

[0005] HER2 is a transmembrane glycoprotein in the epidermal growth factor receptor family. It is a receptor tyrosine kinase and regulates cell survival, proliferation, and growth. HER2 plays an important role in human malignancies. The *ERBB2* gene is amplified or overexpressed in approximately 30% of human breast cancers. Patients with HER2-

5 overexpressing breast cancer have substantially lower overall survival rates and shorter disease-free intervals than patients whose cancer does not overexpress HER2. Moreover, overexpression of HER2 leads to increased breast cancer metastasis. Over-expression of HER2 is also known to occur in many other cancer types, including ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung, and
10 aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

[0006] Multi-specific binding proteins that bind HER2 and one or more immune cell surface proteins have been studied. For example, WO 2018/152518 describes multi-specific binding proteins that bind HER2, NKG2D, and CD16. The present disclosure adds to these developments and provides clinical methods, including dosage regimens, to treat patients
15 with specific HER2-targeting cancer immunotherapies with desired safety and efficacy. Furthermore, the present disclosure adds to the earlier developments in the field by providing formulations comprising such cancer immunotherapies that are sufficiently stable and suitable for administration to patients.

SUMMARY OF THE DISCLOSURE

20 [0007] The present disclosure provides pharmaceutical formulations comprising a multi-specific binding protein having a HER2-binding scFv, an NKG2D-binding Fab, and an antibody Fc domain, the ingredients in the formulation optimized for stability of the multi-specific binding proteins. Also provided are dosage regimens for using the multi-specific binding proteins and pharmaceutical formulations in treating cancer, such as locally advanced
25 or metastatic solid tumor.

[0007a] In one aspect, the present disclosure provides a use of a multi-specific binding protein in the manufacture of a medicament for treating cancer in a subject in need thereof in an initial four-week treatment cycle on Day 1, Day 8, and Day 15 but not on Day 22, wherein the multi-specific binding protein comprises: (a) a Fab that binds NKG2D; (b) an scFv that
30 binds HER2; and (c) an antibody Fc domain that binds CD16.

[0007b] In another aspect, the present disclosure provides a pharmaceutical formulation comprising: (a) a multi-specific binding protein comprising: (i) a Fab that binds NKG2D;

(ii) a single-chain variable fragment (scFv) that binds HER2; and (iii) an antibody Fc domain;

(b) histidine; (c) a sugar or sugar alcohol; and (d) a polysorbate, at pH 5.5 to 6.5.

[0008] Accordingly, in one aspectexample, the present disclosure provides a pharmaceutical formulation at a pH of 5.5 to 6.5 that includes histidine, a polysorbate, a sugar or sugar alcohol, and a multi-specific binding protein that includes an antibody Fc domain, a Fab that binds NKG2D, and a single-chain variable fragment (scFv) that binds HER2.

[0009] In certain embodimentexamples, the concentration of histidine in the pharmaceutical formulation is 10 to 25 mM. In certain embodimentexamples, the concentration of histidine in the pharmaceutical formulation is about 20 mM.

[0010] In certain embodimentexamples, the sugar or sugar alcohol is a disaccharide. In certain embodimentexamples, the disaccharide is sucrose. In certain embodimentexamples, the sugar or sugar alcohol is a sugar alcohol derived from a monosaccharide. In certain embodimentexamples, the sugar alcohol derived from a monosaccharide is sorbitol. In certain embodimentexamples, the concentration of the sugar or sugar alcohol in the pharmaceutical formulation is 200 to 300 mM. In certain embodimentexamples, the concentration of sugar or sugar alcohol in the pharmaceutical formulation is about 250 mM.

[0011] In certain embodimentexamples, the polysorbate is polysorbate 80. In certain embodimentexamples, the concentration of polysorbate 80 in the pharmaceutical formulation is 0.005% to 0.05%. In certain embodimentexamples, the concentration of polysorbate 80 in the pharmaceutical formulation is about 0.01%.

