

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.~~
2. ~~The pharmaceutical formulation of claim 1, wherein the first and/or second antibody Fc domain polypeptides comprise one or more mutation(s) that reduce(s) an effector function of an Fc.~~
3. ~~The pharmaceutical formulation of claim 1 or 2, wherein the concentration of citrate in the pharmaceutical formulation is about 10 mM to about 30 mM.~~
4. ~~The pharmaceutical formulation of claim 3, wherein the concentration of citrate in the pharmaceutical formulation is about 20 mM.~~
5. ~~The pharmaceutical formulation of any one of claims 1-4, wherein the concentration of the sugar in the pharmaceutical formulation is about 3% to about 12% (w/v).~~
6. ~~The pharmaceutical formulation of claim 5, wherein the concentration of the sugar in the pharmaceutical formulation is about 6% (w/v).~~

7. ~~The pharmaceutical formulation of claim 5 or 6, wherein the sugar is a disaccharide.~~
8. ~~The pharmaceutical formulation of claim 7, wherein the disaccharide is sucrose.~~
9. ~~The pharmaceutical formulation of any one of claims 1-8, wherein the concentration of the sugar alcohol in the pharmaceutical formulation is between about 0.5% to about 6% (w/v).~~
10. ~~The pharmaceutical formulation of claim 9, wherein the concentration of the sugar alcohol in the pharmaceutical formulation is about 1% (w/v).~~
11. ~~The pharmaceutical formulation of any one of claims 1-10, wherein the sugar alcohol is derived from a monosaccharide.~~
12. ~~The pharmaceutical formulation of claim 11, wherein the sugar alcohol is mannitol.~~
13. ~~The pharmaceutical formulation of any one of claims 1-12, wherein the concentration of the non-ionic surfactant in the pharmaceutical formulation is between about 0.005% to about 0.02% (w/v).~~
14. ~~The pharmaceutical formulation of claim 13, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is about 0.01% (w/v).~~
15. ~~The pharmaceutical formulation of claim 13 or 14 wherein the non-ionic surfactant is a polysorbate.~~
16. ~~The pharmaceutical formulation of claim 15, wherein the polysorbate is polysorbate 80.~~
17. ~~The pharmaceutical formulation of any one of claims 1-16, wherein the pH is between about 6.1 and about 6.9.~~
18. ~~The pharmaceutical formulation of claim 17, wherein the pH is between about 6.2 and about 6.8.~~

19. — ~~The pharmaceutical formulation of claim 18, wherein the pH is between about 6.3 and about 6.7.~~

20. — ~~The pharmaceutical formulation of claim 19, wherein the pH is between about 6.4 and about 6.6.~~

21. — ~~The pharmaceutical formulation of claim 20, wherein the pH is about 6.5.~~

22. — ~~The pharmaceutical formulation of any one of claims 1-21, further comprising water.~~

23. — ~~The pharmaceutical formulation of claim 22, wherein the water is Water for Injection, USP.~~

24. — ~~The pharmaceutical formulation of any one of claims 1-23, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc-fused protein of about 1 g/L to about 10 g/L.~~

25. — ~~The pharmaceutical formulation of claim 24, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc-fused protein of about 2 g/L to about 8 g/L.~~

26. — ~~The pharmaceutical formulation of claim 25, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc-fused protein of about 4 g/L to about 6 g/L.~~

27. — ~~The pharmaceutical formulation of claim 26, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc-fused protein of about about 5 g/L.~~

28. — ~~The pharmaceutical formulation of any one of claims 1-27, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 0.5 g/L to about 1.5 g/L.~~

29. — ~~The pharmaceutical formulation of claim 28, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 0.75 g/L to about 1.25 g/L.~~

30. — ~~The pharmaceutical formulation of claim 29, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 1 g/L.~~

31. — ~~The pharmaceutical formulation of any one of 1-30, wherein the formulation is designed to be stored at a temperature between about 2°C and about 8°C.~~

32. — ~~The pharmaceutical formulation of any one of claims 1-31, wherein the pharmaceutical formulation is a clear, colorless solution and free of visible particulates.~~

33. — ~~The pharmaceutical formulation of any one of claims 1-32, wherein the formulation has a thermal stability profile as defined by:~~

~~(a) a T_{m1} of greater than about 60°C, greater than about 61°C, greater than about 62°C, greater than about 63°C, greater than about 64°C, greater than about 65°C, or greater than about 66°C; and/or~~

~~(b) a T_{m2} of greater than about 70°C, greater than about 71°C, greater than about 72°C, greater than about 73°C, greater than about 74°C, greater than about 75°C, greater than about 76°C, or greater than about 77°C,
as measured by differential scanning fluorimetry.~~

34. — ~~The pharmaceutical formulation of claim 33, wherein the formulation has a thermal stability profile as defined by a T_{m1} of about 67.0°C and a T_{m2} of about 77.3°C.~~

35. — ~~The pharmaceutical formulation of claim 34, wherein the thermal stability profile of the pharmaceutical formulation, as defined by T_{m1} and/or T_{m2} is changed by less than about 2°C or less than about 1°C when the pharmaceutical formulation is incubated for 1 week at 50°C, as compared to the same pharmaceutical formulation that is incubated for 1 week at 5°C, as measured by differential scanning fluorimetry.~~

36. — ~~The pharmaceutical formulation of any one of 1-35, wherein the formulation has a thermal stability profile as defined by a T_{agg} of greater than about 60°C, greater than about 61°C, greater than about 62°C, greater than about 63°C, greater than about 64°C, greater than about 65°C, greater than about 66°C, or greater than about 67°C, as measured by differential scanning fluorimetry.~~

37. — The pharmaceutical formulation of claim 36, wherein the thermal stability profile of the pharmaceutical formulation, as defined by T_{agg} is changed by less than about 2°C or less than about 1°C when the pharmaceutical formulation is incubated for 1 week at 50°C, as compared to the same pharmaceutical formulation that is incubated for 1 week at 5°C, as measured by differential scanning fluorimetry.

38. — The pharmaceutical formulation of any one of claims 1-37, wherein the pH of the pharmaceutical formulation does not change by more than about 0.2 or about 0.1 in pH value after the pharmaceutical formulation is incubated for 1 week at 5°C.

