

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. NKG2D is a type-II transmembrane protein that is expressed by essentially all natural killer cells where NKG2D serves as an activating receptor. NKG2D is also found on T cells where it acts as a costimulatory receptor. The ability to modulate NK cell function via NKG2D is useful in various therapeutic contexts including malignancy.

[0008] Mutations that lead to epidermal growth factor receptor (EGFR) overexpression or overactivity have been associated with a number of cancers, including non-small cell lung cancer, anal cancers, glioblastoma and epithelial tumors of the head and neck. These somatic mutations involving EGFR lead to its constant activation, which produces uncontrolled cell division. In glioblastoma a more or less specific mutation of EGFR, called EGFR ν III is often observed. Mutations, amplifications or misregulations of EGFR or family members are implicated in other solid tumors, including colorectal cancer, renal cell carcinoma, bladder cancer, cervical cancer, ovarian cancer, pancreatic cancer, and liver cancer.

[0009] Anti-EGFR monoclonal antibodies, such as cetuximab, panitumumab, necitumumab, and zalutumumab, have been developed. However, there remains a need for new and useful antibodies and related therapies for use in treatment of cancer that have greater efficacy and reduced adverse effects.

SUMMARY

[0010] The present application provides multispecific binding proteins that bind to the NKG2D receptor, CD16 receptor on natural killer cells, and EGFR. 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 protein comprising: (a) a first

antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds EGFR; 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 second antigen-binding site comprises: (i) a heavy chain variable domain (VH) comprising complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences of SEQ ID NOs: 136, 146, and 138, respectively; and a light chain variable domain (VL) comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively; (ii) a VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 137, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively; or (iii) a VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 146, and 138, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 142, respectively; wherein the VL of the second antigen-binding site comprises an F87Y substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme. In certain examples, the proteins can agonize NK cells in humans. In some

examples, the proteins can agonize NK cells in humans and in other species such as rodents and cynomolgus monkeys. Formulations containing any one of the proteins disclosed herein; cells containing one or more nucleic acids expressing the proteins, and methods of enhancing tumor cell death using the proteins are also provided.

5 **[0011]** Accordingly, one example of the present application provides a protein comprising (a) a first antigen-binding site that binds NKG2D, (b) a second antigen-binding site that binds EGFR, 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 second antigen-binding site comprises: (i) a heavy chain variable domain (VH) comprising complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences of SEQ ID NOs: 136, 157, and 138, respectively, and a light chain variable domain (VL) comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively; or (ii) a VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 146, and 138, respectively, and a 15 VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 142, respectively.

[0012] In some examples, the VH comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 157, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively.

20 **[0013]** In some examples, the VH comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 146, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively.

[0014] In some examples, the VH comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 137, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 25 sequences of SEQ ID NOs: 140, 141, and 151, respectively.

[0015] In some examples, the VH comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 146, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 142, respectively.

[0016] In some examples, the VH comprises an amino acid sequence at least 90% 30 identical to SEQ ID NO:145 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:150. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:145 and the VL comprises the amino acid sequence of SEQ ID NO:150. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:170 and the VL comprises the amino acid sequence of SEQ ID NO:171.

[0017] In some examples, the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:135 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:150. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:135 and the VL comprises the amino acid sequence of SEQ ID NO:150.

5 **[0018]** In some examples, the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:145 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:147. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:145 and the VL comprises the amino acid sequence of SEQ ID NO:147.

[0019] Another example of the present application protein comprising (a) a first antigen-binding site that binds NKG2D, (b) a second antigen-binding site that binds EGFR, 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 second antigen-binding site comprises a VH comprising an amino acid sequence at least 90% identical to SEQ ID NO:135 and a VL comprising an amino acid sequence at least 90% identical to SEQ ID NO:139, wherein the VH comprises an
10 S62R substitution relative to SEQ ID NO:135 and/or the VL comprises a D92R substitution and/or an F87Y substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme.

[0020] In some examples, the VH comprises an S62R substitution relative to SEQ ID NO:135, numbered under the Chothia numbering scheme. In some examples, the VL
20 comprises a D92R substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme. In some examples, the VH comprises an S62R substitution relative to SEQ ID NO:135 and the VL comprises a D92R substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme.

[0021] In some examples of any one of the examples above, the VL comprises an F87Y
25 substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme.