[0012] In certain embodimentexamples, the concentration of NaCl, if any, is about 10 mM or lower in the pharmaceutical formulation. In certain embodimentexamples, the concentration of NaCl, if any, is about 1 mM or lower in the pharmaceutical formulation.

[0013] In certain embodimentexamples, the pH of the pharmaceutical formulation is 5.8 to 6.2. In certain embodimentexamples, the pH of the pharmaceutical formulation is 5.95 to 6.05.

[0014] In certain embodimentexamples, the concentration of the multi-specific binding protein in the pharmaceutical formulation is about 10 to about 20 mg/mL.

[0015] In certain embodimentexamples, more than 94% of the multi-specific binding protein has native conformation, as determined by size-exclusion chromatography, after

incubation at 50 °C for 3 weeks. In certain [embodimentexamples](#), less than 4% of the multi-specific binding protein forms a high molecular weight complex, as determined by size-exclusion chromatography, after incubation at 50 °C for 3 weeks.

[0016] In another [aspectexample](#), the present disclosure provides use of a pharmaceutical formulation disclosed herein in treating cancer. In certain [embodimentexamples](#), the pharmaceutical formulation is diluted with 0.9% NaCl solution prior to the use.

[0017] In another [aspectexample](#), the present disclosure provides a method for treating cancer, the method comprising administering to a subject in need thereof a multi-specific binding protein in an initial four-week treatment cycle on Day 1, Day 8, and Day 15, wherein the multi-specific binding protein comprises: (a) a Fab that binds NKG2D; (b) an scFv that binds HER2; and (c) an antibody Fc domain.

[0018] In certain [embodimentexamples](#), the method further comprises administering to the subject, after the initial treatment cycle, the multi-specific binding protein in one or more subsequent four-week treatment cycles, wherein the multi-specific binding protein is administered on Day 1 and Day 15 in each subsequent treatment cycle. In certain [embodimentexamples](#), each of the doses comprises the multi-specific binding protein at an amount selected from the group consisting of 5.2×10^{-5} mg/kg, 1.6×10^{-4} mg/kg, 5.2×10^{-4} mg/kg, 1.6×10^{-3} mg/kg, 5.2×10^{-3} mg/kg, 1.6×10^{-2} mg/kg, 5.2×10^{-2} mg/kg, 1.6×10^{-1} mg/kg, 0.52 mg/kg, 1 mg/kg, 1.6 mg/kg, 5.2 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg. In certain [embodimentexamples](#), the multi-specific binding protein is administered by intravenous infusion.

[0019] In certain [embodimentexamples](#), the multi-specific binding protein is used as a monotherapy.

[0020] In certain [embodimentexamples](#), the method further comprises administering to the subject an anti-PD-1 antibody. In certain [embodimentexamples](#), the anti-PD-1 antibody is pembrolizumab. In certain [embodimentexamples](#), 200 mg of pembrolizumab is administered on Day 1 of the initial treatment cycle. In certain [embodimentexample](#), if the subject receives one or more subsequent treatment cycles, 200 mg of pembrolizumab is administered once every three weeks in the subsequent treatment cycles.

[0021] In certain [embodimentexamples](#), the cancer is HER2-positive as determined by immunohistochemistry. In certain [embodimentexample](#), the HER2 level in the cancer is scored at least 1+ as determined by immunohistochemistry. In certain [embodimentexamples](#),

the HER2 level in the cancer is scored 2+ or 3+. In certain [embodimentexamples](#), the HER2 level in the cancer is scored 3+.

[0022] In certain [embodimentexamples](#), the cancer has amplification of the *ERBB2* gene. In certain [embodimentexamples](#), the *ERBB2* gene amplification is determined by fluorescent in situ hybridization. In certain [embodimentexamples](#), the *ERBB2* gene amplification is determined by DNA sequencing.

