

cells – *i.e.*, via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN- γ and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0007] NK cells respond to signals through a variety of activating and inhibitory receptors on their surface. For example, when NK cells encounter healthy self-cells, their activity is inhibited through activation of the killer-cell immunoglobulin-like receptors (KIRs). Alternatively, when NK cells encounter foreign cells or cancer cells, they are activated via their activating receptors (*e.g.*, NKG2D, NCRs, DNAM1). NK cells are also activated by the constant region of some immunoglobulins through CD16 receptors on their surface. The overall sensitivity of NK cells to activation depends on the sum of stimulatory and inhibitory signals.

[0008] HER2 (ErbB2) is a transmembrane glycoprotein, which belongs to 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-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 breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

SUMMARY

[0009] The ~~invention~~disclosure provides multi-specific binding proteins that bind to HER2 on a cancer cell and to the NKG2D receptor and CD16 receptor on natural killer cells. Such proteins can engage more than one kind of NK activating receptor, and may block the binding of natural ligands to NKG2D. In one aspect, the present disclosure provides a multi-specific binding protein comprising: (a) a first antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds NKG2D; (b) a second antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds HER2; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, wherein the first antigen-binding site, the second antigen-binding site, or each of the first and the second antigen-binding sites comprises a heavy chain variable domain and a light chain

variable domain present on the same polypeptide, and wherein the multi-specific binding protein is configured to bind HER2 on a cancer cell and bind NKG2D on a natural killer (NK) cell to activate the NK cell and bind CD16 on the NK cell to activate the NK cell. In

certain embodimentexamples, the proteins can agonize NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspectexamples and embodimentexamples of the inventiondisclosure are described in further detail below.

[0010] Accordingly, one aspectexample of the inventiondisclosure provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds to HER2; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. The antigen-binding sites may each incorporate an

antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.*, arranged as in an antibody, or fused together to form an scFv, or one or more of the antigen-binding sites may be a single domain antibody, such as a V_HH antibody like a camelid antibody or a V_{NAR} antibody like those found in cartilaginous fish.

- 5 [0011] The first antigen-binding site, which binds to NKG2D, in one **embodimentexample**, can incorporate a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:62), CDR2 (SEQ ID NO:63), and
- 10 CDR3 (SEQ ID NO:64) sequences of SEQ ID NO:1. Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:41 and a light chain variable domain related to SEQ ID NO:42. For example, the heavy chain variable domain of the first antigen binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:41, and/or incorporate amino acid
- 15 sequences identical to the CDR1 (SEQ ID NO:65), CDR2 (SEQ ID NO:66), and CDR3 (SEQ ID NO:67) sequences of SEQ ID NO:41. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:42, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:68), CDR2 (SEQ ID NO:69), and CDR3 (SEQ
- 20 ID NO:70) sequences of SEQ ID NO:42. In other **embodimentexamples**, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:43 and a light chain variable domain related to SEQ ID NO:44. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:43, and/or incorporate amino
- 25 acid sequences identical to the CDR1 (SEQ ID NO:71), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73) sequences of SEQ ID NO:43. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:44, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:74), CDR2 (SEQ ID NO:75), and CDR3 (SEQ
- 30 ID NO:76) sequences of SEQ ID NO:44.
- [0012] Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:45 and a light chain variable domain related to SEQ ID NO:46, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:45 and at least 90%

(*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:46 respectively. In another embodimentexample, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:47 and a light chain variable domain related to SEQ ID NO:48, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:47 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:48 respectively.

[0013] The second antigen-binding site can optionally incorporate a heavy chain variable domain related to SEQ ID NO:49 and a light chain variable domain related to SEQ ID NO:53.

For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:49, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:50), CDR2 (SEQ ID NO:51), and CDR3 (SEQ ID NO:52) sequences of SEQ ID NO:49. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:53 and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:54), CDR2 (SEQ ID NO:55), and CDR3 (SEQ ID NO:56) sequences of SEQ ID NO:53.

[0014] Alternatively, the second antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:57 and a light chain variable domain related to SEQ ID NO:58.

For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:57, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:77), CDR2 (SEQ ID NO:78), and CDR3 (SEQ ID NO:79) sequences of SEQ ID NO:57. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:58, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:80), CDR2 (SEQ ID NO:81), and CDR3 (SEQ ID NO:82) sequences of SEQ ID NO:58.

[0015] In another embodimentexample, the second antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:59 and a light chain variable domain related to SEQ ID NO:60. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:59, and/or incorporate amino acid sequences identical to the

CDR1 (SEQ ID NO:83), CDR2 (SEQ ID NO:84), and CDR3 (SEQ ID NO:85) sequences of SEQ ID NO:59. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:60, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:86), CDR2 (SEQ ID NO:87), and CDR3 (SEQ ID NO:88) sequences of SEQ ID NO:60.

