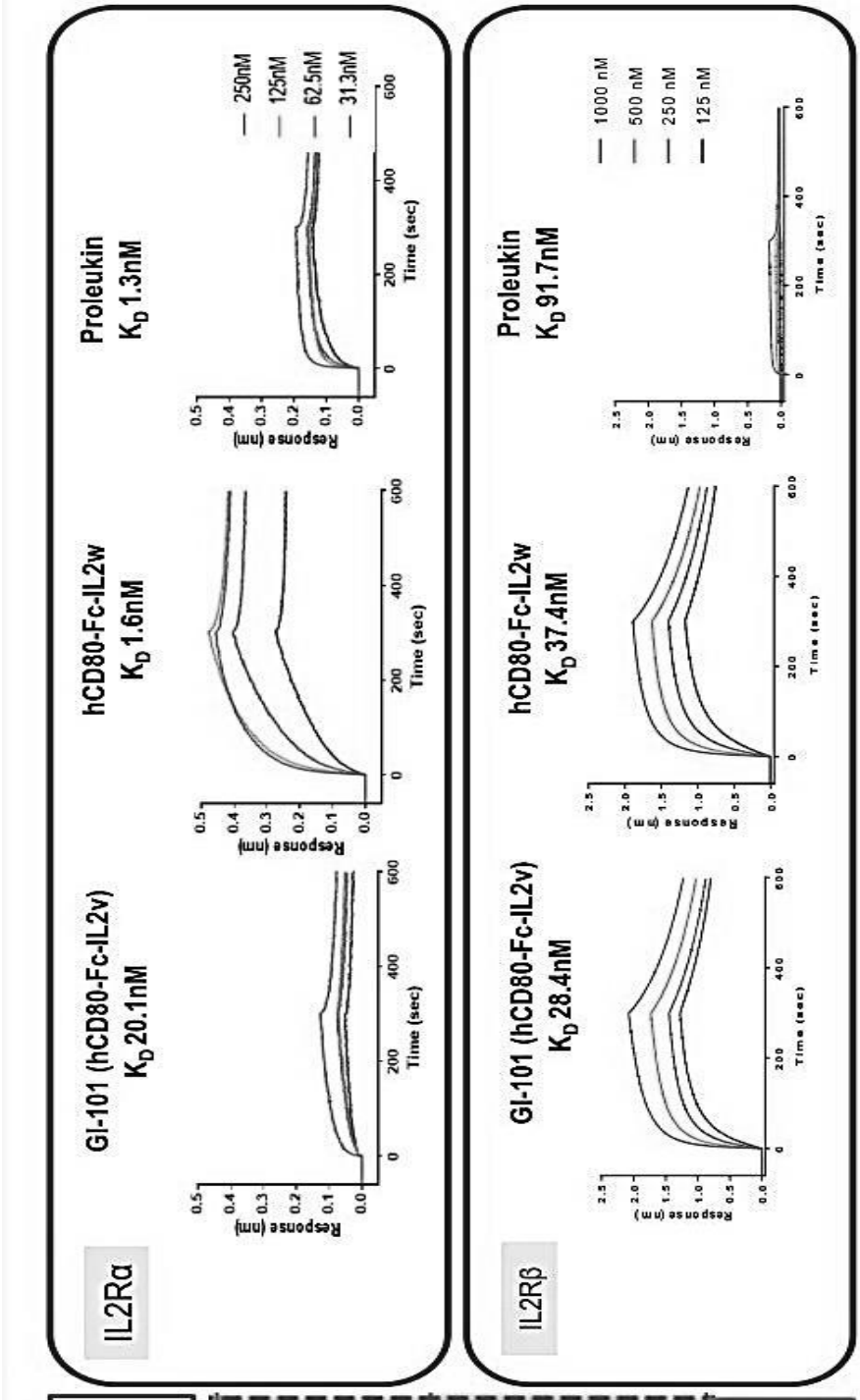
[Fig. 22]


Binding to hPD-L1
GI-101 (hCD80-Fc-IL2v)
K_D 10.5 nM
hCD80-Fc
K_D 19.4 nM
hPD-1
K_D 111 nM
Fc-IL2v
No binding
500 nM
250 nM
125 nM
62.5 nM
31.3 nM
Response (nm)
Time (sec)

[Fig. 23]

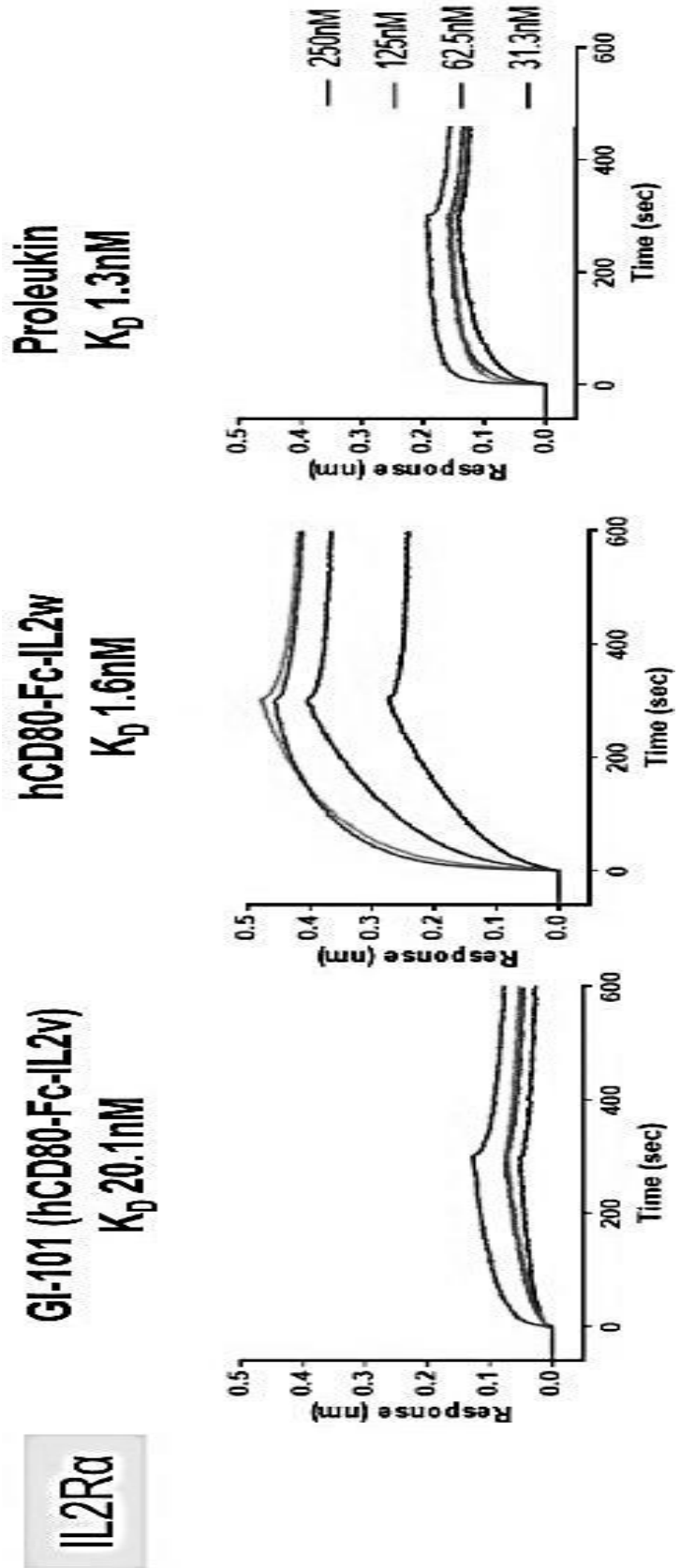

Tumor cell
PD-L1
GI-101
PD-1
T_{eff} Cell
GI-101 dose-dependently inhibits PD-1/PD-L1 interactions
GI-101
IC_{90}
hPD-1
Inhibition Rate (%)
100
80
60
40
20
0
Using PD-L1 fixed to biosensor
0
50
100
150
200
250
300
Conc. (nM)

[Fig. 24]

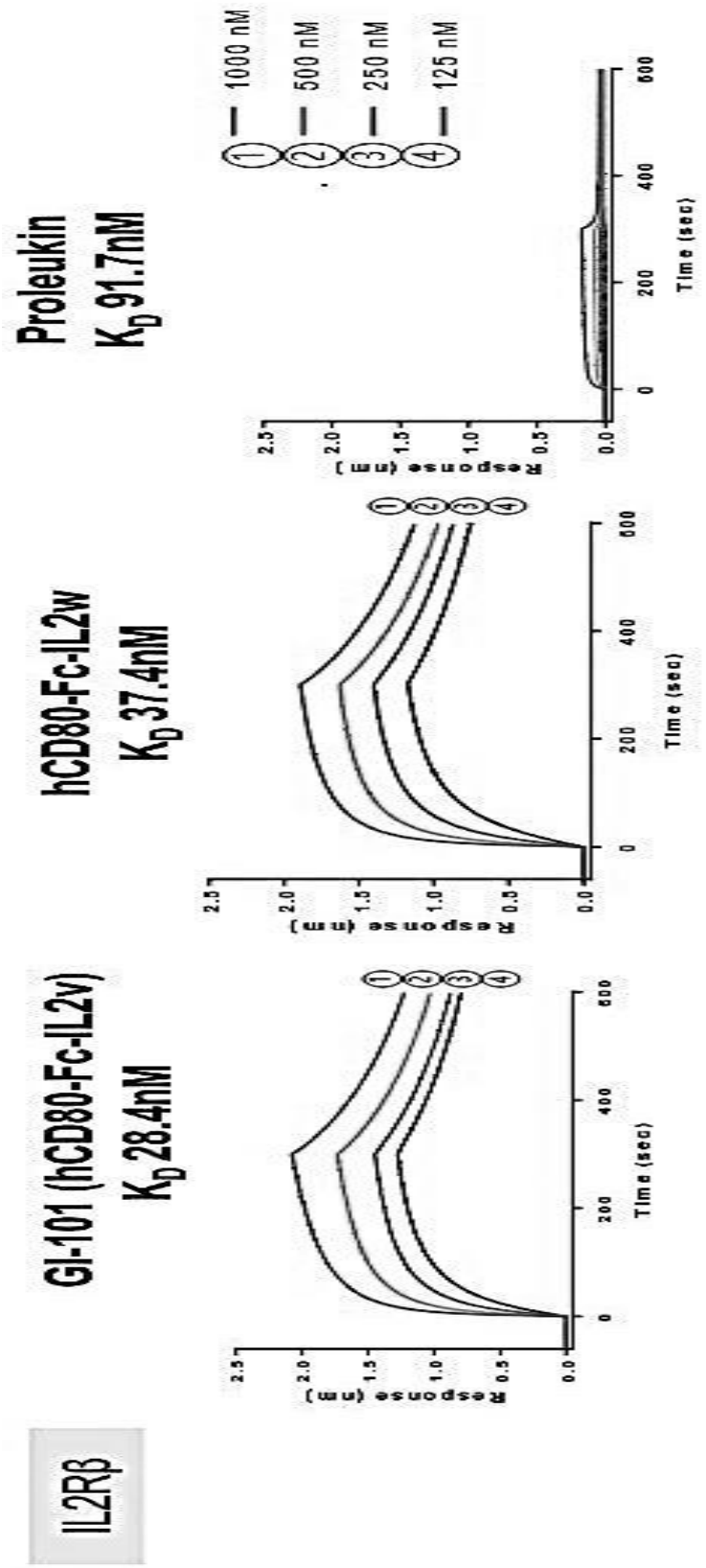


References	Kd (nM)			
Receptor	*hIL2w	**NKTR-214 (IL2)	**NKTR-214 (1-PEG-IL2)	**NKTR-214 (2-PEG-IL2)
hIL2Rα	10 (x14)	8.6 (x28)	189.7 (x9)	486.6 (x8)
hIL2Rβ	144	238.9	1769.5	3951.8

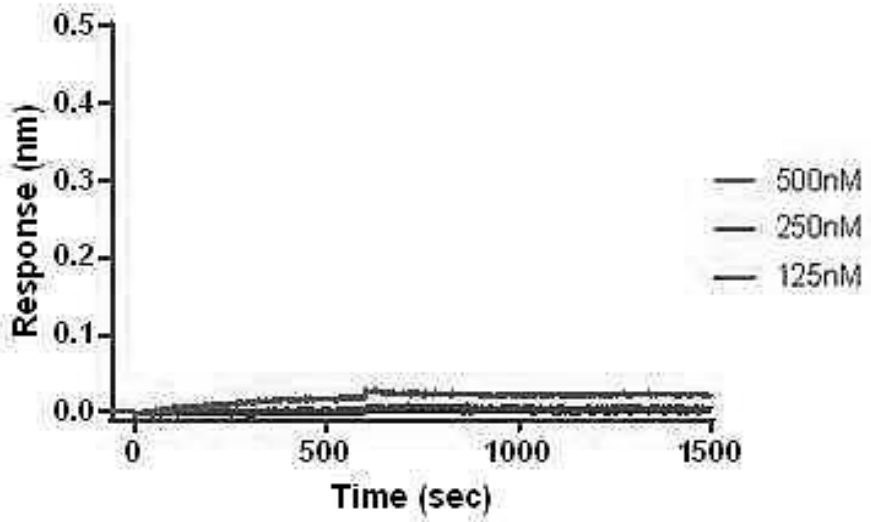
[Fig. 25]


IL2Rα
GI-101 (hCD80-Fc-IL2v)
K_D 20.1nM
hCD80-Fc-IL2w
K_D 1.6nM
Proleukin
K_D 1.3nM
250nM
125nM
62.5nM
31.3nM
Response (nm)
Time (sec)
0.0
0.1
0.2
0.3
0.4
0.5
0
200
400
600

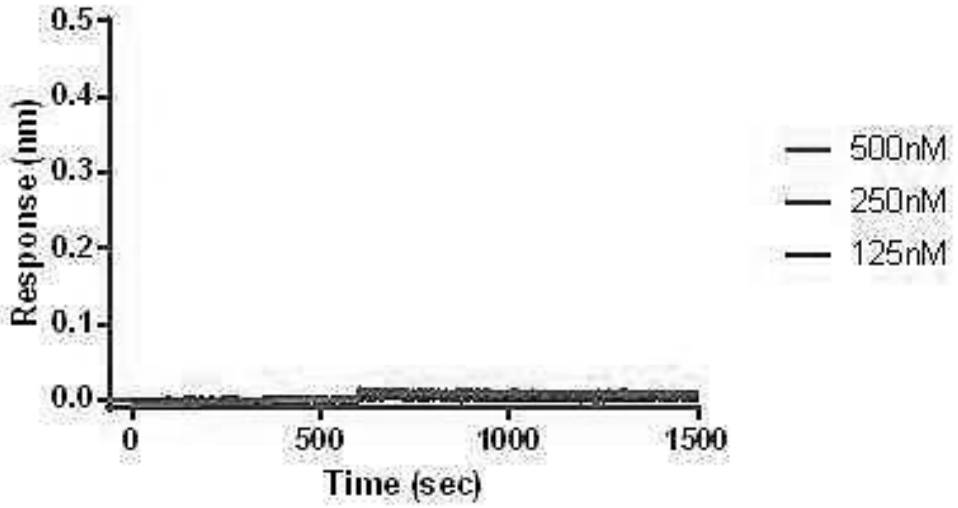
[Fig. 26]


IL2Rβ
GI-101 (hCD80-Fc-IL2v)
K_D 28.4nM
hCD80-Fc-IL2w
K_D 37.4nM
Proleukin
K_D 91.7nM
1000 nM
500 nM
250 nM
125 nM
Response (nm)
Time (sec)

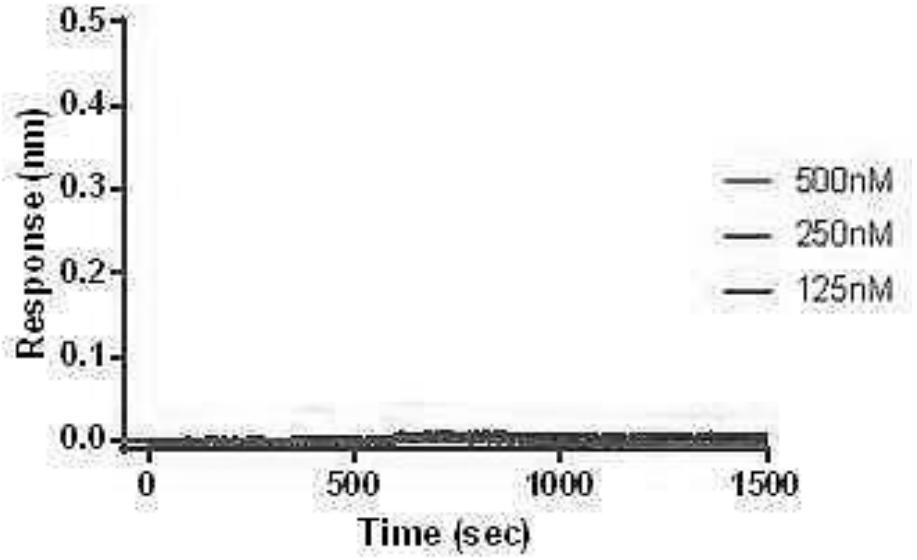
[Fig. 27]


0.5
0.4
0.3
0.2
0.1
0.0
Response (nm)
0
500
1000
1500
Time (sec)
500nM
250nM
125nM

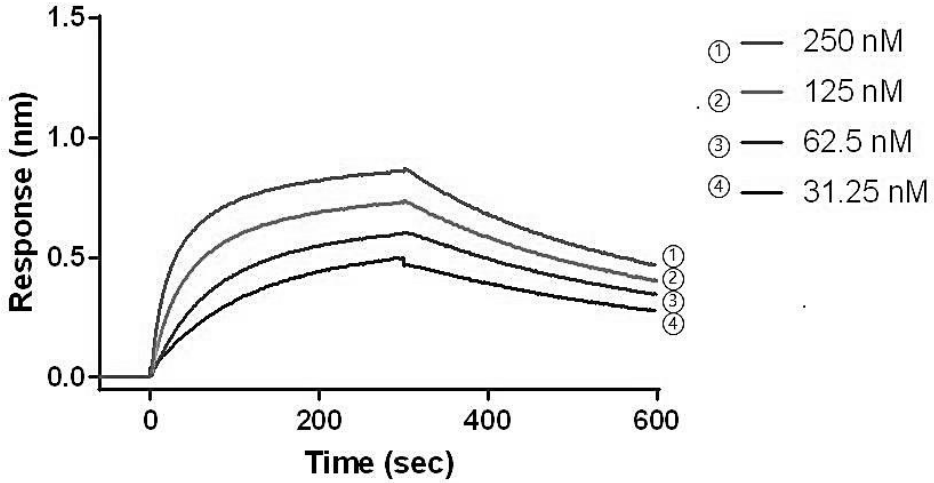
[Fig. 28]


0.5
0.4
0.3
0.2
0.1
0.0
Response (nm)
0
500
1000
1500
Time (sec)
500nM
250nM
125nM

[Fig. 29]

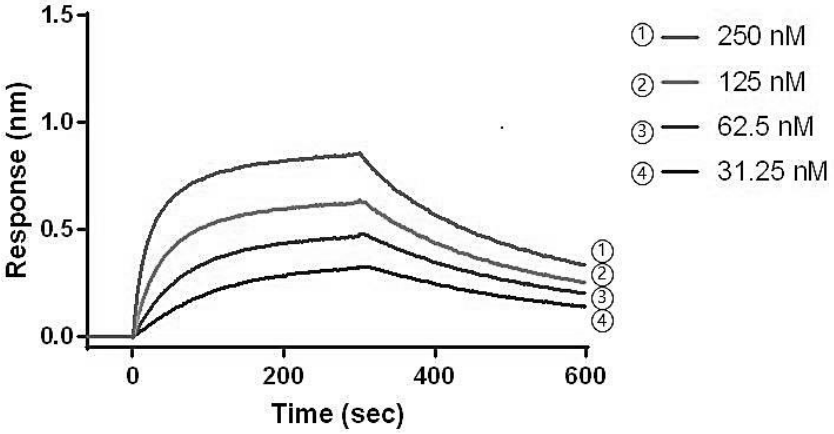


[Fig. 30]



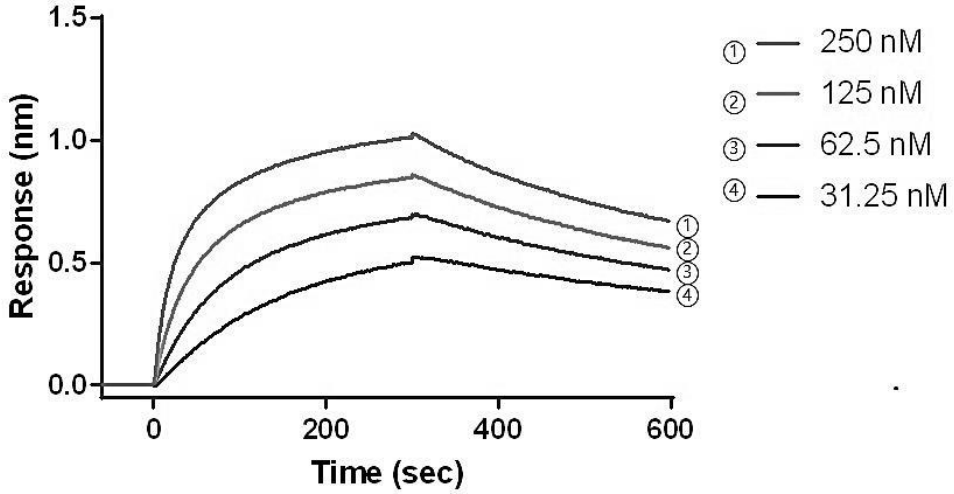
Kon	Koff	Kd
1.30X105	2.01X10-3	1.55X10-8

[Fig. 31]



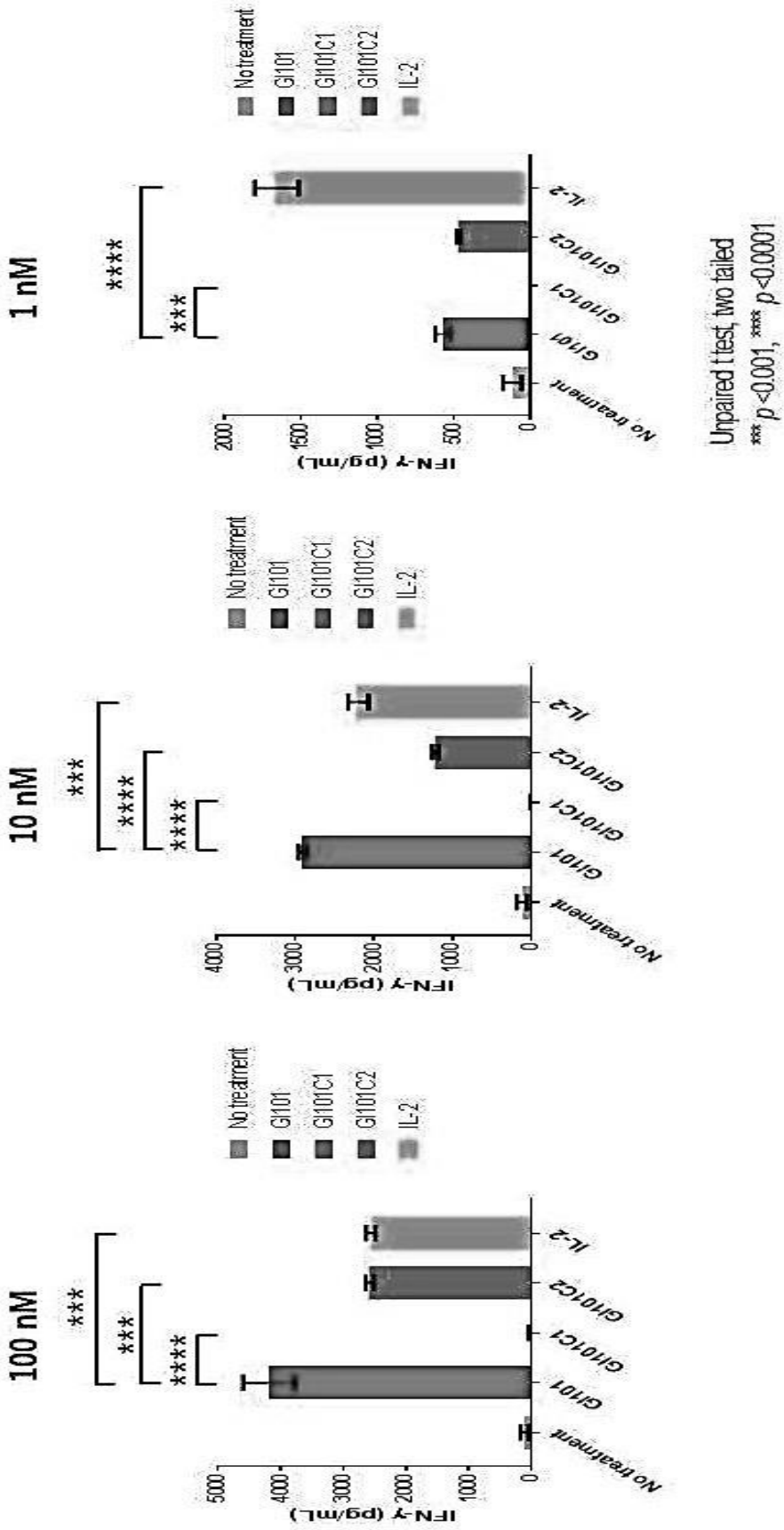
Kon	Koff	Kd
1.32X105	3.11X10-3	2.36X10-8

[Fig. 32]