39. — The pharmaceutical formulation of any one of claims 1-38, wherein the pH of the pharmaceutical formulation does not change by more than about 0.2 or about 0.1 in pH value after the pharmaceutical formulation is incubated for 1 week at 50°C.

40. — The pharmaceutical formulation of any one of claims 1-39, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of less than about 15 nm, less than about 14 nm, less than about 13 nm, or less than about 12 nm, as measured by dynamic light scattering at 25°C.

41. — The pharmaceutical formulation of claim 40, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 11.6 nm.

42. — The pharmaceutical formulation of any one of claims 1-41, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of less than about 20 nm, less than about 19 nm, less than about 18 nm, less than about 17 nm, less than about 16 nm, or less than about 15 nm, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.

43. — The pharmaceutical formulation of claim 42, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 14.4 nm.

44. — ~~The pharmaceutical formulation of any one of claims 1-43, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of less than about 20 nm, less than about 19 nm, less than about 18 nm, less than about 17 nm, or less than about 16 nm, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is subjected to five freeze thaw cycles.~~

45. — ~~The pharmaceutical formulation of claim 44, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 15.3 nm.~~

46. — ~~The pharmaceutical formulation of any one of claims 1-45, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.30, less than about 0.29, less than about 0.28, or less than about 0.27, as measured by dynamic light scattering at 25°C.~~

47. — ~~The pharmaceutical formulation of any one of claims 1-46, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.26.~~

48. — ~~The pharmaceutical formulation of any one of claims 1-47, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.30, less than about 0.29, less than about 0.28, less than about 0.27, or less than about 0.26, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.~~

49. — ~~The pharmaceutical formulation of any one of claims 1-48, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.25.~~

50. — ~~The pharmaceutical formulation of any one of claims 1-49, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.40, less than about 0.35, or less than about 0.34, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is subjected to five freeze thaw cycles.~~

51. — ~~The pharmaceutical formulation of any one of claims 1-50, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.33.~~

52. — ~~The pharmaceutical formulation of any one of claims 1-51, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, or greater than about 99%.~~

53. — ~~The pharmaceutical formulation of claim 52, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 99.0%.~~

54. — ~~The pharmaceutical formulation of any one of claims 1-53, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 75%, greater than about 80%, greater than about 81%, greater than about 82%, greater than about 83%, greater than about 84%, or greater than about 85%, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.~~

55. — ~~The pharmaceutical formulation of claim 54, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 85.2%.~~

56. — ~~The pharmaceutical formulation of any one of claims 1-55, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about 96%, greater than about 97%, or greater than about 98%, after the pharmaceutical formulation is subjected to five freeze-thaw cycles.~~

~~57. The pharmaceutical formulation of claim 56, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 98.9%.~~

~~58. A method comprising administering to a subject in need thereof, the pharmaceutical formulation of any one of claims 1-57, as a single-dose therapy.~~

~~59.1. A method comprising administering pharmaceutical formulation to a subject in need thereof, the pharmaceutical formulation of any one of claims 1-57, in a multiple-dose therapy at an interval of at least three weeks between the doses or at least four weeks between the doses, 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.~~

~~60.2. The pharmaceutical formulation for use method—of claim—159, wherein the pharmaceutical formulation is to be administered to the subject once every three weeks, once every four weeks, or once every six weeks.~~

61. ~~The method of claim 59, wherein the pharmaceutical formulation is administered to the subject once every four weeks.~~

62. ~~The method of claim 59, wherein the pharmaceutical formulation is administered to the subject once every six weeks.~~

63.3. ~~The pharmaceutical formulation for use method of any one of claim 1 or 2s 59-62, wherein further comprising stopping the multiple-dose therapy is stopped if the subject develops progressive disease, unacceptable toxicity, or meets a criterion for withdrawal.~~

64.4. ~~The pharmaceutical formulation for use method of any one of claims 59-63 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.~~

65.5. ~~The pharmaceutical formulation for use method of claim 4-64, wherein: the total duration of the multi-dose therapy is equal to or less than 24 months.~~

(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.~~

66. ~~The method of claim 64, wherein the total treatment duration is greater than 24 months.~~

67.6. ~~The pharmaceutical formulation for use method of any one of claims 1-5 58-66, wherein the pharmaceutical formulation is to be administered by subcutaneous injection.~~

68.7. ~~The pharmaceutical formulation for use method of any one of claims 1-6 58-67, 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: between about 0.05 µg/kg to about 1.75 µg/kg, based on the subject's weight.~~

(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.

~~69. The method of any one of claims 58-68, wherein the pharmaceutical formulation is administered to the subject in an amount sufficient to provide the heterodimeric Fe-fused protein at a dosage of 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.~~

~~70. The method of any one of claims 58-67, wherein the pharmaceutical formulation is administered to the subject in an amount sufficient to provide the heterodimeric Fe-fused protein at a dosage of greater than 0.00 µg/kg and less than about 0.05 µg/kg, based on the subject's weight.~~

~~71. The method of any one of claims 58-67, wherein the pharmaceutical formulation is administered to the subject in an amount sufficient to provide the heterodimeric Fe-fused protein at a dosage of greater than about 1.75 µg/kg, based on the subject's weight.~~

72.8. The pharmaceutical formulation for use method of any one of claims 1-7-58-71, 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.

~~73. The method of claim 72, wherein the subject has a locally advanced or metastatic solid tumor.~~

~~74. The method of claim 72 or 73, wherein the presence of the cancer in the subject is confirmed using the Response Evaluation Criteria for Solid Tumors (RECIST), version 1.1.~~

~~75.9.~~ The pharmaceutical formulation for use ~~method of any one of claims 8-72-74~~, 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.

~~76.10.~~ The pharmaceutical formulation for use ~~method of any one of claims 8-9-72-75~~, wherein the subject is anti-PD-1 refractory.

~~77.11.~~ The pharmaceutical formulation for use ~~method of claim 9-75~~, wherein the subject has melanoma.

~~78.12.~~ The pharmaceutical formulation for use ~~method of claim 11-77~~, wherein: ~~the subject has previously been treated with an anti-PD-1 antibody for at least 6 weeks.~~

- (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.