[0016] In some embodimentexamples, the second antigen-binding site incorporates a light chain variable domain having an amino acid sequence identical to the amino acid sequence of the light chain variable domain present in the first antigen-binding site.

[0017] In some embodimentexamples, the protein incorporates a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains, and/or amino acid sequences at least 90% identical to amino acid sequence 234-332 of a human IgG antibody.

[0018] In another aspect, the present disclosure provides a formulation comprising the multi-specific binding protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure provides a cell comprising one or more nucleic acids encoding the multi-specific binding protein as described herein. Formulations containing one of these proteins; cells containing one or more nucleic acids expressing these proteins, and methods of enhancing tumor cell death using these proteins are also provided.

[0019] In yet another aspect, the present disclosure provides a use of the multi-specific binding protein as described herein or the formulation as described herein in the manufacture of a medicament for treating cancer. Another aspectexample of the inventiondisclosure involves a method of treating cancer in a patient. The method comprises administering to a patient in need thereof a therapeutically effective amount of the multi-specific binding protein described herein. Exemplary cancers for treatment using the multi-specific binding proteins include, for example, breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1** is a representation of a multi-specific binding protein that contains an NKG2D-binding domain (right arm), a tumor associated antigen-binding domain (left arm) and an Fc domain or a portion thereof that binds to CD16.

[0021] FIG. 2 is a representation of a multi-specific binding protein that contains an NKG2D-binding domain in a scFv format (right arm), a tumor associated antigen-binding domain (left arm) and an Fc domain or a portion thereof that binds to CD16.

[0022] FIG. 3 are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to human recombinant NKG2D in an ELISA assay.

[0023] FIG. 4 are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to cynomolgus recombinant NKG2D in an ELISA assay.

CLAIMS

WHAT IS CLAIMED IS:

1. A multi-specific binding protein comprising:
 - (a) a first antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds NKG2D,;
 - (b) a second antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds HER2; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, ~~or a third antigen-binding site that binds CD16~~

wherein the first antigen-binding site, the second antigen-binding site, or each of the first and the second antigen-binding sites comprises a heavy chain variable domain and a light chain variable domain present on the same polypeptide, and

wherein the multi-specific binding protein is configured to bind HER2 on a cancer cell and bind NKG2D on a natural killer (NK) cell to activate the NK cell and bind CD16 on the NK cell to activate the NK cell.

2. The multi-specific binding protein of claim 1, wherein the first antigen-binding site binds to NKG2D in humans, and non-human primates, ~~and rodents.~~

~~3. The protein of claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.~~

- ~~43. A~~ The multi-specific binding protein ~~according to of~~ claim ~~31 or 2~~, wherein the heavy chain variable domain and the light chain variable domain of the first antigen-binding site are present on the same polypeptide.

~~5. The protein according to any one of claims 3-4, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.~~

64. The multi-specific binding protein according to any one of claims 1-35, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

5. The multi-specific binding protein according to claim 4, consisting essentially of:

(i) a first immunoglobulin heavy chain consisting essentially of a single-chain variable fragment (scFv) fused via a linker or an antibody hinge to the N-terminus of a first Fc domain;

(ii) a second immunoglobulin heavy chain consisting essentially of a heavy chain variable domain and a CH1 heavy chain domain, linked to the N-terminus of a second Fc domain; and

(iii) an immunoglobulin light chain consisting essentially of a light chain variable domain and a constant light chain domain,

wherein:

(a) the scFv comprises a heavy chain variable domain and a light chain variable domain that pair to bind NKG2D, and the heavy chain variable domain of (ii) and the light chain variable domain of (iii) pair to bind HER2;

or

(b) the scFv comprises a heavy chain variable domain and a light chain variable domain that pair to bind HER2, and the heavy chain variable domain of (ii) and the light chain variable domain of (iii) pair to bind NKG2D,

and wherein the first and second Fc domains form a dimer that binds CD16.

6. The multi-specific binding protein according to claim 5, wherein the heavy chain variable domain and the light chain variable domain of the scFv pair to bind NKG2D, and the heavy chain variable domain of (ii) and the light chain variable domain of (iii) pair to bind HER2.

7. The multi-specific binding protein according to any one of claims 1-6, wherein the heavy chain variable domain and the light chain variable domain that pair to bind NKG2D block a natural ligand from binding to and activating NKG2D.

