

Claims

1. A method for increasing a lymphocyte count in a subject in need thereof, comprising administering
- (i) a modified interleukin-7 of the following formula (I):
- A - IL-7 formula (I)
- wherein A is an oligopeptide consisting of 1 to 10 amino acid residues, and
the IL-7 is a polypeptide which is capable of binding to IL-7 receptor; or
- (ii) an interleukin-7 fusion protein comprising
- (a) the modified interleukin-7,
- (b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and
- (c) a third domain which prolongs the half-life of the interleukin-7 fusion protein,
to the subject at a dose of greater than about 600 µg/kg.
2. The method of claim 1, wherein the subject is suffering from a cancer; infection; chronic failure of the right ventricle of the heart; Hodgkin's disease; a leak or rupture in the thoracic duct; side effects of prescription medications including anticancer agents (*e.g.*, chemotherapy), antiviral agents, or glucocorticoids; malnutrition resulting from diets that are low in protein, radiation therapy, uremia, autoimmune disorders, immune deficiency syndromes, thymectomy, or a combination thereof; or idiopathic, acute radiation syndrome(ARS) or a combination thereof.
3. The method of claim 1 or 2, the IL-7 has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 6.

4. The method of claim 3, wherein the A is linked to the N-terminal of the IL-7.
5. The method of claim 3, wherein A is methionine, glycine, methionine-methionine, glycine-glycine, methionine-glycine, glycine-methionine, methionine-methionine-methionine, methionine-methionine-glycine, methionine-glycine-methionine, glycine-methionine-methionine, methionine-glycine-glycine, glycine-methionine-glycine, glycine-glycine-methionine, or glycine-glycine-glycine.
6. The method of claim 5, wherein the third domain is linked to the N-terminal or C-terminal of the first domain or the second domain.
7. The method of any one of claims 4-6, wherein the third domain is any one selected from the group consisting of an Fc region of immunoglobulin or a part thereof, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, and a combination thereof.
8. The method of claim 7, wherein the third domain comprises an Fc region of a modified immunoglobulin.
9. The method of claim 8, wherein the modified immunoglobulin is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE and a combination thereof.

10. The method of claim 9, wherein the Fc region of the modified immunoglobulin comprises a hinge region, a CH2 domain, and a CH3 domain from the N-terminal to the C-terminal direction,

wherein the hinge region comprises a human IgD hinge region,

the CH2 domain comprises a part of the amino acid residues of CH2 domain of human IgD and human IgG4, and

the CH3 domain comprises a part of the amino acid residues of the human IgG4 CH3 domain.

11. The method of claim 10, wherein the Fc region of the modified immunoglobulin is represented by the following Formula (I):

Formula (I)

$N'-(Z1)_p-Y-Z2-Z3-Z4-C'$

wherein N' is the N-terminal of a polypeptide and C' is the C-terminal of a polypeptide;

p is an integer of 0 or 1;

Z1 is an amino acid sequence having 5 to 9 consecutive amino acid residues from the amino acid residue at position 98 toward the N-terminal, among the amino acid residues at positions from 90 to 98 of SEQ ID NO: 7;

Y is an amino acid sequence having 5 to 64 consecutive amino acid residues from the amino acid residue at position 162 toward the N-terminal, among the amino acid residues at positions from 99 to 162 of SEQ ID NO: 7;

Z2 is an amino acid sequence having 4 to 37 consecutive amino acid residues from the amino acid residue at position 163 toward the C-terminal, among the amino acid residues at positions from 163 to 199 of SEQ ID NO: 7;

Z3 is an amino acid sequence having 71 to 106 consecutive amino acid residues from the amino acid residue at position 220 toward the N-terminal, among the amino acid residues at positions from 115 to 220 of SEQ ID NO: 8; and

Z4 is an amino acid sequence having 80 to 107 consecutive amino acid residues from the amino acid residue at position 221 toward the C-terminal, among the amino acid residues at positions from 221 to 327 of SEQ ID NO: 8.

12. The method of claim 1, wherein the third domain has an amino acid sequence selected from the group consisting of SEQ ID NOS: 9 to 14.

13. The method of claim 2, wherein the cancer is a solid tumor, a cancer of lymphatic system, or leukemia.

14. The method of claim 13, wherein the solid tumor is synovial sarcoma, infiltrating duct carcinoma, rectal cancer, colon cancer, ovary cancer, ascending colon cancer, anal cancer, invasive ductal carcinoma, adenocarcinoma, rectal cancer with paraaortic in metastasis, neuroendocrine carcinoma (cervix), sigmoid colon cancer, or glioblastoma.

15. The method of any one of claims 1, 2, 13, or 14, wherein the subject has previously received, concurrently receives, or will receive one or more of cancer treatments including surgery, radiation, and/or chemotherapy.

16. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose in a range from greater than about 600 $\mu\text{g/kg}$ to about 2,000 $\mu\text{g/kg}$.

17. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of about 720 $\mu\text{g/kg}$ or above, about 960 $\mu\text{g/kg}$ or above, about 1,200 $\mu\text{g/kg}$ or above, about 1,700 $\mu\text{g/kg}$ or above, or about 2,000 $\mu\text{g/kg}$.

18. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of about 720 $\mu\text{g/kg}$ or above, about 840 $\mu\text{g/kg}$ or above, or about 1,440 $\mu\text{g/kg}$ or above.

19. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of about 720 $\mu\text{g/kg}$ or above, about 840 $\mu\text{g/kg}$ or above, about 960 $\mu\text{g/kg}$ or above, about 1,200 $\mu\text{g/kg}$ or above, about 1,440 $\mu\text{g/kg}$ or above, about 1,700 $\mu\text{g/kg}$ or above, or about 2,000 $\mu\text{g/kg}$.

20. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times at an interval of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, or 15 weeks.

21. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times at an interval of 10 days, 20 days, 30 days, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, or 100 days.

22. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered parenthetically, intramuscularly, subcutaneously, ophthalmic, intravenously, intraperitoneally, intradermally, intraorbitally, intracerebrally, intracranially, intraspinally, intraventricular, intrathecally, intracisternally, intracapsularly, or intratumorally.

23. The method of claims 1 or 2, comprising administering the (ii) interleukin-7 fusion protein.

24. The method of claim 23, wherein the (ii) interleukin-7 fusion protein comprises the amino acid sequence of SEQ ID NO: 24.

25. The method of claims 1 or 2, wherein the subject has a lymphocyte count of about 1000 lymphocyte cells or less/ μ l of blood, as determined according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

26. The method of claim 25, wherein the lymphocyte is T-cell.

27. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times in an amount of about 720 μ g/kg at an

interval of about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 week, or about 6 weeks.

28. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times in an amount of about 840 $\mu\text{g/kg}$ at an interval of about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, or about 6 weeks.

29. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times in an amount of about 960 $\mu\text{g/kg}$ at an interval of about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, or about 9 weeks.

30. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times in an amount of about 1,200 $\mu\text{g/kg}$ at an interval of about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, or about 10 weeks.

31. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered twice or more times in an amount of about 1,440 $\mu\text{g/kg}$ at an interval of about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 2 months, or about 3 months.

32. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of greater than about 600 $\mu\text{g/kg}$, greater than

about 700 µg/kg, greater than about 800 µg/kg, greater than about 900 µg/kg, greater than about 1,000 µg/kg, greater than about 1,100 µg/kg, greater than about 1,200 µg/kg, greater than about 1,300 µg/kg, greater than about 1,400 µg/kg, greater than about 1,500 µg/kg, greater than about 1,600 µg/kg, greater than about 1,700 µg/kg, greater than about 1,800 µg/kg, greater than about 1,900 µg/kg, or greater than about 2,000 µg/kg.

33. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of between about 610 µg/kg and about 1,200 µg/kg, between about 650 µg/kg and about 1,200 µg/kg, between about 700 µg/kg and about 1,200 µg/kg, between about 750 µg/kg and about 1,200 µg/kg, between about 800 µg/kg and about 1,200 µg/kg, between about 850 µg/kg and about 1,200 µg/kg, between about 900 µg/kg and about 1,200 µg/kg, between about 950 µg/kg and about 1,200 µg/kg, between about 1,000 µg/kg and about 1,200 µg/kg, between about 1,050 µg/kg and about 1,200 µg/kg, between about 1,100 µg/kg and about 1,200 µg/kg, between about 1,200 µg/kg and about 2,000 µg/kg, between about 1,300 µg/kg and about 2,000 µg/kg, between about 1,500 µg/kg and about 2,000 µg/kg, between about 1,700 µg/kg and about 2,000 µg/kg, between about 610 µg/kg and about 1,000 µg/kg, between about 650 µg/kg and about 1,000 µg/kg, between about 700 µg/kg and about 1,000 µg/kg, between about 750 µg/kg and about 1,000 µg/kg, between about 800 µg/kg and about 1,000 µg/kg, between about 850 µg/kg and about 1,000 µg/kg, between about 900 µg/kg and about 1,000 µg/kg, or between about 950 µg/kg and about 1,000 µg/kg.

34. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of between about 700 µg/kg and about 900 µg/kg, between about 750 µg/kg and about 950 µg/kg, between about 700 µg/kg and about 850 µg/kg, between about 750 µg/kg and about 850 µg/kg, between about 700 µg/kg and about 800 µg/kg, between about 800 µg/kg and about 900 µg/kg, between about 750 µg/kg and about 850 µg/kg, or between about 850 µg/kg and about 950 µg/kg.

35. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dose of about 650 µg/kg, about 680 µg/kg, about 700 µg/kg, about 720 µg/kg, about 740 µg/kg, about 750 µg/kg, about 760 µg/kg, about 780 µg/kg, about 800 µg/kg, about 820 µg/kg, about 840 µg/kg, about 850 µg/kg, about 860 µg/kg, about 880 µg/kg,

about 900 µg/kg, about 920 µg/kg, about 940 µg/kg, about 950 µg/kg, about 960 µg/kg, about 980 µg/kg, about 1,000 µg/kg, about 1,020 µg/kg, about 1,040 µg/kg, about 1,060 µg/kg, about 1,080 µg/kg, about 1,100 µg/kg, about 1,120 µg/kg, about 1,140 µg/kg, about 1,160 µg/kg, about 1,180 µg/kg, about 1,200 µg/kg, about 1,220 µg/kg, about 1,240 µg/kg, about 1,260 µg/kg, about 1,280 µg/kg, about 1,300 µg/kg, about 1,320 µg/kg, about 1,340 µg/kg, about 1,360 µg/kg, about 1,380 µg/kg, about 1,400 µg/kg, about 1,420 µg/kg, about 1,440 µg/kg, about 1,460 µg/kg, about 1,480 µg/kg, about 1,500 µg/kg, about 1,520 µg/kg, about 1,540 µg/kg, about 1,560 µg/kg, about 1,580 µg/kg, about 1,600 µg/kg, about 1,620 µg/kg, about 1,640 µg/kg, about 1,660 µg/kg, about 1,680 µg/kg, about 1,700 µg/kg, about 1,720 µg/kg, about 1,740 µg/kg, about 1,760 µg/kg, about 1,780 µg/kg, about 1,800 µg/kg, about 1,820 µg/kg, about 1,840 µg/kg, about 1,860 µg/kg, about 1,880 µg/kg, about 1,900 µg/kg, about 1,920 µg/kg, about 1,940 µg/kg, about 1,960 µg/kg, about 1,980 µg/kg, or about 2,000 µg/kg.

36. The method of claims 1 or 2, wherein the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein is administered at a dosing frequency of once a week, once in every two weeks, once in every three weeks, once in every four weeks, once in every five weeks, once in every six weeks, once in every seven weeks, once in every eight weeks, once in every nine weeks, once in every 10 weeks, once in every 11 weeks, once in every 12 weeks, once in every 13 weeks, once in every 14 weeks, or once in every 15 weeks.

37. The method of claim 26, wherein the T-cell is CD4⁺ and/or CD8⁺ T-cell.

38. The method of claim 26, wherein the T-cell is CD4⁺/CD8⁺ T-cell.

39. The method of claim 25, wherein the the subject has a lymphocyte count of about 800 lymphocyte cells or less/ µl of blood.

40. The method of claim 25, wherein the the subject has a lymphocyte count of about 500 lymphocyte cells or less/ µl of blood.

41. The method of claim 25, wherein the the subject has a lymphocyte count of about 200 lymphocyte cells or less/ μ l of blood.

42. The method of claims 1 or 2, wherein the subject has been, is concurrently, or will be administered with an anti-cancer agent.

43. The method of claim 42, wherein the anti-cancer agent is an anti-cancer chemical compound.

44. The method of claims 25 or 42, wherein a number of tumor infiltrating lymphocytes (TILs) in the tumor is increased after the administration of the (i) modified interleukin-7 or the (ii) interleukin-7 fusion protein compared to a number of TILs in a tumor before the administration.

45. The method of claim 44, wherein the TILs are CD4⁺ TILs.

46. The method of claim 44, wherein the TILs are CD8⁺ TILs.

47. The method of claim 44, wherein the number of TILs is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% after the administration.

48. A use of the following (i) and/or (ii) for increasing a lymphocyte count in a subject in need thereof, comprising administering

(i) a modified interleukin-7 of the following formula (I):

A - IL-7 formula (I)

wherein A is an oligopeptide consisting of 1 to 10 amino acid residues, and

the IL-7 is a polypeptide which is capable of binding to IL-7 receptor; and/or

(ii) an interleukin-7 fusion protein comprising

(a) the modified interleukin-7,

(b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and

(c) a third domain which prolongs the half-life of the interleukin-7 fusion protein, to the subject at a dose of greater than about 600 µg/kg.

49. A use of the following (i) and/or (ii) in manufacturing a medicament for use in increasing a lymphocyte count in a subject in need thereof, said medicament being administered to the patient at a dose of greater than about 600 µg/kg,

(i) a modified interleukin-7 of the following formula (I):

A - IL-7 formula (I)

wherein A is an oligopeptide consisting of 1 to 10 amino acid residues, and

the IL-7 is a polypeptide which is capable of binding to IL-7 receptor; and/or

(ii) an interleukin-7 fusion protein comprising

(a) the modified interleukin-7,

(b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and

(c) a third domain which prolongs the half-life of the interleukin-7 fusion protein.

50. A pharmaceutical composition for increasing a lymphocyte count in a subject in need thereof, comprising as an active ingredient,

(i) a modified interleukin-7 of the following formula (I):

A - IL-7 formula (I)

wherein A is an oligopeptide consisting of 1 to 10 amino acid residues, and

the IL-7 is a polypeptide which is capable of binding to IL-7 receptor; and/or

(ii) an interleukin-7 fusion protein comprising

(a) the modified interleukin-7,

(b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and

(c) a third domain which prolongs the half-life of the interleukin-7 fusion protein, wherein said pharmaceutical composition is administered to the subject at a dose of greater than about 600 µg/kg.