[0023] In certain [embodimentexamples](#), the cancer is a solid tumor. In certain [embodimentexamples](#), the cancer is a locally advanced or metastatic solid tumor. In certain [embodimentexamples](#), the cancer is selected from the group consisting of gastric cancer, colorectal cancer, non-small cell lung cancer (NSCLC), head and neck cancer, biliary tract cancer, glioblastoma, sarcoma, uterine cancer, cervical cancer, ovarian cancer, esophageal cancer, squamous carcinoma of the skin, prostate cancer, carcinoma of the salivary gland, breast cancer, pancreatic cancer, and gallbladder cancer. In certain [embodimentexamples](#), the cancer is urothelial bladder cancer or metastatic breast cancer.

[0024] The following features can be incorporated into any of the [embodimentexamples](#) recited above:

[0025] In certain [embodimentexamples](#), the Fab comprises a heavy chain variable domain and a light chain variable domain, wherein (a) the heavy chain variable domain comprises complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 188, respectively; and (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.

[0026] In certain [embodimentexamples](#), (a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 169, respectively; and (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively. In certain [embodimentexamples](#), the heavy chain variable domain of the Fab comprises an amino acid sequence at least 90% identical to SEQ ID NO:94, and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98. In certain [embodimentexamples](#), the heavy chain variable

domain of the Fab comprises the amino acid sequence of SEQ ID NO:94, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:98.

[0027] In certain [embodimentexamples](#), the scFv comprises a heavy chain variable domain and a light chain variable domain, wherein (a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 115, 116, and 117, respectively; and (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 119, 120, and 121, respectively. In certain [embodimentexamples](#), the heavy chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:195, and the light chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:196. In certain [embodimentexamples](#), the heavy chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:195, and the light chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:196.

[0028] In certain [embodimentexamples](#), the light chain variable domain of the scFv is linked to the heavy chain variable domain of the scFv via a flexible linker. In certain [embodimentexamples](#), the flexible linker comprises the amino acid sequence of SEQ ID NO:143. In certain [embodimentexamples](#), the flexible linker consists of the amino acid sequence of SEQ ID NO:143. In certain [embodimentexamples](#), the light chain variable domain of the scFv is positioned to the N-terminus of the heavy chain variable domain of the scFv.

[0029] In certain [embodimentexamples](#), the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv. In certain [embodimentexamples](#), the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain.

[0030] In certain [embodimentexamples](#), the scFv comprises the amino acid sequence of SEQ ID NO:139.

[0031] In certain [embodimentexamples](#), the antibody Fc domain comprises a first antibody Fc sequence linked to the Fab and a second antibody Fc sequence linked to the scFv. In certain [embodimentexamples](#), the first antibody Fc sequence is linked to the heavy chain portion of the Fab. In certain [embodimentexamples](#), the scFv is linked to the second antibody Fc sequence via a hinge comprising Ala-Ser.

[0032] In certain embodimentexamples, the first and second antibody Fc sequences each comprise a hinge and a CH2 domain of a human IgG1 antibody. In certain

embodimentexamples, the first and second antibody Fc sequences each comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a wild-type human IgG1 antibody.

[0033] In certain embodimentexamples, the first and second antibody Fc sequences comprise different mutations promoting heterodimerization. In certain embodimentexamples, the first antibody Fc sequence is a human IgG1 Fc sequence comprising K360E and K409W substitutions. In certain embodimentexamples, the second antibody Fc sequence is a human IgG1 Fc sequence comprising Q347R, D399V, and F405T substitutions.

[0034] In certain embodimentexamples, the multi-specific binding protein comprises (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:141; (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:140; and (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:142.

[0035] Other embodimentexamples and details of the disclosure are presented herein below.