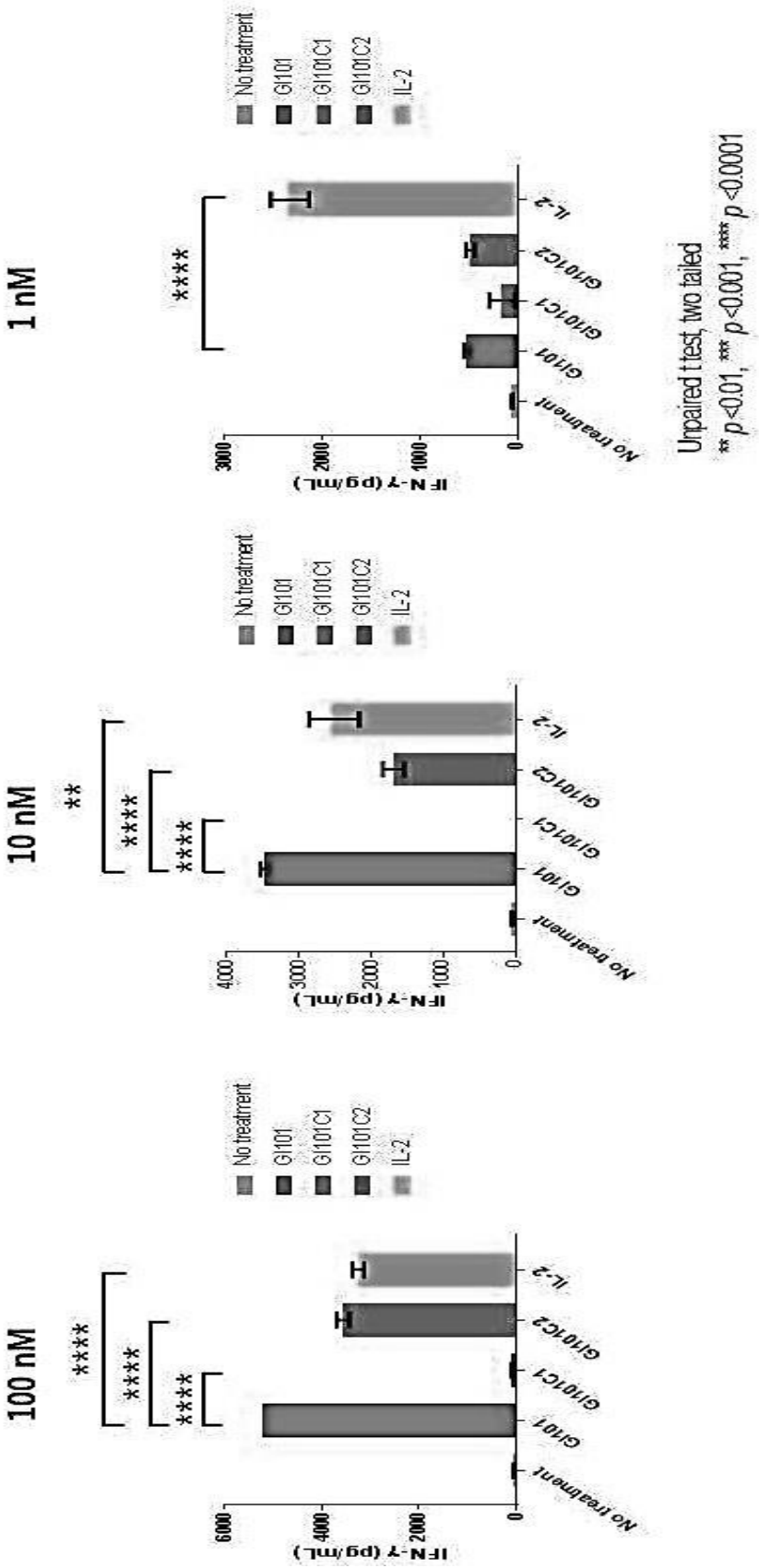


Kon	Koff	Kd
1.10X105	1.27X10-3	1.15X10-8

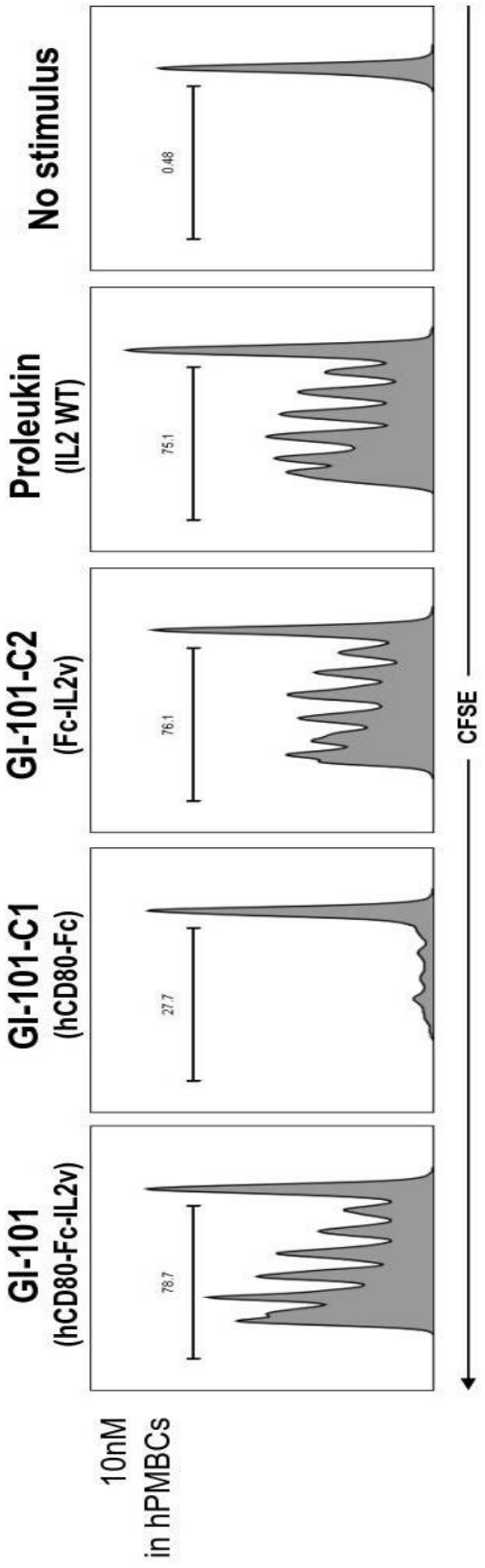
[Fig. 33]


1 nM
10 nM
100 nM
No treatment
GI101
GI101C1
GI101C2
IL-2
IFN-γ (pg/mL)
No treatment
GI101
GI101C1
GI101C2
IL-2
Unpaired t test, two tailed
*** p<0.001, **** p<0.0001

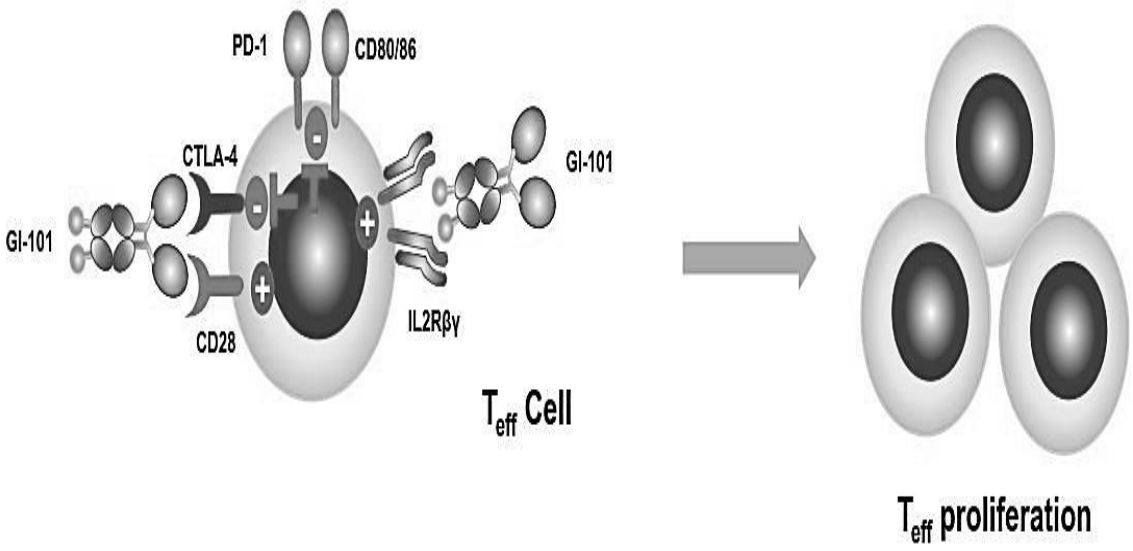
[Fig. 34]


100 nM
10 nM
1 nM
No treatment
GI101
GI101C1
GI101C2
IL-2
IFN-γ (pg/mL)
No treatment
GI101
GI101C1
GI101C2
IL-2
Unpaired t test, two tailed
** p<0.01, *** p<0.001, **** p<0.0001

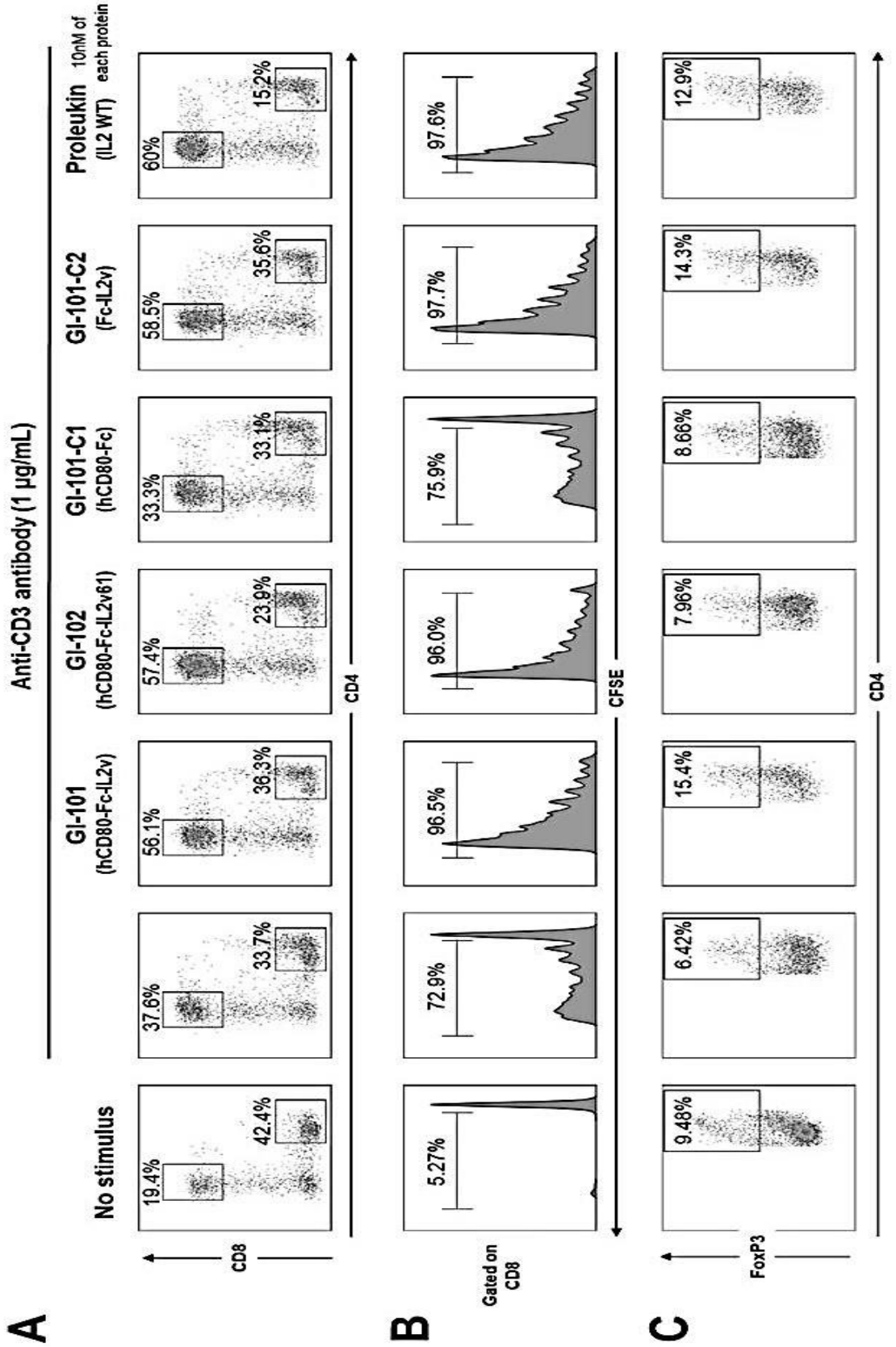
[Fig. 35]


10nM
in hPMBCs
GI-101
(hCD80-Fc-IL2v)
78.7
GI-101-C1
(hCD80-Fc)
27.7
GI-101-C2
(Fc-IL2v)
76.1
Proleukin
(IL2 WT)
75.1
No stimulus
0.48
CFSE

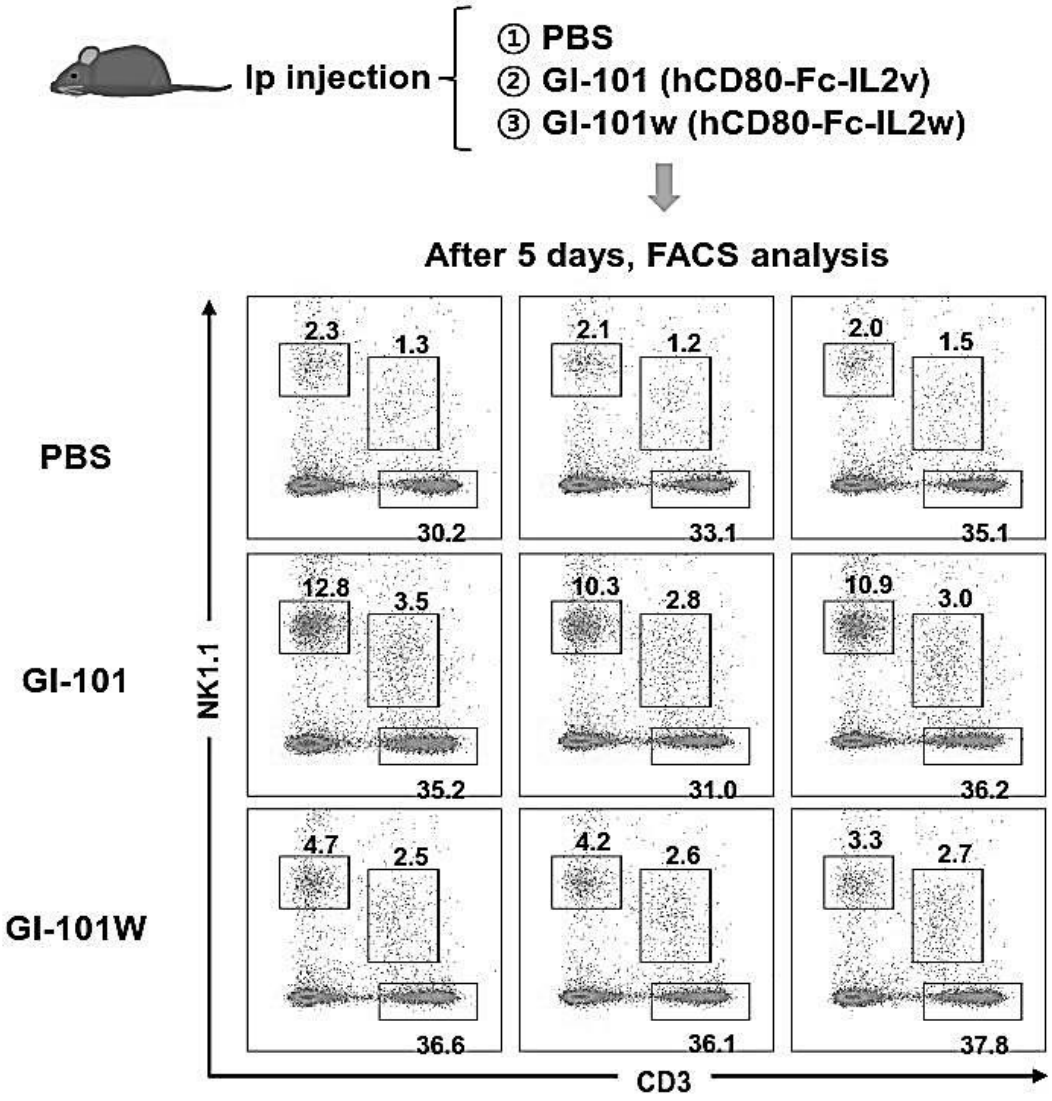
[Fig. 36]


PD-1
CD80/86
CTLA-4
GI-101
GI-101
CD28
IL2Rβγ
T_{eff} Cell
T_{eff} proliferation

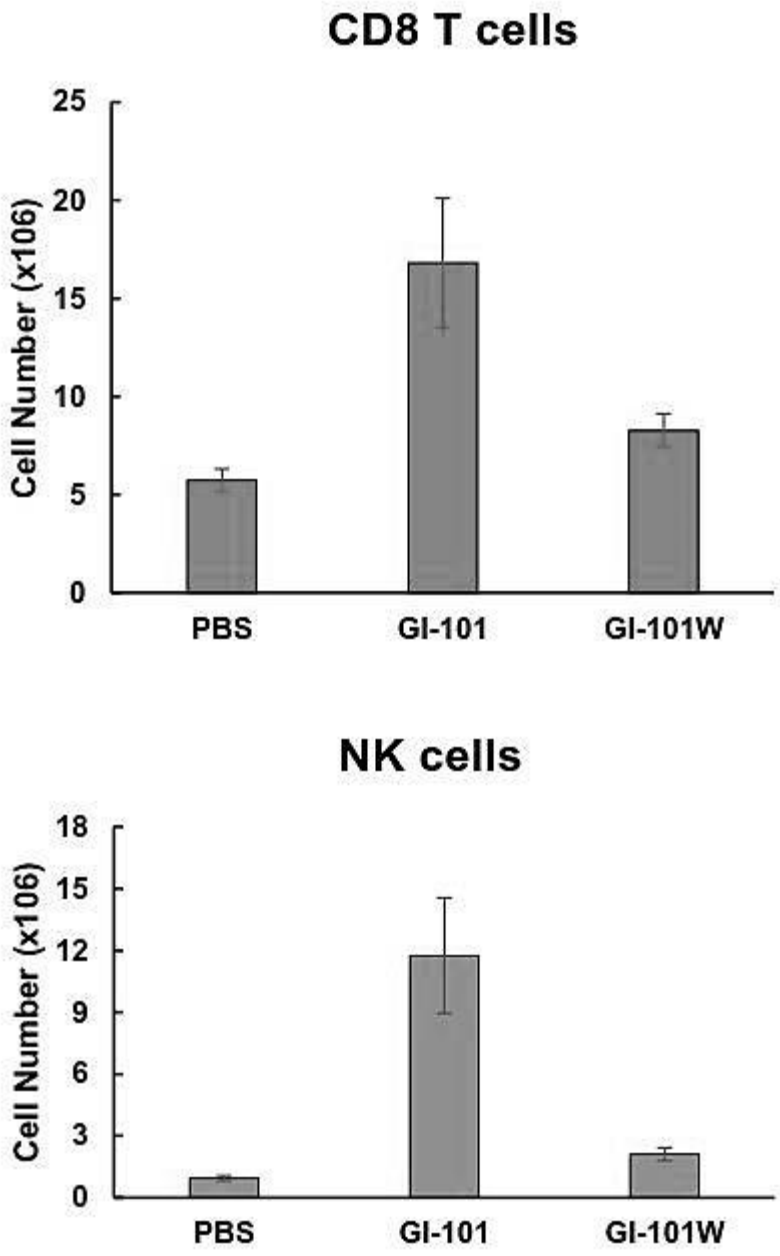
[Fig. 37]


Anti-CD3 antibody (1 μg/mL)
No stimulus
GI-101 (hCD80-Fc-IL2v)
GI-102 (hCD80-Fc-IL2v61)
GI-101-C1 (hCD80-Fc)
GI-101-C2 (Fc-IL2v)
Proleukin (IL2 WT) 10nM of each protein
A
CD8
CD4
19.4%
42.4%
37.6%
33.7%
56.1%
36.3%
57.4%
23.9%
33.3%
33.1%
58.5%
35.6%
60%
15.2%
B
Gated on CD8
CFSE
5.27%
72.9%
96.5%
96.0%
75.9%
97.7%
97.6%
C
FoxP3
CD4
9.48%
6.42%
15.4%
7.96%
8.66%
14.3%
12.9%

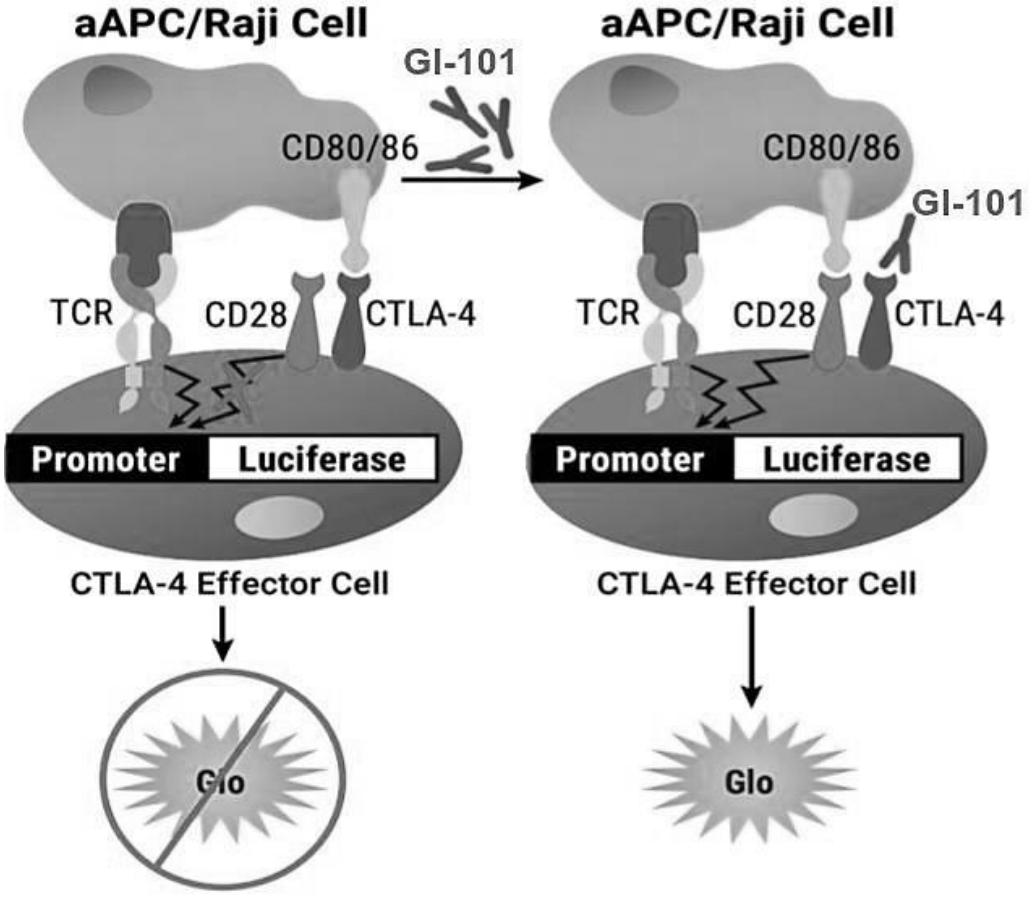
[Fig. 38]


Ip injection
① PBS
② GI-101 (hCD80-Fc-IL2v)
③ GI-101w (hCD80-Fc-IL2w)
After 5 days, FACS analysis
PBS
GI-101
GI-101W
NK1.1
CD3
2.3
1.3
30.2
2.1
1.2
33.1
2.0
1.5
35.1
12.8
3.5
35.2
10.3
2.8
31.0
10.9
3.0
36.2
4.7
2.5
36.6
4.2
2.6
36.1
3.3
2.7
37.8

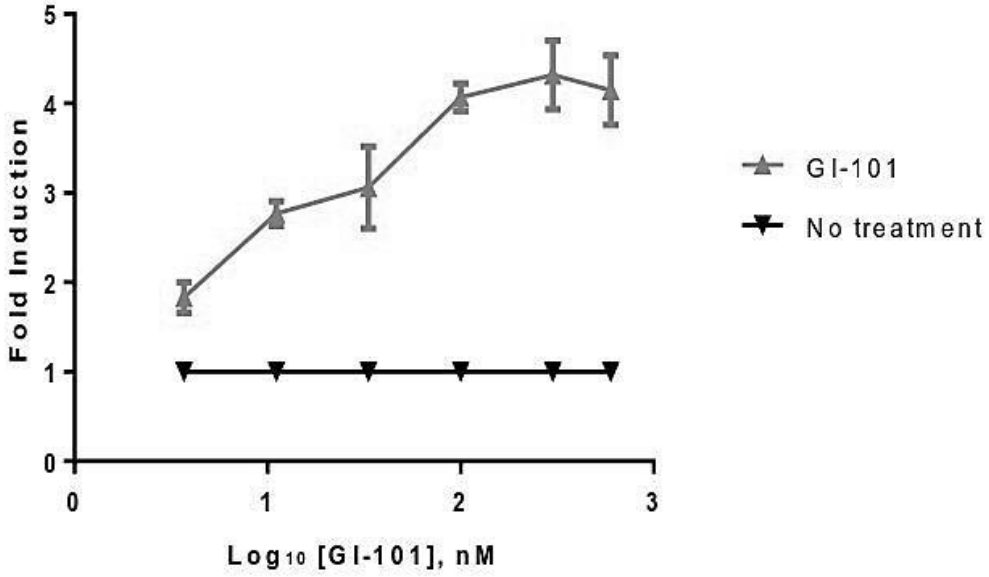
[Fig. 39]


CD8 T cells
Cell Number (x106)
25
20
15
10
5
0
PBS
GI-101
GI-101W
NK cells
Cell Number (x106)
18
15
12
9
6
3
0
PBS
GI-101
GI-101W