[0022] In some examples, the second antigen-binding site comprises a single-chain fragment variable (scFv), and wherein the scFv comprises an amino acid sequence at least 90% identical to, or comprising, a sequence selected from the group consisting of SEQ ID NOs: 152, 154, 148, and 158.

30 **[0023]** In some examples, the protein of the present disclosure comprises a polypeptide comprising an amino acid sequence at least 90% identical to, or comprising, a sequence selected from the group consisting of SEQ ID NO: 167, 168, and 166.

[0024] In some examples, the second antigen-binding site binds human EGFR with a dissociation constant (K_D) smaller than or equal to 5 nM, as measured by surface plasmon resonance (SPR). In some examples, the second antigen-binding site binds rhesus macaque EGFR with a dissociation constant (K_D) smaller than or equal to 6 nM, as measured by surface plasmon resonance (SPR).

[0025] In some examples, the first antigen-binding site that binds NKG2D is a Fab fragment, and the second antigen-binding site that binds EGFR is an scFv. In some examples, the first antigen-binding site that binds NKG2D is an scFv, and the second antigen-binding site that binds EGFR is a Fab fragment. In some examples, the protein further comprises an additional antigen-binding site that binds EGFR. In some examples, the first antigen-binding site that binds NKG2D is an scFv, and the second and the additional antigen-binding sites that bind EGFR are each a Fab fragment. In some examples, the first antigen-binding site that binds NKG2D is an scFv, and the second and the additional antigen-binding sites that bind EGFR are each an scFv. In some examples, the amino acid sequences of the second and the additional antigen-binding sites are identical.

[0026] In some examples, the scFv that binds NKG2D is linked to an antibody constant domain or a portion thereof sufficient to bind CD16, via a hinge comprising Ala-Ser or Gly-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain. In some examples, each scFv that binds EGFR is linked to an antibody constant domain or a portion thereof sufficient to bind CD16, via a hinge comprising Ala-Ser or Gly-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain. In some examples, the hinge further comprises an amino acid sequence Thr-Lys-Gly.

[0027] In some examples, within the scFv that binds NKG2D, the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv.

In some examples, within each scFv that binds EGFR, the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv. In some examples, the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain, numbered under the Kabat numbering scheme. In some examples, within the scFv that binds NKG2D, the heavy chain variable domain is linked to the light chain variable domain via a flexible linker. In some examples, within each scFv that binds EGFR, the heavy chain variable domain is linked to the light chain variable domain via a flexible linker. In some examples, the flexible linker comprises (G4S)₄ (SEQ ID NO: 119).

[0028] In some examples, within the scFv that binds NKG2D, the heavy chain variable domain is positioned at the C-terminus of the light chain variable domain. In some examples, within each scFv that binds EGFR, the heavy chain variable domain is positioned at the C-terminus of the light chain variable domain. In some examples, within the scFv that binds

5 NKG2D, the heavy chain variable domain is positioned at the N-terminus of the light chain variable domain.

[0029] In some examples, within each scFv that binds EGFR, the heavy chain variable domain is positioned at the N-terminus of the light chain variable domain. In some examples, the Fab fragment that binds NKG2D is not positioned between an antigen-binding site and

10 the Fc or the portion thereof. In some examples, no Fab fragment that binds EGFR is positioned between an antigen-binding site and the Fc or the portion thereof.

[0030] In some examples, the first antigen-binding site that binds NKG2D comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 81, 82, and 112, respectively; and a VL comprising CDR1, CDR2, and CDR3

15 comprising the amino acid sequences of SEQ ID NOs: 86, 77, and 87, respectively. In some examples, the first antigen-binding site that binds NKG2D comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 81, 82, and 97, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 86, 77, and 87, respectively. In some examples, the VH of the

20 first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:95, and the VL of the first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:85. In some examples, the VH of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:95, and the VL of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:85.

[0031] In some examples, the antibody Fc domain comprises a hinge and a CH2 domain. In some examples, the antibody Fc domain is a human IgG1 antibody Fc domain. In some examples, the antibody Fc domain or the portion thereof comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody or SEQ ID NO:118.