WHAT IS CLAIMED IS:

1. ~~A pharmaceutical formulation comprising:~~

~~(a) a multi-specific binding protein comprising:~~

~~(i) a Fab that binds NKG2D;~~

~~(ii) a single-chain variable fragment (scFv) that binds HER2; and~~

~~(iii) an antibody Fc domain;~~

~~(b) histidine;~~

~~(c) a sugar or sugar alcohol; and~~

~~(d) a polysorbate,~~

~~at pH 5.5 to 6.5.~~

2. ~~The pharmaceutical formulation of claim 1, wherein the concentration of histidine in the pharmaceutical formulation is 10 to 25 mM.~~

3. ~~The pharmaceutical formulation of claim 2, wherein the concentration of histidine in the pharmaceutical formulation is about 20 mM.~~

4. ~~The pharmaceutical formulation of any one of claims 1-3, wherein the sugar or sugar alcohol is a disaccharide.~~

5. ~~The pharmaceutical formulation of claim 4, wherein the disaccharide is sucrose.~~

6. ~~The pharmaceutical formulation of any one of claims 1-3, wherein the sugar or sugar alcohol is a sugar alcohol derived from a monosaccharide.~~

7. ~~The pharmaceutical formulation of claim 6, wherein the sugar alcohol derived from a monosaccharide is sorbitol.~~

- ~~8. The pharmaceutical formulation of any one of claims 4-7, wherein the concentration of the sugar or sugar alcohol in the pharmaceutical formulation is 200 to 300 mM.~~
- ~~9. The pharmaceutical formulation of claim 8, wherein the concentration of the sugar or sugar alcohol in the pharmaceutical formulation is about 250 mM.~~
- ~~10. The pharmaceutical formulation of any one of claims 1-9, wherein the polysorbate is polysorbate 80.~~
- ~~11. The pharmaceutical formulation of claim 10, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is 0.005% to 0.05%.~~
- ~~12. The pharmaceutical formulation of claim 11, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is about 0.01%.~~
- ~~13. The pharmaceutical formulation of any one of claims 1-12, wherein the concentration of NaCl, if any, is about 10 mM or lower in the pharmaceutical formulation.~~
- ~~14. The pharmaceutical formulation of claim 13, wherein the concentration of NaCl, if any, is about 1 mM or lower in the pharmaceutical formulation.~~
- ~~15. The pharmaceutical formulation of any one of claims 1-14, wherein the pH is 5.8 to 6.2.~~
- ~~16. The pharmaceutical formulation of any one of claims 1-15, wherein the pH is 5.95 to 6.05.~~
- ~~17. The pharmaceutical formulation of any one of claims 1-16, wherein the Fab comprises a heavy chain variable domain and a light chain variable domain, and wherein~~
- ~~—— (a) the heavy chain variable domain comprises complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 188, respectively; and~~
- ~~—— (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.~~

~~18. The pharmaceutical formulation of claim 17, wherein~~

~~— (a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 169, respectively; and~~

~~— (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.~~

~~19. The pharmaceutical formulation of any one of claims 1-18, wherein the heavy chain variable domain of the Fab comprises an amino acid sequence at least 90% identical to SEQ ID NO:94, and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98.~~

~~20. The pharmaceutical formulation of any one of claims 1-19, wherein the heavy chain variable domain of the Fab comprises the amino acid sequence of SEQ ID NO:94, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:98.~~

~~21. The pharmaceutical formulation of any one of claims 1-20, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain, and wherein~~

~~— (a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 115, 116, and 117, respectively; and~~

~~— (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 119, 120, and 121, respectively.~~

~~22. The pharmaceutical formulation of claim 21, wherein the heavy chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:195, and the light chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:196.~~

~~23. The pharmaceutical formulation of claim 21 or 22, wherein the heavy chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:195, and the light chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:196.~~