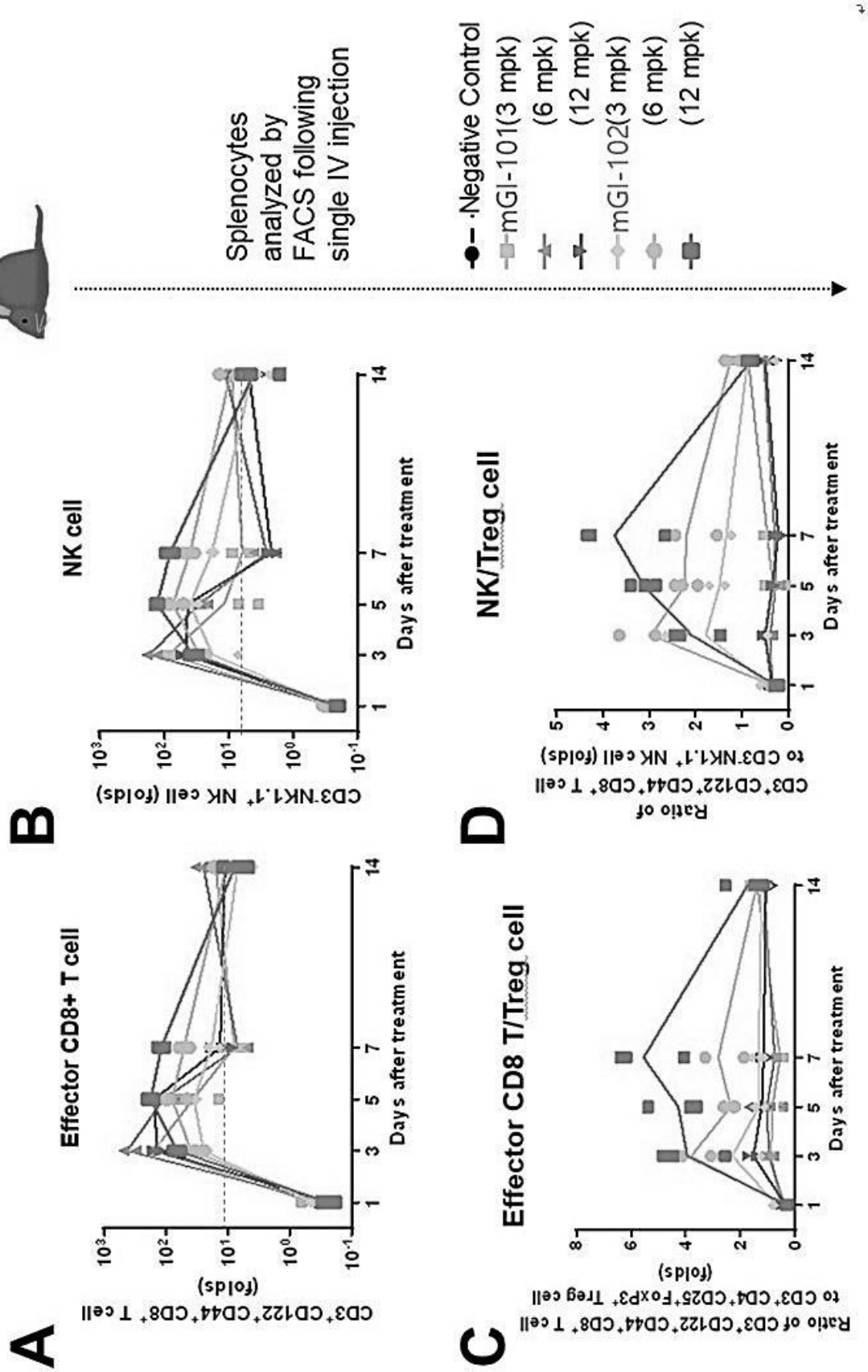
[Fig. 40]


aAPC/Raji Cell
GI-101
CD80/86
TCR
CD28
CTLA-4
Promoter
Luciferase
CTLA-4 Effector Cell
Glo
aAPC/Raji Cell
CD80/86
GI-101
TCR
CD28
CTLA-4
Promoter
Luciferase
CTLA-4 Effector Cell
Glo

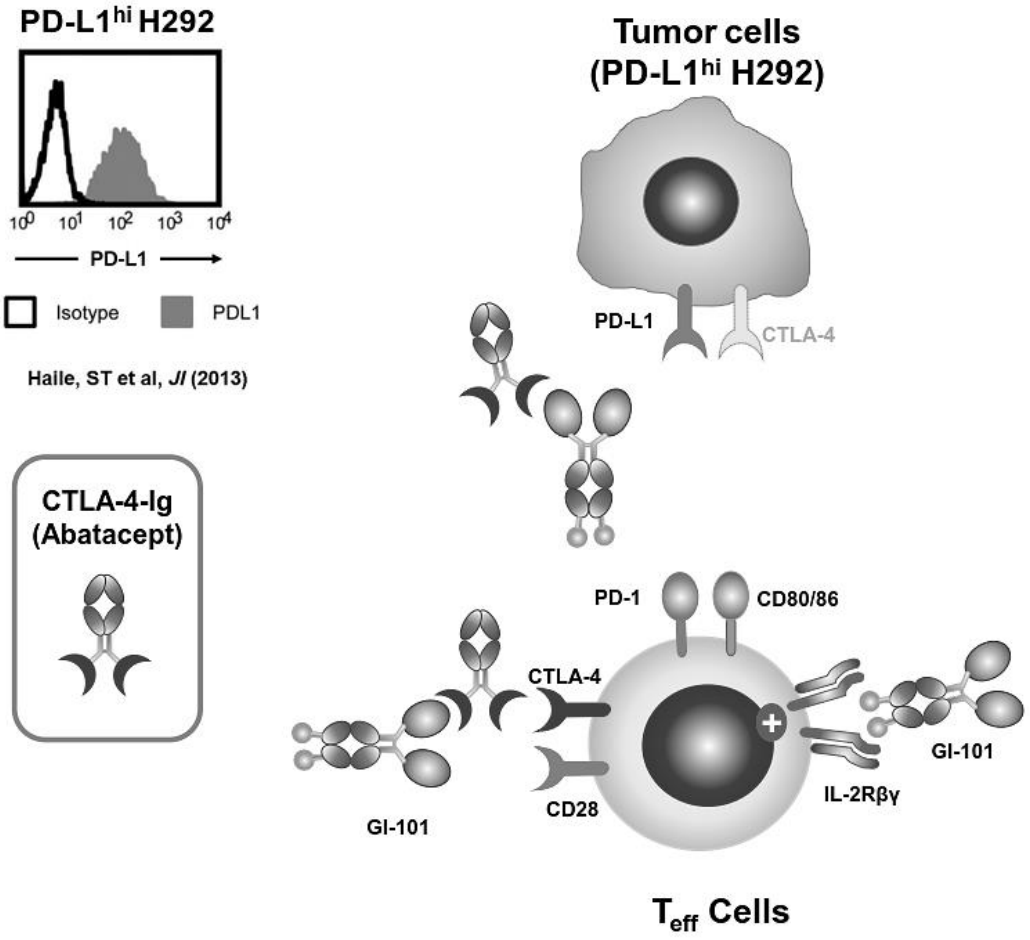
[Fig. 41]


Fold Induction
0
1
2
3
4
5
Log10 [GI-101], nM
0
1
2
3
GI-101
No treatment

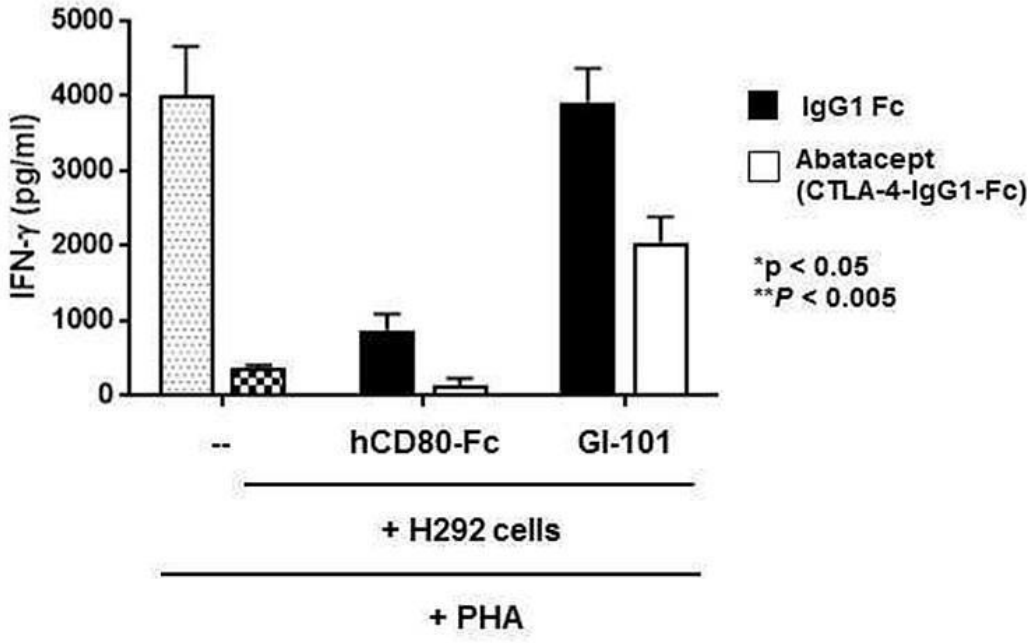
[Fig. 42]


A
Effector CD8+ T cell
CD3+CD122+CD44+CD8+ T cell (folds)
Days after treatment
B
NK cell
CD3-NK1.1+ NK cell (folds)
Days after treatment
Splenocytes analyzed by FACS following single IV injection
Negative Control
mGI-101(3 mpk)
(6 mpk)
(12 mpk)
mGI-102(3 mpk)
(6 mpk)
(12 mpk)
C
Effector CD8 T/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3+CD4+CD25+FoxP3+ Treg cell (folds)
Days after treatment
D
NK/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3-NK1.1+ NK cell (folds)
Days after treatment

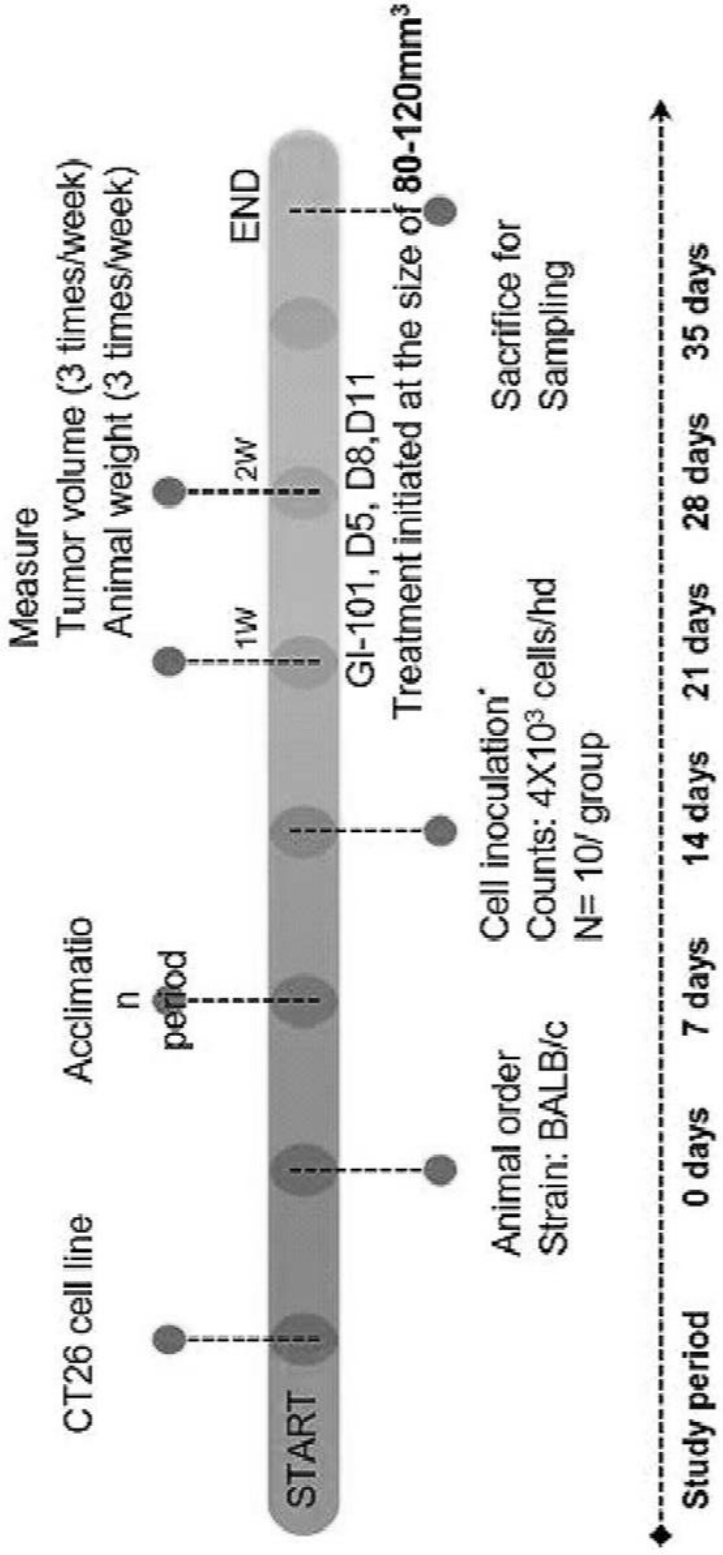
[Fig. 43]


PD-L1hi H292
10^0 10^1 10^2 10^3 10^4
PD-L1
Isotype
PDL1
Haile, ST et al, JI (2013)
CTLA-4-Ig
(Abatacept)
Tumor cells
(PD-L1hi H292)
PD-L1
CTLA-4
PD-1
CD80/86
CTLA-4
GI-101
CD28
IL-2Rβγ
GI-101
Teff Cells

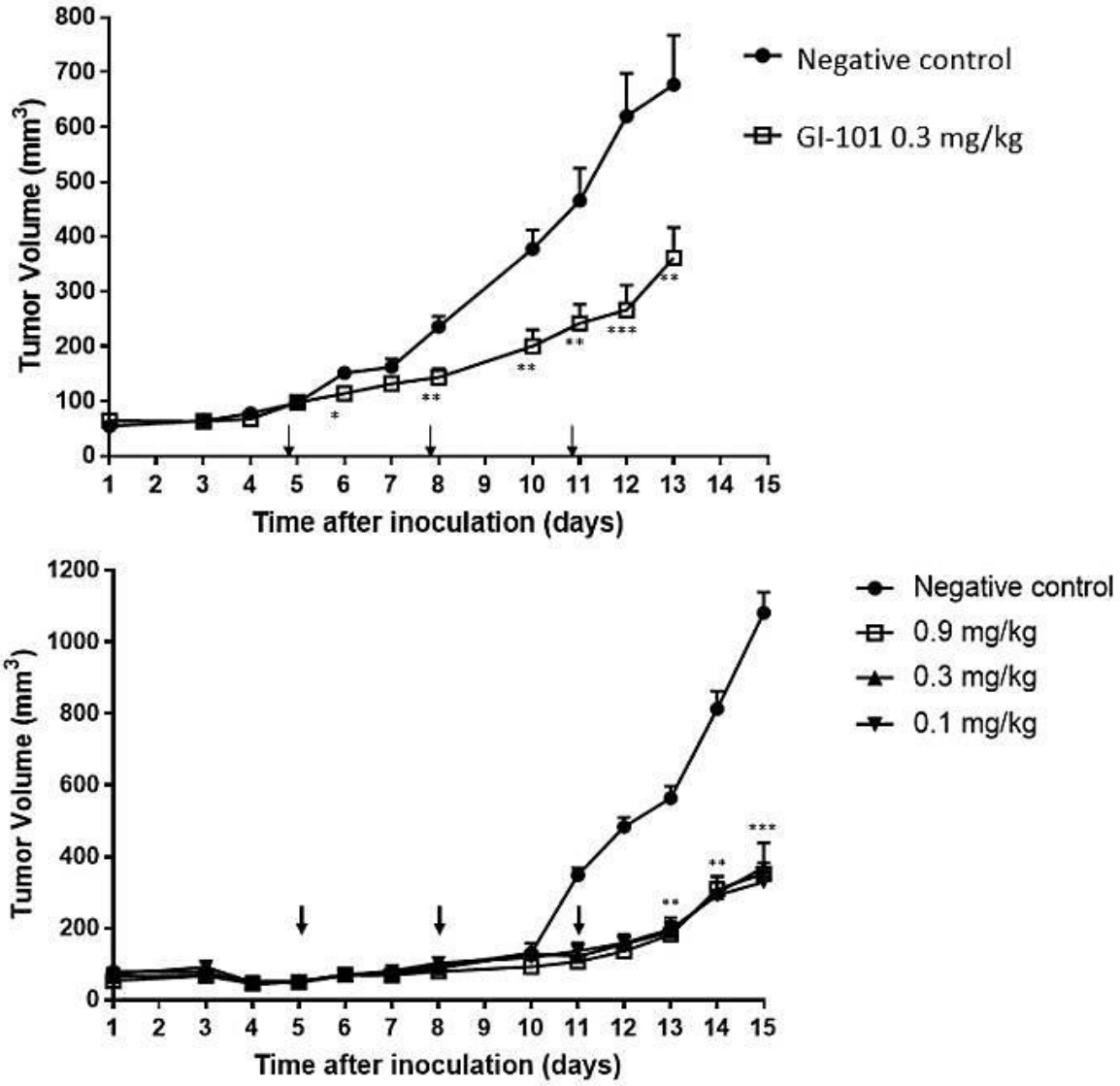
[Fig. 44]


IFN-γ (pg/ml)
5000
4000
3000
2000
1000
0
--
hCD80-Fc
GI-101
+ H292 cells
+ PHA
IgG1 Fc
Abatacept
(CTLA-4-IgG1-Fc)
*p < 0.05
**P < 0.005

[Fig. 45]

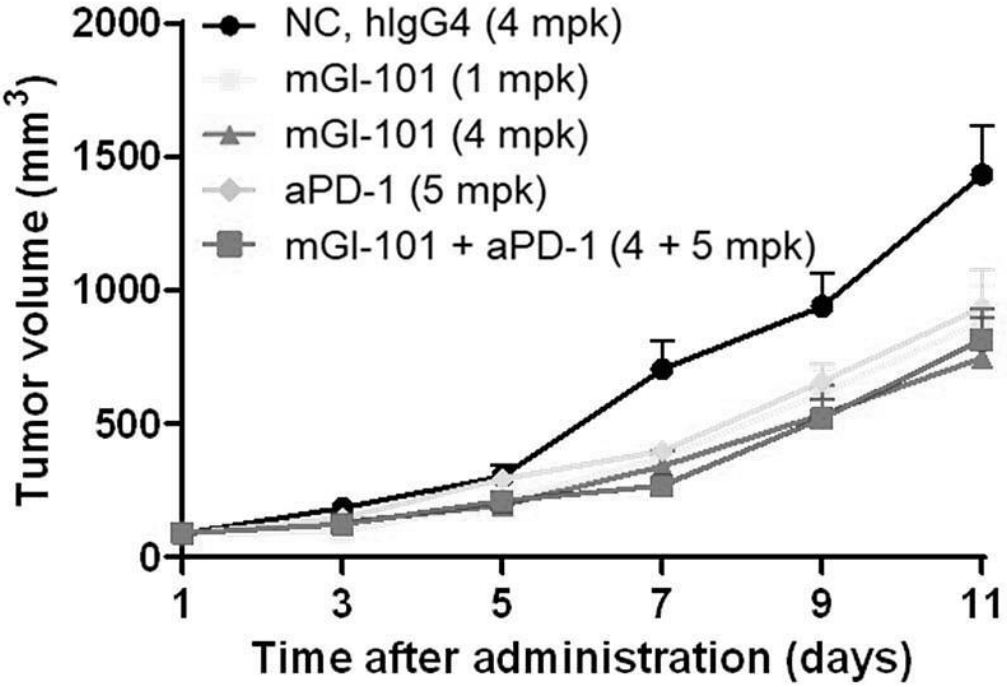

CT26 cell line
Acclimatio
n
period
Animal order
Strain: BALB/c
START
Cell inoculation*
Counts: 4X10³ cells/hd
N= 10/ group
Measure
Tumor volume (3 times/week)
Animal weight (3 times/week)
1W
2W
END
GI-101, D5, D8,D11
Treatment initiated at the size of 80-120mm³
Sacrifice for
Sampling
Study period
0 days
7 days
14 days
21 days
28 days
35 days

[Fig. 46]

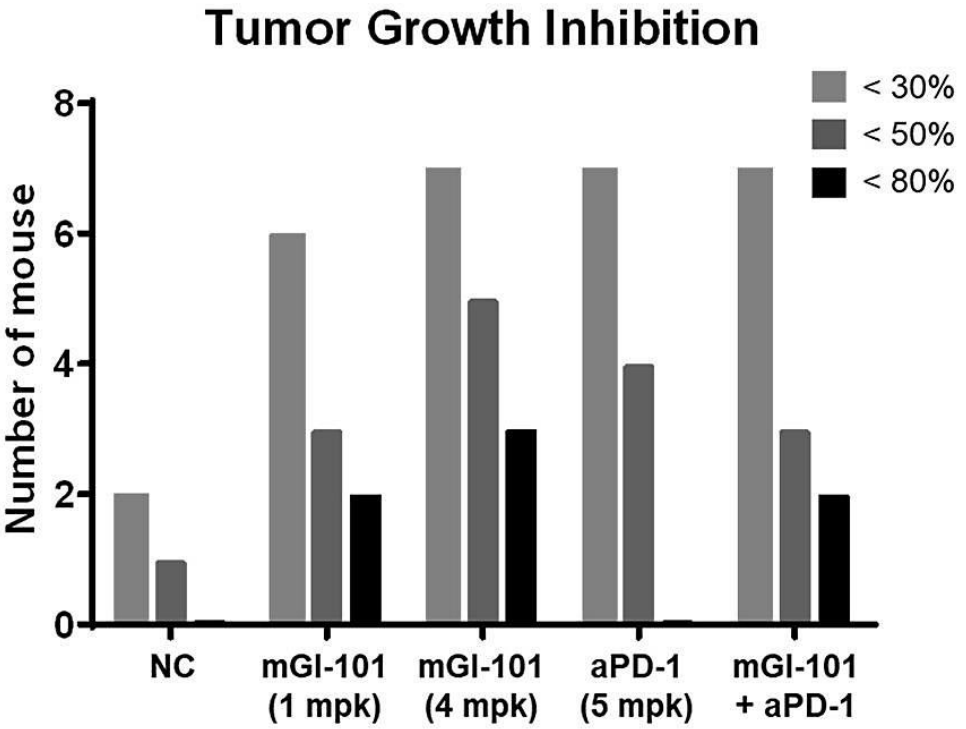


* p<0.05, ** p<0.005, *** p<0.001
Each point represents mean + SE (n=10)

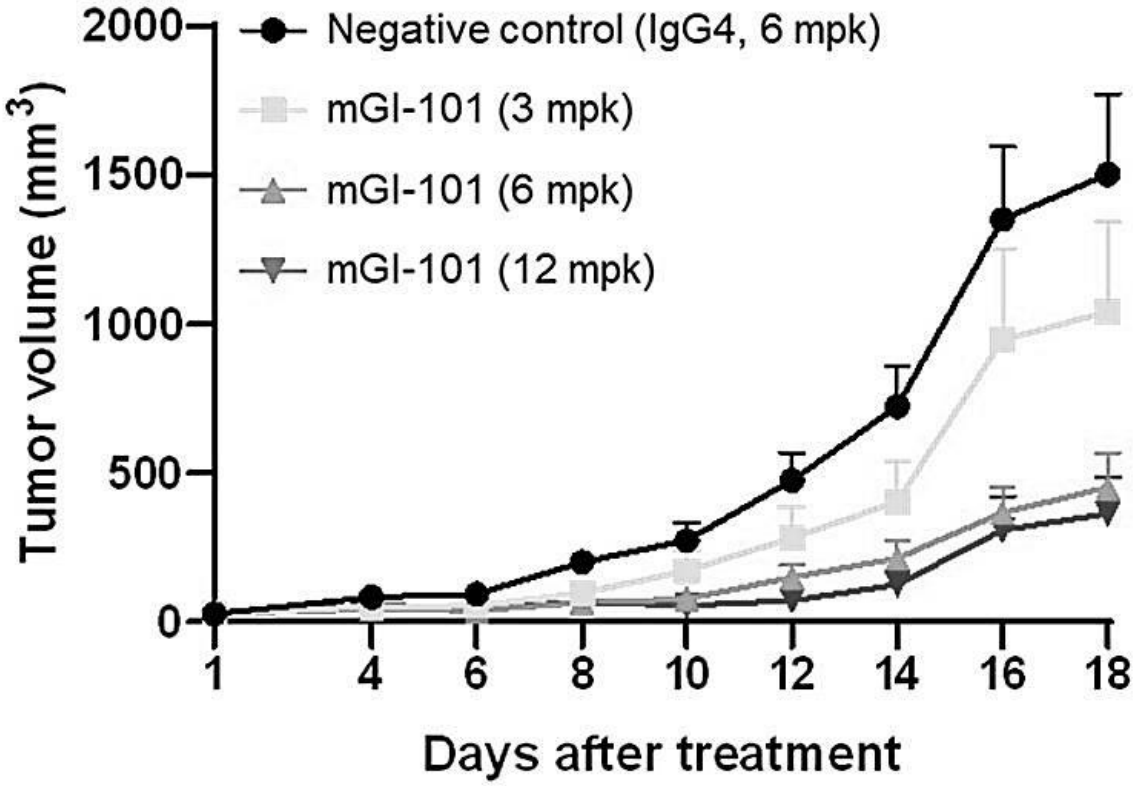
[Fig. 47]


NC, hIgG4 (4 mpk)
mGI-101 (1 mpk)
mGI-101 (4 mpk)
aPD-1 (5 mpk)
mGI-101 + aPD-1 (4 + 5 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
3
5
7
9
11
Time after administration (days)

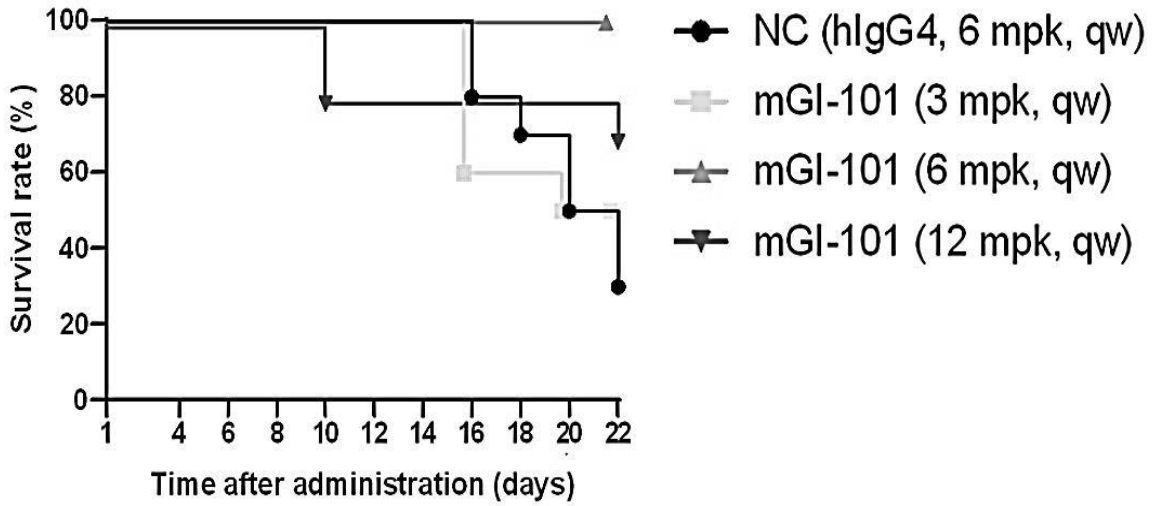
[Fig. 48]


Tumor Growth Inhibition
< 30%
< 50%
< 80%
Number of mouse
8
6
4
2
0
NC
mGI-101 (1 mpk)
mGI-101 (4 mpk)
aPD-1 (5 mpk)
mGI-101 + aPD-1

