

WHAT IS CLAIMED IS:

1. A pharmaceutical formulation comprising:
 - (a) a heterodimeric Fc-fused protein comprising:
 - (i) a first polypeptide comprising a first antibody Fc domain polypeptide and a first subunit of a multisubunit cytokine; and
 - (ii) a second polypeptide comprising a second antibody Fc domain polypeptide and a second, different subunit of the multisubunit cytokine,
 - (b) citrate;
 - (c) a sugar;
 - (d) a sugar alcohol; and
 - (e) a non-ionic surfactant,at pH 6.0 to 7.0
wherein the first and second antibody Fc domain polypeptides each comprise different mutations promoting heterodimerization, and
wherein the first subunit and second, different subunit of the multisubunit cytokine are bound to each other,
for use in therapy,
wherein the pharmaceutical formulation is to be administered to a subject in need thereof in a multiple-dose therapy at an interval of at least three weeks between the doses or at least four weeks between the doses.
2. The pharmaceutical formulation for use of claim 1, wherein the pharmaceutical formulation is to be administered to the subject once every three weeks, once every four weeks, or once every six weeks.
3. The pharmaceutical formulation for use of claim 1 or 2, wherein the multiple-dose therapy is stopped if the subject develops progressive disease, unacceptable toxicity, or meets a criterion for withdrawal.
4. The pharmaceutical formulation for use of any one of claims 1-3, wherein if the subject experiences a complete response (CR) during the multiple-dose therapy, then the multiple-dose therapy is to be further administered for at least 12 months after the confirmation of the complete response.

5. The pharmaceutical formulation for use of claim 4, wherein:
 - (a) the total duration of the multiple-dose therapy is equal to or less than 24 months; or
 - (b) the total duration of the multiple-dose therapy is greater than 24 months.
6. The pharmaceutical formulation for use of any one of claims 1-5, wherein the pharmaceutical formulation is to be administered by subcutaneous injection.
7. The pharmaceutical formulation for use of any one of claims 1-6, wherein the pharmaceutical formulation is to be administered to the subject in an amount sufficient to provide the heterodimeric Fc-fused protein at a dosage of:
 - (a) between 0.05 µg/kg to 1.75 µg/kg, based on the subject's weight;
 - (b) about 0.05 µg/kg, about 0.10 µg/kg, about 0.20 µg/kg, about 0.40 µg/kg, about 0.60 µg/kg, about 0.80 µg/kg, about 1.00 µg/kg, about 1.20 µg/kg, about 1.40 µg/kg, or about 1.75 µg/kg, based on the subject's weight;
 - (c) greater than 0.00 µg/kg and less than 0.05 µg/kg, based on the subject's weight; and/or
 - (d) greater than 1.75 µg/kg, based on the subject's weight.
8. The pharmaceutical formulation for use of any one of claims 1-7, wherein the subject has cancer;
optionally wherein the cancer is a locally advanced or metastatic solid tumor;
further optionally wherein the presence of the cancer in the subject is confirmed using the Response Evaluation Criteria for Solid Tumors (RECIST), version 1.1.
9. The pharmaceutical formulation for use of claim 8, wherein the cancer is selected from the group consisting of: melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell carcinoma (HNSCC), classical Hodgkin lymphoma, primary mediastinal large B-Cell lymphoma, bladder cancer, urothelial carcinoma, micro-satellite instability high cancer, colorectal cancer, gastric cancer, oesophageal cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma (RCC), endometrial carcinoma, cutaneous T cell lymphoma, and triple negative breast cancer.

10. The pharmaceutical formulation for use of any one of claims 8-9, wherein the subject is anti-PD-1 refractory.
11. The pharmaceutical formulation for use of claim 9, wherein the subject has melanoma.
12. The pharmaceutical formulation for use of claim 11, wherein:
 - (a) the subject has previously been treated with an anti-PD-1 antibody for at least 6 weeks;
 - (b) the subject has been confirmed of progression of disease at least 4 weeks after the initial diagnosis of progression of disease while receiving an anti-PD-1 antibody, optionally wherein the progression of the disease is confirmed by radiological or clinical observation; and/or
 - (c) if the subject has a tumor comprising a BRAF activating mutation, then the subject has previously been treated with a BRAF inhibitor.
13. The pharmaceutical formulation for use of claim 9, wherein the subject has RCC; optionally wherein the RCC has clear cell histology.
14. The pharmaceutical formulation for use of claim 13, wherein the subject has:
 - (a) previously been treated with an anti-PD-1/PD-L1 antibody and/or an anti-vascular endothelial growth factor therapy; or
 - (b) previously received three or fewer lines of therapy.
15. The pharmaceutical formulation for use of claim 9, wherein the subject has urothelial carcinoma.
16. The pharmaceutical formulation for use of claim 15, wherein the subject has:
 - (a) locally advanced or metastatic transitional cell carcinoma of the urothelium;
 - (b) previously been treated with a single treatment comprising a platinum-containing regimen and has shown radiographic progression recurrence within 6 months after the last administration of the platinum-containing regimen;
 - (c) previously received two or less lines of therapy; or
 - (d) not previously received a checkpoint inhibitor therapy as a monotherapy or in combination with a platinum based chemotherapy; optionally wherein the checkpoint inhibitor therapy comprises an anti-PD-1 antibody or anti-PD-L1 antibody.