- ~~24. —The pharmaceutical formulation of any one of claims 21-23, wherein the light chain variable domain of the scFv is linked to the heavy chain variable domain of the scFv via a flexible linker.~~
- ~~25. —The pharmaceutical formulation of claim 24, wherein the flexible linker comprises the amino acid sequence of SEQ ID NO:143.~~
- ~~26. —The pharmaceutical formulation of claim 24 or 25, wherein the flexible linker consists of the amino acid sequence of SEQ ID NO:143.~~
- ~~27. —The pharmaceutical formulation of any one of claims 21-26, wherein the light chain variable domain of the scFv is positioned to the N-terminus of the heavy chain variable domain of the scFv.~~
- ~~28. —The pharmaceutical formulation of any one of claims 21-27, wherein the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv.~~
- ~~29. —The pharmaceutical formulation of claim 28, wherein the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain.~~
- ~~30. —The pharmaceutical formulation of any one of claims 21-29, wherein the scFv comprises the amino acid sequence of SEQ ID NO:139.~~
- ~~31. —The pharmaceutical formulation of any one of claims 1-30, wherein the antibody Fc domain comprises a first antibody Fc sequence linked to the Fab and a second antibody Fc sequence linked to the scFv.~~
- ~~32. —The pharmaceutical formulation of claim 31, wherein the first antibody Fc sequence is linked to the heavy chain portion of the Fab.~~
- ~~33. —The pharmaceutical formulation of claim 31 or 32, wherein the scFv is linked to the second antibody Fc sequence via a hinge comprising Ala-Ser.~~

~~34. The pharmaceutical formulation of any one of claims 31-33, wherein the first and second antibody Fc sequences each comprise a hinge and a CH2 domain of a human IgG1 antibody.~~

~~35. The pharmaceutical formulation of claim 34, wherein the first and second antibody Fc sequences each comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a wild-type human IgG1 antibody.~~

~~36. The pharmaceutical formulation of any one of claims 31-35, wherein the first and second antibody Fc sequences comprise different mutations promoting heterodimerization.~~

~~37. The pharmaceutical formulation of claim 36, wherein the first antibody Fc sequence is a human IgG1 Fc sequence comprising K360E and K409W substitutions.~~

~~38. The pharmaceutical formulation of claim 36 or 37, wherein the second antibody Fc sequence is a human IgG1 Fc sequence comprising Q347R, D399V, and F405T substitutions.~~

~~39. The pharmaceutical formulation of any one of claims 1-38, wherein the multi-specific binding protein comprises:~~

~~—— (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:141;~~

~~—— (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:140; and~~

~~—— (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:142.~~

~~40. The pharmaceutical formulation of any one of claims 1-39, wherein the concentration of the multi-specific binding protein in the pharmaceutical formulation is about 10 to about 20 mg/mL.~~

~~41. The pharmaceutical formulation of any one of claims 1-40, wherein more than 94% of the multi-specific binding protein has native conformation, as determined by size-exclusion chromatography, after incubation at 50 °C for 3 weeks.~~

~~42. The pharmaceutical formulation of any one of claims 1-41, wherein less than 4% of the multi-specific binding protein form a high molecular weight complex, as determined by size-exclusion chromatography, after incubation at 50 °C for 3 weeks.~~

~~43. The pharmaceutical formulation of any one of claims 1-42 for use in treating cancer.~~

~~44. The pharmaceutical formulation for use of claim 43, wherein the pharmaceutical formulation is diluted with 0.9% NaCl solution prior to the use.~~

~~451. A method for treating cancer, the method comprising administering to a subject in need thereof a~~Use of a multi-specific binding protein in the manufacture of a medicament for treating cancer in a subject in need thereof in an initial four-week treatment cycle on Day 1, Day 8, and Day 15 but not on Day 22, wherein the multi-specific binding protein comprises:

(a) a Fab that binds NKG2D;

(b) an scFv that binds HER2; and

(c) an antibody Fc domain that binds CD16.

~~462. The method-use of claim 451, wherein the treatment~~ further comprising ~~administering to the subject, that~~ after the initial treatment cycle, the multi-specific binding protein is to be administered to the subject in one or more subsequent four-week treatment cycles, wherein the multi-specific binding protein is to be administered on Day 1 and Day 15 in each subsequent treatment cycle.