~~79.— The method of claim 78, wherein 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.~~

~~80.— The method of claim 79, wherein progression of disease is confirmed by radiological or clinical observation.~~

~~81.— The method of claim 77, wherein, if the subject has a tumor comprising a BRAF activating mutation, then the subject has previously been treated with a BRAF inhibitor.~~

~~82.13.~~ The pharmaceutical formulation for use method of claim 9-75, wherein the subject has RCC; optionally wherein the RCC has clear cell histology.

~~83. The method of claim 82, wherein the RCC has clear cell histology.~~

~~84.14.~~ The pharmaceutical formulation for use method of claim 13-82, wherein the subject patient has; previously been treated with an anti-PD-1/PD-L1 antibody and/or an anti-vascular endothelial growth factor therapy.

(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.

~~85. The method of claim 82, wherein the subject has previously received three or fewer lines of therapy.~~

~~86.15.~~ The pharmaceutical formulation for use method of claim 9-75, wherein the subject has urothelial carcinoma.

~~87.16.~~ The pharmaceutical formulation for use method of claim 15-86, wherein the subject has; locally advanced or metastatic transitional cell carcinoma of the urothelium.

(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.

~~88. The method of claim 86, wherein the subject has 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.~~

89. ~~The method of claim 86, wherein the subject has previously received two or less lines of therapy.~~

90. ~~The method of claim 86, wherein the subject has not previously received a checkpoint inhibitor (e.g., anti-PD-1 or anti-PD-L1 antibody) therapy as a monotherapy or in combination with a platinum-based chemotherapy.~~

91.17. The pharmaceutical formulation for use ~~method~~ of any one of claims 1-16-58-90, wherein the pharmaceutical formulation is to be administered to the subject as a monotherapy.

92.18. The pharmaceutical formulation for use ~~method~~ of any one of claims 1-16-58-90, wherein the pharmaceutical formulation is to be administered to the subject as a combination therapy.

93.19. The pharmaceutical formulation for use ~~method~~ of claim 18-92, wherein further comprising administering to an anti-PD-1 antibody is to be further administered to the subject an anti-PD-1 antibody.

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.

94.21. The pharmaceutical formulation for use ~~method~~ of claim 20-93, wherein the anti-PD-1 antibody is nivolumab or pembrolizumab.

95.22. The pharmaceutical formulation for use ~~method~~ of any one of claims 19-21-94, wherein: pembrolizumab is administered intravenously.

(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.

96. ~~The method of claim 94 or 95, wherein pembrolizumab is administered at a dose of 200 mg.~~

97. ~~The method of any one of claims 94-96, wherein administration of pembrolizumab precedes each administration of the pharmaceutical formulation.~~

98. ~~The method of claim 97, wherein the pharmaceutical formulation is administered within 1 hour after completion of administration of pembrolizumab.~~

99. ~~The method of claim 92, wherein the anti-PD-1 antibody is nivolumab.~~

100. ~~The method of claim 99, wherein nivolumab is administered intravenously.~~

101. ~~The method of claim 99 or 100, wherein nivolumab is administered at a dose of 480 mg.~~

102. ~~The method of any one of claims 99-101, wherein administration of nivolumab precedes each administration of the pharmaceutical formulation.~~

103. ~~The method of claim 102, wherein the pharmaceutical formulation is administered within 1 hour after completion of administration of nivolumab.~~

104.23. The pharmaceutical formulation for use of claims 21 or 22~~method of any one of claims 99-103~~, 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.

105.24. The pharmaceutical formulation for use~~method of claim 23-104~~, wherein the cancer is:~~melanoma.~~

(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.

~~106.—The method of claim 105, wherein the melanoma is unresectable.~~

~~107.—The method of claim 104, wherein the cancer is colorectal cancer.~~

~~108.—The method of claim 107, wherein the colorectal cancer is microsatellite instability-high (MSI-H) or mismatch repair deficient metastatic (dMMR) colorectal cancer.~~

~~109.25._____The pharmaceutical formulation for use method of any one of claims 21 or 22 92-98, wherein the anti-PD-1 antibody is pembrolizumab and further comprising performing a surgical intervention is to be further performed to lyse cancer cells, remove a tumor, or debulk a tumor in the subject.~~

~~110.26._____The pharmaceutical formulation for use method of claim 25-109, wherein the surgical intervention comprises: cryotherapy.~~

~~(a) _____cryotherapy;~~

~~(b) _____hyperthermic therapy; or~~

~~(c) _____radiotherapy; optionally wherein the radiotherapy is a stereotactic body radiation therapy (SBRT).~~

~~111.—The method of claim 109, wherein the surgical intervention comprises hyperthermic therapy.~~

~~112.—The method of claim 109, wherein the surgical intervention comprises administering to the subject a radiotherapy.~~

~~113.—The method of claim 112, wherein the radiotherapy is a stereotactic body radiation therapy (SBRT).~~

~~114.27._____The pharmaceutical formulation for use method of any one of claims 18-26-92-113, wherein an NK cell-targeting therapy is to be further administered comprising~~

~~administering to the subject an NK cell targeting therapy; optionally wherein the NK cell-targeting therapy comprises a multi-specific binding protein.~~

~~115.—The method of claim 114, wherein the subject is administered a multi-specific binding protein.~~

~~116.28._____The pharmaceutical formulation for use method of any one of claims 18-27-92-115, wherein: further comprising administering to the subject a chimeric antigen receptor therapy.~~

~~(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.~~

~~117.—The method of any one of claims 92-116, further comprising administering to the subject a cytokine therapy.~~

~~118.—The method of any one of claims 92-117, further comprising administering to the subject an innate immune system agonist therapy.~~

~~119.—The method of any one of claims 92-118, further comprising administering to the subject a chemotherapy.~~

~~120.—The method of any one of claims 92-119, further comprising administering to the subject a targeted antigen therapy.~~

~~121.—The method of any one of claims 92-120, further comprising administering to the subject an oncolytic virus therapy.~~

~~122.—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.~~

~~123. The method of claim 122, wherein~~

~~(1) if the CRP concentration in the subject's blood is higher than a threshold CRP concentration, then the subject is identified as being at risk for developing an adverse drug reaction; and~~

