

[0006] Natural killer (NK) cells are a component of the innate immune system and make up approximately 15% of circulating lymphocytes. NK cells infiltrate virtually all tissues and were originally characterized by their ability to kill tumor cells effectively without the need for prior sensitization. Activated NK cells kill target cells by means similar to cytotoxic T 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 relates to multi-specific binding proteins that bind to and kill human cancer cells expressing epidermal growth factor receptor 2 (HER2 or ErbB2). 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 certain ~~embodiment~~examples, the proteins can agonize NK cells in humans, and in other species

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

[0009a] In one aspect, the present disclosure provides a multispecific binding protein comprising: a Fab fragment that binds NKG2D, a single-chain variable fragment (scFv) that binds HER2 and an antibody Fc domain or a portion thereof sufficient to bind CD16, comprising: (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. In another aspect, the present disclosure provides a use of a protein as described herein in the manufacture of a medicament for treating cancer, comprising that the protein is to be administered to a patient.

[0010] In one aspectexample, the present inventiondisclosure provides a protein (*e.g.*, a multi-specific binding protein) comprising: (a) a first antigen-binding site comprising an Fab fragment that binds NKG2D; (b) a second antigen-binding site comprising a single-chain variable fragment (scFv) 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.

[0011] In certain embodimentexamples, the scFv is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16, or the third antigen-binding site that binds CD16, via a hinge comprising Ala-Ser. In certain embodimentexamples, the scFv is linked to the antibody Fc domain.

[0012] In certain embodimentexamples, the scFv comprises a heavy chain variable domain and a light chain variable domain. 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.

[0013] 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 is positioned to the N-terminus or C-terminus to the heavy chain variable domain. In certain embodimentexamples, the light chain variable domain is positioned to the N-terminus of the heavy chain variable domain.

[0014] In certain embodiment examples, the Fab fragment is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16, or the third antigen-binding site that binds CD16.

[0015] In certain embodiment examples, the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain comprising 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 169, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively. In certain embodiment examples, the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences

represented by the amino acid sequences of SEQ ID NOs: 95, 96, and 97, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.

[0016] In certain ~~embodiment~~examples, a protein of the present ~~invention~~disclosure comprising a first antigen-binding that binds NKG2D, comprises:

(a) a heavy chain variable domain comprising 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 169, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(b) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 173, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(c) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 95, 96, and 97, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(d) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 166, 88, and 167, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 91, 92, and 93, respectively;

(e) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 162, 72, and 170, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 107, 108, and 109, respectively;

(f) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 162, 72, and 163, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3

sequences represented by the amino acid sequences of SEQ ID NOs: 75, 76, and 77, respectively;

(g) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 164, 80, and 165, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 75, 76, and 85, respectively;

(h) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 176, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(i) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 179, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(j) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 182, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

(k) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 185, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively; or

(l) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 188, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively; and

a second antigen-binding site comprising an scFv that binds HER2, comprises:

(a) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 115, 116, and 117, respectively, and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 119, 120, and 121,

respectively;

(b) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 123, 124, and 125, respectively, and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 127, 128, and 129, respectively; or

(c) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 131, 132, and 133, respectively, and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 135, 136, and 137, respectively.

[0017] In some embodiment examples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:94 and a light chain variable domain related to SEQ ID NO:98. 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:94, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:97 or 169) sequences of SEQ ID NO:94. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0018] In some embodiment examples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:144 and a light chain variable domain related to SEQ ID NO:98. 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:144, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:172 or 173) sequences of SEQ ID NO:144. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0019] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:174 and a light chain variable domain related to SEQ ID NO:98. 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:174, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:175 or 176) sequences of SEQ ID NO:174. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0020] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:177 and a light chain variable domain related to SEQ ID NO:98. 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:177, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:178 or 179) sequences of SEQ ID NO:177. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0021] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:180 and a light chain variable domain related to SEQ ID NO:98. 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:180, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:181 or 182) sequences of SEQ ID NO:180. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0022] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:183 and a light chain variable domain related to SEQ ID NO:98. 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:183, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:184 or 185) sequences of SEQ ID NO:183. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0023] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:186 and a light chain variable domain related to SEQ ID NO:98. 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:186, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or 168), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:187 or 188) sequences of SEQ ID NO:186. 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:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0024] In some embodimentexamples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:86 and a light chain variable domain related to SEQ ID NO:90. 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:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or 166), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:89 or 167) sequences of SEQ ID NO:86. 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:90, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:91), CDR2 (SEQ ID NO:92), and CDR3 (SEQ ID NO:93) sequences of SEQ ID NO:90.