~~473. The method-use of claim 45-1 or 462, wherein each of the doses comprises~~ the multi-specific binding protein is to be administered by intravenous infusion at an amount selected from the group consisting of 5.2×10^{-5} mg/kg, 1.6×10^{-4} mg/kg, 5.2×10^{-4} mg/kg, 1.6×10^{-3} mg/kg, 5.2×10^{-3} mg/kg, 1.6×10^{-2} mg/kg, 5.2×10^{-2} mg/kg, 1.6×10^{-1} mg/kg, 0.52 mg/kg, 1.0 mg/kg, 1.6 mg/kg, 5.2 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg in each dose.

~~48. The method of any one of claims 45-47, wherein the multi-specific binding protein is administered by intravenous infusion.~~

~~49. The method of any one of claims 45-48, wherein the multi-specific binding protein is used as a monotherapy.~~

~~504.~~ The ~~method-use~~ of any one of claims ~~45-48~~1-3, further comprising ~~administering to that~~
~~the subject~~ an anti-PD-1 antibody is to be administered to the subject.

~~51.~~ The method of claim 50, wherein the anti-PD-1 antibody is pembrolizumab.

~~52.~~ The method of claim 51, wherein 200 mg of pembrolizumab is administered on Day 1 of
the initial treatment cycle.

~~53.~~ The method of claim 51 or 52, wherein if the subject receives one or more subsequent
treatment cycles, 200 mg of pembrolizumab is administered once every three weeks in the
subsequent treatment cycles.

~~545.~~ The ~~method-use~~ of any one of claims ~~45-53~~1-4, wherein the cancer is HER2-positive
according to the ASCO/CAP guidelines determined by immunohistochemistry.

~~6.~~ The use of any one of claims 1-5, wherein the cancer is a locally advanced or metastatic
solid tumor.

~~55.~~ The method of any one of claims 45-53, wherein the HER2 level in the cancer is scored
at least 1+ ~~as determined by immunohistochemistry~~.

~~56.~~ The method of claim 54 or 55, wherein the HER2 level in the cancer is scored 2+ or 3+.

~~577.~~ The ~~method-use~~ of any one of claims ~~1-6-54 or 55~~1-6-54 or 55, wherein the HER2 level in the cancer
is scored 3+ as determined by immunohistochemistry.

~~8.~~ The use of any one of claims 1-6, wherein the HER2 level in the cancer is scored 2+ or
3+ as determined by immunohistochemistry.

~~9.~~ The use of claim 8, wherein the cancer has amplification of the *ERBB2* gene.

~~5810.~~ The ~~method-use~~ of any one of claims ~~45-57~~1-7, wherein the cancer has amplification of
the *ERBB2* gene.

~~59.~~ The method of claim 58, wherein *ERBB2* gene amplification is determined by
fluorescent in-situ hybridization.

~~60. The method of claim 58, wherein ERBB2 gene amplification is determined by DNA sequencing.~~

~~61. The method of any one of claims 45-60, wherein the cancer is a solid tumor.~~

~~62. The method of claim 61, wherein the cancer is a locally advanced or metastatic solid tumor.~~

~~6311. The method-use of any one of claims 621-10, wherein the cancer is urothelial bladder cancer-or.~~

~~12. The use of any one of claims 1-10, wherein the cancer is metastatic breast cancer.~~

~~13. The use of claim 7 or 9, wherein the cancer is gastric cancer.~~

~~14. The use of claim 7 or 9, wherein the cancer is esophageal cancer.~~

~~15. The use of claim 7 or 9, wherein the cancer is breast cancer.~~

~~64. The method of claim 61 or 62, wherein the cancer is selected from the group consisting of gastric cancer, colorectal cancer, non-small cell lung cancer (NSCLC), head and neck cancer, biliary tract cancer, glioblastoma, sarcoma, uterine cancer, cervical cancer, ovarian cancer, esophageal cancer, squamous carcinoma of the skin, prostate cancer, carcinoma of the salivary gland, breast cancer, pancreatic cancer, and gallbladder cancer.~~