8. The multi-specific binding protein according to claim 7, wherein the natural ligand is ULBP6 or MICA.

~~7. — The protein according to claim 5 or 6, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.~~

89. The multi-specific binding protein according to any one of ~~the preceding~~ claims 1-6, wherein the heavy chain variable domain and the light chain variable domain that pair to bind NKG2D comprise: the first antigen-binding site comprises

(a) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:1;_i

~~9. — The protein according to any of claims 1-7, wherein the first antigen-binding site comprises-~~(b) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:41 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:42;_i

~~10. — The protein according to any of claims 1-7, wherein the first antigen-binding site comprises-~~(c) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:43 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:44;_i

~~11. — The protein according to any of claims 1-7, wherein the first antigen-binding site comprises-~~(d) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:45 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:46;_i

~~12. — The protein according to any of claims 1-7, wherein the first antigen-binding site comprises-~~(e) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:47 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:48;_i

~~13. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises (f) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:94 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:95; or-~~

~~14. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises (g) a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:102 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:103.~~

~~15. The protein of claim 1 or 2, wherein the first antigen-binding site is a single domain antibody.~~

~~16. The protein of claim 15, wherein the single domain antibody is a V_HH fragment or a V_{NAR} fragment.~~

~~17. The protein according to any one of claims 1-2 or 15-16, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.~~

~~18. The protein according to claim 17, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.~~

~~1910. The multi-specific bindingA protein according to any one of the preceding claims 1-9, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 50, 51, and 52, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 54, 55, and 56, respectively.~~

~~optionally wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:49 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:53.~~

~~20. The protein according to any of the preceding claims, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:50;~~
~~a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:51;~~
~~and~~

~~—— a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:52.~~

~~21. The protein according to claim 20, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:54;~~

~~a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:55; and~~

~~—— a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:56.~~

2211. The multi-specific bindingA protein according to any one of claims 1-189, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 77, 78, and 79, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 80, 81, and 82, respectively,

optionally wherein the heavy chain variable domain ~~of the second antigen-binding site~~ comprises an amino acid sequence at least 90% identical to SEQ ID NO:57 and the light chain variable domain ~~of the second antigen-binding site~~ comprises an amino acid sequence at least 90% identical to SEQ ID NO:58.

~~23. The protein according to any one of claims 1-18 or 22, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:77;~~

~~—— a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:78;~~
~~and~~

~~—— a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:79.~~

~~24. The protein according to claim 23, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

- ~~a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:80;~~
- ~~— a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:81; and~~
- ~~— a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:82.~~

2512. The multi-specific bindingA protein according to any one of claims 1-189, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 83, 84, and 85, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 86, 87, and 88, respectively.

optionally wherein the heavy chain variable domain ~~of the second antigen-binding site~~ comprises an amino acid sequence at least 90% identical to SEQ ID NO:59 and the light chain variable domain ~~of the second antigen-binding site~~ comprises an amino acid sequence at least 90% identical to SEQ ID NO:60.

~~26. The protein according to any one of claims 1-18 or 25, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

- ~~a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:83;~~
- ~~— a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:84;~~
- ~~and~~
- ~~— a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:85.~~

~~27. The protein according to any one of claim 26, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

- ~~a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:86;~~
- ~~— a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:87; and~~

~~—— a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:88.~~

~~28. — The protein according to any one of claims 1-4 or 8-16, wherein the second antigen-binding site is a single domain antibody.~~

~~29. — The protein of claim 28, wherein the second antigen binding site is a V_HH fragment or a V_{NAR} fragment.~~

~~3013. The multi-specific bindingA protein according to any one of the preceding claims 1-12, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises comprising hinge and CH2 domains of an antibody Fc domain, optionally.~~

~~31. — The protein according to claim 30, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.~~

~~3214. The multi-specific binding protein according to claim 30-13or 31, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, optionally.~~

~~33. — The protein according to any one of claims 30-32, wherein the Fc domain comprises an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, T350, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439, numbered according to the EU index as in Kabat.~~

~~3415. A formulation comprising the multi-specific bindingA protein according to any one of the preceding claims 1-14 and a pharmaceutically acceptable carrier.~~

~~3516. A cell comprising one or more nucleic acids encoding the expressing a multi-specific binding protein according to any one of claims 1-3314.~~

~~36. — A method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to a protein according to any one of claims 1-33.~~

~~3717. A method of treating cancer, wherein the method comprises administering~~Use of thea
~~multi-specific binding~~ protein according to any one of claims 1-~~33-14~~ or ~~a the~~ formulation
according to claim ~~34~~15 in the manufacture of a medicament for treating cancer~~to a patient~~.

~~3818.~~ The ~~use~~method of claim ~~3717~~, wherein the cancer is selected from the group
consisting of breast cancer, ovarian cancer, esophageal cancer, bladder cancer, ~~and~~ gastric
cancer, salivary duct carcinoma, adenocarcinoma of the lung, and an aggressive forms of uterine
cancer, optionally wherein the aggressive form of uterine cancer is~~such as~~ uterine
serous endometrial carcinoma.