~~(2) if the CRP concentration in the subject's blood is about the same or lower than the threshold C-reactive protein concentration, the subject is not identified as being at risk for developing an adverse drug reaction.~~

~~124. The method of claim 122, wherein if the CRP concentration in the subject's blood is higher than the threshold CRP concentration, then (1) the administration of the pharmaceutical formulation is paused; (2) the heterodimeric Fc fused protein is administered at a lower dose; or (3) a remedial action is taken to reduce or alleviate the formulation's toxicity effects in the subject.~~

~~125.29. The pharmaceutical formulation for use of any one of claims ~~1-28~~ 1-57 or the method of any one of claims ~~58-124~~, 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.~~

~~126.30. The pharmaceutical formulation for use or the method of claim ~~29~~ 29-125, wherein the multisubunit cytokine is a human IL12.~~

~~127.~~ The pharmaceutical formulation ~~or the method of claim 126~~, wherein the human IgG1 Fc domain polypeptides ~~comprise one or more mutation(s) that reduce(s) an effector function of an Fc.~~

~~128.~~ 31. The pharmaceutical formulation for use ~~or the method of claim 29 or 30~~ ~~127~~, 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.

~~129.~~ 32. The pharmaceutical formulation for use ~~or the method of claim 31~~ ~~128~~, wherein the first and second antibody Fc domain polypeptides each comprise mutations L234A, L235A, and P329A.

~~130.~~ 33. The pharmaceutical formulation for use ~~or the method of claim 32~~ ~~129~~, 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.

~~131.~~ 34. The pharmaceutical formulation for use ~~or the method of claim 33~~ ~~130~~, 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.

~~132.~~ 35. The pharmaceutical formulation for use ~~or the method of claim 34~~ ~~131~~, 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.

~~133.~~ 36. The pharmaceutical formulation for use ~~or the method of claim 35~~ ~~132~~, 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.

~~134.~~ 37. The pharmaceutical formulation for use ~~or the method of claim 36~~ ~~133~~, 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.

~~135-38.~~ 38. The pharmaceutical formulation ~~for use or the method of claim 37-134~~, 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.

136.49. _____ 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.

~~137. — The kit of claim 136, wherein the one or more vessels collectively comprise about 0.5 mg to about 2 mg of heterodimeric Fc fused protein.~~

~~138. — The kit of claim 137, wherein the one or more vessels collectively comprise about 1 mg of heterodimeric Fc fused protein.~~

~~139. — The kit of claim 138, wherein the kit comprises one vessel comprising about 1 mg of heterodimeric Fc fused protein.~~

~~140. — The kit of any one of claims 136-139, wherein the pharmaceutical formulation is a lyophilized formulation or a liquid formulation.~~

~~141. — The kit of claim 140, wherein the pharmaceutical formulation is a liquid formulation supplied in a volume of 1 mL.~~

142. ~~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 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.~~

143. ~~The use of a heterodimeric Fc fused protein of claim 142, wherein the liquid pharmaceutical formulation comprises about 1.0 g/L of the heterodimeric Fc fused protein.~~

144. ~~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 0.1 mg — about 2 mg of 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.~~

145. ~~The use of a heterodimeric Fc fused protein of claim 144, wherein the liquid pharmaceutical formulation comprises about 1 mg of heterodimeric Fc fused protein.~~

146. ~~The use of a heterodimeric Fc fused protein of any one of claims 142-145, wherein the medicament is contained in one vessel.~~

~~147. The use of a heterodimeric Fc fused protein of any one of claims 142-146, wherein each vessel contains 1 mg of heterodimeric Fc fused protein.~~

~~148. The use of any one of claims 146-147, wherein the medicament is administered to the subject on day 1, every 3 weeks.~~

~~149. The use of any one of claims 146-147, wherein the medicament is administered to the subject on day 1, every 4 weeks.~~

~~150. The use of any one of claims 146-148, wherein the medicament is administered subcutaneously.~~

~~151. The use of any one of claims 146-150, wherein the medicament is administered in a volume of about 0.1 mL to about 1 mL.~~

~~152. The use of claim 151, wherein the medicament is administered in a volume of about 1 mL.~~

~~153. The use of any one of claims 146-152, wherein the medicament is administered to a maximum of two injection sites.~~

~~154. The use of claim 153, wherein a second injection is completed within 10 minutes after a first injection.~~

~~155. The use of any one of claims 146-154, wherein the medicament is administered at a dose of about 0.05 mg/kg to about 1.75 mg/kg.~~

~~156. The use of claim 155, wherein the medicament is administered at a dose of about 1 mg/kg.~~

~~157. The use of any one of claims 146-156, wherein the medicament is diluted prior to administration in a solution of 0.9% saline (sodium chloride for injection) and 0.01% polysorbate 80.~~

~~158.—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.~~

~~159.—The method of claim 158, wherein the acetic acid is added to the solution comprising the heterodimeric Fc fused protein for about 60 minutes.~~

~~160.—The method of claim 158 or 159, wherein the acetic acid adjusts and maintains the pH of the solution to about 3.65.~~

~~161.—The method of claim 158, wherein the CHO cell culture expressing the heterodimeric Fc fused protein is maintained in suspension.~~

~~162.—The method of claim 161, wherein the CHO cell culture expressing the heterodimeric Fc fused protein is cultured for 7-21 days in a bioreactor.~~

~~163.—The method of claim 161 or 162, wherein the CHO cell culture expressing the heterodimeric Fc fused protein is cultured for 14 days in a bioreactor.~~

~~164.—The method of any one of claims 158-163, wherein the CHO cell culture expressing the heterodimeric Fc fused protein is harvested by depth filtration to yield a CHO harvest medium.~~

165. ~~The method of claim 164, wherein the depth filtration is a two-stage single-use depth filtration consisting of DOHC and XOHC filters.~~

166. ~~The method of claim 164 or 165, wherein the heterodimeric Fc-fused protein is purified from the CHO harvest medium using Protein A capture chromatography, mixed-mode chromatography, and cation-exchange chromatography to yield the solution comprising the heterodimeric Fc-fused protein.~~