~~65. The method of any one of claims 45-64, wherein the Fab comprises a heavy chain variable domain and a light chain variable domain, and wherein~~

~~—— (a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 188, respectively; and~~

~~—— (b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.~~

~~6616. The method-use of any one of claims 651-15, wherein:~~

(a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 169, respectively; and

(b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

~~67. —The method of any one of claims 45–66, optionally~~ wherein the heavy chain variable domain of the Fab comprises an amino acid sequence at least 90% identical to SEQ ID NO:94, and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98.

~~68. —The method of any one of claims 45–67, wherein the heavy chain variable domain of the Fab comprises the amino acid sequence of SEQ ID NO:94, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:98.~~

~~69~~17. The ~~method~~use of any one of claims ~~45–68~~1–16, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain, and wherein:

(a) the heavy chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 115, 116, and 117, respectively; and

(b) the light chain variable domain comprises CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 119, 120, and 121, respectively;

~~70. —The method of claim 69, optionally~~ wherein the heavy chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:195, and the light chain variable domain of the scFv comprises an amino acid sequence at least 90% identical to SEQ ID NO:196.

~~71. —The method of claim 69 or 70, wherein the heavy chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:195, and the light chain variable domain of the scFv comprises the amino acid sequence of SEQ ID NO:196.~~

~~7218.~~ The ~~method-use~~ of ~~any one of claims 69-71~~17, wherein the light chain variable domain of the scFv is linked to the N-terminus of the heavy chain variable domain of the scFv via a flexible linker comprising the amino acid sequence of SEQ ID NO:143.

~~73.~~—The method of claim 72, wherein the flexible linker comprises the amino acid sequence of SEQ ID NO:143.

~~74.~~—The method of claim 72 or 73, wherein the flexible linker consists of the amino acid sequence of SEQ ID NO:143.

~~75.~~—The method of any one of claims 69-74, wherein the light chain variable domain of the scFv is positioned to the N-terminus of the heavy chain variable domain of the scFv.

~~7619.~~ The ~~method-use~~ of ~~any one of claims 69-75~~17 or 18, wherein the heavy chain variable domain of the scFv comprises a cysteine residue at position 44 and forms a disulfide bridge with the light chain variable domain of the scFv comprises a cysteine residue at position 100, the amino acid positions numbered under Kabat, and wherein the two cysteine residues form a disulfide bridge.

~~77.~~—The method of claim 76, wherein the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain.

~~78.~~—The method of any one of claims 69-77, wherein the scFv comprises the amino acid sequence of SEQ ID NO:139.

~~7920.~~ The ~~method-use~~ of any one of claims ~~451-78~~19, wherein the antibody Fc domain comprises a first antibody Fc sequence linked to the heavy chain portion of the Fab and a second antibody Fc sequence linked to the scFv via a hinge comprising Ala-Ser, and wherein the first and second antibody Fc sequences comprise different mutations promoting heterodimerization.

~~80.~~—The method of claim 79, wherein the first antibody Fc sequence is linked to the heavy chain portion of the Fab.

~~81.~~—The method of claim 79 or 80, wherein the scFv is linked to the second antibody Fc sequence via a hinge comprising Ala-Ser.

~~8221.~~ The ~~method-use~~ of ~~any one of~~ claims ~~79-81~~20, wherein the first and second antibody Fc sequences each comprise a hinge and a CH2 domain of a human IgG1 antibody.