[0025] In some embodiment examples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:102 and a light chain variable domain related to SEQ ID NO:106. 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:102, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71 or 162), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:105 or 170) sequences of SEQ ID NO:102. 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:106, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:107), CDR2 (SEQ ID NO:108), and CDR3 (SEQ ID NO:109) sequences of SEQ ID NO:106.

[0026] In some embodiment examples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:70 and a light chain variable domain related to SEQ ID NO:74. 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:70, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71 or 162), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73 or 163) sequences of SEQ ID NO:70. 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:74, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:75), CDR2 (SEQ ID NO:76), and CDR3 (SEQ ID NO:77) sequences of SEQ ID NO:74.

[0027] In some embodiment examples, the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:70 and a light chain variable domain related to SEQ ID NO:74. 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:70, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71 or 162), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73 or 163) sequences of SEQ ID NO:70. 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:74, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:75), CDR2 (SEQ ID NO:76), and CDR3 (SEQ ID NO:77) sequences of SEQ ID NO:74.

[0028] In some [embodimentexamples](#), the first antigen-binding site comprises a heavy chain variable domain related to SEQ ID NO:78 and a light chain variable domain related to SEQ ID NO:82. 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:78, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:79 or 164), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81 or 165) sequences of SEQ ID NO:78. 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:82, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:75), CDR2 (SEQ ID NO:76), and CDR3 (SEQ ID NO:77) sequences of SEQ ID NO:82.

[0029] In certain [embodimentexamples](#), the first antigen-binding site binds to NKG2D with a K_D of 2 nM to 120 nM, as measured by surface plasmon resonance. In certain [embodimentexamples](#), the protein binds to NKG2D with a K_D of 2 nM to 120 nM, as measured by surface plasmon resonance.

[0030] In some [embodimentexamples](#), the second antigen-binding site binding to HER2 comprises a heavy chain variable domain related to SEQ ID NO:195 and a light chain variable domain related to SEQ ID NO:196. 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:195, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:115), CDR2 (SEQ ID NO:116), and CDR3 (SEQ ID NO:117) sequences of SEQ ID NO:195. 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:196, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:119), CDR2 (SEQ ID NO:120), and CDR3 (SEQ ID NO:121) sequences of SEQ ID NO:196. In some [embodimentexamples](#), the second antigen-binding site binding to HER2 comprises an scFv comprising the amino acid sequence of SEQ ID NO:139.

[0031] Alternatively, the second antigen-binding site binding to HER2 comprises a heavy chain variable domain related to SEQ ID NO:197 and a light chain variable domain related to SEQ ID NO:198. 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:197, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:123), CDR2 (SEQ ID NO:124), and CDR3 (SEQ ID

NO:125) sequences of SEQ ID NO:197. 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:198, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:127), CDR2 (SEQ ID NO:128), and CDR3 (SEQ ID NO:129) sequences of SEQ ID NO:198. In some [embodimentexamples](#), the second antigen-binding site binding to HER2 comprises an scFv comprising the amino acid sequence of SEQ ID NO:189.

[0032] Alternatively, the second antigen-binding site binding to HER2 comprises a heavy chain variable domain related to SEQ ID NO:199 and a light chain variable domain related to SEQ ID NO:200. 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:199, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:131), CDR2 (SEQ ID NO:132), and CDR3 (SEQ ID NO:133) sequences of SEQ ID NO:199. 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:200, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:135), CDR2 (SEQ ID NO:136), and CDR3 (SEQ ID NO:137) sequences of SEQ ID NO:200. In some [embodimentexamples](#), the second antigen-binding site binding to HER2 comprises an scFv comprising the amino acid sequence of SEQ ID NO:171.