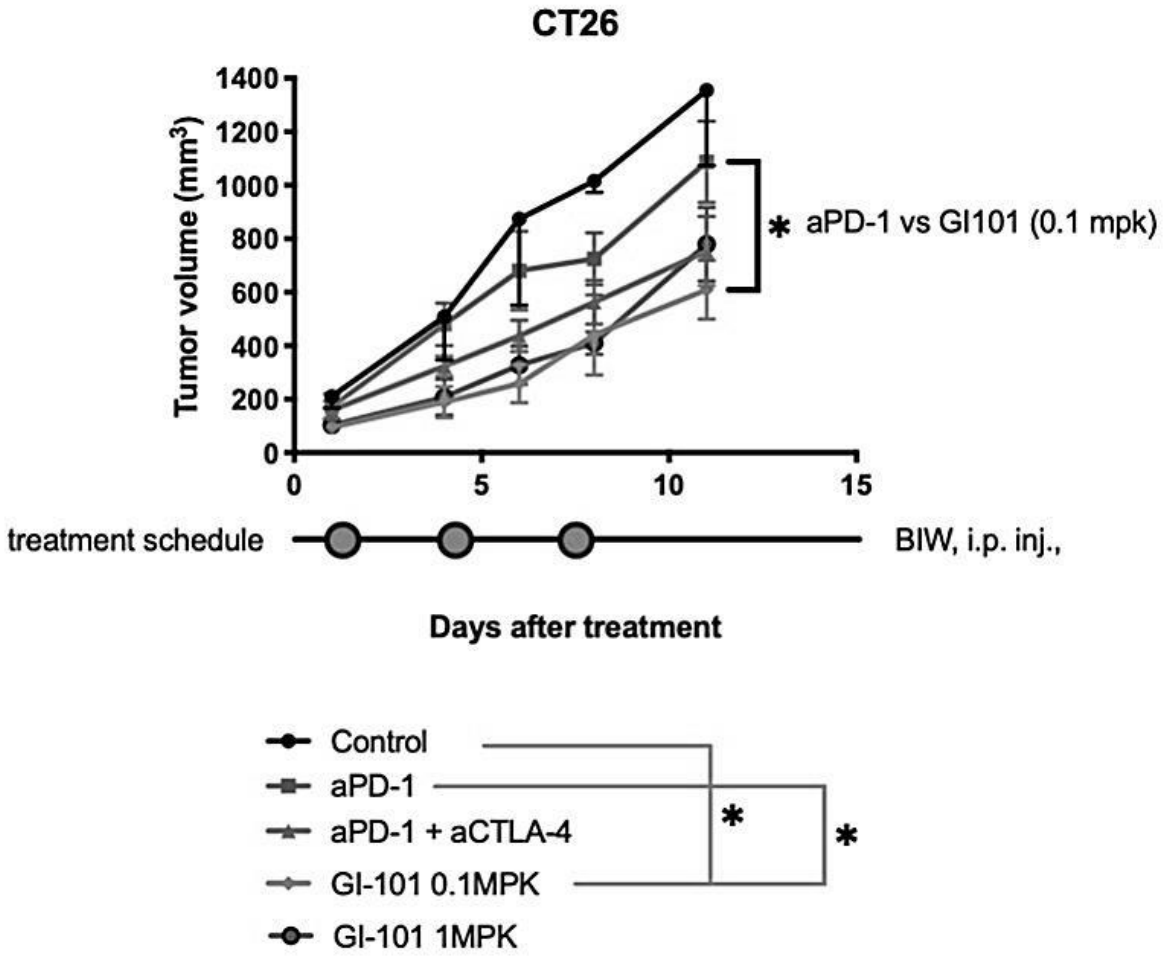
[Fig. 49]


Negative control (IgG4, 6 mpk)
mGI-101 (3 mpk)
mGI-101 (6 mpk)
mGI-101 (12 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
4
6
8
10
12
14
16
18
Days after treatment

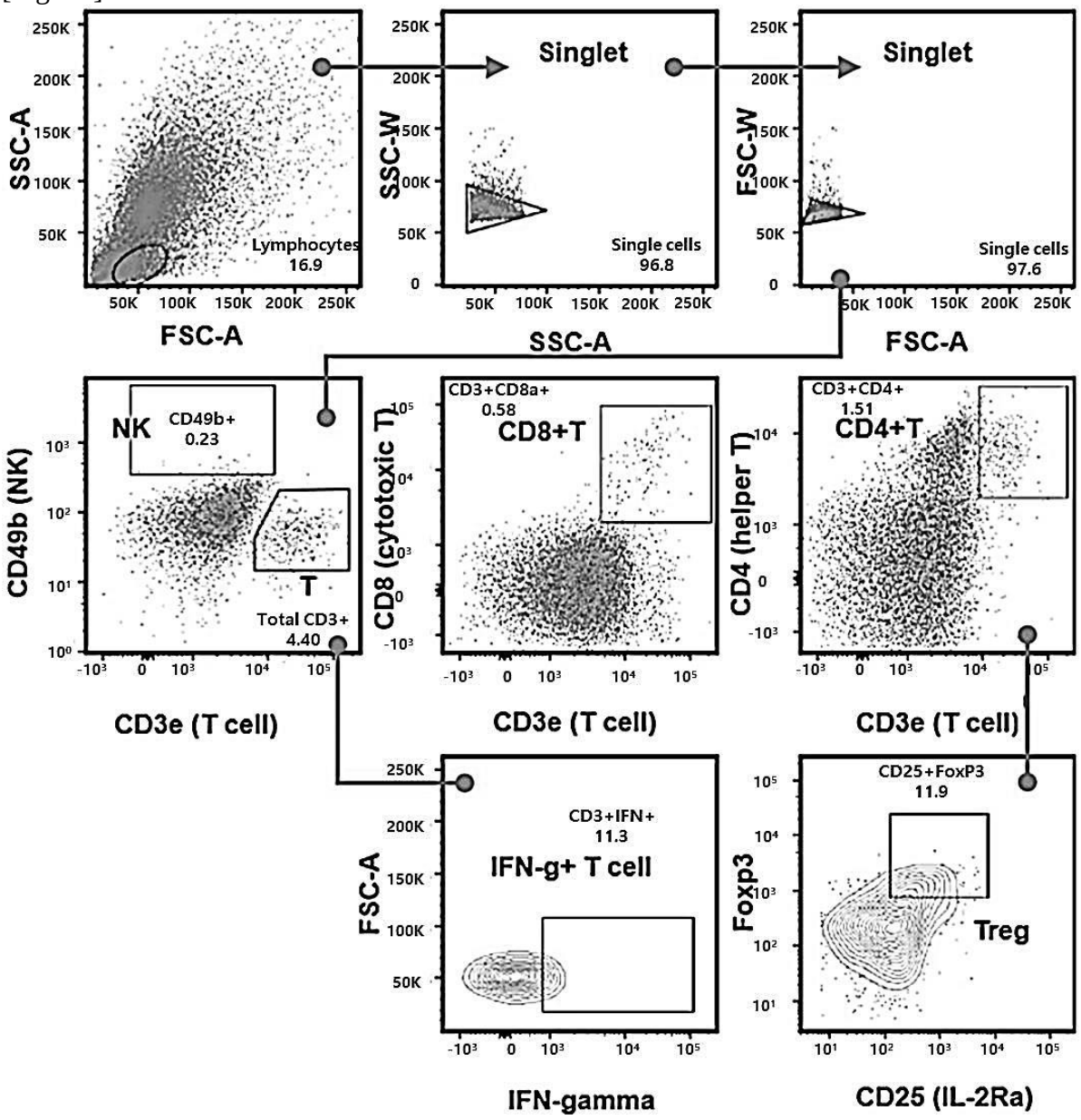
[Fig. 50]


NC (hIgG4, 6 mpk, qw)
mGI-101 (3 mpk, qw)
mGI-101 (6 mpk, qw)
mGI-101 (12 mpk, qw)
Survival rate (%)
100
80
60
40
20
0
1
4
6
8
10
12
14
16
18
20
22
Time after administration (days)

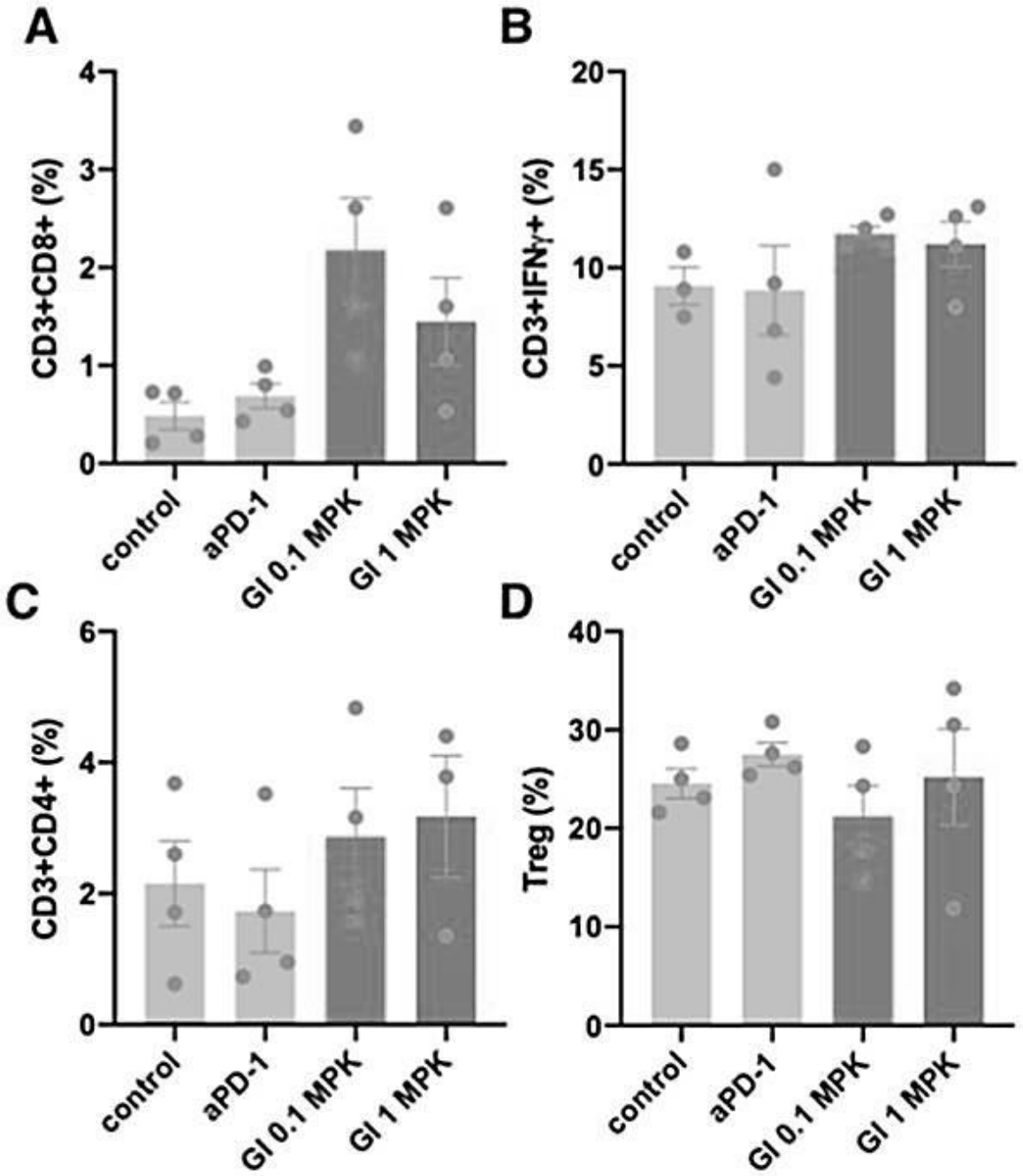
[Fig. 51]


CT26
Tumor volume (mm3)
1400
1200
1000
800
600
400
200
0
0
5
10
15
* aPD-1 vs GI101 (0.1 mpk)
treatment schedule
BIW, i.p. inj.,
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
*
*

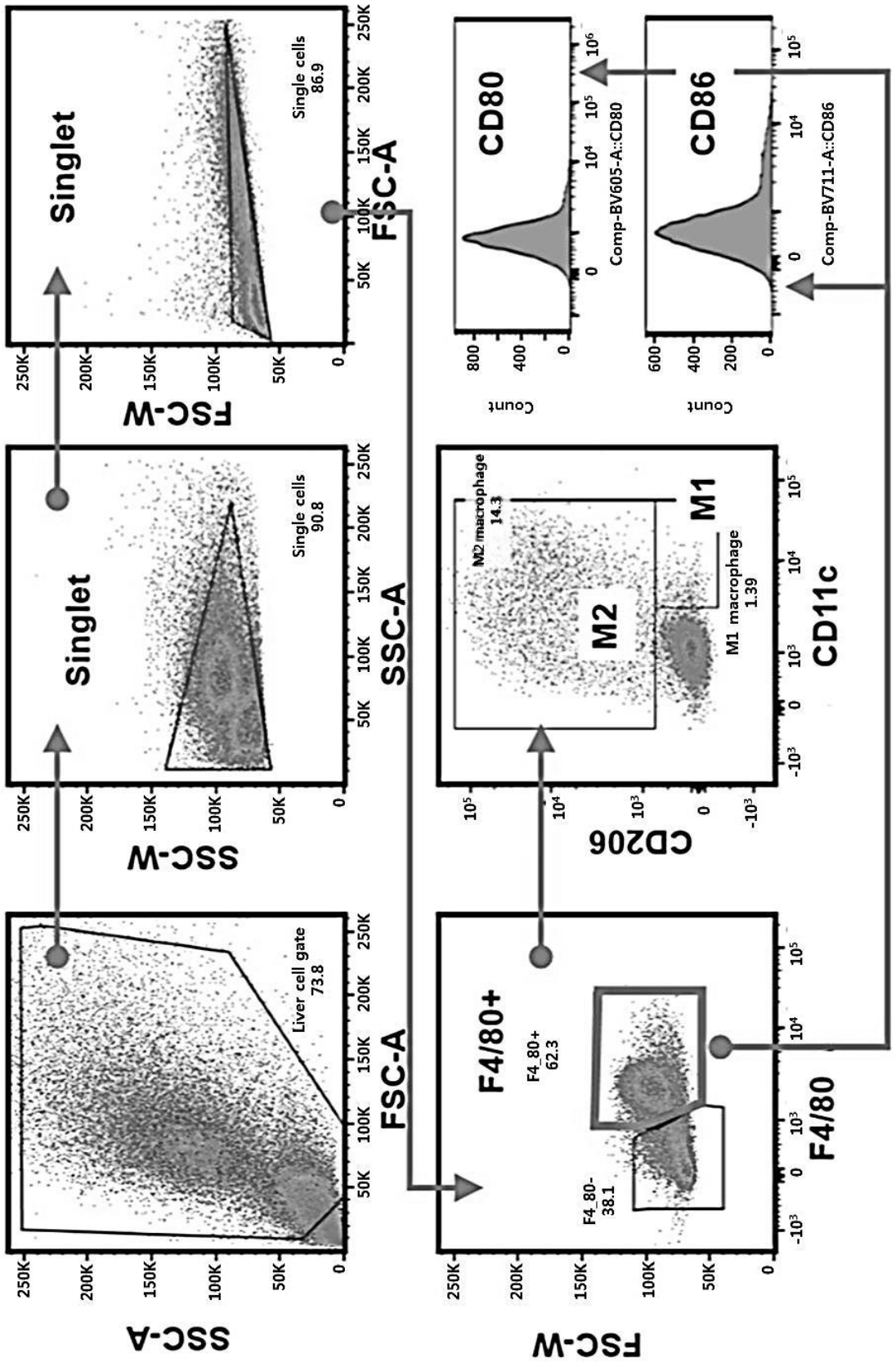
[Fig. 52]


SSC-A
FSC-A
Lymphocytes
16.9
Singlet
SSC-W
Single cells
96.8
Singlet
FSC-W
Single cells
97.6
FSC-A
CD49b (NK)
CD3e (T cell)
NK
CD49b+
0.23
T
Total CD3+
4.40
CD8 (cytotoxic T)
CD3+CD8a+
0.58
CD8+T
CD4 (helper T)
CD3+CD4+
1.51
CD4+T
FSC-A
IFN-gamma
CD3+IFN+
11.3
IFN-g+ T cell
Foxp3
CD25 (IL-2Ra)
CD25+FoxP3
11.9
Treg

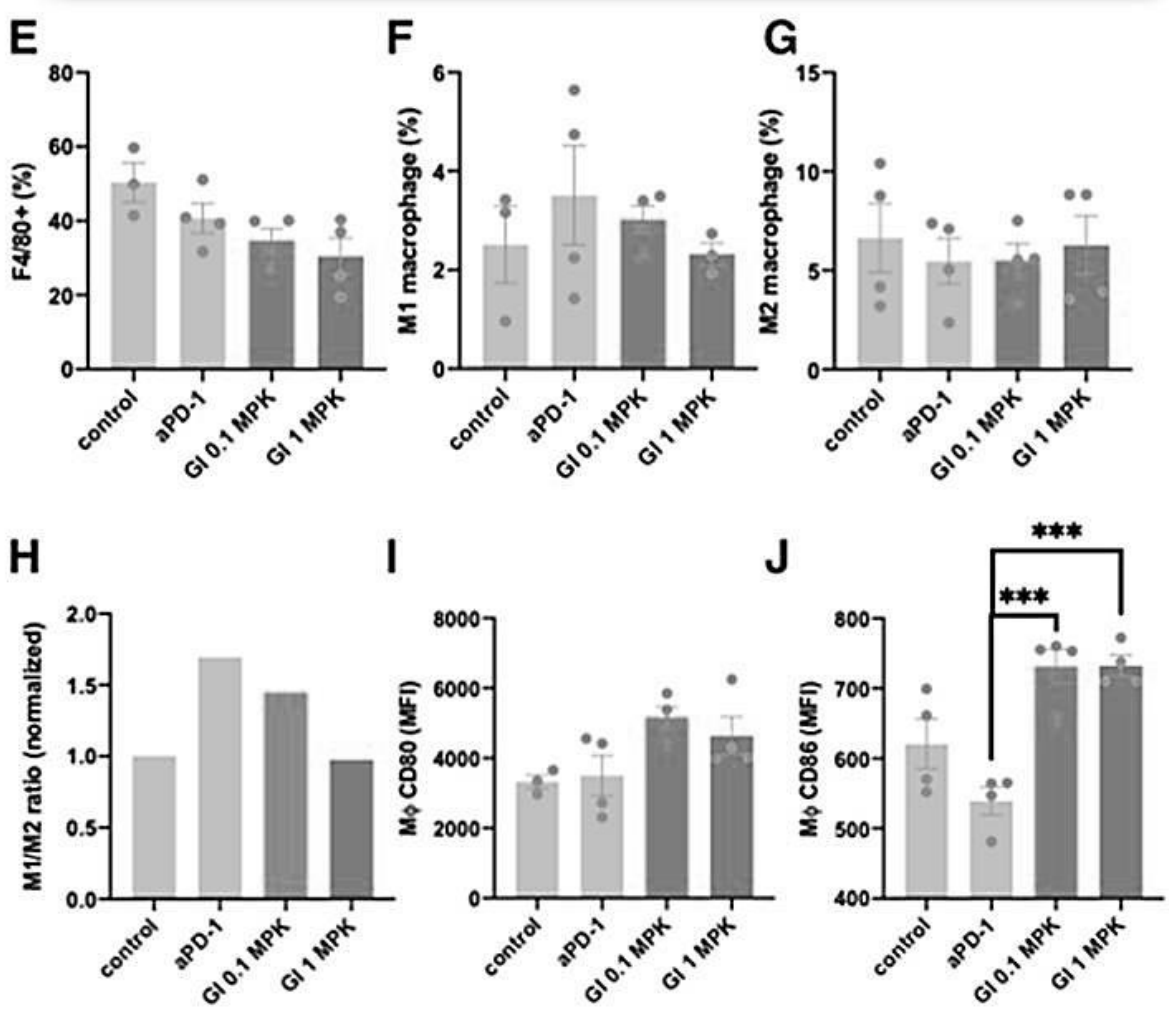
[Fig. 53]


A
CD3+CD8+ (%)
4
3
2
1
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
CD3+IFNγ+ (%)
20
15
10
5
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
CD3+CD4+ (%)
6
4
2
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
D
Treg (%)
40
30
20
10
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK

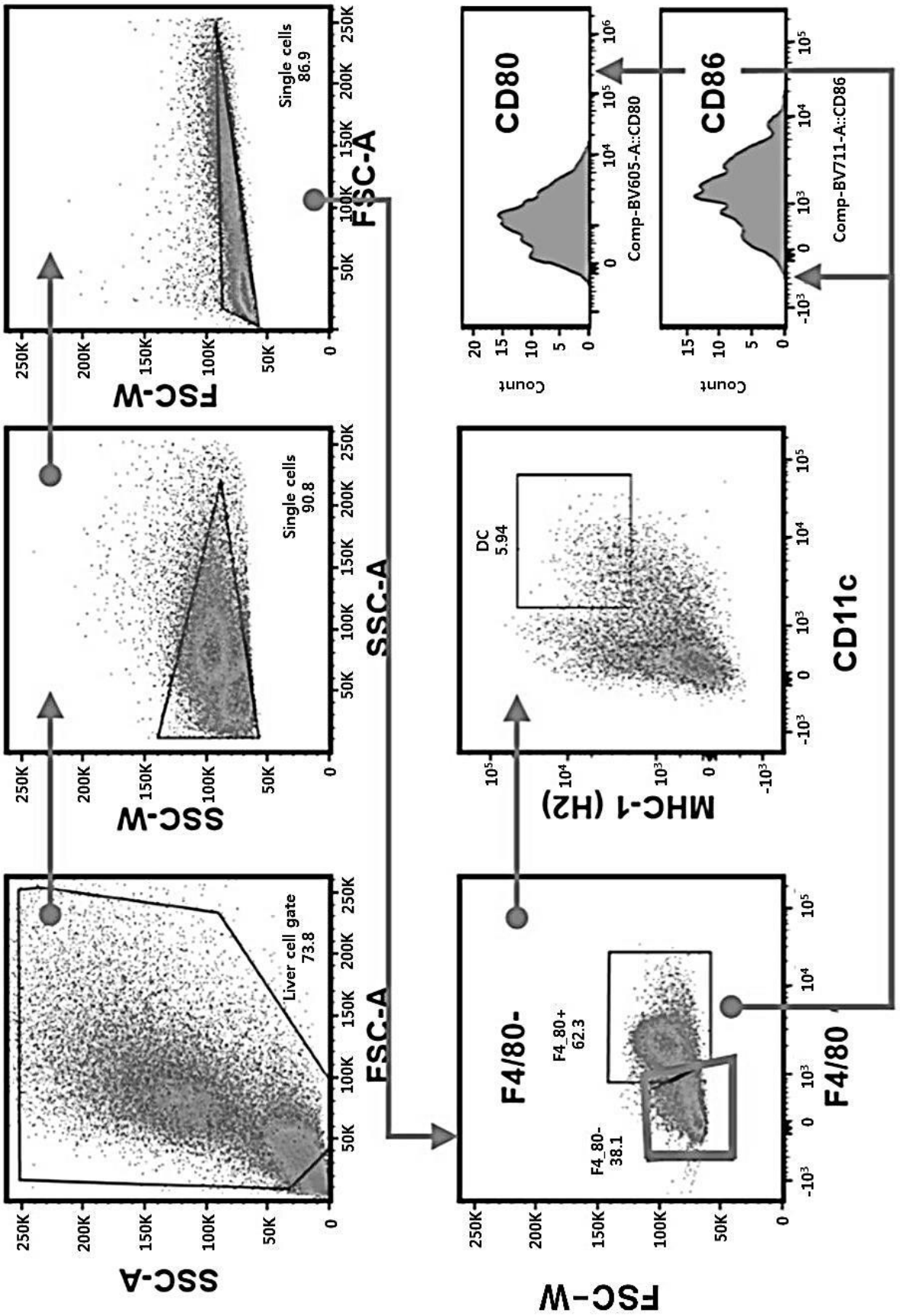
[Fig. 54]


SSC-A
FSC-A
Liver cell gate
73.8
SSC-W
FSC-A
Singlet
Single cells
90.8
FSC-W
FSC-A
Singlet
Single cells
86.9
FSC-W
F4/80
F4/80+
F4_80+
62.3
F4_80-
38.1
CD206
CD11c
M2 macrophage
14.3
M2
M1
M1 macrophage
1.39
Count
CD80
Comp-BV605-A::CD80
Count
CD86
Comp-BV711-A::CD86

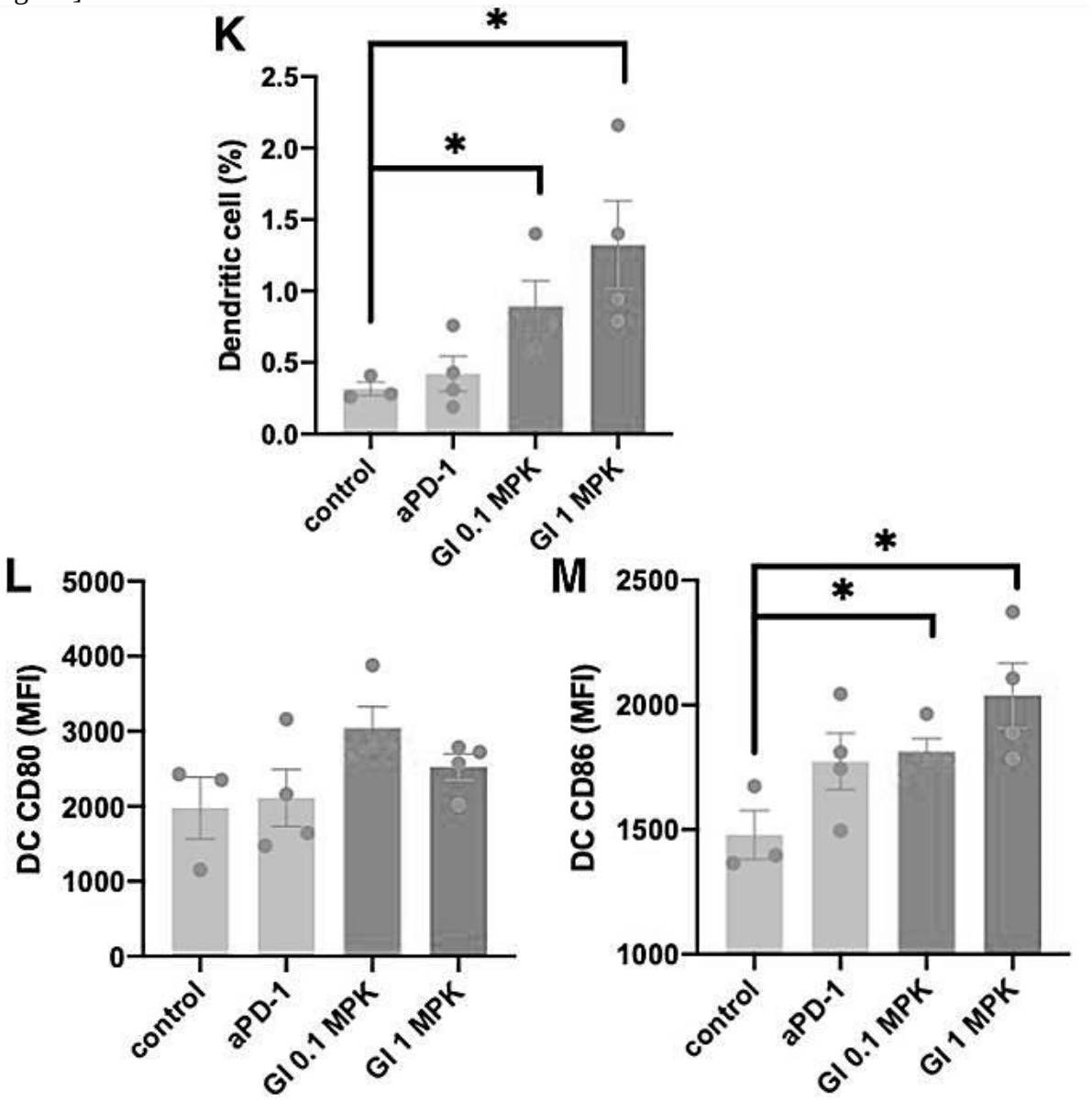
[Fig. 55]