~~83.—The method of claim 82, wherein the first and second antibody Fc sequences each comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a wild-type human IgG1 antibody.~~

~~84.—The method of any one of claims 79-83, wherein the first and second antibody Fc sequences comprise different mutations promoting heterodimerization.~~

~~85.—The method of claim 84, wherein the first antibody Fc sequence is a human IgG1 Fc sequence comprising K360E and K409W substitutions.~~

~~86.—The method of claim 84 or 85, wherein the second antibody Fc sequence is a human IgG1 Fc sequence comprising Q347R, D399V, and F405T substitutions.~~

~~8722.~~ The ~~method-use~~ of any one of claims ~~45-86~~1-21, wherein the multi-specific binding protein comprises:

- (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:141;
- (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:140; and
- (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:142.

~~8823.~~ The ~~method-use~~ of any one of claims ~~45-87~~1-22, wherein the multi-specific binding protein is-has been formulated in a pharmaceutical formulation of pH 5.5 to 6.5 comprising histidine, a sugar or sugar alcohol, and a polysorbate,

optionally wherein the pharmaceutical formulation is diluted with 0.9% NaCl solution prior to administration to the subject in need thereof.

~~89.—The method of claim 88, wherein the concentration of histidine in the pharmaceutical formulation is 10 to 25 mM.~~

90. —The method of claim 89, wherein the concentration of histidine in the pharmaceutical formulation is about 20 mM.

91. —The method of any one of claims 88-90, wherein the sugar or sugar alcohol in the pharmaceutical formulation is a disaccharide.

92. —The method of claim 91, wherein the disaccharide is sucrose.

93. —The method of any one of claims 88-92, wherein the sugar or sugar alcohol in the pharmaceutical formulation is a sugar alcohol derived from a monosaccharide.

94. —The method of claim 93, wherein the sugar alcohol derived from a monosaccharide is sorbitol.

95. —The method of any one of claims 88-94, wherein the concentration of the sugar or sugar alcohol in the pharmaceutical formulation is 200 to 300 mM.

96. —The method of claim 95, wherein the concentration of the sugar or sugar alcohol in the pharmaceutical formulation is about 250 mM.

97. —The method of any one of claims 88-96, wherein the polysorbate in the pharmaceutical formulation is polysorbate 80.

98. —The method of claim 97, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is 0.005% to 0.05%.

99. —The method of claim 98, wherein the concentration of polysorbate 80 in the pharmaceutical formulation is about 0.01%.

100. —The method of any one of claims 88-99, wherein the concentration of NaCl, if any, is about 10 mM or lower in the pharmaceutical formulation.

101. —The method of claim 100, wherein the concentration of NaCl, if any, is about 1 mM or lower in the pharmaceutical formulation.

~~102.—The method of any one of claims 88-101, wherein the pH of the pharmaceutical formulation is 5.8 to 6.2.~~

~~103.—The method of any one of claims 88-102, wherein the pH of the pharmaceutical formulation is 5.95 to 6.05.~~

~~104~~24. The ~~method-use~~ of ~~any one of claims 88-103~~23, wherein the concentration of the multi-specific binding protein in the pharmaceutical formulation is ~~about~~ 10 to ~~about~~ 20 mg/mL.

~~105.—The method of any one of claims 88-104, wherein more than 94% of the multi-specific binding protein in the pharmaceutical formulation has native conformation, as determined by size-exclusion chromatography, after incubation at 50 °C for 3 weeks.~~

~~106.—The method of any one of claims 88-105, wherein less than 4% of the multi-specific binding protein in the pharmaceutical formulation form a high molecular weight complex, as determined by size-exclusion chromatography, after incubation at 50 °C for 3 weeks.~~

~~107.—The method of any one of claims 88-106, wherein the pharmaceutical formulation is diluted with 0.9% NaCl solution prior to administering to the subject in need thereof.~~

425. A pharmaceutical formulation comprising:

_____ (a) a multi-specific binding protein comprising:

_____ (i) a Fab that binds NKG2D;

_____ (ii) a single-chain variable fragment (scFv) that binds HER2; and

_____ (iii) an antibody Fc domain;

_____ (b) histidine;

_____ (c) a sugar or sugar alcohol; and

_____ (d) a polysorbate,

at pH 5.5 to 6.5.