[0032] In some examples, at least one polypeptide chain of the antibody Fc domain

30 comprises one or more mutations, relative to SEQ ID NO:118, at one or more positions selected from Q347, Y349, 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 numbering system. In some examples, at least one polypeptide chain

of the antibody Fc domain comprises one or more mutations, relative to SEQ ID NO:118, selected from Q347E, Q347R, Y349S, Y349K, Y349T, Y349D, Y349E, Y349C, L351K, L351D, L351Y, S354C, E356K, E357Q, E357L, E357W, K360E, K360W, Q362E, S364K, S364E, S364H, S364D, T366V, T366I, T366L, T366M, T366K, T366W, T366S, L368E, L368A, L368D, K370S, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, T394F, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I, Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E, numbered according to the EU numbering system.

[0033] In some examples, one polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:118, at one or more positions selected from Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and K439; and the other polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:118, at one or more positions selected from Q347, Y349, L351, S354, E356, E357, S364, T366, L368, K370, N390, K392, T394, D399, D401, F405, Y407, K409, T411, and K439, numbered according to the EU numbering system. In some examples, one polypeptide chain of the antibody heavy chain constant region comprises K360E and K409W substitutions relative to SEQ ID NO:118; and the other polypeptide chain of the antibody heavy chain constant region comprises Q347R, D399V and F405T substitutions relative to SEQ ID NO:118, numbered according to the EU numbering system.

[0034] In some examples, one polypeptide chain of the antibody heavy chain constant region comprises a Y349C substitution relative to SEQ ID NO:118; and the other polypeptide chain of the antibody heavy chain constant region comprises an S354C substitution relative to SEQ ID NO:118, numbered according to the EU numbering system.

[0035] Another example of the present application provides a protein comprising (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:167, (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:164, and (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165.

[0036] Another example of the present application provides a protein comprising (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:168, (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:164, and (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165.

[0037] Another example of the present application provides a protein comprising (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:166, (b) a second

polypeptide comprising the amino acid sequence of SEQ ID NO:164, and (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165.

[0038] Another aspect of the present application provides a formulation comprising a protein as disclosed herein and a pharmaceutically acceptable carrier.

5 **[0039]** Another aspect of the present application provides a cell comprising one or more nucleic acids encoding a protein as disclosed herein. In some examples, the cell comprising one or more nucleic acids encoding a protein comprising an amino acid sequence of SEQ ID NO: 167 or SEQ ID NO: 169.

[0040] Another example of the present application provides a purified protein as
10 disclosed herein. In some examples, the protein is purified using a method selected from the group consisting of: centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

[0041] Another example of the present application provides a method of enhancing tumor
15 cell death, comprising exposing the tumor cell and a natural killer cell to an effective amount of a protein as disclosed herein or a formulation as disclosed herein, wherein the tumor cell expresses EGFR.

[0042] In yet another aspect, the present disclosure provides a use of a protein as
20 described herein in the manufacture of a medicament for treating a cancer. Another example of the present application provides a method of treating cancer, wherein the method comprises administering to a patient in need thereof an effective amount of a protein as disclosed herein or a formulation as disclosed herein. In some examples, the cancer is a solid tumor. In some examples, the cancer is lung cancer, breast cancer, kidney cancer, colorectal cancer, gastric cancer, brain cancer, glioma, bladder cancer, head and neck cancer, bladder
25 cancer, pancreatic cancer, and liver cancer, cervical cancer, ovarian cancer or prostate cancer. In some examples, the cancer expresses EGFR.

[0043] These and other examples and advantages of the TriNKETs described in the present application are illustrated by the following figures, detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

30 **[0044]** **FIG. 1** is a representation of a heterodimeric, multispecific binding antibody, *e.g.*, a trispecific binding protein (TriNKET). Each arm can represent either the NKG2D-binding domain, or the binding domain corresponding to a tumor-associated antigen. In some

embodiments, the NKG2D binding domain and the tumor-associated antigen binding domains can share a common light chain.

WHAT IS CLAIMED IS:

1. A protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds EGFR; 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 second antigen-binding site comprises:

- (i) a heavy chain variable domain (VH) comprising complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences of SEQ ID NOs: 136, 146, and 138, respectively; and a light chain variable domain (VL) comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively;
- (ii) a VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 137, and 138, respectively; and the VL comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 151, respectively; or
- (iii) a VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 136, 146, and 138, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140, 141, and 142, respectively;

wherein the VL of the second antigen-binding site comprises an F87Y substitution relative to SEQ ID NO:139, numbered under the Chothia numbering scheme.