E
F4/80+ (%)
F
M1 macrophage (%)
G
M2 macrophage (%)
H
M1/M2 ratio (normalized)
I
Mϕ CD80 (MFI)
J
Mϕ CD86 (MFI)
***
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK

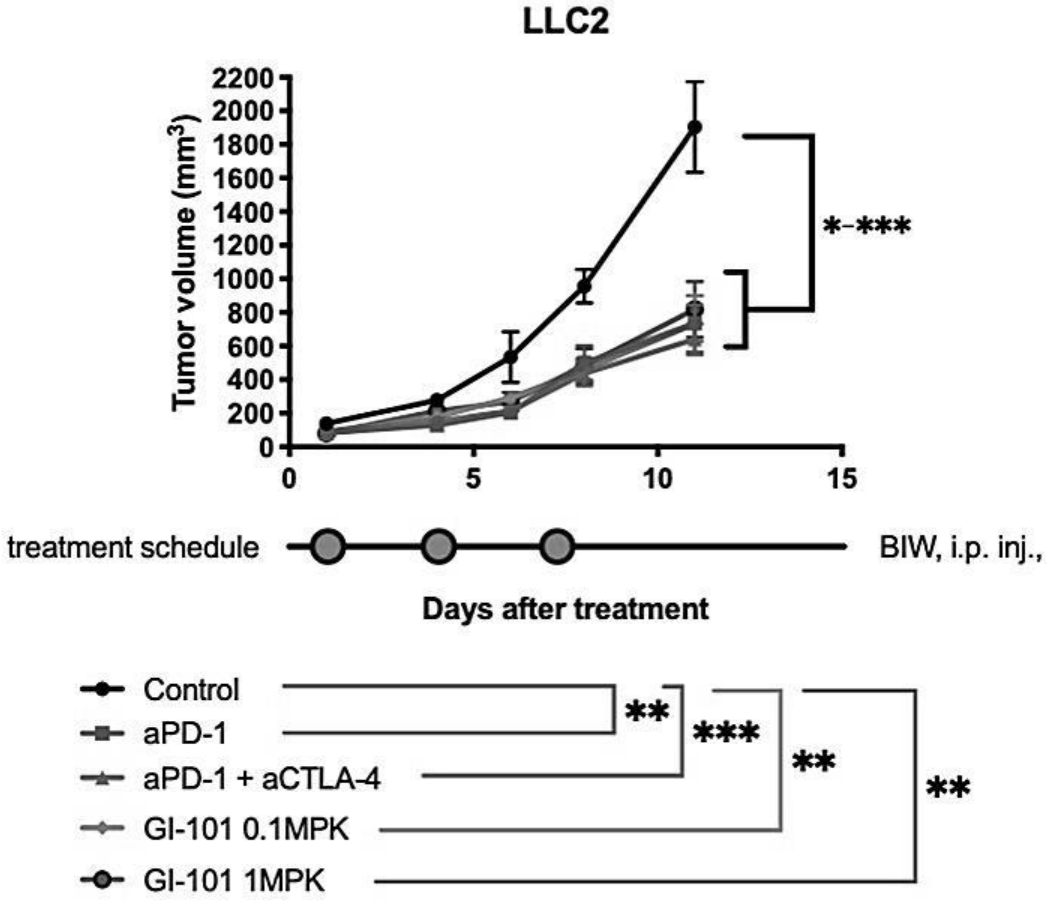
[Fig. 56]


SSC-A
FSC-A
Liver cell gate
73.8
FSC-W
FSC-A
Single cells
86.9
SSC-W
SSC-A
Single cells
90.8
FSC-W
F4/80
F4/80-
F4_80+
62.3
F4_80-
38.1
MHC-1 (H2)
CD11c
DC
5.94
Count
Comp-BV605-A::CD80
CD80
Count
Comp-BV711-A::CD86
CD86

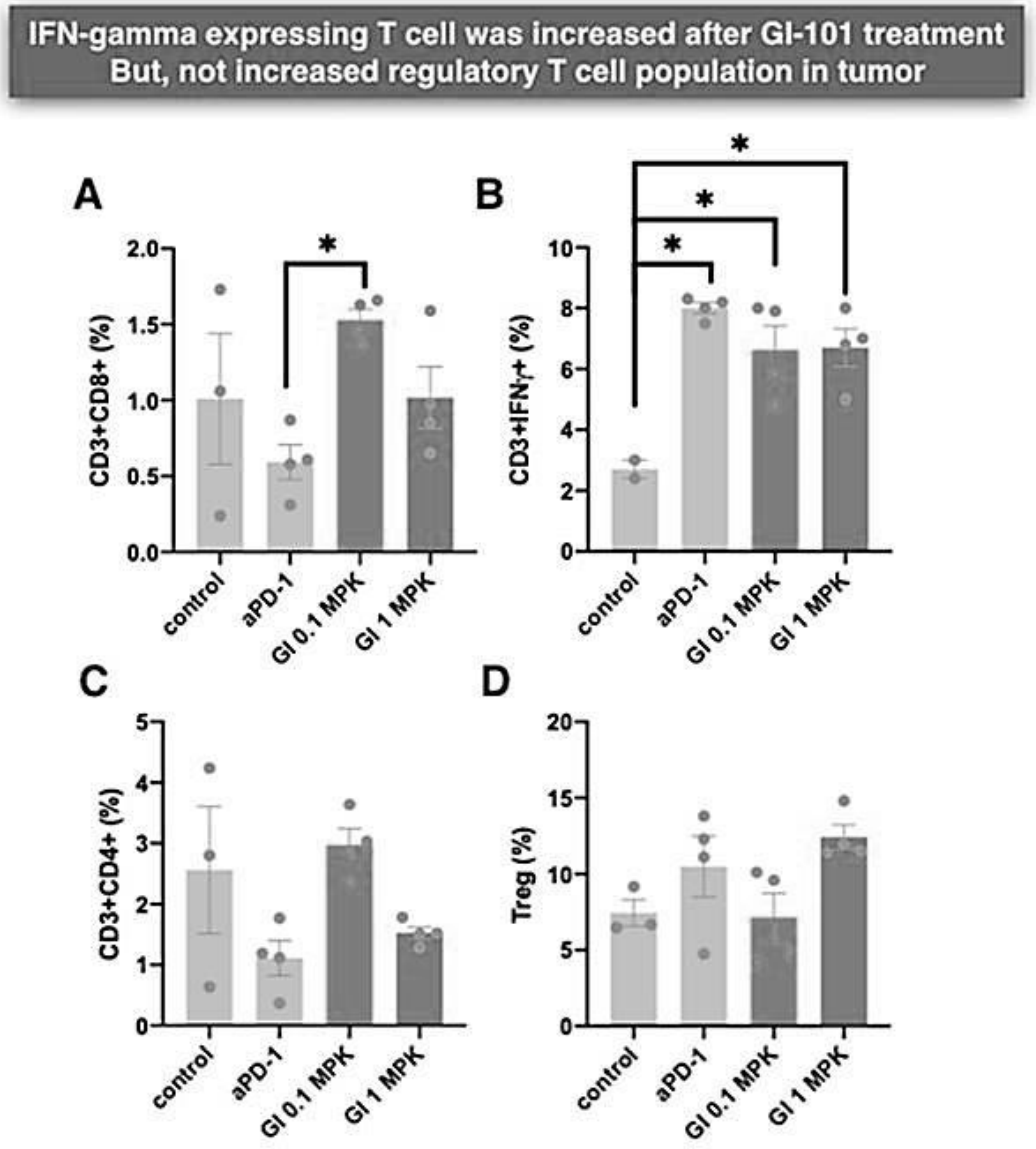
[Fig. 57]


K
Dendritic cell (%)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
L
DC CD80 (MFI)
5000
4000
3000
2000
1000
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
M
DC CD86 (MFI)
2500
2000
1500
1000
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK

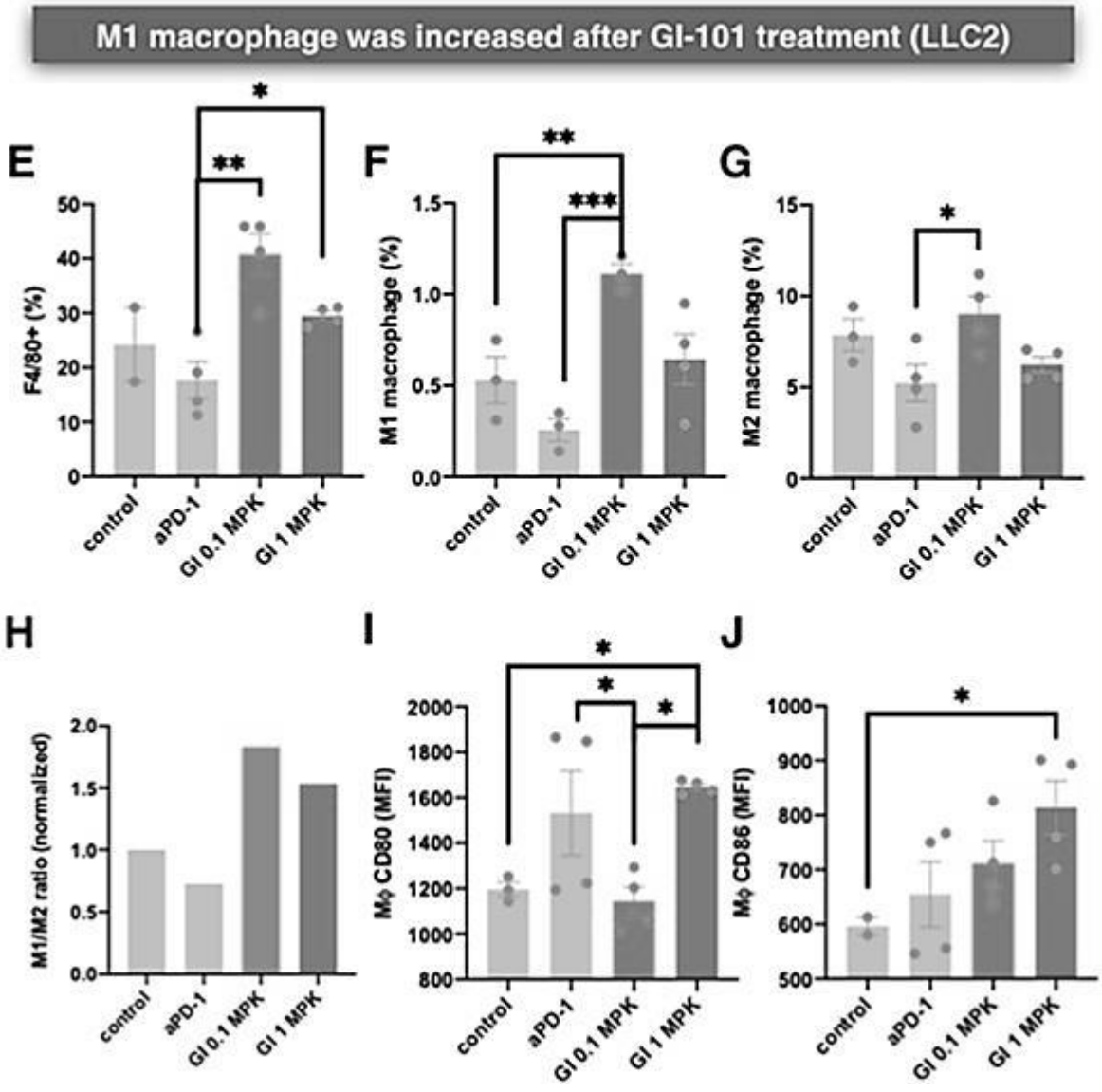
[Fig. 58]


LLC2
Tumor volume (mm3)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
*-***
treatment schedule
BIW, i.p. inj.,
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
**
***
**
**

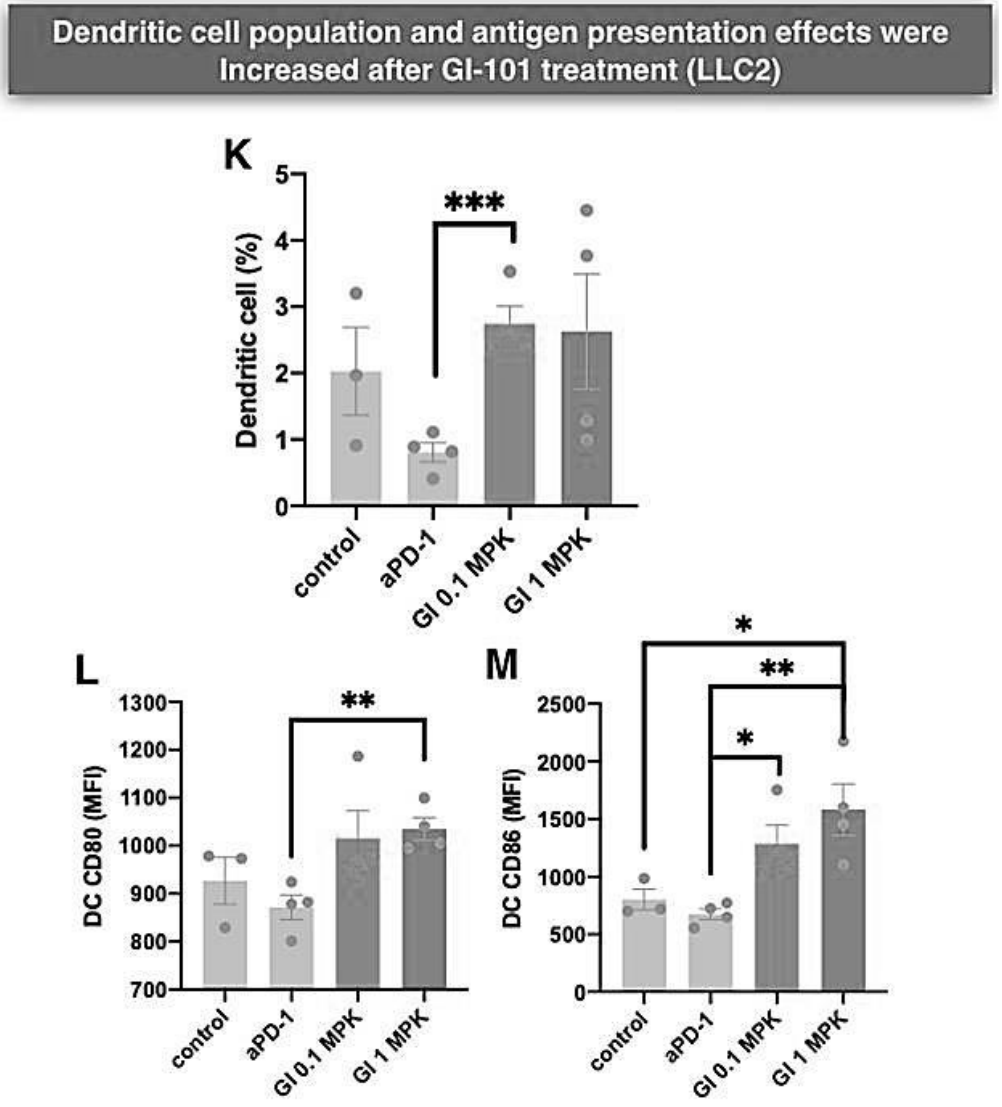
[Fig. 59]


IFN-gamma expressing T cell was increased after GI-101 treatment
But, not increased regulatory T cell population in tumor
A
CD3+CD8+ (%)
2.0
1.5
1.0
0.5
0.0
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
CD3+IFNγ+ (%)
10
8
6
4
2
0
*
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
CD3+CD4+ (%)
5
4
3
2
1
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
D
Treg (%)
20
15
10
5
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK

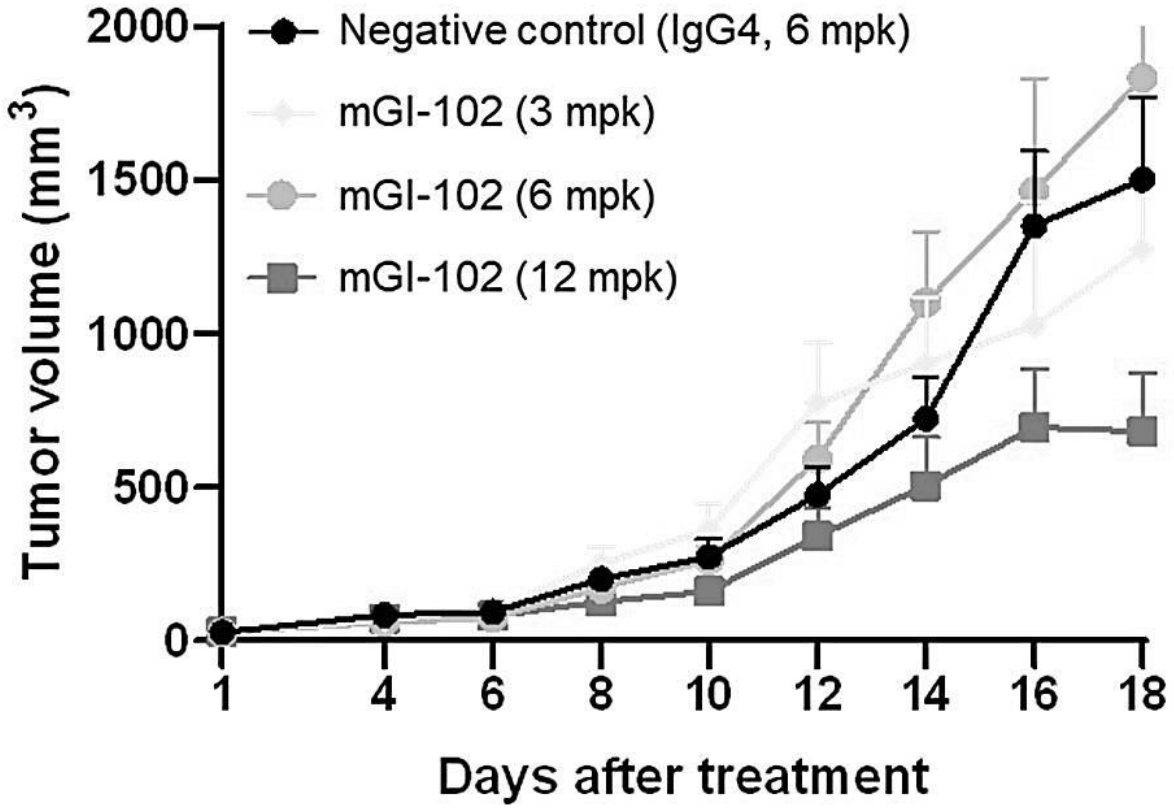
[Fig. 60]


M1 macrophage was increased after GI-101 treatment (LLC2)
E
F4/80+ (%)
F
M1 macrophage (%)
G
M2 macrophage (%)
H
M1/M2 ratio (normalized)
I
MΦ CD80 (MFI)
J
MΦ CD86 (MFI)
control
aPD-1
GI 0.1 MPK
GI 1 MPK

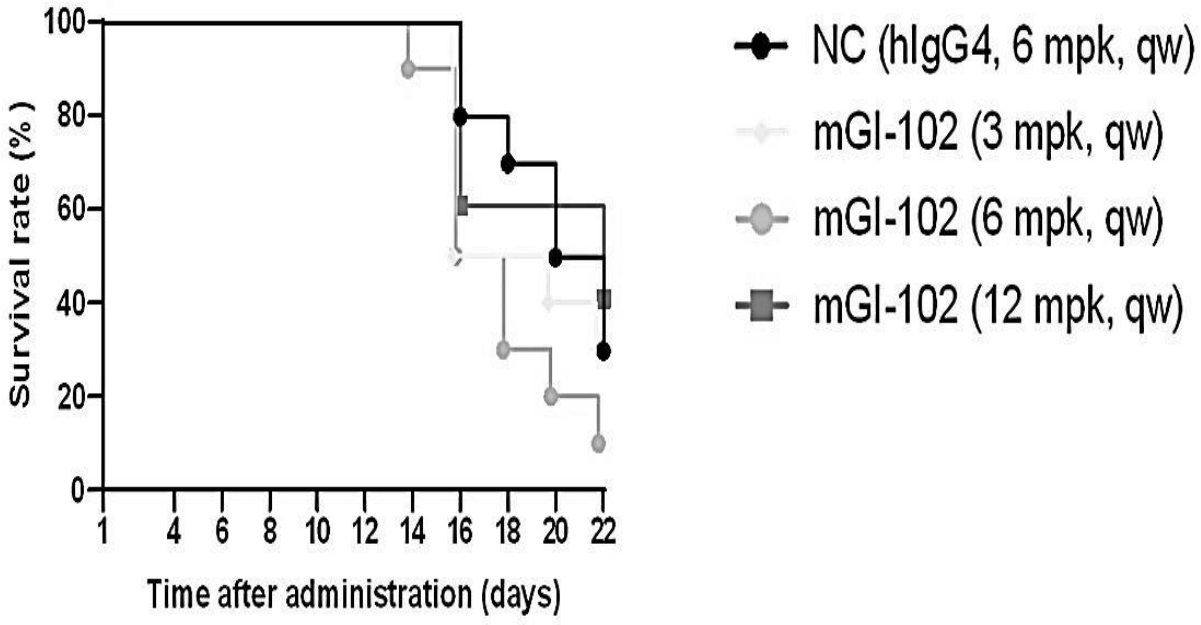
[Fig. 61]


Dendritic cell population and antigen presentation effects were Increased after GI-101 treatment (LLC2)
K
Dendritic cell (%)
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK
L
DC CD80 (MFI)
**
M
DC CD86 (MFI)
*
**
*

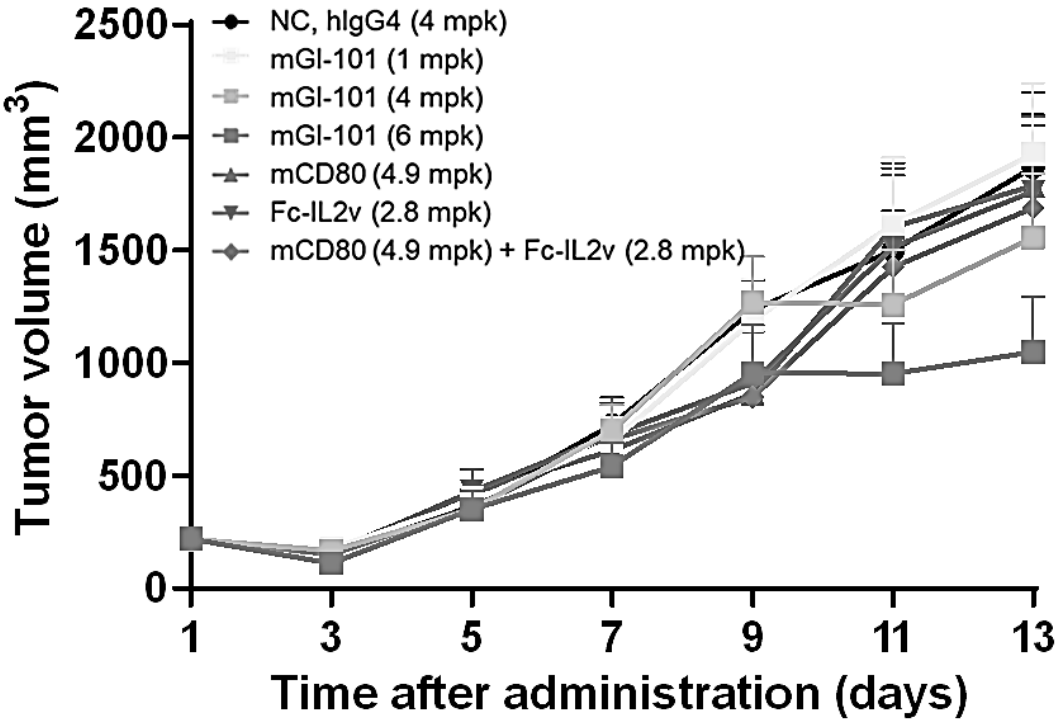
[Fig. 62]


Negative control (IgG4, 6 mpk)
mGI-102 (3 mpk)
mGI-102 (6 mpk)
mGI-102 (12 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
4
6
8
10
12
14
16
18
Days after treatment