[0033] In certain [embodimentexamples](#), the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody. In certain [embodimentexamples](#), the antibody Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

[0034] In certain [embodimentexample](#), the antibody Fc domain comprises an Fc domain comprising 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, K439.

[0035] In certain [embodimentexamples](#), the antibody Fc domain comprises an Fc domain of a human IgG1 comprising Q347R, D399V, and F405T substitutions, *e.g.*, in an Fc domain linked to an scFv. In certain [embodimentexamples](#), the antibody Fc domain comprises an Fc domain of a human IgG1 comprising K360E and K409W substitutions, *e.g.*, in an Fc domain linked to an Fab fragment.

[0036] In certain [embodimentexamples](#), the antibody Fc domain comprises an Fc domain of a human IgG1 comprising a T366W substitution, *e.g.*, in an Fc domain linked to an Fab fragment. In certain [embodimentexamples](#), the antibody Fc domain comprises an Fc domain of a human IgG1 comprising T366S, L368A, and Y407V substitutions, *e.g.*, in an Fc domain linked to an scFv.

[0037] In certain [embodimentexamples](#), the protein comprises a sequence of SEQ ID NO:141, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:194, SEQ ID NO:155, or SEQ ID NO:148. In certain [embodimentexamples](#), this sequence represents the heavy chain portion of the Fab fragment linked to the antibody Fc domain.

[0038] In certain [embodimentexamples](#), the protein comprises a sequence of SEQ ID NO:140 or SEQ ID NO:146. In certain [embodimentexamples](#), this sequence represents the scFv linked to the antibody Fc domain.

[0039] In another [aspectexample](#), the instant disclosure provides a protein (*e.g.*, a multi-specific binding protein) comprising: (a) a first polypeptide comprising 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:141; (b) a second polypeptide comprising 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:140, SEQ ID NO:190, or SEQ ID NO:192; and (c) a third polypeptide comprising 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:142. In certain [embodimentexamples](#), the second polypeptide comprises 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:140.

[0040] In another [aspectexample](#), the instant disclosure provides a protein (*e.g.*, a multi-specific binding protein) comprising: (a) a first polypeptide comprising an amino acid sequence at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:141; (b) a second polypeptide comprising an amino acid sequence at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:140, SEQ ID NO:190, or SEQ ID NO:192; and (c) a third polypeptide comprising an amino acid sequence at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:142. In certain [embodimentexamples](#), the second polypeptide comprises an amino acid sequence at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:140.

[0041] In another [aspectexample](#), the instant disclosure provides a protein (*e.g.*, a multi-specific binding protein) comprising: (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, SEQ ID NO:190, or SEQ ID NO:192; and (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:142. In certain embodiment examples, the 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.

[0042] In another aspect, the instant disclosure provides a formulation comprising a protein disclosed herein and a pharmaceutically acceptable carrier.

[0043] In another aspect, the instant disclosure provides a cell comprising one or more nucleic acids expressing a protein disclosed herein.

[0044] In another aspect example, the instant disclosure provides a method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to a protein disclosed herein.

[0045] In another aspect example, the instant disclosure provides a method of treating cancer, wherein the method comprises administering a protein or a formulation disclosed herein to a patient in need thereof. In certain embodiment examples, the cancer is selected from the group consisting of breast cancer, thyroid cancer, gastric cancer, renal cell carcinoma, adenocarcinoma of the lung, prostate cancer, cholangiocarcinoma, uterine cancer, pancreatic cancer, colorectal cancer, ovarian cancer, cervical cancer, head and neck cancer, lung squamous, mesothelioma, liver cancer, mesothelioma, sarcoma, and gall bladder cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] **FIG. 1** illustrates a trispecific antibody (TriNKET) that contains an HER2-binding scFv, a NKG2D-targeting Fab, and a heterodimerized antibody constant region/domain (“CD domain”) that binds CD16 (scFv-Fab format). In an exemplary embodiment, the Fc domain linked to the Fab fragment comprises mutations of K360E and K409W and the Fc domain linked to the scFv comprises matching mutations Q347R, D399V, and F405T for forming an Fc heterodimer (shown as a triangular lock-and-key format in the Fc domains in FIG. 1). The antibody format is referred herein as F3’-TriNKET. In another exemplary embodiment, the Fc domain linked to the Fab fragment comprises the mutations of Q347R, D399V, and F405T, and the Fc domain linked to the scFv comprises matching mutations K360E and K409W for forming a heterodimer.