2. The protein of claim 1, wherein:

- (i) the second antigen-binding site is as defined in claim 1(i) and the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:145 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:150, optionally wherein (a) the VH comprises the

amino acid sequence of SEQ ID NO:145 and the VL comprises the amino acid sequence of SEQ ID NO:150, or (b) the VH comprises the amino acid sequence of SEQ ID NO:170 and the VL comprises the amino acid sequence of SEQ ID NO:171;

(ii) the second antigen-binding site is as defined in claim 1(ii) and the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:135 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:150, optionally wherein the VH comprises the amino acid sequence of SEQ ID NO:135 and the VL comprises the amino acid sequence of SEQ ID NO:150; or

(iii) the second antigen-binding site is as defined in claim 1(iii) and the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:145 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:147, optionally wherein the VH comprises the amino acid sequence of SEQ ID NO:145 and the VL comprises the amino acid sequence of SEQ ID NO:147.

3. The protein of claim 1 or 2, wherein the second antigen-binding site comprises a single-chain fragment variable (scFv), and wherein the scFv comprises an amino acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 152, 154, and 148; optionally wherein the scFv comprises the amino acid sequence of SEQ ID NO: 152, 154, or 148.

4. The protein of any one of claims 1-3, wherein the protein comprises a polypeptide comprising an amino acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NO: 167, 168, and 166; optionally wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 167, 168, or 166.

5. The protein of any one of claims 1-4, wherein the second antigen-binding site binds human EGFR with a dissociation constant (K_D) smaller than or equal to 5 nM, as measured by surface plasmon resonance (SPR), and/or the second antigen-binding site binds rhesus macaque EGFR with a dissociation constant (K_D) smaller than or equal to 6 nM, as measured by surface plasmon resonance (SPR).

6. The protein of any one of claims 1, 2 and 5, wherein the first antigen-binding site that binds NKG2D is a Fab fragment, and the second antigen-binding site that binds EGFR is an scFv.

7. The protein of claim 6, wherein within each scFv that binds EGFR:

(i) the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv, optionally wherein the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain, numbered under the Kabat numbering scheme; and/or

(ii) the heavy chain variable domain is linked to the light chain variable domain via a flexible linker, optionally wherein the flexible linker comprises the (G₄S)₄ amino acid sequence set forth in SEQ ID NO:119; and/or

(iii) the heavy chain variable domain is positioned at the C-terminus of the light chain variable domain.

8. The protein according to any one of claims 1-7, wherein the first antigen-binding site that binds NKG2D comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 81, 82, and 112, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 86, 77, and 87, respectively.

9. The protein according to any one of claims 1-7, wherein the first antigen-binding site that binds NKG2D comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 81, 82, and 97, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 86, 77, and 87, respectively.

10. The protein of claim 8 or 9, wherein the VH of the first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:95, and the VL of the first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:85, optionally wherein the VH of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:95, and the VL of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:85.

11. The protein according to any one of claims 1-10, wherein the antibody Fc domain comprises a hinge and a CH2 domain of a human IgG1 antibody Fc domain, optionally wherein the antibody Fc domain or the portion thereof comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody or SEQ ID NO:118.
12. The protein of claim 11, wherein the antibody Fc domain is a human IgG1 antibody Fc domain comprising two polypeptide chains, wherein:
- (i) one of the polypeptide chains comprises K360E and K409W substitutions relative to SEQ ID NO:118; and the other polypeptide chain comprises Q347R, D399V and F405T substitutions relative to SEQ ID NO:118, numbered according to the EU numbering system, and/or
 - (ii) one of the polypeptide chains comprises a Y349C substitution relative to SEQ ID NO:118; and the other polypeptide chain comprises an S354C substitution relative to SEQ ID NO:118, numbered according to the EU numbering system.
13. The protein of claim 1, comprising:
- (i) (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:167;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:164; and
 - (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165;
 - (ii) (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:168;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:164; and
 - (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165; or
 - (iii) (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:166;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:164; and
 - (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:165.

14. A formulation comprising a protein according to any one of claims 1-13 and a pharmaceutically acceptable carrier.
15. A cell comprising one or more nucleic acids encoding a protein of any one of claims 1-13.
16. Use of a protein of any one of claims 1-13 in the manufacture of a medicament for treating a cancer.