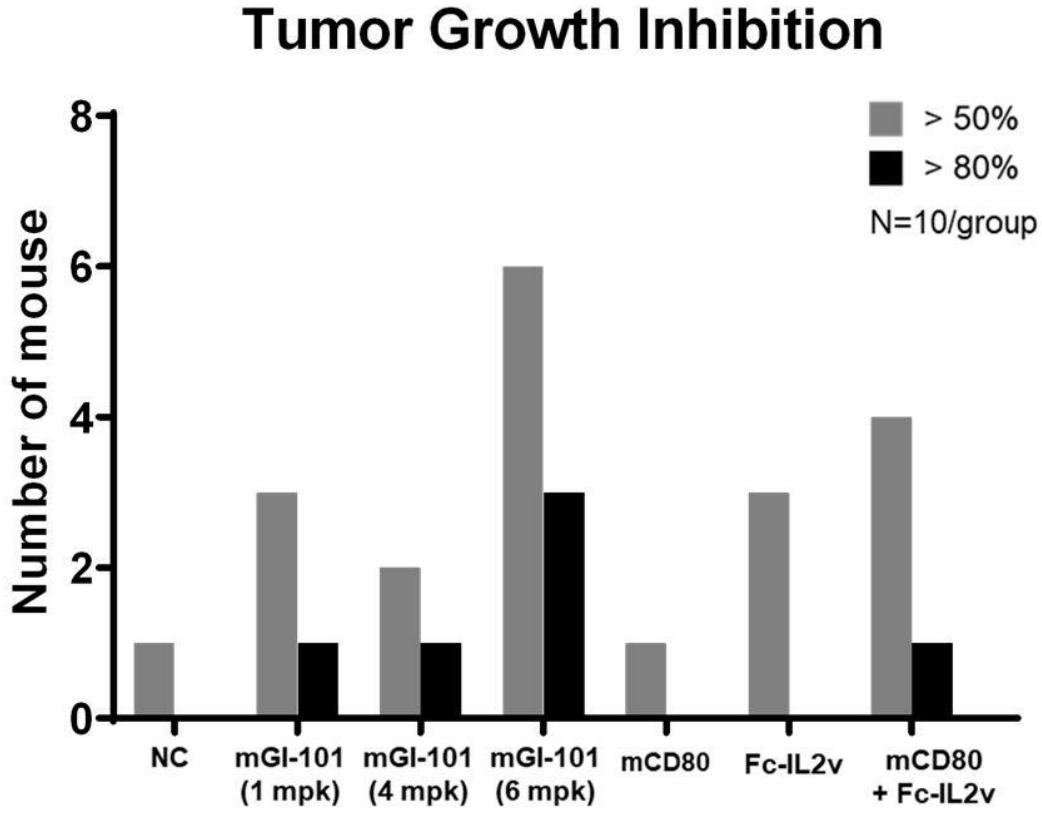
[Fig. 63]


NC (hIgG4, 6 mpk, qw)
mGI-102 (3 mpk, qw)
mGI-102 (6 mpk, qw)
mGI-102 (12 mpk, qw)
Survival rate (%)
100
80
60
40
20
0
1
4
6
8
10
12
14
16
18
20
22
Time after administration (days)

[Fig. 64]


NC, hIgG4 (4 mpk)
mGI-101 (1 mpk)
mGI-101 (4 mpk)
mGI-101 (6 mpk)
mCD80 (4.9 mpk)
Fc-IL2v (2.8 mpk)
mCD80 (4.9 mpk) + Fc-IL2v (2.8 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
3
5
7
9
11
13
Time after administration (days)

[Fig. 65]


Tumor Growth Inhibition
> 50%
> 80%
N=10/group
Number of mouse
8
6
4
2
0
NC
mGI-101 (1 mpk)
mGI-101 (4 mpk)
mGI-101 (6 mpk)
mCD80
Fc-IL2v
mCD80 + Fc-IL2v

[Fig. 66]

A 2-Week Intravenous Dose Toxicity Study of GI-101 in Cynomolgus Monkeys

Table 1 Clinical observations

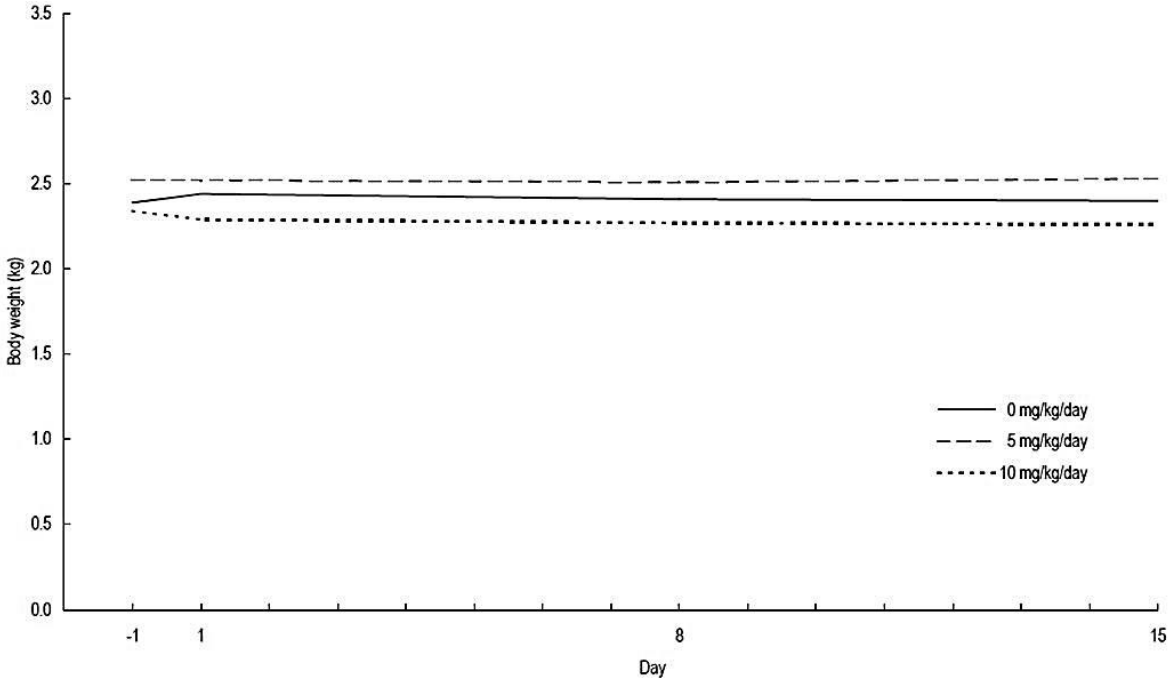
Sex: Male

Group	Animal No.	Day -1 am	Day 1 Pre	Day 1 0.5 h	Day 2 am	Day 3 am	Day 4 am	Day 5 am	Day 6 am	Day 7 am	Day 8 Pre	Day 8 0.5 h	Day 9 am	Day 10 am	Day 11 am	Day 12 am	Day 13 am	Day 14 am	Day of necropsy am
0 mg/kg/day	CJ1M01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ1M02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ1M03	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5 mg/kg/day	CJ2M01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ2M02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ2M03	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10 mg/kg/day	CJ3M01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ3M02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	CJ3M03	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

General Footnote: Pre: Pre-dosing 0.5 h: 0.5 hours post-dosing

NA: No clinical or fecal abnormalities

[Fig. 67]



[Fig. 68]

Body weight (kg)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
-1	Mean	2.39	2.52	2.34
	S.D.	0.10	0.31	0.16
	N	3	3	3
1	Mean	2.44	2.52	2.29
	S.D.	0.07	0.31	0.16
	N	3	3	3
8	Mean	2.41	2.51	2.27
	S.D.	0.12	0.34	0.08
	N	3	3	3
15	Mean	2.40	2.53	2.26
	S.D.	0.12	0.34	0.11
	N	3	3	3

Statistical Test: Generalised Anova/Ancova Test Transformation: Identity (No Transformation)

[Fig. 69]

A 2-Week Intravenous Dose Toxicity Study of GI-101 in Cynomolgus Monkeys

Table 3 Food consumption

Sex: Male

Unit: (g/day)

Group	Animal No.	Day														
		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	CJ1M01	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
mg/kg/day	CJ1M02	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	CJ1M03	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	S.D.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	CJ2M01	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
mg/kg/day	CJ2M02	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	CJ2M03	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	S.D.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	CJ3M01	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
mg/kg/day	CJ3M02	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	CJ3M03	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	S.D.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

[Fig. 70]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
%Retic. (%)	-2	Mean	0.75	1.40	0.93
		S.D.	0.07	0.52	0.30
		N	3	3	3
	15	Mean	1.67	3.10 d1	3.14 d1
		S.D.	0.61	0.55	0.32
		N	3	3	3
#Retic (10^9/L)	-2	Mean	44.0	81.9	55.1
		S.D.	7.0	28.4	19.7
		N	3	3	3
	15	Mean	91.5	171.6 d1	161.5 d1
		S.D.	33.6	32.3	17.2
		N	3	3	3
PLT (10^3/μL)	-2	Mean	404	380	380
		S.D.	25	90	28
		N	3	3	3
	15	Mean	501	522	601
		S.D.	29	135	85
		N	3	3	3

[Fig. 71]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
WBC (10^3/μL)	-2	Mean	10.59	8.18	8.29
		S.D.	1.63	1.43	2.11
		N	3	3	3
	15	Mean	8.09	12.08	16.52
		S.D.	0.25	0.74	6.81
		N	3	3	3
%Neut (%)	-2	Mean	38.1	23.1	23.6
		S.D.	23.2	3.2	13.1
		N	3	3	3
	15	Mean	35.9	14.5	22.4
		S.D.	14.3	2.1	14.2
		N	3	3	3
%Lymph (%)	-2	Mean	57.7	71.6	69.8
		S.D.	21.7	2.8	13.7
		N	3	3	3
	15	Mean	59.6	81.2	69.5
		S.D.	13.1	1.4	15.0
		N	3	3	3

[Fig. 72]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
#Neut (10^3/μL)	-2	Mean	4.24	1.86	1.77
		S.D.	3.09	0.22	0.52
		N	3	3	3
	15	Mean	2.93	1.76	3.11
		S.D.	1.26	0.35	0.63
		N	3	3	3
#Lymph (10^3/μL)	-2	Mean	5.92	5.87	5.98
		S.D.	1.78	1.24	2.54
		N	3	3	3
	15	Mean	4.80	9.80	12.05
		S.D.	0.94	0.48	6.47
		N	3	3	3
#Mono (10^3/μL)	-2	Mean	0.36	0.37	0.45
		S.D.	0.10	0.09	0.08
		N	3	3	3
	15	Mean	0.32	0.33	0.77 d[1]
		S.D.	0.11	0.06	0.26
		N	3	3	3

[Fig. 73]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
AST (U/L)	-2	Mean	36	45	30
		S.D.	19	21	7
		N	3	3	3
	15	Mean	24	34	33
		S.D.	5	5	8
		N	3	3	3
ALT (U/L)	-2	Mean	58	72	34
		S.D.	51	71	8
		N	3	3	3
	15	Mean	34	52	36
		S.D.	6	44	5
		N	3	3	3
ALP (U/L)	-2	Mean	1511	1658	1972
		S.D.	542	258	357
		N	3	3	3
	15	Mean	1395	1444	1565
		S.D.	365	346	235
		N	3	3	3

[Fig. 74]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
LD (U/L)	-2	Mean	255	289	292
		S.D.	6	57	47
		N	3	3	3
	15	Mean	236	304	361 dd[1]
		S.D.	15	47	38
		N	3	3	3
CK (U/L)	-2	Mean	132	140	182
		S.D.	23	4	61
		N	3	3	3
	15	Mean	120	128	140
		S.D.	31	19	23
		N	3	3	3
GLU (mg/dL)	-2	Mean	98	91	112
		S.D.	17	4	15
		N	3	3	3
	15	Mean	87	88	104
		S.D.	9	17	5
		N	3	3	3

[Fig. 75]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
BIL (mg/dL)	-2	Mean	0.10	0.15 d[1]	0.10
		S.D.	0.02	0.03	0.01
		N	3	3	3
	15	Mean	0.11	0.15	0.10
		S.D.	0.04	0.03	0.03
		N	3	3	3
UN (mg/dL)	-2	Mean	19.1	15.4	18.8
		S.D.	7.2	2.3	4.5
		N	3	3	3
	15	Mean	16.2	13.9	14.2
		S.D.	5.6	0.7	2.4
		N	3	3	3
CRE (mg/dL)	-2	Mean	0.73	0.69	0.73
		S.D.	0.07	0.17	0.10
		N	3	3	3
	15	Mean	0.72	0.66	0.65
		S.D.	0.06	0.13	0.11
		N	3	3	3

[Fig. 76]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
CHO (mg/dL)	-2	Mean	109	162	147
		S.D.	23	59	47
		N	3	3	3
	15	Mean	108	159	143
		S.D.	24	46	34
		N	3	3	3
TG (mg/dL)	-2	Mean	44	27	40
		S.D.	24	12	2
		N	3	3	3
	15	Mean	35	29	34
		S.D.	2	14	14
		N	3	3	3
PL (mg/dL)	-2	Mean	180	236	220
		S.D.	48	43	65
		N	3	3	3
	15	Mean	164	216	195
		S.D.	31	52	40
		N	3	3	3

[Fig. 77]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
IP (mg/dL)	-2	Mean	5.16	5.14	5.00
		S.D.	1.16	0.91	0.90
		N	3	3	3
	15	Mean	5.52	5.94	5.66
		S.D.	0.61	0.61	0.94
		N	3	3	3
CA (mg/dL)	-2	Mean	9.63	9.82	9.79
		S.D.	0.59	0.55	0.19
		N	3	3	3
	15	Mean	9.45	9.48	9.31
		S.D.	0.57	0.25	0.03
		N	3	3	3
NA (mEq/L)	-2	Mean	152.8	154.5	153.9
		S.D.	2.4	4.2	2.5
		N	3	3	3
	15	Mean	151.8	153.5	153.4
		S.D.	3.3	2.0	3.3
		N	3	3	3

[Fig. 78]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
K (mEq/L)	-2	Mean	4.28	4.17	3.90
		S.D.	0.69	0.29	0.40
		N	3	3	3
	15	Mean	3.99	4.09	3.85
		S.D.	0.50	0.18	0.14
		N	3	3	3
CL (mEq/L)	-2	Mean	112.3	111.1	110.7
		S.D.	2.2	3.9	2.6
		N	3	3	3
	15	Mean	111.5	109.6	110.2
		S.D.	0.7	3.7	2.8
		N	3	3	3
TP (g/dL)	-2	Mean	7.20	7.36	7.53
		S.D.	0.59	0.42	0.22
		N	3	3	3
	15	Mean	6.99	7.22	7.30
		S.D.	0.62	0.37	0.11
		N	3	3	3

[Fig. 79]

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
ALB (g/dL)	-2	Mean	4.18	4.11	4.14
		S.D.	0.34	0.46	0.19
		N	3	3	3
	15	Mean	4.09	3.98	3.88
		S.D.	0.36	0.47	0.17
		N	3	3	3
A/G	-2	Mean	1.39	1.27	1.22
		S.D.	0.06	0.19	0.04
		N	3	3	3
	15	Mean	1.41	1.24	1.13
		S.D.	0.04	0.22	0.07
		N	3	3	3

[Fig. 80]

Sex: Male

Group	Animal No.	Tumor necrosis factor-α (pg/mL)				Interferon-γ (pg/mL)				Interleukin-1β (pg/mL)				Interleukin-2 (pg/mL)				Interleukin-4 (pg/mL)			
		Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15
0	CJ1M01	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
mg/kg/day	CJ1M02	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ1M03	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Mean	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	CJ2M01	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
mg/kg/day	CJ2M02	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ2M03	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Mean	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	CJ3M01	BLQ	BLQ	BLQ	BLQ	BLQ	5.51	4.60	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
mg/kg/day	CJ3M02	BLQ	BLQ	BLQ	BLQ	BLQ	12.74	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ3M03	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Mean	N.C.	N.C.	N.C.	N.C.	N.C.	9.13	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

General Footnote: Day 8: Prior to dosing

BLQ: Below the lower limit of quantification (4.9 pg/mL for Interleukin-4, 2.4 pg/mL for the others)

When plasma concentrations were BLQ in 1 of the 3 animals, the mean of the 2 remaining animals was calculated.

The mean was regarded as N.C. when plasma concentrations in 2 of the 3 animals were BLQ.

N.C.: Not calculated

[Fig. 81]

Sex: Male

Group	Animal No.	Interleukin-6 (pg/mL)				Interleukin-8 (pg/mL)				Interleukin-10 (pg/mL)				Interleukin-12 (pg/mL)			
		Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15	Pretest	Day 3	Day 8	Day 15
0 mg/kg/day	CJ1M01	BLQ	2.95	BLQ	3.34	4047.41	4861.65	7894.37	6783.89	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ1M02	BLQ	BLQ	BLQ	BLQ	3418.18	1382.07	6035.41	4096.95	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ1M03	BLQ	2.78	BLQ	BLQ	809.60	905.96	972.78	981.31	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Mean	N.C.	2.87	N.C.	N.C.	2758.40	2383.23	4967.52	3954.05	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	1716.78	2159.54	3582.23	2903.93	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	2	0	1	3	3	3	3	0	0	0	0	0	0	0	0
5 mg/kg/day	CJ2M01	BLQ	BLQ	BLQ	BLQ	4800.40	3355.74	5986.48	5511.93	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ2M02	BLQ	BLQ	BLQ	BLQ	2633.61	2388.95	4778.15	5266.00	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ2M03	BLQ	2.91	BLQ	BLQ	7482.97	6571.61	9663.74	8892.13	BLQ	BLQ	BLQ	BLQ	7.07	6.31	BLQ	BLQ
	Mean	N.C.	N.C.	N.C.	N.C.	4972.33	4105.43	6809.46	6556.69	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	2429.25	2189.79	2544.64	2026.29	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	1	0	0	3	3	3	3	0	0	0	0	1	1	0	0
10 mg/kg/day	CJ3M01	BLQ	5.26	BLQ	BLQ	8312.98	2753.66	7101.75	8973.45	BLQ	19.41	BLQ	BLQ	3.41	BLQ	BLQ	2.96
	CJ3M02	BLQ	BLQ	BLQ	BLQ	7136.73	2722.78	9985.51	9298.79	BLQ	108.34	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	CJ3M03	BLQ	2.41	BLQ	BLQ	5832.12	4900.91	8873.95	9812.62	BLQ	39.40	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Mean	N.C.	3.84	N.C.	N.C.	7093.94	3459.12	8653.74	9361.62 [d']	N.C.	55.72	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S.D.	N.C.	N.C.	N.C.	N.C.	1240.98	1248.73	1454.44	423.10	N.C.	46.66	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	N	0	2	0	0	3	3	3	3	0	3	0	0	1	0	0	1

General Footnote: Day 8: Prior to dosing

BLQ: Below the lower limit of quantification (12.2 pg/mL for Interleukin-10, 2.4 pg/mL for the others)

When plasma concentrations were BLQ in 1 of the 3 animals, the mean of the 2 remaining animals was calculated.

The mean was regarded as N.C. when plasma concentrations in 2 of the 3 animals were BLQ.

N.C.: Not calculated

1 [d - Test: Dunnett 2 Sided $p < 0.05$]

[Fig. 82]

Sex: Male

Group	Animal No.	Ratio in lymphocytes (%)																			
		T cell				CD4 T cell				CD8 T cell				Regulatory T cell				NK cell			
		Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15
0 mg/kg/day	CJ1M01	49.2	57.1	57.0	53.3	28.0	34.9	34.6	32.9	15.1	15.3	16.0	13.5	1.3	1.5	1.5	1.6	38.4	33.5	34.4	37.1
	CJ1M02	69.4	68.5	67.3	55.2	27.3	29.6	25.4	19.0	35.7	31.7	35.6	30.4	0.8	0.9	0.8	0.7	18.3	10.9	20.3	30.5
	CJ1M03	55.4	70.8	61.2	51.6	27.5	38.7	29.9	22.3	22.5	26.9	26.3	24.8	1.3	1.7	1.3	1.5	31.7	16.6	29.0	35.5
	Mean	58.0	65.5	61.8	53.4	27.6	34.4	30.0	24.7	24.4	24.6	26.0	22.9	1.1	1.4	1.2	1.3	29.5	20.3	27.9	34.4
	S.D.	10.3	7.3	5.2	1.8	0.4	4.6	4.6	7.3	10.4	8.4	9.8	8.6	0.3	0.4	0.4	0.5	10.2	11.8	7.1	3.4
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5 mg/kg/day	CJ2M01	62.6	86.7	60.8	59.7	25.0	55.3	22.7	17.1	32.2	25.1	32.2	35.4	1.0	4.4	2.0	2.0	26.0	4.4	28.2	29.6
	CJ2M02	52.7	75.9	55.3	51.9	22.7	41.0	24.3	19.4	24.8	28.2	25.6	25.6	0.8	3.6	2.6	1.6	27.2	6.3	28.9	34.4
	CJ2M03	61.0	78.0	62.9	59.3	17.2	31.0	16.2	17.2	38.0	39.4	40.8	36.4	0.8	3.5	2.5	2.6	19.0	6.9	17.8	21.3
			d[1]																		
	Mean	58.8	80.2	59.7	57.0	21.6	42.4	21.1	17.9	31.7	30.9	32.9	32.5	0.9	3.8	2.4	2.1	24.1	5.9	25.0	28.4
	S.D.	5.3	5.7	3.9	4.4	4.0	12.2	4.3	1.3	6.6	7.5	7.6	6.0	0.1	0.5	0.3	0.5	4.4	1.3	6.2	6.6
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10 mg/kg/day	CJ3M01	69.3	80.8	73.6	69.8	15.2	32.7	18.3	18.0	43.8	34.8	45.4	44.1	0.8	4.1	3.0	2.5	17.1	6.5	14.1	15.1
	CJ3M02	65.2	86.4	65.4	66.0	18.4	28.4	13.7	16.6	39.0	48.8	42.1	37.3	0.6	2.8	1.6	1.9	16.0	3.8	14.8	15.3
	CJ3M03	70.2	89.7	76.8	74.0	19.2	46.7	23.8	28.2	44.9	33.8	44.9	37.5	1.0	7.6	4.6	5.9	17.5	3.0	10.6	16.5
			d[1]		dd[2]	dd[2]		d[1]		d[1]		d[1]	d[1]		d[1]					d[1]	dd[2]
	Mean	68.2	85.6	71.9	69.9	17.6	35.9	18.6	20.9	42.6	39.1	44.1	39.6	0.8	4.8	3.1	3.4	16.9	4.4	13.2	15.6
	S.D.	2.7	4.5	5.9	4.0	2.1	9.6	5.1	6.3	3.1	8.4	1.8	3.9	0.2	2.5	1.5	2.2	0.8	1.8	2.3	0.8
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D3, D8 and D15: Days 3, 8 (prior to dosing) and 15

1 [d - Test: Dunnett 2 Sided p < 0.05]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

[Fig. 83]

Sex: Male

		Lymphocytes		Absolute count (10^3/μL)									
	Animal	(10^3/μL)		T cell		CD4 T cell		CD8 T cell		Regulatory T cell		NK cell	
Group	No.	Pre	D15	Pre	D15	Pre	D15	Pre	D15	Pre	D15	Pre	D15
0	CJ1M01	7.40	5.60	3.64	2.98	2.07	1.84	1.12	0.76	0.10	0.09	2.84	2.08
mg/kg/day	CJ1M02	6.42	5.03	4.46	2.78	1.75	0.96	2.29	1.53	0.05	0.04	1.17	1.53
	CJ1M03	3.94	3.77	2.18	1.95	1.08	0.84	0.89	0.93	0.05	0.06	1.25	1.34
	Mean	5.92	4.80	3.43	2.57	1.63	1.21	1.43	1.07	0.07	0.06	1.75	1.65
	S.D.	1.78	0.94	1.15	0.55	0.51	0.55	0.75	0.40	0.03	0.03	0.94	0.38
	N	3	3	3	3	3	3	3	3	3	3	3	3
5	CJ2M01	5.01	9.42	3.14	5.62	1.25	1.61	1.61	3.33	0.05	0.19	1.30	2.79
mg/kg/day	CJ2M02	7.29	10.34	3.84	5.37	1.65	2.01	1.81	2.65	0.06	0.17	1.98	3.56
	CJ2M03	5.32	9.63	3.25	5.71	0.92	1.66	2.02	3.51	0.04	0.25	1.01	2.05
											dd[2]		
	Mean	5.87	9.80	3.41	5.57	1.27	1.76	1.81	3.16	0.05	0.20	1.43	2.80
	S.D.	1.24	0.48	0.38	0.18	0.37	0.22	0.21	0.45	0.01	0.04	0.50	0.76
	N	3	3	3	3	3	3	3	3	3	3	3	3
10	CJ3M01	8.38	13.41	5.81	9.36	1.27	2.41	3.67	5.91	0.07	0.34	1.43	2.02
mg/kg/day	CJ3M02	6.25	17.73	4.08	11.70	1.15	2.94	2.44	6.61	0.04	0.34	1.00	2.71
	CJ3M03	3.32	5.01	2.33	3.71	0.64	1.41	1.49	1.88	0.03	0.30	0.58	0.83
					d[1]				d[1]		dd[2]		
	Mean	5.98	12.05	4.07	8.26	1.02	2.25	2.53	4.80	0.05	0.33	1.00	1.85
	S.D.	2.54	6.47	1.74	4.11	0.33	0.78	1.09	2.55	0.02	0.02	0.43	0.95
	N	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D15: Day 15

1 [d - Test: Dunnett 2 Sided p < 0.05] 2 [dd - Test: Dunnett 2 Sided p < 0.01]

[Fig. 84]

Sex: Male

		Ratio to baseline (pretest, %)																			
	Animal	T cell				CD4 T cell				CD8 T cell				Regulatory T cell				NK cell			
Group	No.	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15
0	CJ1M01	1.00	1.16	1.16	1.08	1.00	1.25	1.24	1.18	1.00	1.01	1.06	0.89	1.00	1.15	1.15	1.23	1.00	0.87	0.90	0.97
mg/kg/day	CJ1M02	1.00	0.99	0.97	0.80	1.00	1.08	0.93	0.70	1.00	0.89	1.00	0.85	1.00	1.13	1.00	0.88	1.00	0.60	1.11	1.67
	CJ1M03	1.00	1.28	1.10	0.93	1.00	1.41	1.09	0.81	1.00	1.20	1.17	1.10	1.00	1.31	1.00	1.15	1.00	0.52	0.91	1.12
	Mean	1.00	1.14	1.08	0.94	1.00	1.25	1.09	0.90	1.00	1.03	1.08	0.95	1.00	1.20	1.05	1.09	1.00	0.66	0.97	1.25
	S.D.	0.00	0.15	0.10	0.14	0.00	0.17	0.16	0.25	0.00	0.16	0.09	0.13	0.00	0.10	0.09	0.18	0.00	0.18	0.12	0.37
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	CJ2M01	1.00	1.38	0.97	0.95	1.00	2.21	0.91	0.68	1.00	0.78	1.00	1.10	1.00	4.40	2.00	2.00	1.00	0.17	1.08	1.14
mg/kg/day	CJ2M02	1.00	1.44	1.05	0.98	1.00	1.81	1.07	0.85	1.00	1.14	1.03	1.03	1.00	4.50	3.25	2.00	1.00	0.23	1.06	1.26
	CJ2M03	1.00	1.28	1.03	0.97	1.00	1.80	0.94	1.00	1.00	1.04	1.07	0.96	1.00	4.38	3.13	3.25	1.00	0.36	0.94	1.12
															dd[2]	d[1]			d[1]		
	Mean	1.00	1.37	1.02	0.97	1.00	1.94	0.97	0.84	1.00	0.99	1.03	1.03	1.00	4.43	2.79	2.42	1.00	0.25	1.03	1.17
	S.D.	0.00	0.08	0.04	0.02	0.00	0.23	0.09	0.16	0.00	0.19	0.04	0.07	0.00	0.06	0.69	0.72	0.00	0.10	0.08	0.08
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	CJ3M01	1.00	1.17	1.06	1.01	1.00	2.15	1.20	1.18	1.00	0.79	1.04	1.01	1.00	5.13	3.75	3.13	1.00	0.38	0.82	0.88
mg/kg/day	CJ3M02	1.00	1.33	1.00	1.01	1.00	1.54	0.74	0.90	1.00	1.25	1.08	0.96	1.00	4.67	2.67	3.17	1.00	0.24	0.93	0.96
	CJ3M03	1.00	1.28	1.09	1.05	1.00	2.43	1.24	1.47	1.00	0.75	1.00	0.84	1.00	7.60	4.60	5.90	1.00	0.17	0.61	0.94
							d[1]								dd[2]	dd[2]	d[1]		d[1]		
	Mean	1.00	1.26	1.05	1.02	1.00	2.04	1.06	1.18	1.00	0.93	1.04	0.94	1.00	5.80	3.67	4.07	1.00	0.26	0.79	0.93
	S.D.	0.00	0.08	0.05	0.02	0.00	0.46	0.28	0.29	0.00	0.28	0.04	0.09	0.00	1.58	0.97	1.59	0.00	0.11	0.16	0.04
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D3, D8 and D15: Days 3, 8 (prior to dosing) and 15