[0047] **FIG. 2** is a representation of a “knob-in-hole” (KiH) TriNKET, which includes an scFv that binds HER2 linked to an Fc domain via a hinge comprising Ala-Ser, and an NKG2D-binding Fab fragment including (a) a heavy chain portion comprising a heavy chain

CLAIMS

WHAT IS CLAIMED IS:

1. A multispecific binding protein comprising:
 - 5 (a) ~~—a first antigen-binding site comprising an~~ Fab fragment that binds NKG2D₂;
 - (b) ~~—a second antigen-binding site comprising a~~ single-chain variable fragment (scFv) that binds HER2₂; and
 - (c) ~~—an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.~~
- 10 2. ~~—The protein according to claim 1, wherein the scFv is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16, or the third antigen-binding site that binds CD16, via a hinge comprising Ala-Ser.~~
3. ~~—The protein according to claim 2, wherein the scFv is linked to the antibody Fc domain.~~
- 15 4. ~~—The protein according to any one of claims 1-3, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain.~~
5. ~~—The protein according to claim 4, wherein the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv.~~
6. ~~—The protein according to claim 5, wherein the disulfide bridge is formed between C44~~
20 ~~of the heavy chain variable domain and C100 of the light chain variable domain.~~
7. ~~—The protein according to any one of claims 4-6, wherein the light chain variable domain of the scFv is linked to the heavy chain variable domain of the scFv via a flexible linker.~~
8. ~~—The protein according to claim 7, wherein the flexible linker comprises the amino acid~~
25 ~~sequence of SEQ ID NO:143.~~
9. ~~—The protein according to claim 8, wherein the flexible linker consists of the amino acid sequence of SEQ ID NO:143.~~
10. ~~—The protein according to any one of claims 4-9, wherein the light chain variable domain is positioned to the N-terminus or C-terminus to the heavy chain variable domain.~~

11. —The protein according to claim 10, wherein the light chain variable domain is positioned to the N-terminus of the heavy chain variable domain.

12. —The protein according to any one of claims 1-11, wherein the Fab fragment is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16, or the third antigen-binding site that binds CD16.

13. —A protein according to any one of claims 1-12, wherein the first antigen-binding site that binds NKG2D comprises:

——(a) a heavy chain variable domain comprising 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 169, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(b) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 173, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(c) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 95, 96, and 97, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(d) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 166, 88, and 167, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 91, 92, and 93, respectively;

——(e) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 162, 72, and 170, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 107, 108, and 109, respectively;

——(f) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 162, 72, and 163, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by

the amino acid sequences of SEQ ID NOs: 75, 76, and 77, respectively;

——(g) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 164, 80, and 165, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 75, 76, and 85, respectively;

——(h) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 176, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(i) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 179, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(j) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 182, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively;

——(k) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 185, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively; or

——(l) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 168, 96, and 188, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 99, 100, and 101, respectively.

14.——The protein according to any one of claims 1-13, wherein the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain at least 90% identical to an amino acid sequence selected from SEQ ID NO:86 and SEQ ID NO:94.