167. ~~The method of claim 166, wherein Protein A capture chromatography comprises: equilibrating a Protein A resin with 20 mM Tris, 150 mM NaCl at pH 7.5;~~

~~loading CHO harvest medium onto the Protein A resin;~~

~~washing the loaded Protein A resin with 20 mM Tris, 150 mM NaCl at pH 7.5;~~

~~washing the loaded Protein A resin with 50 mM acetate at pH 5.4; and~~

~~eluting the heterodimeric Fc-fused protein from the Protein A resin with 50 mM acetate, 100 mM arginine at pH 3.7 and collecting by 280 nm UV starting at 1.25 AU/cm ascending and ending at 1.25 AU/cm descending.~~

168. ~~The method of claim 167, wherein the acetic acid is added at a concentration of 0.5M to the solution comprising the heterodimeric Fc-fused protein eluted from the Protein A resin, wherein the acetic acid acidifies the pH of the solution to pH 3.65 for 60 minutes, followed by neutralization of the solution to pH 5.2 by adding 2M Tris.~~

169. ~~The method of claim 168, wherein following acidification and neutralization of the solution, the solution comprising the heterodimeric Fc-fused protein is passed through a 0.2 μ m filter.~~

170. ~~The method of claim 169, wherein the filtered solution comprising the heterodimeric Fc-fused protein eluted from the Protein A resin is passed through X0SP depth filtration.~~

171. ~~The method of claim 170, wherein mixed-mode chromatography comprises: equilibrating a mixed-mode chromatography column with 50 mM acetate at pH 5.2;~~

~~loading the solution passed through X0SP filtration onto the mixed-mode chromatography column;~~

washing the loaded mixed mode chromatography column with 50 mM acetate at pH 5.2; and

eluting the heterodimeric Fc-fused protein from the mixed mode chromatography column with 50 mM Acetate, 250 mM NaCl at pH 5.2 and collecting by 280 nm UV starting at 0.625 AU/cm ascending and ending at 1.50 AU/cm descending.

172.—The method of claim 171, wherein the solution comprising the heterodimeric Fc-fused protein eluted from the mixed mode chromatography column is passed through a 0.2 µm filter.

173.—The method of claim 172, wherein cation exchange chromatography comprises:
equilibrating a cation exchange chromatography resin with 50 mM Tris at pH 7.4;

loading the filtered solution eluted from the mixed mode chromatography column onto the cation exchange chromatography resin;

washing the loaded cation exchange chromatography resin with 50 mM Tris at pH 7.4;
and

eluting the heterodimeric Fc-fused protein from the cationic exchange chromatography resin with a gradient of 50 mM Tris at pH 7.4 and 50 mM Tris, 0.5 M NaCl at pH 7.4, and collecting by 280 nm UV starting at 2.5 AU/cm ascending and ending at 4.5 AU/cm descending.

174.—The method of claim 173, wherein the solution comprising the heterodimeric Fc-fused protein eluted from the cation exchange chromatography resin is passed through a 0.2 µm filter.

175.—The method of claim 174, wherein the filtered solution comprising the heterodimeric Fc-fused protein eluted from the cation exchange chromatography resin is nanofiltrated through a prefilter, a 20 nm nominal filter, and a 0.2 µm membrane.

176.—The method of claim 175, wherein the nanofiltrated solution comprising the heterodimeric Fc-fused protein is ultrafiltrated and diafiltrated, wherein ultrafiltration and diafiltration comprises:

equilibrating an ultrafiltration system with 50 mM Tris, 265 mM NaCl at pH 7.4;
concentrating the nanofiltrated solution comprising the heterodimeric Fc-fused protein to a concentration of about 5.0 g/L;

exchanging the buffer using 7 diavolumes of 20 mM citrate at pH 6.5;
concentration the diafiltrated solution comprising the heterodimeric Fc fused protein to a concentration of about 11.0 g/L;

diluting the concentration solution comprising the heterodimeric Fc fused protein to a concentration of about 5 g/L to about 10 g/L with 20 mM citrate at pH 6.5; and

adding 20 mM citrate, 18% (w/v) sucrose, 3% (w/v) mannitol, 0.03% (w/v) polysorbate-80 at pH 6.5 to achieve a final concentration of the ultrafiltration/diafiltration retentate solution comprising the heterodimeric Fc fused protein of 20 mM citrate, 6% (w/v) sucrose, 1% (w/v) mannitol, 0.01% (w/v) polysorbate-80.

177.—The method of claim 176, wherein the ultrafiltrated/diafiltrated solution comprising the heterodimeric Fc fused protein is passed through a 0.2 μ m membrane to yield a bulk drug substance.

178.—The method of claim 177, wherein the bulk drug substance is diluted to an 80% drug product solution in a 0.2 μ m filtered buffer comprising 20 mM citrate, 6% (w/v) sucrose, 1% (w/v) mannitol, and 0.01% (w/v) polysorbate-80 at pH 6.5.

179.—The method of claim 177 or 178, wherein the bulk drug substance or 80% drug product is diluted to a concentration for administration of 1 mg/mL of the heterodimeric Fc fused protein in a 0.2 μ m filtered buffer comprising 20 mM citrate, 6% (w/v) sucrose, 1% (w/v) mannitol, and 0.01% (w/v) polysorbate-80 at pH 6.5.

180.—A method of treating cancer in a subject who has received treatment with a checkpoint inhibitor antibody for at least 6 weeks, the method comprising administering 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.