1 [d - Test: Dunnett 2 Sided p < 0.05] 2 [dd - Test: Dunnett 2 Sided p < 0.01]

[Fig. 85]

Sex: Male

		Ratio in each cell type (%)																									
	Animal	Ki67 + T cell				Ki67 + CD4 T cell				Ki67 + CD8 T cell				Ki67 + Treg				Ki67 + ICOS + Treg				ICOS + Treg				Ki67 + NK cell			
Group	No.	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15
0	CJ1M01	9.6	7.6	9.1	10.3	9.3	5.8	7.7	7.9	6.3	5.8	7.2	9.2	30.6	23.4	23.9	23.2	28.5	20.7	20.3	21.1	54.9	40.6	37.5	38.2	7.0	19.0	16.3	9.6
mg/kg/day	CJ1M02	10.9	8.2	7.6	10.0	7.5	7.1	7.3	10.7	11.7	7.3	6.5	7.5	14.7	16.8	28.0	24.4	13.8	13.6	24.7	20.3	23.3	17.6	32.9	25.4	20.6	9.6	11.2	8.5
	CJ1M03	10.1	7.2	12.6	16.8	7.5	5.3	7.2	15.1	12.3	8.2	17.2	16.6	34.5	24.1	28.7	46.8	29.4	20.1	25.5	38.2	40.8	29.5	32.1	46.2	15.4	15.7	39.9	25.4
	Mean	10.2	7.7	9.8	12.4	8.1	6.1	7.4	11.2	10.1	7.1	10.3	11.1	26.6	21.4	26.9	31.5	23.9	18.1	23.5	26.5	39.7	29.2	34.2	36.6	14.3	14.8	22.5	14.5
	S.D.	0.7	0.5	2.6	3.8	1.0	0.9	0.3	3.6	3.3	1.2	6.0	4.8	10.5	4.0	2.6	13.3	8.8	3.9	2.8	10.1	15.8	11.5	2.9	10.5	6.9	4.8	15.3	9.5
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	CJ2M01	10.4	16.9	40.5	30.5	9.6	13.3	16.2	15.2	10.2	21.5	53.3	34.4	40.4	41.9	68.9	62.0	35.5	33.4	51.5	45.2	49.5	42.4	57.0	52.0	8.6	27.8	54.1	45.3
mg/kg/day	CJ2M02	6.6	14.6	36.9	24.6	5.4	13.7	22.7	11.5	5.2	13.3	46.4	28.4	29.2	39.2	65.0	43.4	25.0	32.0	55.4	30.8	41.7	39.4	65.1	42.0	11.5	39.8	58.6	52.3
	CJ2M03	9.9	13.4	48.9	31.6	11.7	11.7	28.6	17.2	8.1	13.4	54.6	36.3	39.4	28.2	61.7	49.8	32.3	22.8	40.4	25.3	39.4	28.4	45.6	28.1	8.4	13.1	57.1	45.5
			dd[1]	dd[1]	dd[1]		d[2]	dd[1]			d[2]	dd[1]	dd[1]		d[2]	dd[1]				dd[1]				d[2]				d[2]	d[2]
	Mean	9.0	15.0	42.1	28.9	8.9	12.9	22.5	14.6	7.8	16.1	51.4	33.0	36.3	36.4	65.2	51.7	30.9	29.4	49.1	33.8	43.5	36.7	55.9	40.7	9.5	26.9	56.6	47.7
	S.D.	2.1	1.8	6.2	3.8	3.2	1.1	6.2	2.9	2.5	4.7	4.4	4.1	6.2	7.3	3.6	9.4	5.4	5.8	7.8	10.3	5.3	7.4	9.8	12.0	1.7	13.4	2.3	4.0
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	CJ3M01	11.4	20.8	51.0	32.9	12.3	16.8	32.8	18.4	9.6	23.7	56.5	36.9	41.9	39.2	68.8	54.5	37.6	28.7	51.3	22.0	52.6	33.4	58.0	27.9	6.0	12.6	42.2	24.0
mg/kg/day	CJ3M02	9.1	21.7	57.2	29.5	8.9	23.1	35.7	13.3	7.5	17.9	63.2	32.5	35.3	51.8	68.3	47.4	32.4	38.3	54.5	31.2	48.8	47.4	67.9	44.2	12.7	34.2	57.8	44.0
	CJ3M03	9.8	23.0	54.6	32.3	10.6	23.6	28.0	20.4	8.3	19.4	65.3	37.1	38.7	45.3	66.5	61.4	35.5	29.2	42.5	36.6	53.7	36.0	49.1	48.4	10.8	32.6	77.5	51.2
			dd[1]	dd[1]	dd[1]		dd[1]	dd[1]			dd[1]	dd[1]	dd[1]		dd[1]	dd[1]			d[2]	dd[1]				d[2]				d[2]	d[2]
	Mean	10.1	21.8	54.3	31.6	10.6	21.2	32.2	17.4	8.5	20.3	61.7	35.5	38.6	45.4	67.9	54.4	35.2	32.1	49.4	29.9	51.7	38.9	58.3	40.2	9.8	26.5	59.2	39.7
	S.D.	1.2	1.1	3.1	1.8	1.7	3.8	3.9	3.7	1.1	3.0	4.6	2.6	3.3	6.3	1.2	7.0	2.6	5.4	6.2	7.4	2.6	7.4	9.4	10.8	3.5	12.0	17.7	14.1
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D3, D8 and D15: Days 3, 8 (prior to dosing) and 15 Treg: Regulatory T cell

1 [dd - Test: Dunnett 2 Sided $p < 0.01$]

2 [d - Test: Dunnett 2 Sided $p < 0.05$]

[Fig. 86]

Sex: Male

Group	Animal No.	Absolute count in each cell type (10^3/μL)													
		Ki67 + T cell		Ki67 + CD4 T cell		Ki67 + CD8 T cell		Ki67 + Treg		Ki67 + ICOS + Treg		ICOS + Treg		Ki67 + NK cell	
		Pre	D15	Pre	D15	Pre	D15	Pre	D15	Pre	D15	Pre	D15	Pre	D15
0 mg/kg/day	CJ1M01	0.35	0.31	0.19	0.15	0.07	0.07	0.03	0.02	0.03	0.02	0.05	0.03	0.20	0.20
	CJ1M02	0.49	0.28	0.13	0.10	0.27	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.24	0.13
	CJ1M03	0.22	0.33	0.08	0.13	0.11	0.15	0.02	0.03	0.01	0.02	0.02	0.03	0.19	0.34
	Mean	0.35	0.31	0.13	0.13	0.15	0.11	0.02	0.02	0.02	0.02	0.03	0.02	0.21	0.22
	S.D.	0.14	0.03	0.06	0.03	0.11	0.04	0.01	0.01	0.01	0.01	0.02	0.01	0.03	0.11
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5 mg/kg/day	CJ2M01	0.33	1.71	0.12	0.24	0.16	1.15	0.02	0.12	0.02	0.09	0.02	0.10	0.11	1.26
	CJ2M02	0.25	1.32	0.09	0.23	0.09	0.75	0.02	0.07	0.02	0.05	0.03	0.07	0.23	1.86
	CJ2M03	0.32	1.80	0.11	0.29	0.16	1.27	0.02	0.12	0.01	0.06	0.02	0.07	0.08	0.93
					d[1]				dd[2]		d[1]		d[1]		d[1]
	Mean	0.30	1.61	0.11	0.25	0.14	1.06	0.02	0.10	0.02	0.07	0.02	0.08	0.14	1.35
	S.D.	0.04	0.26	0.02	0.03	0.04	0.27	0.00	0.03	0.01	0.02	0.01	0.02	0.08	0.47
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10 mg/kg/day	CJ3M01	0.66	3.08	0.16	0.44	0.35	2.18	0.03	0.19	0.03	0.07	0.04	0.09	0.09	0.48
	CJ3M02	0.37	3.45	0.10	0.39	0.18	2.15	0.01	0.16	0.01	0.11	0.02	0.15	0.13	1.19
	CJ3M03	0.23	1.20	0.07	0.29	0.12	0.70	0.01	0.18	0.01	0.11	0.02	0.15	0.06	0.42
			d[1]		dd[2]		d[1]		dd[2]		dd[2]		dd[2]		
	Mean	0.42	2.58	0.11	0.37	0.22	1.68	0.02	0.18	0.02	0.10	0.03	0.13	0.09	0.70
	S.D.	0.22	1.21	0.05	0.08	0.12	0.85	0.01	0.02	0.01	0.02	0.01	0.03	0.04	0.43
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D15: Day 15 Treg: Regulatory T cell

1 [d - Test: Dunnett 2 Sided p < 0.05]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

[Fig. 87]

Sex: Male

		Ratio to baseline (pretest, %)																											
	Animal	Ki67 + T cell				Ki67 + CD4 T cell				Ki67 + CD8 T cell				Ki67 + Treg				Ki67 + ICOS + Treg				ICOS + Treg				Ki67 + NK cell			
Group	No.	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15	Pre	D3	D8	D15
0	CJ1M01	1.00	0.79	0.95	1.07	1.00	0.62	0.83	0.85	1.00	0.92	1.14	1.46	1.00	0.76	0.78	0.76	1.00	0.73	0.71	0.74	1.00	0.74	0.68	0.70	1.00	2.71	2.33	1.37
mg/kg/day	CJ1M02	1.00	0.75	0.70	0.92	1.00	0.95	0.97	1.43	1.00	0.62	0.56	0.64	1.00	1.14	1.90	1.66	1.00	0.99	1.79	1.47	1.00	0.76	1.41	1.09	1.00	0.47	0.54	0.41
	CJ1M03	1.00	0.71	1.25	1.66	1.00	0.71	0.96	2.01	1.00	0.67	1.40	1.35	1.00	0.70	0.83	1.36	1.00	0.68	0.87	1.30	1.00	0.72	0.79	1.13	1.00	1.02	2.59	1.65
	Mean	1.00	0.75	0.97	1.22	1.00	0.76	0.92	1.43	1.00	0.74	1.03	1.15	1.00	0.87	1.17	1.26	1.00	0.80	1.12	1.17	1.00	0.74	0.96	0.97	1.00	1.40	1.82	1.14
	S.D.	0.00	0.04	0.28	0.39	0.00	0.17	0.08	0.58	0.00	0.16	0.43	0.45	0.00	0.24	0.63	0.46	0.00	0.17	0.58	0.38	0.00	0.02	0.39	0.24	0.00	1.17	1.12	0.65
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	CJ2M01	1.00	1.63	3.89	2.93	1.00	1.39	1.69	1.58	1.00	2.11	5.23	3.37	1.00	1.04	1.71	1.53	1.00	0.94	1.45	1.27	1.00	0.86	1.15	1.05	1.00	3.23	6.29	5.27
mg/kg/day	CJ2M02	1.00	2.21	5.59	3.73	1.00	2.54	4.20	2.13	1.00	2.56	8.92	5.46	1.00	1.34	2.23	1.49	1.00	1.28	2.22	1.23	1.00	0.94	1.56	1.01	1.00	3.46	5.10	4.55
	CJ2M03	1.00	1.35	4.94	3.19	1.00	1.00	2.44	1.47	1.00	1.65	6.74	4.48	1.00	0.72	1.57	1.26	1.00	0.71	1.25	0.78	1.00	0.72	1.16	0.71	1.00	1.56	6.80	5.42
			d[1]	dd[2]	dd[2]						dd[2]	dd[2]	dd[2]															dd[2]	dd[2]
	Mean	1.00	1.73	4.81	3.28	1.00	1.64	2.78	1.73	1.00	2.11	6.96	4.44	1.00	1.03	1.84	1.43	1.00	0.98	1.64	1.09	1.00	0.84	1.29	0.92	1.00	2.75	6.06	5.08
	S.D.	0.00	0.44	0.86	0.41	0.00	0.80	1.29	0.35	0.00	0.46	1.86	1.05	0.00	0.31	0.35	0.15	0.00	0.29	0.51	0.27	0.00	0.11	0.23	0.19	0.00	1.04	0.87	0.47
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	CJ3M01	1.00	1.82	4.47	2.89	1.00	1.37	2.67	1.50	1.00	2.47	5.89	3.84	1.00	0.94	1.64	1.30	1.00	0.76	1.36	0.59	1.00	0.63	1.10	0.53	1.00	2.10	7.03	4.00
mg/kg/day	CJ3M02	1.00	2.38	6.29	3.24	1.00	2.60	4.01	1.49	1.00	2.39	8.43	4.33	1.00	1.47	1.93	1.34	1.00	1.18	1.68	0.96	1.00	0.97	1.39	0.91	1.00	2.69	4.55	3.46
	CJ3M03	1.00	2.35	5.57	3.30	1.00	2.23	2.64	1.92	1.00	2.34	7.87	4.47	1.00	1.17	1.72	1.59	1.00	0.82	1.20	1.03	1.00	0.67	0.91	0.90	1.00	3.02	7.18	4.74
			dd[2]	dd[2]	dd[2]			d[1]			dd[2]	dd[2]	dd[2]															dd[2]	dd[2]
	Mean	1.00	2.18	5.44	3.14	1.00	2.07	3.11	1.64	1.00	2.40	7.40	4.21	1.00	1.19	1.76	1.41	1.00	0.92	1.41	0.86	1.00	0.76	1.13	0.78	1.00	2.60	6.25	4.07
	S.D.	0.00	0.32	0.92	0.22	0.00	0.63	0.78	0.25	0.00	0.07	1.33	0.33	0.00	0.27	0.15	0.16	0.00	0.23	0.24	0.24	0.00	0.19	0.24	0.22	0.00	0.47	1.48	0.64
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

General Footnote: Pre: Pretest D3, D8 and D15: Days 3, 8 (prior to dosing) and 15 Treg: Regulatory T cell

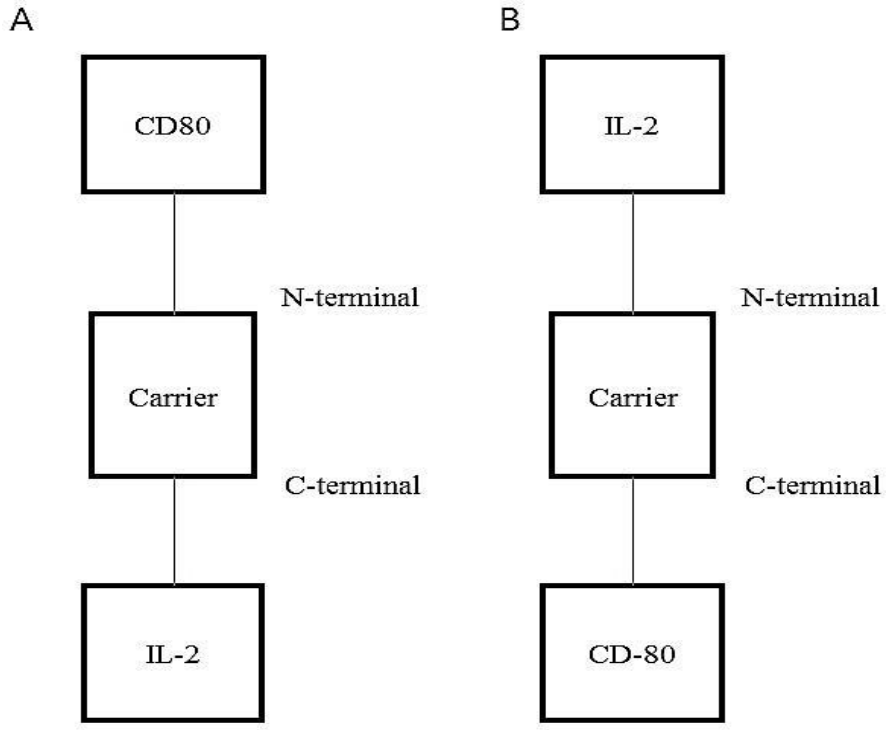
1 [d - Test: Dunnett 2 Sided $p < 0.05$] 2 [dd - Test: Dunnett 2 Sided $p < 0.01$]

[Fig. 88]

Day(s): 15 Relative to Start Date

Sex: Male		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Liver (g)	Mean	41.5	42.7	42.1
	S.D.	0.9	7.1	5.6
	N	3	3	3
Liver (%)	Mean	1.73	1.68	1.86
	S.D.	0.09	0.10	0.18
	N	3	3	3
Spleen (g)	Mean	1.727	3.358	3.886 d¹
	S.D.	0.520	0.758	1.087
	N	3	3	3
Spleen (%)	Mean	0.072	0.132	0.171 d¹
	S.D.	0.023	0.025	0.040
	N	3	3	3
Pancreas (g)	Mean	4.27	4.27	3.24
	S.D.	0.54	1.20	0.38
	N	3	3	3

[Fig. 89]



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 65                  70                  75                  80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
                 85                  90                  95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205
```

<210> 3
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> hinge

<400> 3

```
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
  1               5                  10                  15

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro
```

```
            20                  25                  30


<210>    4
<211>    216
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    immunoglobulin fc


<400>    4
Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
  1               5                  10                  15

Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
             20                  25                  30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
         35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
     50                  55                  60

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
 65                  70                  75                  80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                 85                  90                  95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Leu Gly
```

210 215

<210> 5
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 5
Gly Gly Gly Gly Ser
1 5

<210> 6
<211> 133
<212> PRT
<213> Artificial Sequence

<220>
<223> hIL-2M

<400> 6
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
50 55 60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 7
<211> 617
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein comprising variants of IL-2 and fragments of CD80

<400> 7

```
Met Asp Ala Met Leu Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

Ala Val Phe Val Ser Pro Ser His Ala Val Ile His Val Thr Lys Glu
             20                  25                  30

Val Lys Glu Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu
         35                  40                  45

Glu Leu Ala Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val
     50                  55                  60

Leu Thr Met Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn
 65                  70                  75                  80

Arg Thr Ile Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala
                 85                  90                  95

Leu Arg Pro Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr
            100                 105                 110

Glu Lys Asp Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser
        115                 120                 125

Val Lys Ala Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro
    130                 135                 140

Thr Ser Asn Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro
145                 150                 155                 160

Glu Pro His Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile
                165                 170                 175

Asn Thr Thr Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser
            180                 185                 190

Ser Lys Leu Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu
        195                 200                 205

Ile Lys Tyr Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr
    210                 215                 220
```

```
Thr Lys Gln Glu His Phe Pro Asp Asn Gly Ser Gly Gly Gly Gly Ser
225                 230                 235                 240

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Glu Ser Lys Tyr Gly
                245                 250                 255

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
            260                 265                 270

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg
        275                 280                 285

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
    290                 295                 300

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
305                 310                 315                 320

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
                325                 330                 335

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
            340                 345                 350

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
        355                 360                 365

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
    370                 375                 380

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
385                 390                 395                 400

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                405                 410                 415

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
            420                 425                 430

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
        435                 440                 445

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Leu His Glu Ala
    450                 455                 460

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly
465                 470                 475                 480

Gly Gly Gly Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
                485                 490                 495

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
```

```
            500                 505                 510

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe
        515                 520                 525

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
    530                 535                 540

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
545                 550                 555                 560

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
                565                 570                 575

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
            580                 585                 590

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
        595                 600                 605

Cys Gln Ser Ile Ile Ser Thr Leu Thr
    610                 615


<210>    8
<211>    1857
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    nucleotiedes coding fusion protein (GI101)


<400>    8
atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg         60

tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc        120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa        180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac        240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct        300

gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag        360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac        420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct        480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg        540

tctcaggacc ccgaaaccga gctgtacgct gtgtcctcca agctggactt caacatgacc        600
```

accaaccaca gcttcatgtg cctgattaag tacggccacc tgagagtgaa ccagaccttc 660

aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct 720

ggcggaggtg gaagcggagg cggaggatct gctgagtcta agtatggccc tccttgtcct 780

ccatgtcctg ctccagaagc tgctggcgga ccctctgtgt tcctgtttcc tccaaagcct 840

aaggaccagc tcatgatctc tcggacaccc gaagtgacct gcgtggtggt ggatgtgtct 900

caagaggacc ctgaggtgca gttcaattgg tacgtggacg gcgtggaagt gcacaacgcc 960

aagaccaagc ctagagagga acagttcaac tccacctaca gagtggtgtc cgtgctgacc 1020

gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctagatgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc tcttggaggt 1440

ggtggcggtt ctgcccctac cagctcctct accaagaaaa cccagctcca gttggagcat 1500

ctgctgctgg acctccagat gattctgaac gggatcaaca actataagaa ccccaagctg 1560

accgccatgc tgaccgctaa gttctacatg cccaagaagg ccaccgagct gaagcacctc 1620

cagtgcctgg aagaagaact gaagcccctg gaagaggtgc tgaatctggc ccagtccaag 1680

aacttccacc tgaggccacg ggacctgatc agcaacatca acgtgatcgt gctggaactg 1740

aagggctccg agacaacctt tatgtgcgag tacgccgacg agacagccac catcgtggaa 1800

tttctgaacc ggtggatcac cttctgccag agcatcatct ccacactgac ctgatga 1857

<210> 9
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI101)

<400> 9

```
Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
  1               5                  10                  15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
             20                  25                  30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
         35                  40                  45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
     50                  55                  60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
 65                  70                  75                  80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
                 85                  90                  95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
```

```
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430

Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
    450                 455                 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465                 470                 475                 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
                485                 490                 495

Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
            500                 505                 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
        515                 520                 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
    530                 535                 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545                 550                 555                 560
```

```
Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
                565                 570                 575

Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
            580                 585                 590
```

<210> 10
<211> 133
<212> PRT
<213> Artificial Sequence

<220>
<223> hIL-2

<400> 10

```
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
  1               5                  10                  15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
             20                  25                  30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
         35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
     50                  55                  60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
 65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                 85                  90                  95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130
```

<210> 11
<211> 288
<212> PRT
<213> Artificial Sequence

<220>

<223> CD80

<400> 11
Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
  1   5   10   15

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
             20   25   30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
         35   40   45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
     50   55   60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
 65   70   75   80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
                 85   90   95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
            100   105   110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
        115   120   125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
    130   135   140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
145   150   155   160

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
                165   170   175

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
            180   185   190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
        195   200   205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
    210   215   220

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
225   230   235   240

Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
                245   250   255

Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala Pro Arg Cys Arg

```
            260                 265                 270

Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
        275                 280                 285
```

<210> 12
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> modified Fc

<400> 12

```
Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys
  1               5                  10                  15

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
             20                  25                  30

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
         35                  40                  45

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
     50                  55                  60

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 65                  70                  75                  80

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                 85                  90                  95

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            100                 105                 110

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        115                 120                 125

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    130                 135                 140

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
145                 150                 155                 160

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                165                 170                 175

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            180                 185                 190
```

```
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        195                 200                 205

Leu Ser Leu Ser Leu Gly Lys
    210                 215
```

<210> 13
<211> 306
<212> PRT
<213> Artificial Sequence

<220>
<223> mCD80

<400> 13

```
Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
  1               5                  10                  15

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
             20                  25                  30

Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
         35                  40                  45

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
     50                  55                  60

Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
 65                  70                  75                  80

Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
                 85                  90                  95

Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
            100                 105                 110

Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
        115                 120                 125

Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
    130                 135                 140

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
145                 150                 155                 160

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
                165                 170                 175

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
            180                 185                 190
```

```
Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
        195                 200                 205

Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
    210                 215                 220

Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
225                 230                 235                 240

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
                245                 250                 255

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
            260                 265                 270

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
        275                 280                 285

Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
    290                 295                 300

Phe Leu
305
```

<210> 14
<211> 1848
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (mGI101)

<400> 14

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atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg        60

tctccttctc acgctgtgga cgagcagctc tccaagtccg tgaaggataa ggtcctgctg       120

ccttgccggt acaactctcc tcacgaggac gagtctgagg accggatcta ctggcagaaa       180

cacgacaagg tggtgctgtc cgtgatcgcc ggaaagctga aagtgtggcc tgagtacaag       240

aacaggaccc tgtacgacaa caccacctac agcctgatca tcctgggcct cgtgctgagc       300

gatagaggca cctattcttg cgtggtgcag aagaaagagc ggggcaccta cgaagtgaag       360

cacctggctc tggtcaagct gtccatcaag gccgacttca gcacccctaa catcaccgag       420

tctggcaacc cttccgccga caccaagaga atcacctgtt tcgcctctgg cggcttccct       480

aagcctcggt tctcttggct ggaaaacggc agagagctgc ccggcatcaa taccaccatt       540
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tctcaggacc cagagtccga gctgtacacc atctccagcc agctcgactt taacaccacc 600

agaaaccaca ccatcaagtg cctgattaag tacggcgacg cccacgtgtc cgaggacttt 660

acttgggaga aacctcctga ggaccctcct gactctggat ctggcggcgg aggttctggc 720

ggaggtggaa gcggaggcgg aggatctgct gagtctaagt atggccctcc ttgtcctcca 780

tgtcctgctc cagaagctgc tggcggaccc tctgtgttcc tgtttcctcc aaagcctaag 840

gaccagctca tgatctctcg gacccctgaa gtgacctgcg tggtggtgga tgtgtctcaa 900

gaggaccctg aggtgcagtt caattggtac gtggacggcg tggaagtgca caacgccaag 960

accaagccta gagaggaaca gttcaactcc acctatagag tggtgtccgt gctgaccgtg 1020

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gtcaagggct tctacccttc cgacattgcc gtggaatggg agtccaatgg ccagcctgag 1260

aacaactaca agaccacacc tcctgtgctg gactccgacg gctccttctt tctgtactct 1320

cgcctgaccg tggacaagtc taggtggcaa gagggcaacg tgttctcctg ctctgtgctg 1380

cacgaggctc tgcacaacca ctacacccag aagtccctgt ctctgtctct tggaggtggt 1440

ggcggttctg cccctacctc cagctctacc aagaaaaccc agctccagtt ggagcatctg 1500

ctgctggacc tccagatgat cctgaatggc atcaacaatt acaagaaccc caagctgacc 1560

gccatgctga ccgctaagtt ctacatgccc aagaaggcca ccgagctgaa gcacttgcag 1620

tgcctggaag aggaactgaa gcccctggaa gaagtgctga atctggccca gtccaagaac 1680

ttccacctga ggcctaggga cctgatctcc aacatcaacg tgatcgtgct ggaactgaaa 1740

ggctccgaga caaccttcat gtgcgagtac gccgacgaga cagccaccat cgtggaattt 1800

ctgaaccggt ggatcacctt ctgccagagc atcatctcca cactgacc 1848

<210> 15
<211> 616
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (mGI101)

<400> 15