15.——The protein according to any one of claims 1-14, wherein the first antigen-binding site that binds NKG2D comprises:

——(a) a heavy chain variable domain at least 90% identical to SEQ ID NO:94 and a light chain variable domain at least 90% identical to SEQ ID NO:98;

~~—— (b) a heavy chain variable domain at least 90% identical to SEQ ID NO:144 and a light chain variable domain at least 90% identical to SEQ ID NO:98; or~~
~~—— (c) a heavy chain variable domain at least 90% identical to SEQ ID NO:86 and a light chain variable domain at least 90% identical to SEQ ID NO:90.~~

16. ~~The protein according to any one of claims 1-15, wherein the first antigen-binding site that binds NKG2D comprises:~~

~~—— (a) a heavy chain variable domain at least 95% identical to SEQ ID NO:94 and a light chain variable domain at least 95% identical to SEQ ID NO:98;~~

~~—— (b) a heavy chain variable domain at least 95% identical to SEQ ID NO:144 and a light chain variable domain at least 95% identical to SEQ ID NO:98; or~~

~~—— (c) a heavy chain variable domain at least 95% identical to SEQ ID NO:86 and a light chain variable domain at least 95% identical to SEQ ID NO:90.~~

17. ~~The protein according to any one of claims 1-16, wherein the first antigen-binding site that binds NKG2D comprises:~~

~~—— (a) a heavy chain variable domain identical to SEQ ID NO:94 and a light chain variable domain identical to SEQ ID NO:98;~~

~~—— (b) a heavy chain variable domain identical to SEQ ID NO:144 and a light chain variable domain identical to SEQ ID NO:98; or~~

~~—— (c) a heavy chain variable domain identical to SEQ ID NO:86 and a light chain variable domain identical to SEQ ID NO:90.~~

18. ~~A protein according to any one of claims 1-17, wherein the protein binds to NKG2D with a K_D of 2 nM to 120 nM, as measured by surface plasmon resonance.~~

19. ~~The protein according to any one of claims 1-18, wherein the second antigen-binding site that binds HER2 comprises:~~

~~—— (a) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 115, 116, and 117, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 119, 120, and 121, respectively;~~

~~—— (b) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 123, 124, and 125, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences~~

represented by the amino acid sequences of SEQ ID NOs: 127, 128, and 129, respectively; or

~~—— (c) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 131, 132, and 133, respectively, and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences~~

~~represented by the amino acid sequences of SEQ ID NOs: 135, 136, and 137, respectively.~~

20. ~~The protein according to any one of claims 1-19, wherein the second antigen-binding site that binds HER2 comprises:~~

~~—— (a) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:195 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:196;~~

~~—— (b) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:197 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:198; or~~

~~—— (c) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:199 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:200.~~

21. ~~The protein according to any one of claims 1-20, wherein the second antigen-binding site that binds HER2 comprises:~~

~~—— (a) a heavy chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:195 and a light chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:196;~~

~~—— (b) a heavy chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:197 and a light chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:198; or~~

~~—— (c) a heavy chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:199 and a light chain variable domain comprising an amino acid sequence at least 95% identical to SEQ ID NO:200.~~

22. ~~The protein according to any one of claims 1-21, wherein the second antigen-binding site that binds HER2 comprises:~~

~~—— (a) a heavy chain variable domain comprising an amino acid sequence identical to SEQ ID NO:195 and a light chain variable domain comprising an amino acid sequence identical to SEQ ID NO:196;~~

~~——(b) a heavy chain variable domain comprising an amino acid sequence identical to SEQ ID NO:197 and a light chain variable domain comprising an amino acid sequence identical to SEQ ID NO:198; or~~

~~——(c) a heavy chain variable domain comprising an amino acid sequence identical to SEQ ID NO:199 and a light chain variable domain comprising an amino acid sequence identical to SEQ ID NO:200.~~

~~23.——The protein according to any one of claims 1-22, wherein the second antigen-binding site that binds HER2 comprises the amino acid sequence of SEQ ID NO:139, SEQ ID NO:189, or SEQ ID NO:171.~~

~~24.——The protein according to claim 23, wherein the second antigen-binding site that binds HER2 comprises the amino acid sequence of SEQ ID NO:139.~~

~~25.——The protein according to any one of claims 1-24, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.~~

~~26.——The protein according to claim 25, wherein the antibody Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.~~

~~27.——The protein according to claim 25 or 26, wherein the antibody Fc domain comprises 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, K439.~~

~~28.——The protein according to any one of claims 1-27, wherein the antibody Fc domain is an Fc domain of a human IgG1 comprising Q347R, D399V, and F405T substitutions.~~

~~29.——The protein according to claim 28, wherein the antibody Fc domain comprising the substitutions is linked to the scFv.~~

~~30.——The protein according to any one of claims 1-29, wherein the antibody Fc domain is an Fc domain of a human IgG1 comprising K360E and K409W substitutions.~~

31. — The protein according to claim 30, wherein the antibody Fc domain comprising the substitutions is linked to the Fab fragment.