~~181. The method of claim 180, wherein the checkpoint inhibitor antibody is an anti-programmed cell death protein 1 (PD-1) antibody.~~

~~182. The method of claim 180 or 181, wherein the cancer is melanoma.~~

~~183. The method of claim 182, wherein the melanoma is unresectable or metastatic.~~

~~184. The method of claim 182 or 183, wherein the subject is confirmed to have progressive disease at least 4 weeks after the initial diagnosis of progressive disease while receiving the anti PD-1 antibody.~~

~~185. The method of any one of claims 182-184, wherein the subject is confirmed to have progressive disease at least 4 weeks after the initial diagnosis of progressive disease while receiving the anti PD-1 antibody.~~

~~186. The method of any one of claims 184 or 185, wherein progressive disease is confirmed by radiological or clinical observation.~~

~~187. A method of 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, the method comprising administering 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.~~

~~188. The method of claim 187, wherein the checkpoint inhibitor antibody is an anti-PD-1 antibody or an anti-PD-L1 antibody.~~

~~189.—The method of claim 187 or 188, wherein the cancer is advanced renal cell carcinoma (RCC).~~

~~190.—The method of claim 189, wherein the RCC is unresectable or metastatic.~~

~~191.—The method of claim 189 or 190, wherein the RCC has a clear cell component.~~

~~192.—The method of any one of claims 189-191, wherein the subject received no more than 3 previous lines of therapy.~~

~~193.—The method of any one of claims 189-192, wherein the subject has not received treatment with a checkpoint inhibitor.~~

~~194.—The method of claim 193, wherein the checkpoint inhibitor comprises an anti PD-1 antibody or anti PD-L1 antibody as a monotherapy or in combination with a platinum based chemotherapy.~~

~~195.—A method of treating cancer in a subject who has received treatment with only one platinum containing regimen, the method comprising administering 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.~~

~~196.—The method of claim 195, wherein the platinum containing regimen is platinum in combination with an agent selected from gemcitabine, methotrexate, vinblastine, and doxorubicin.~~

197. ~~The method of claim 195 or 196, wherein the cancer is locally advanced or metastatic transitional cell urothelial carcinoma.~~

198. ~~The method of claim 197, wherein the urothelial carcinoma includes one or more of the group consisting of the renal pelvis, ureters, urinary urothelium, and urethra.~~

199. ~~The method of claim 197 or 198, wherein the urothelial carcinoma is inoperable.~~

200. ~~The method of any one of claims 197-199, wherein the urothelial carcinoma is characterized with radiographic progression or with recurrence within 6 months after the last administration of a platinum-containing regimen as an adjuvant.~~

201. ~~The method of any one of claims 197-200, wherein the urothelial carcinoma is considered failure of a first-line, platinum-containing regimen.~~

202. ~~The method of any one of claims 197-201, wherein the subject has received no more than 2 lines of therapy (including the platinum-containing regimen) for the treatment of the urothelial carcinoma prior to administration of the pharmaceutical formulation.~~

203. ~~The method of any one of claims 197-202, wherein the subject has not received treatment with a checkpoint inhibitor (CPI) as a first-line therapy.~~

204. ~~The method of claim 203, wherein the checkpoint inhibitor is an anti-PD-1 antibody or anti-PD-L1 antibody.~~

205. ~~The method of claim 203 or 204, wherein the checkpoint inhibitor is a monotherapy or in combination with a platinum-based chemotherapy.~~

206. ~~The method of any one of claims 195-205, wherein the pharmaceutical formulation is administered in combination with pembrolizumab.~~

207. ~~The method of claim 206, wherein pembrolizumab is administered once every 3 weeks.~~

~~208. The method of claim 206 or 207, wherein pembrolizumab is administered before administration of the pharmaceutical formulation.~~

~~209. The method of claim 208, wherein the pharmaceutical formulation is administered within one hour after the completion of administration of pembrolizumab.~~

~~210. The method of any one of claims 206-209, wherein pembrolizumab is administered at a dose of 200 mg.~~

~~211. The method of any one of claims 206-210, wherein pembrolizumab is administered intravenously.~~

~~212. The method of any one of claims 206-211, 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, urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, oesophageal cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma, and endometrial carcinoma.~~

~~213. The method of any one of claims 195-205, wherein the pharmaceutical formulation is administered in combination with nivolumab.~~

~~214. The method of claim 213, wherein nivolumab is administered before administration of the pharmaceutical formulation.~~

~~215. The method of claim 214, wherein the pharmaceutical formulation is administered within one hour after the completion of administration of nivolumab.~~

~~216. The method of any one of claims 213-215, wherein nivolumab is administered at a dose of about 480 mg.~~

~~217. The method of any one of claims 213-216, wherein nivolumab is administered intravenously.~~

218. — ~~The method of any one of claims 213-217, wherein the cancer is selected from the group consisting of: melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), renal cell carcinoma, classical Hodgkin lymphoma, head and neck squamous cell carcinoma (HNSCC), colorectal cancer, hepatocellular carcinoma, bladder cancer, and oesophageal cancer.~~

219. — ~~The method of claim 218, wherein the cancer is melanoma.~~

220. — ~~The method of claim 219, wherein the melanoma is unresectable or metastatic.~~

221. — ~~The method of claim 218, wherein the cancer is colorectal cancer.~~

222. — ~~The method of claim 221, wherein the colorectal cancer is microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer.~~

223. — ~~The method of any one of claims 180-211, wherein the pharmaceutical formulation is administered to the subject on day 1, every 3 weeks.~~

224. — ~~The method of any one of claims 180-222, wherein the pharmaceutical formulation is administered to the subject on day 1, every 4 weeks.~~

225. — ~~The method of any one of claims 180-223, wherein the pharmaceutical formulation is administered subcutaneously.~~

226. — ~~The method of any one of claims 180-225, wherein the pharmaceutical formulation is administered in a volume of about 0.1 mL to about 1 mL.~~

227. — ~~The method of claim 226, wherein the pharmaceutical formulation is administered in a volume of about 1 mL.~~

228. — ~~The method of any one of claims 180-227, wherein the pharmaceutical formulation is administered to a maximum of two injection sites.~~

229. ~~The method of claim 228, wherein a second injection is completed within 10 minutes after a first injection.~~

230. ~~The method of any one of claims 180-229, wherein the pharmaceutical formulation is administered at a dose of about 0.05 mg/kg to about 1.75 mg/kg.~~

231. ~~The method of claim 230, wherein the pharmaceutical formulation is administered at a dose of about 1 mg/kg.~~

232. ~~The method of any one of claims 180-231, wherein the pharmaceutical formulation is diluted prior to administration in a solution of 0.9% saline (sodium chloride for injection) and 0.01% polysorbate 80.~~

233. ~~The method of any one of claims 180-232, wherein the presence of the cancer is determined using the Response Evaluation Criteria for Solid Tumors (RECIST), version 1.1.~~