17. The pharmaceutical formulation for use of any one of claims 1-16, wherein the pharmaceutical formulation is to be administered to the subject as a monotherapy.

18. The pharmaceutical formulation for use of any one of claims 1-16, wherein the pharmaceutical formulation is to be administered to the subject as a combination therapy.

19. The pharmaceutical formulation for use of claim 18, wherein an anti-PD-1 antibody is to be further administered to the subject.

20. The pharmaceutical formulation for use of claim 19, wherein:

- (a) the anti-PD-1 antibody is to be administered intravenously;
- (b) the anti-PD-1 antibody is to be administered preceding each pharmaceutical formulation is to be administered; and/or
- (c) the pharmaceutical formulation is to be administered within 1 hour after completion of the anti-PD-1 antibody is to be administered.

21. The pharmaceutical formulation for use of claim 20, wherein the anti-PD-1 antibody is nivolumab or pembrolizumab.

22. The pharmaceutical formulation for use of any one of claims 19-21, wherein:

- (a) the anti-PD-1 antibody is nivolumab and is to be administered at a dose of 480 mg; or
- (b) the anti-PD-1 antibody is pembrolizumab and is to be administered at a dose of 200 mg.

23. The pharmaceutical formulation for use of claims 21 or 22, wherein the anti-PD-1 antibody is nivolumab and the cancer is selected from the group consisting of: melanoma, NSCLC, SCLC, RCC, classical Hodgkin lymphoma, HNSCC, urothelial carcinoma, colorectal cancer, hepatocellular carcinoma, bladder cancer, and oesophageal cancer.

24. The pharmaceutical formulation for use of claim 23, wherein the cancer is:

- (a) melanoma; optionally wherein the melanoma is unresectable; or
- (b) colorectal cancer; optionally the colorectal cancer is microsatellite instability-high (MSI-H) or mismatch repair deficient metastatic (dMMR) colorectal cancer.

25. The pharmaceutical formulation for use of claim 21 or 22, wherein the anti-PD-1 antibody is pembrolizumab and a surgical intervention is to be further performed to lyse cancer cells, remove a tumor, or debulk a tumor in the subject.

26. The pharmaceutical formulation for use of claim 25, wherein the surgical intervention comprises:

- (a) cryotherapy;
- (b) hyperthermic therapy; or
- (c) radiotherapy; optionally wherein the radiotherapy is a stereotactic body radiation therapy (SBRT).

27. The pharmaceutical formulation for use of any one of claims 18-26, wherein an NK cell-targeting therapy is to be further administered to the subject; optionally wherein the NK cell-targeting therapy comprises a multi-specific binding protein.

28. The pharmaceutical formulation for use of any one of claims 18-27, wherein:

- (a) a chimeric antigen receptor therapy;
- (b) a cytokine therapy;
- (c) an innate immune system agonist therapy;
- (d) a targeted antigen therapy; and/or
- (e) an oncolytic virus therapy,

is to be administered to the subject.

29. The pharmaceutical formulation for use of any one of claims 1-28, wherein the first and second antibody Fc domain polypeptides are human IgG1 Fc domain polypeptides; optionally wherein the human IgG1 Fc domain polypeptides comprise one or more mutation(s) that reduce(s) an effector function of an Fc.

30. The pharmaceutical formulation for use of claim 29, wherein the multisubunit cytokine is a human IL12.

31. The pharmaceutical formulation for use of claim 29 or 30, wherein the first and second antibody Fc domain polypeptides comprise mutations selected from L234A, L235A or L235E, G237A, P329A, A330S, and P331S, numbered according to the EU numbering system.

32. The pharmaceutical formulation for use of claim 31, wherein the first and second antibody Fc domain polypeptides each comprise mutations L234A, L235A, and P329A.

33. The pharmaceutical formulation for use of claim 32, wherein the first subunit of a multisubunit cytokine is a p40 subunit of IL12 and the second subunit of a multisubunit cytokine is a p35 subunit of IL12.

34. The pharmaceutical formulation for use of claim 33, wherein the first subunit of a multisubunit cytokine comprises the amino acid sequence of SEQ ID NO: 127 and the second subunit of a multisubunit cytokine comprises the amino acid sequence of SEQ ID NO: 128.

35. The pharmaceutical formulation for use of claim 34, wherein the second subunit of a multisubunit cytokine is fused to the second antibody Fc domain by a linker comprising the amino acid sequence of SEQ ID NO: 108.

36. The pharmaceutical formulation for use of claim 35, wherein

(a) the first antibody Fc domain comprises mutations L234A, L235A, P329A, Y349C, K360E, and K409W, and

(b) the second antibody Fc domain comprises mutations L234A, L235A, P329A, Q347R, S354C, D399V, and F405T.