32. — The protein according to any one of claims 1-27, wherein the antibody Fc domain is an Fc domain of a human IgG1 comprising a T366W substitution.

5 33. — The protein according to claim 32, wherein the antibody Fc domain comprising the substitution is linked to the Fab fragment.

34. — The protein according to any one of claims 1-27 and 32-33, wherein the antibody Fc domain is an Fc domain of a human IgG1 comprising T366S, L368A, and Y407V substitutions.

10 35. — The protein according to claim 34, wherein the antibody Fc domain comprising the substitutions is linked to the scFv.

36. — The protein according to any of claims 1-35, comprising a sequence of SEQ ID NO:141, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:194, SEQ ID NO:155, or SEQ ID NO:148.

15 37. — The protein according to any one of claims 1-36, wherein the scFv linked to the antibody Fc domain is represented by the amino acid sequence of SEQ ID NO:140 or SEQ ID NO:146.

38. — A protein comprising:

20 — (a) a first polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO:141;

— (b) a second polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO:140, SEQ ID NO:190, or SEQ ID NO:192; and

— (c) a third polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO:142.

25 39. — The protein according to claim 38, wherein the second polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO:140.

40. — A protein comprising:

— (a) a first polypeptide comprising an amino acid sequence at least 95% identical to

SEQ ID NO:141;

~~—(b) a second polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:140, SEQ ID NO:190, or SEQ ID NO:192; and~~

~~—(c) a third polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:142.~~

~~41. The protein according to claim 40, wherein the second polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:140.~~

~~42. A protein comprising:~~

~~(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, SEQ ID NO:190, or SEQ ID NO:192; and~~

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

~~43. The protein according to claim 42, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO:140.~~

~~44. A formulation comprising a protein according to any one of the preceding claims 1~~
and a pharmaceutically acceptable carrier.

~~45. A cell comprising one or more nucleic acids expressing a protein according to any one of claims 1-43.~~

~~46. 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 1-43.~~

~~47. A method of treating cancer, wherein the method comprises administering~~
Use of a protein according to ~~any one of claims 1-43 or a formulation according to claim 44 in the manufacture of a medicament for treating cancer, comprising that the protein is to be~~
administered to a patient.

~~48. The method-use~~ of claim ~~47~~, wherein the cancer is selected from the group consisting of breast cancer, thyroid cancer, gastric cancer, renal cell carcinoma, adenocarcinoma of the lung, prostate cancer, cholangiocarcinoma, uterine cancer, pancreatic

cancer, colorectal cancer, ovarian cancer, cervical cancer, head and neck cancer, lung squamous, mesothelioma, liver cancer, mesothelioma, sarcoma, and gall bladder cancer.

6. The use of claim 4, wherein the cancer is gastric cancer.

5 7. The use of claim 4, wherein the cancer is esophageal cancer.

8. The use of claim 4, wherein the cancer is breast cancer.

9. The use of claim 4, wherein the cancer is bladder cancer.

10

10. The use of claim 4, wherein the cancer is lung cancer.

11. The use of claim 4, wherein the cancer is adenocarcinoma of the lung.

15 12. The use of claim 4, wherein the cancer is lung squamous cell carcinoma.

13. The use of claim 4, further comprising that an immune checkpoint inhibitor that inhibits programmed cell death protein 1 (PD1) is to be administered.

20 14. The use of claim 4, further comprising that an immune checkpoint inhibitor that inhibits PDL1 is to be administered.

15. The use of claim 4, wherein further comprising that an immune checkpoint inhibitor that inhibits LAG3 is to be administered.

25