234. ~~The method of any one of claims 180-233, wherein a subject who has a confirmed complete response is treated with the pharmaceutical formulation for at least 12 months after confirmation unless a criterion for discontinuation is met.~~

235. ~~A method of treating a subject whose blood concentration of C-reactive protein (CRP) is monitored, the method comprising administering to the subject 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.~~

236. ~~The method of claim 235, wherein~~

~~(1) — if the CRP concentration in the subject's blood is higher than a threshold CRP concentration, then the subject is identified as being at risk for developing an adverse drug reaction; and~~

~~(2) — if the CRP concentration in the subject's blood is about the same or lower than the threshold C reactive protein concentration, the subject is not identified as being at risk for developing an adverse drug reaction.~~

~~237. — The method of claim 235, wherein if the CRP concentration in the subject's blood is higher than the threshold CRP concentration, then (1) the administration of the pharmaceutical formulation is paused; (2) the heterodimeric Fc fused protein is administered at a lower dose; or (3) a remedial action is taken to reduce or alleviate the formulation's toxicity effects in the subject.~~

~~238. — A method of treating cancer in a subject in need thereof, the method comprising subcutaneous administration of a pharmaceutical formulation comprising a heterodimeric Fc-fused protein and 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; 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.~~

~~239. — The kit of any one of claims 136-141, the use of any one of claims 142-157, or the method of any one of claims 158-238, wherein the first Fc region and second Fc region are human IgG1 Fc regions.~~

~~240. — The kit, the use, or the method of claim 239, wherein human IgG1 Fc regions comprise one or more mutation(s) that reduce(s) an effector function of an Fc.~~

~~241. The kit, the use, or the method of claim 240, wherein the first Fc region and second Fc region comprise one or more mutation(s) selected from L234A, L235A or L235E, G237A, P329A, A330S, and P331S, numbered according to the EU numbering system.~~

~~242. The kit, the use, or the method of claim 241, wherein the first Fc region and second Fc region each comprise mutations L234A, L235A, and P329A.~~

~~243. The kit, the use, or the method of claim 242, wherein the p40 subunit of IL12 comprises the amino acid sequence of SEQ ID NO: 127 and the p35 subunit of IL-12 comprises the amino acid sequence of SEQ ID NO: 128.~~

~~244. The kit, the use, or the method of claim 243, wherein the p35 subunit of IL-12 is fused to the second Fc region by a linker comprising the amino acid sequence of SEQ ID NO: 108.~~

~~245. The kit, the use, or the method of claim 244, wherein~~

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

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

~~246. The kit, the use, or the method of claim 245, wherein~~

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

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

~~247. The kit, the use, or the method of claim 246, wherein the first Fc region linked to the p40 subunit of IL12 comprises the amino acid sequence of SEQ ID NO:290 and the second Fc region linked to the p35 subunit of IL-12 comprises the amino acid sequence of SEQ ID NO:291.~~

~~248. The kit, the use, or the method of any one of claims 239-247, wherein the pharmaceutical formulation comprises: (a) citrate; (b) a sugar; (c) a sugar alcohol; and (d) a non-ionic surfactant, further wherein the pH of the formulation is between about 6.0 and about 7.0.~~

249.—~~The kit, the use, or the method of claim 248, wherein the concentration of citrate in the pharmaceutical formulation is about 10 mM to about 30 mM.~~

250.—~~The kit, the use, or the method of claim 249, wherein the concentration of citrate in the pharmaceutical formulation is about 20 mM.~~

251.—~~The kit, the use, or the method of any one of claims 248-250, wherein the concentration of the sugar in the pharmaceutical formulation is about 3% to about 12% (w/v).~~

252.—~~The kit, the use, or the method of claim 251, wherein the concentration of the sugar in the pharmaceutical formulation is about 6% (w/v).~~

253.—~~The kit, the use, or the method of claim 251 or 252, wherein the sugar is a disaccharide.~~

254.—~~The kit, the use, or the method of claim 253, wherein the disaccharide is sucrose.~~

255.—~~The kit, the use, or the method of any one of claims 248-254, wherein the concentration of the sugar alcohol in the pharmaceutical formulation is between about 0.5% to about 6% (w/v).~~

256.—~~The kit, the use, or the method of claim 255, wherein the concentration of the sugar alcohol in the pharmaceutical formulation is about 1% (w/v).~~

257.—~~The kit, the use, or the method of any one of claims 248-256, wherein the sugar alcohol is derived from a monosaccharide.~~

258.—~~The kit, the use, or the method of claim 257, wherein the sugar alcohol is mannitol.~~

259.—~~The kit, the use, or the method of any one of claims 248-258, wherein the concentration of the non-ionic surfactant in the pharmaceutical formulation is between about 0.005% to about 0.02% (w/v).~~

260. — ~~The kit, the use, or the method of claim 259, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is about 0.01% (w/v).~~

261. — ~~The kit, the use, or the method of claim 259 or 260, wherein the non-ionic surfactant is a polysorbate.~~

262. — ~~The kit, the use, or the method of claim 261, wherein the polysorbate is polysorbate 80.~~

263. — ~~The kit, the use, or the method of any one of claims 248-262, wherein the pH is between about 6.1 and about 6.9.~~

264. — ~~The kit, the use, or the method of claim 263, wherein the pH is between about 6.2 and about 6.8.~~

265. — ~~The kit, the use, or the method of claim 264, wherein the pH is between about 6.3 and about 6.7.~~

266. — ~~The kit, the use, or the method of claim 265, wherein the pH is between about 6.4 and about 6.6.~~

267. — ~~The kit, the use, or the method of claim 266, wherein the pH is about 6.5.~~

268. — ~~The kit, the use, or the method of any one of claims 248-267, wherein the pharmaceutical formulation further comprising water.~~

269. — ~~The kit, the use, or the method of claim 268, wherein the water is Water for Injection, USP.~~

270. — ~~The kit, the use, or the method of any one of claims 248-269, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc-fused protein of about 1 g/L to about 10 g/L.~~

~~271. The kit, the use, or the method of claim 270, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc fused protein of about 2 g/L to about 8 g/L.~~

~~272. The kit, the use, or the method of claim 271, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc fused protein of about 4 g/L to about 6 g/L.~~