37. The pharmaceutical formulation for use of claim 36, wherein

(a) the first antibody Fc domain comprises the amino acid sequence of SEQ ID NO:215, and

(b) the second antibody Fc domain comprises the amino acid sequence of SEQ ID NO:216.

38. The pharmaceutical formulation for use of claim 37, wherein the first antibody Fc domain peptide comprises the amino acid sequence of SEQ ID NO:290 and the second antibody Fc domain peptide comprises the amino acid sequence of SEQ ID NO:291.

39. A pharmaceutical formulation comprising:

(a) a heterodimeric Fc-fused protein comprising:

(i) a first polypeptide comprising a first antibody Fc domain polypeptide and a first subunit of a multisubunit cytokine; and

(ii) a second polypeptide comprising a second antibody Fc domain polypeptide and a second, different subunit of the multisubunit cytokine,

(b) citrate;

(c) a sugar;

(d) a sugar alcohol; and

(e) a non-ionic surfactant,

at pH 6.0 to 7.0

wherein the first and second antibody Fc domain polypeptides each comprise different mutations promoting heterodimerization, and

wherein the first subunit and second, different subunit of the multisubunit cytokine are bound to each other,

for use in therapy

wherein the pharmaceutical formulation is to be administered as a single-dose therapy.

40. A pharmaceutical formulation comprising a heterodimeric Fc-fused protein and a pharmaceutically acceptable carrier to the subject, wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization for use in treating cancer in a subject who has received treatment with a checkpoint inhibitor antibody for at least 6 weeks.

41. A pharmaceutical formulation comprising a heterodimeric Fc-fused protein and a pharmaceutically acceptable carrier to the subject, wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment

crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization for use in treating cancer in a subject who has received treatment with a checkpoint inhibitor antibody or an anti-vascular endothelial growth factor therapy as a monotherapy, or in combination.

42. A pharmaceutical formulation comprising a heterodimeric Fc-fused protein and a pharmaceutically acceptable carrier to the subject, wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization for use in treating cancer in a subject who has received treatment with only one platinum-containing regimen.

43. Use of a heterodimeric Fc-fused protein in the manufacture of a medicament for treating a cancer, wherein the medicament is manufactured in a liquid pharmaceutical formulation comprising about 0.5 g/L to about 1.5 g/L or about 0.1 mg – about 2 mg of the heterodimeric Fc-fused protein contained in one or more vessels,

wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization.

44. A pharmaceutical formulation comprising a heterodimeric Fc-fused protein and pharmaceutically for use in treating a cancer, wherein the medicament is to be administered subcutaneously to the subject, and

wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization; and the pharmaceutical formulation comprises citrate; a sugar; a sugar alcohol; and a non-ionic surfactant, and the pH of the formulation is between 5.5 and 7.0.

45. A method of manufacturing a heterodimeric Fc-fused protein for the preparation of a pharmaceutical formulation thereof, the method comprising adding acetic acid to a solution comprising the heterodimeric Fc-fused protein obtained from a Chinese Hamster Ovary (CHO) cell culture expressing the heterodimeric Fc-fused protein for 30 minutes to 90 minutes, wherein the acetate adjusts and maintains the pH of the solution at pH 3.55 to 3.75, and

wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization.

46. A pharmaceutical formulation comprising a heterodimeric Fc-fused protein and a pharmaceutically acceptable carrier, wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and

the second Fc region each comprise one or more mutations promoting heterodimerization for use in treating a subject whose blood concentration of C-reactive protein (CRP) is monitored.

47. A method of detecting toxicity in a subject receiving a pharmaceutical formulation comprising measuring the concentration of C-reactive protein (CRP) in the subject's blood, wherein the pharmaceutical formulation comprises a heterodimeric Fc-fused protein and a pharmaceutically acceptable carrier, and wherein the heterodimeric Fc-fused protein comprises a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization.

48. A pharmaceutical formulation comprising:

(a) a heterodimeric Fc-fused protein comprising:

(i) a first polypeptide comprising a first antibody Fc domain polypeptide and a first subunit of a multisubunit cytokine; and

(ii) a second polypeptide comprising a second antibody Fc domain polypeptide and a second, different subunit of the multisubunit cytokine,

(b) citrate;

(c) a sugar;

(d) a sugar alcohol; and

(e) a non-ionic surfactant,

at pH 6.0 to 7.0,

wherein the first and second antibody Fc domain polypeptides each comprise different mutations promoting heterodimerization, and

wherein the first subunit and second, different subunit of the multisubunit cytokine are bound to each other.

49. A kit comprising one or more vessels comprising a pharmaceutical formulation, wherein the pharmaceutical formulation comprises:

(a) a heterodimeric Fc-fused protein comprising a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations promoting heterodimerization; and

(b) a pharmaceutically acceptable carrier,
and wherein the one or more vessels collectively comprise about 0.1 mg – about 2 mg of heterodimeric Fc-fused protein.