```
Met Asp Ala Met Leu Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

Ala Val Phe Val Ser Pro Ser His Ala Val Asp Glu Gln Leu Ser Lys
             20                  25                  30

Ser Val Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His
         35                  40                  45

Glu Asp Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val
     50                  55                  60

Val Leu Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys
 65                  70                  75                  80

Asn Arg Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly
                 85                  90                  95

Leu Val Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys
            100                 105                 110

Glu Arg Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser
        115                 120                 125

Ile Lys Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro
    130                 135                 140

Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro
145                 150                 155                 160

Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile
                165                 170                 175

Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser
            180                 185                 190

Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu
        195                 200                 205

Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys
    210                 215                 220

Pro Pro Glu Asp Pro Pro Asp Ser Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Glu Ser Lys Tyr Gly Pro
                245                 250                 255

Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
            260                 265                 270
```

```
Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr
        275                 280                 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
    290                 295                 300

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305                 310                 315                 320

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
                325                 330                 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
            340                 345                 350

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
        355                 360                 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
    370                 375                 380

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385                 390                 395                 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
                405                 410                 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
            420                 425                 430

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
        435                 440                 445

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu
    450                 455                 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly Gly
465                 470                 475                 480

Gly Gly Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln
                485                 490                 495

Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn
            500                 505                 510

Asn Tyr Lys Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Tyr
        515                 520                 525

Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu
    530                 535                 540

Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn
```

```
545                 550                 555                 560

Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
                565                 570                 575

Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
            580                 585                 590

Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys
        595                 600                 605

Gln Ser Ile Ile Ser Thr Leu Thr
    610                 615
```

<210> 16
<211> 1437
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI101C1)

<400> 16

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atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg        60

tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc       120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa       180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac       240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct       300

gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag       360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac       420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct       480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg       540

tctcaggacc ccgaaaccga gctgtacgct gtgtcctcca agctggactt caacatgacc       600

accaaccaca gcttcatgtg cctgattaag tacggccacc tgagagtgaa ccagaccttc       660

aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct       720

ggcggaggtg gaagcggagg cggaggatct gctgagtcta agtatggccc tccttgtcct       780

ccatgtcctg ctccagaagc tgctggcgga ccctctgtgt tcctgtttcc tccaaagcct       840
```

aaggaccagc tcatgatctc tcggacaccc gaagtgacct gcgtggtggt ggatgtgtct 900

caagaggacc ctgaggtgca gttcaattgg tacgtggacg gcgtggaagt gcacaacgcc 960

aagaccaagc ctagagagga acagttcaac tccacctaca gagtggtgtc cgtgctgacc 1020

gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctaggtgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc cctgggc 1437

<210> 17
<211> 454
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI101C1)

<400> 17

```
Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
  1               5                  10                  15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
             20                  25                  30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
         35                  40                  45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
     50                  55                  60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
 65                  70                  75                  80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
                 85                  90                  95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
```

```
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400
```

```
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430

Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly
    450
```

<210> 18
<211> 1176
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI101C2)

<400> 18

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atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg         60

tctccatctc acgccgctga gtctaagtac ggccctcctt gtcctccatg tcctgctcca        120

gaagctgctg gcggaccctc tgtgttcctg tttcctccaa agcctaagga ccagctcatg        180

atctctcgga cccctgaagt gacctgcgtg gtggtggatg tgtctcaaga ggaccctgag        240

gtgcagttca attggtacgt ggacggcgtg gaagtgcaca acgccaagac caagcctaga        300

gaggaacagt tcaactccac ctacagagtg gtgtccgtgc tgaccgtgct gcaccaggat        360

tggctgaacg gcaaagagta caagtgcaag gtgtccaaca agggcctgcc ttccagcatc        420

gaaaagacca tctccaaggc taagggccag cctagggaac cccaggttta caccctgcct        480

ccaagccaag aggaaatgac caagaaccag gtgtccctga cctgcctggt caagggcttc        540

taccttccg acattgccgt ggaatgggag tccaatggcc agcctgagaa caactacaag        600

accacacctc ctgtgctgga ctccgacggc tccttctttc tgtactctcg cctgaccgtg        660

gacaagtcta ggtggcaaga gggcaacgtg ttctcctgct ctgtgctgca cgaggccctg        720

cacaatcact acacccagaa gtccctgtct ctgtctcttg gcggaggcgg aggatctgct        780

cctacctcca gctccaccaa gaaaacccag ctccagttgg agcatctgct gctggacctc        840

cagatgatcc tgaatggcat caacaattac aagaacccca agctgaccgc catgctgacc        900
```

gctaagttct acatgcccaa gaaggccacc gagctgaagc acctccagtg cctggaagag 960

gaactgaagc ccctggaaga agtgctgaat ctggcccagt ccaagaactt ccacctgagg 1020

cctagggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaagg ctccgagaca 1080

accttcatgt gcgagtacgc cgacgagaca gccaccatcg tggaatttct gaaccggtgg 1140

atcaccttct gccagtccat catctccaca ctgacc 1176

<210> 19
<211> 367
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI101C2)

<400> 19
Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu
1 5 10 15

Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
20 25 30

Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
35 40 45

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
50 55 60

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
65 70 75 80

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
85 90 95

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
100 105 110

Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
115 120 125

Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn
130 135 140

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
145 150 155 160

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
165 170 175

```
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg
            180                 185                 190

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys
        195                 200                 205

Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    210                 215                 220

Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser Ser
225                 230                 235                 240

Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
                245                 250                 255

Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Ala
            260                 265                 270

Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys
        275                 280                 285

His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu
    290                 295                 300

Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile
305                 310                 315                 320

Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
                325                 330                 335

Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
            340                 345                 350

Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
        355                 360                 365
```

<210> 20
<211> 1434
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (mGI101C1)

<400> 20

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atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg        60

tctccttctc acgctgtgga cgagcagctc tccaagtccg tgaaggataa ggtcctgctg       120
```

ccttgccggt acaactctcc tcacgaggac gagtctgagg accggatcta ctggcagaaa 180

cacgacaagg tggtgctgtc cgtgatcgcc ggaaagctga aagtgtggcc tgagtacaag 240

aacaggaccc tgtacgacaa caccacctac agcctgatca tcctgggcct cgtgctgagc 300

gatagaggca cctattcttg cgtggtgcag aagaaagagc ggggcaccta cgaagtgaag 360

cacctggctc tggtcaagct gtccatcaag gccgacttca gcacccctaa catcaccgag 420

tctggcaacc cttccgccga caccaagaga atcacctgtt tcgcctctgg cggcttccct 480

aagcctcggt tctcttggct ggaaaacggc agagagctgc ccggcatcaa taccaccatt 540

tctcaggacc cagagtccga gctgtacacc atctccagcc agctcgactt taacaccacc 600

agaaaccaca ccatcaagtg cctgattaag tacggcgacg cccacgtgtc cgaggacttt 660

acttgggaga aacctcctga ggaccctcct gactctggat ctggcggcgg aggttctggc 720

ggaggtggaa gcggaggcgg aggatctgct gagtctaagt atggccctcc ttgtcctcca 780

tgtcctgctc cagaagctgc tggcggaccc tctgtgttcc tgtttcctcc aaagcctaag 840

gaccagctca tgatctctcg gacccctgaa gtgacctgcg tggtggtgga tgtgtctcaa 900

gaggaccctg aggtgcagtt caattggtac gtggacggcg tggaagtgca caacgccaag 960

accaagccta gagaggaaca gttcaactcc acctatagag tggtgtccgt gctgaccgtg 1020

ctgcaccagg attggctgaa cggcaaagag tacaagtgca aggtgtccaa caagggcctg 1080

ccttccagca tcgaaaagac catcagcaag gctaagggcc agcctaggga accccaggtt 1140

tacaccctgc ctccaagcca agaggaaatg accaagaacc aggtgtccct gacctgcctg 1200

gtcaagggct tctacccttc cgacattgcc gtggaatggg agtccaatgg ccagcctgag 1260

aacaactaca agaccacacc tcctgtgctg gactccgacg gctccttctt tctgtactct 1320

cgcctgaccg tggacaagtc taggtggcaa gagggcaacg tgttctcctg ctctgtgctg 1380

cacgaggctc tgcacaacca ctacacccag aagtccctgt ctctgtccct gggc 1434

<210> 21
<211> 478
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (mGI101C1)

<400> 21

```
Met Asp Ala Met Leu Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

Ala Val Phe Val Ser Pro Ser His Ala Val Asp Glu Gln Leu Ser Lys
             20                  25                  30

Ser Val Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His
         35                  40                  45

Glu Asp Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val
     50                  55                  60

Val Leu Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys
 65                  70                  75                  80

Asn Arg Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly
                 85                  90                  95

Leu Val Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys
            100                 105                 110

Glu Arg Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser
        115                 120                 125

Ile Lys Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro
    130                 135                 140

Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro
145                 150                 155                 160

Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile
                165                 170                 175

Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser
            180                 185                 190

Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu
        195                 200                 205

Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys
    210                 215                 220

Pro Pro Glu Asp Pro Pro Asp Ser Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Glu Ser Lys Tyr Gly Pro
                245                 250                 255

Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
            260                 265                 270
```

```
Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr
        275                 280                 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
    290                 295                 300

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305                 310                 315                 320

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
                325                 330                 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
            340                 345                 350

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
        355                 360                 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
    370                 375                 380

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385                 390                 395                 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
                405                 410                 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
            420                 425                 430

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
        435                 440                 445

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu
    450                 455                 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
465                 470                 475


<210>    22
<211>    133
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    variants of IL-2 (3M, M45)


<400>    22
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
  1               5                  10                  15
```

```
Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
             20                  25                  30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Ala Met Pro Lys
         35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
     50                  55                  60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
 65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                 85                  90                  95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130


<210>    23
<211>    133
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    variants of IL-2 (3M, M61)


<400>    23
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
  1               5                  10                  15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
             20                  25                  30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys
         35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Arg Glu Leu Lys
     50                  55                  60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
 65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                 85                  90                  95
```

```
Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130
```

<210> 24
<211> 133
<212> PRT
<213> Artificial Sequence

<220>
<223> variants of IL-2 (3M, M72)

<400> 24

```
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
  1               5                  10                  15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
             20                  25                  30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys
         35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
     50                  55                  60

Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu
 65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                 85                  90                  95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130
```

<210> 25
<211> 1851
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI102-M45)

<400> 25
atggatgcta tgctgagagg cctgtgttgc gtgctgctgc tgtgtggcgc tgtgttcgtg 60

tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc 120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa 180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac 240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct 300

gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag 360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac 420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct 480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg 540

tctcaggacc ccgaaaccga gctgtacgct gtgtcctcca agctggactt caacatgacc 600

accaaccaca gcttcatgtg cctgattaag tacggccacc tgagagtgaa ccagaccttc 660

aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct 720

ggcggaggtg gaagcggagg cggaggatct gctgagtcta agtatggccc tccttgtcct 780

ccatgtcctg ctccagaagc tgctggcgga ccctctgtgt tcctgtttcc tccaaagcct 840

aaggaccagc tcatgatctc tcggacaccc gaagtgacct gcgtggtggt ggatgtgtct 900

caagaggacc ctgaggtgca gttcaattgg tacgtggacg gcgtggaagt gcacaacgcc 960

aagaccaagc ctagagagga acagttcaac tccacctaca gagtggtgtc cgtgctgacc 1020

gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctagatgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc tcttggaggt 1440

ggtggcggtt ctgcccctac cagctcctct accaagaaaa cccagctcca gttggagcat 1500

ctgctgctgg acctccagat gattctgaac gggatcaaca actataagaa ccccaagctg 1560

accgccatgc tgaccgctaa gttcgccatg cccaagaagg ccaccgagct gaagcacctc 1620

cagtgcctgg aagaagaact gaagcccctg gaagaggtgc tgaatctggc ccagtccaag 1680

aacttccacc tgaggccacg ggacctgatc agcaacatca acgtgatcgt gctggaactg 1740

aagggctccg agacaacctt tatgtgcgag tacgccgacg agacagccac catcgtggaa 1800

tttctgaacc ggtggatcac cttctgccag agcatcatct ccacactgac c 1851

<210> 26
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI102-M45)

<400> 26
Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
1 5 10 15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
100 105 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
115 120 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
130 135 140

```
Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430
```

```
Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
    450                 455                 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465                 470                 475                 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
                485                 490                 495

Ala Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu
            500                 505                 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
        515                 520                 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
    530                 535                 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545                 550                 555                 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
                565                 570                 575

Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
            580                 585                 590
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<210> 27
<211> 1851
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI102-M61)

<400> 27

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tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc       120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa       180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac       240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct       300
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gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag 360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac 420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct 480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg 540

tctcaggacc ccgaaaccga gctgtacgct gtgtcctcca agctggactt caacatgacc 600

accaaccaca gcttcatgtg cctgattaag tacggccacc tgagagtgaa ccagaccttc 660

aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct 720

ggcggaggtg gaagcggagg cggaggatct gctgagtcta agtatggccc tccttgtcct 780

ccatgtcctg ctccagaagc tgctggcgga ccctctgtgt tcctgtttcc tccaaagcct 840

aaggaccagc tcatgatctc tcggacaccc gaagtgacct gcgtggtggt ggatgtgtct 900

caagaggacc ctgaggtgca gttcaattgg tacgtggacg gcgtggaagt gcacaacgcc 960

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gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctagatgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc tcttggaggt 1440

ggtggcggtt ctgcccctac cagctcctct accaagaaaa cccagctcca gttggagcat 1500

ctgctgctgg acctccagat gattctgaac gggatcaaca actataagaa ccccaagctg 1560

accgccatgc tgaccgctaa gttctacatg cccaagaagg ccaccgagct gaagcacctc 1620

cagtgcctgg aaagggaact gaagcccctg gaagaggtgc tgaatctggc ccagtccaag 1680

aacttccacc tgaggccacg ggacctgatc agcaacatca acgtgatcgt gctggaactg 1740

aagggctccg agacaacctt tatgtgcgag tacgccgacg agacagccac catcgtggaa 1800

tttctgaacc ggtggatcac cttctgccag agcatcatct ccacactgac c 1851

<210> 28
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI102-M61)

<400> 28

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Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
  1               5                  10                  15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
             20                  25                  30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
         35                  40                  45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
     50                  55                  60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
 65                  70                  75                  80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
                 85                  90                  95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220
```

```
Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430

Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
    450                 455                 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465                 470                 475                 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
                485                 490                 495

Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
            500                 505                 510
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Lys His Leu Gln Cys Leu Glu Arg Glu Leu Lys Pro Leu Glu Glu Val
        515                 520                 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
    530                 535                 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545                 550                 555                 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
                565                 570                 575

Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
            580                 585                 590
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<210> 29
<211> 1857
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI102-M72)

<400> 29

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tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc       120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa       180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac       240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct       300

gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag       360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac       420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct       480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg       540

tctcaggacc ccgaaaccga gctgtacgct gtgtcctcca agctggactt caacatgacc       600

accaaccaca gcttcatgtg cctgattaag tacggccacc tgagagtgaa ccagaccttc       660

aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct       720
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ggcggaggtg gaagcggagg cggaggatct gctgagtcta agtatggccc tccttgtcct 780

ccatgtcctg ctccagaagc tgctggcgga ccctctgtgt tcctgtttcc tccaaagcct 840

aaggaccagc tcatgatctc tcggacaccc gaagtgacct gcgtggtggt ggatgtgtct 900

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gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctagatgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc tcttggaggt 1440

ggtggcggtt ctgcccctac agctcctct accaagaaaa cccagctcca gttggagcat 1500

ctgctgctgg acctccagat gattctgaac gggatcaaca actataagaa ccccaagctg 1560

accgccatgc tgaccgctaa gttctacatg cccaagaagg ccaccgagct gaagcacctc 1620

cagtgcctgg aagaagaact gaagcccctg gaagaggtgc tgaatggggc ccagtccaag 1680

aacttccacc tgaggccacg ggacctgatc agcaacatca acgtgatcgt gctggaactg 1740

aagggctccg agacaacctt tatgtgcgag tacgccgacg agacagccac catcgtggaa 1800

tttctgaacc ggtggatcac cttctgccag agcatcatct ccacactgac ctgatga 1857

<210> 30
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI102-M72)

<400> 30
Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
1 5 10 15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp

```
            20                  25                  30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
         35                  40                  45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
     50                  55                  60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
 65                  70                  75                  80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
                 85                  90                  95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300
```

```
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430

Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
    450                 455                 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465                 470                 475                 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
                485                 490                 495

Ala Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
            500                 505                 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
        515                 520                 525

Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
    530                 535                 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545                 550                 555                 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
                565                 570                 575

Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
            580                 585                 590
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<210> 31
<211> 1851
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (GI101w)

<400> 31
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tctccttctc acgctgtgat ccacgtgacc aaagaagtga aagaggtcgc cacactgtcc 120

tgcggccaca acgtttcagt ggaagaactg gcccagacca ggatctactg gcagaaagaa 180

aagaaaatgg tgctgaccat gatgtccggc gacatgaaca tctggcctga gtacaagaac 240

cggaccatct tcgacatcac caacaacctg tccatcgtga ttctggccct gaggccttct 300

gatgagggca cctatgagtg cgtggtgctg aagtacgaga aggacgcctt caagcgcgag 360

cacctggctg aagtgacact gtccgtgaag gccgactttc ccacaccttc catctccgac 420

ttcgagatcc ctacctccaa catccggcgg atcatctgtt ctacctctgg cggctttcct 480

gagcctcacc tgtcttggct ggaaaacggc gaggaactga acgccatcaa caccaccgtg 540

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aactggaaca ccaccaagca agagcacttc cctgacaatg gatctggcgg cggaggttct 720

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gtgctgcacc aggattggct gaacggcaaa gagtacaagt gcaaggtgtc caacaagggc 1080

ctgccttcca gcatcgaaaa gaccatctcc aaggctaagg gccagcctag ggaaccccag 1140

gtttacaccc tgcctccaag ccaagaggaa atgaccaaga accaggtgtc cctgacctgc 1200

ctggtcaagg gcttctaccc ttccgacatt gccgtggaat gggagtccaa tggccagcct 1260

gagaacaact acaagaccac acctcctgtg ctggactccg acggctcctt ctttctgtac 1320

tctcgcctga ccgtggacaa gtctagatgg caagagggca acgtgttctc ctgctctgtg 1380

ctgcacgagg ccctgcacaa tcactacacc cagaagtccc tgtctctgtc tcttggaggt 1440

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acccgcatgc tgacctttaa gttctacatg cccaagaagg ccaccgagct gaagcacctc 1620

cagtgcctgg aagaagaact gaagcccctg gaagaggtgc tgaatctggc ccagtccaag 1680

aacttccacc tgaggccacg ggacctgatc agcaacatca acgtgatcgt gctggaactg 1740

aagggctccg agacaacctt tatgtgcgag tacgccgacg agacagccac catcgtggaa 1800

tttctgaacc ggtggatcac cttctgccag agcatcatct ccacactgac c 1851

<210> 32
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (GI101w)

<400> 32
Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
1 5 10 15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser

```
            100                 105                 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
        115                 120                 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
    130                 135                 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145                 150                 155                 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
                165                 170                 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
            180                 185                 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
        195                 200                 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Ala Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
                325                 330                 335

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380
```

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Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385                 390                 395                 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                405                 410                 415

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
            420                 425                 430

Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445

Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
    450                 455                 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465                 470                 475                 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
                485                 490                 495

Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
            500                 505                 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
        515                 520                 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
    530                 535                 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545                 550                 555                 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
                565                 570                 575

Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
            580                 585                 590
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<210> 33
<211> 1848
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotiedes coding fusion protein (mGI102-M61)

<400> 33
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tctccttctc acgctgtgga cgagcagctc tccaagtccg tgaaggataa ggtcctgctg 120

ccttgccggt acaactctcc tcacgaggac gagtctgagg accggatcta ctggcagaaa 180

cacgacaagg tggtgctgtc cgtgatcgcc ggaaagctga aagtgtggcc tgagtacaag 240

aacaggaccc tgtacgacaa caccacctac agcctgatca tcctgggcct cgtgctgagc 300

gatagaggca cctattcttg cgtggtgcag aagaaagagc ggggcaccta cgaagtgaag 360

cacctggctc tggtcaagct gtccatcaag gccgacttca gcacccctaa catcaccgag 420

tctggcaacc cttccgccga caccaagaga atcacctgtt tcgcctctgg cggcttccct 480

aagcctcggt tctcttggct ggaaaacggc agagagctgc ccggcatcaa taccaccatt 540

tctcaggacc cagagtccga gctgtacacc atctccagcc agctcgactt taacaccacc 600

agaaaccaca ccatcaagtg cctgattaag tacggcgacg cccacgtgtc cgaggacttt 660

acttgggaga aacctcctga ggaccctcct gactctggat ctggcggcgg aggttctggc 720

ggaggtggaa gcggaggcgg aggatctgct gagtctaagt atggccctcc ttgtcctcca 780

tgtcctgctc cagaagctgc tggcggaccc tctgtgttcc tgtttcctcc aaagcctaag 840

gaccagctca tgatctctcg gacccctgaa gtgacctgcg tggtggtgga tgtgtctcaa 900

gaggaccctg aggtgcagtt caattggtac gtggacggcg tggaagtgca caacgccaag 960

accaagccta gagaggaaca gttcaactcc acctatagag tggtgtccgt gctgaccgtg 1020

ctgcaccagg attggctgaa cggcaaagag tacaagtgca aggtgtccaa caagggcctg 1080

ccttccagca tcgaaaagac catcagcaag gctaagggcc agcctaggga accccaggtt 1140

tacaccctgc ctccaagcca agaggaaatg accaagaacc aggtgtccct gacctgcctg 1200

gtcaagggct tctacccttc cgacattgcc gtggaatggg agtccaatgg ccagcctgag 1260

aacaactaca agaccacacc tcctgtgctg gactccgacg gctccttctt tctgtactct 1320

cgcctgaccg tggacaagtc taggtggcaa gagggcaacg tgttctcctg ctctgtgctg 1380

cacgaggctc tgcacaacca ctacacccag aagtccctgt ctctgtctct tggaggtggt 1440

ggcggttctg cccctacctc cagctctacc aagaaaaccc agctccagtt ggagcatctg 1500

ctgctggacc tccagatgat cctgaatggc atcaacaatt acaagaaccc caagctgacc 1560

gccatgctga ccgctaagtt ctacatgccc aagaaggcca ccgagctgaa gcacttgcag 1620

tgcctggaaa gggaactgaa gcccctggaa gaagtgctga atctggccca gtccaagaac 1680

ttccacctga ggcctaggga cctgatctcc aacatcaacg tgatcgtgct ggaactgaaa 1740

ggctccgaga caaccttcat gtgcgagtac gccgacgaga cagccaccat cgtggaattt 1800

ctgaaccggt ggatcacctt ctgccagagc atcatctcca cactgacc 1848

<210> 34
<211> 616
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein (mGI102-M61)

<400> 34
Met Asp Ala Met Leu Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
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Ala Val Phe Val Ser Pro Ser His Ala Val Asp Glu Gln Leu Ser Lys
20 25 30

Ser Val Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His
35 40 45

Glu Asp Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val
50 55 60

Val Leu Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys
65 70 75 80

Asn Arg Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly
85 90 95

Leu Val Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys
100 105 110

Glu Arg Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser
115 120 125

Ile Lys Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro
130 135 140

Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro
145 150 155 160

Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile
165 170 175

Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser

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            180                 185                 190

Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu
        195                 200                 205

Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys
    210                 215                 220

Pro Pro Glu Asp Pro Pro Asp Ser Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Glu Ser Lys Tyr Gly Pro
                245                 250                 255

Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
            260                 265                 270

Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr
        275                 280                 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
    290                 295                 300

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305                 310                 315                 320

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
                325                 330                 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
            340                 345                 350

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
        355                 360                 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
    370                 375                 380

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385                 390                 395                 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
                405                 410                 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
            420                 425                 430

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
        435                 440                 445

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu
    450                 455                 460
```

```
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly Gly
465                 470                 475                 480

Gly Gly Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln
                485                 490                 495

Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn
            500                 505                 510

Asn Tyr Lys Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Tyr
        515                 520                 525

Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Arg
    530                 535                 540

Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn
545                 550                 555                 560

Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
                565                 570                 575

Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
            580                 585                 590

Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys
        595                 600                 605

Gln Ser Ile Ile Ser Thr Leu Thr
    610                 615


<210>    35
<211>    153
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    wild type hIL-2


<400>    35
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
  1               5                  10                  15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
             20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
         35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
     50                  55                  60
```

```
Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
 65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145                 150


<210>    36
<211>    158
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    IL-2 with signal sequence


<400>    36
Met Asp Ala Met Leu Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

Ala Val Phe Val Ser Pro Ser His Ala Ala Pro Thr Ser Ser Ser Thr
             20                  25                  30

Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met
         35                  40                  45

Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met
     50                  55                  60

Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His
 65                  70                  75                  80

Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn
                85                  90                  95

Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser
            100                 105                 110

Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe
        115                 120                 125
```

```
Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn
    130                 135                 140

Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
145                 150                 155
```

<210> 37
<211> 474
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence coding IL-2 with signal sequence

<400> 37

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tctccttctc acgctgcccc taccagctcc tctaccaaga aaacccagct ccagttggag        120

catctgctgc tggacctcca gatgattctg aacgggatca acaactataa gaaccccaag        180

ctgacccgca tgctgacctt taagttctac atgcccaaga aggccaccga gctgaagcac        240

ctccagtgcc tggaagaaga actgaagccc ctggaagagg tgctgaatct ggcccagtcc        300

aagaacttcc acctgaggcc acgggacctg atcagcaaca tcaacgtgat cgtgctggaa        360

ctgaagggct ccgagacaac ctttatgtgc gagtacgccg acgagacagc caccatcgtg        420

gaatttctga accggtggat caccttctgc cagagcatca tctccacact gacc              474
```