~~273. The kit, the use, or the method of claim 272, wherein the pharmaceutical formulation comprises a bulk concentration of heterodimeric Fc fused protein of about 5 g/L.~~

~~274. The kit, the use, or the method of any one of claims 248-273, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 0.5 g/L to about 1.5 g/L.~~

~~275. The kit, the use, or the method of claim 274, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 0.75 g/L to about 1.25 g/L.~~

~~276. The kit, the use, or the method of claim 275, wherein the pharmaceutical formulation comprises a concentration of the protein for administration of about 1 g/L.~~

~~277. The kit, the use, or the method of any one of 248-276, wherein the formulation is designed to be stored at a temperature between 2°C and 8°C.~~

~~278. The kit, the use, or the method of any one of claims 248-277, wherein the pharmaceutical formulation is a clear, colorless solution and free of visible particulates.~~

~~279. The kit, the use, or the method of any one of claims 248-278, wherein the pharmaceutical formulation has a thermal stability profile as defined by:~~

~~(a) a T_{m1} of greater than about 60°C, greater than about 61°C, greater than about 62°C, greater than about 63°C, greater than about 64°C, greater than about 65°C, or greater than about 66°C; and/or~~

~~(b) a T_{m2} of greater than about 70°C, greater than about 71°C, greater than about 72°C, greater than about 73°C, greater than about 74°C, greater than about 75°C, greater than about 76°C, or greater than about 77°C,~~

as measured by differential scanning fluorimetry.

280. — ~~The kit, the use, or the method of claim 279, wherein the formulation has a thermal stability profile as defined by a T_{m1} of about 67.0°C and a T_{m2} of about 77.3°C.~~

281. — ~~The kit, the use, or the method of claim 280, wherein the thermal stability profile of the pharmaceutical formulation, as defined by T_{m1} and/or T_{m2} is changed by less than about 2°C or less than about 1°C when the pharmaceutical formulation is incubated for 1 week at 50°C, as compared to the same pharmaceutical formulation that is incubated for 1 week at 5°C, as measured by differential scanning fluorimetry.~~

282. — ~~The kit, the use, or the method of any one of 248-281, wherein the formulation has a thermal stability profile as defined by a T_{agg} of greater than 60°C, greater than about 61°C, greater than about 62°C, greater than about 63°C, greater than about 64°C, greater than about 65°C, greater than about 66°C, or greater than about 67°C, as measured by differential scanning fluorimetry.~~

283. — ~~The kit, the use, or the method of claim 282, wherein the thermal stability profile of the pharmaceutical formulation, as defined by T_{agg} is changed by less than about 2°C or less than about 1°C when the pharmaceutical formulation is incubated for 1 week at 50°C, as compared to the same pharmaceutical formulation that is incubated for 1 week at 5°C, as measured by differential scanning fluorimetry.~~

284. — ~~The kit, the use, or the method of any one of claims 248-283, wherein the pH of the pharmaceutical formulation does not change by more than about 0.2 or about 0.1 in pH value after the pharmaceutical formulation is incubated for 1 week at 5°C.~~

285. — ~~The kit, the use, or the method of any one of claims 248-284, wherein the pH of the pharmaceutical formulation does not change by more than about 0.2 or about 0.1 in pH value after the pharmaceutical formulation is incubated for 1 week at 50°C.~~

286. — ~~The kit, the use, or the method of any one of claims 248-285, wherein the heterodimeric Fe-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of~~

less than about 15 nm, less than about 14 nm, less than about 13 nm, or less than about 12 nm, as measured by dynamic light scattering at 25°C.

287.—The kit, the use, or the method of claim 286, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 11.6 nm.

288.—The kit, the use, or the method of any one of claims 248–287, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of less than about 20 nm, less than about 19 nm, less than about 18 nm, less than about 17 nm, less than about 16 nm, or less than about 15 nm, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.

289.—The kit, the use, or the method of claim 288, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 14.4 nm.

290.—The kit, the use, or the method of any one of claims 248–289, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of less than about 20 nm, less than about 19 nm, less than about 18 nm, less than about 17 nm, or less than about 16 nm, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is subjected to five freeze thaw cycles.

291.—The kit, the use, or the method of claim 290, wherein the heterodimeric Fc-fused protein in the pharmaceutical formulation has a Z-average hydrodynamic diameter of about 15.3 nm.

292.—The kit, the use, or the method of any one of claims 248–291, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.30, less than about 0.29, less than about 0.28, or less than about 0.27, as measured by dynamic light scattering at 25°C.

293.—The kit, the use, or the method of any one of claims 248–292, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.26.

294. — ~~The kit, the use, or the method of any one of claims 248-293, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.30, less than about 0.29, less than about 0.28, less than about 0.27, or less than about 0.26, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.~~

295. — ~~The kit, the use, or the method of any one of claims 248-294, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.25.~~

296. — ~~The kit, the use, or the method of any one of claims 248-295, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is less than about 0.40, less than about 0.35, or less than about 0.34, as measured by dynamic light scattering at 25°C, after the pharmaceutical formulation is subjected to five freeze-thaw cycles.~~

297. — ~~The kit, the use, or the method of any one of claims 248-296, wherein the polydispersity index of the heterodimeric Fc-fused protein in the pharmaceutical formulation is about 0.33.~~

298. — ~~The kit, the use, or the method of any one of claims 248-297, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, or greater than about 99%.~~

299. — ~~The kit, the use, or the method of claim 298, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 99.0%.~~

300. — ~~The kit, the use, or the method of any one of claims 248-299, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 75%, greater than about 80%, greater than about 81%, greater than about 82%, greater than about 83%, greater than~~

~~about 84%, or greater than about 85%, after the pharmaceutical formulation is incubated for 2 weeks at 50°C.~~

~~301. The kit, the use, or the method of claim 300, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 85.2%.~~

~~302. The kit, the use, or the method of any one of claims 248-301, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about 96%, greater than about 97%, or greater than about 98%, after the pharmaceutical formulation is subjected to five freeze thaw cycles.~~

~~303. The kit, the use, or the method of claim 302, wherein the purity profile of the pharmaceutical formulation, as measured by the area of the main peak as a percentage of total detected area in a SEC-HPLC analysis, is about 98.9%.~~