

receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN-gamma and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0006] 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.

SUMMARY

[0007] In one aspect, the present disclosure refers to the use of a protein or a formulation comprising the protein in the preparation of a medicament for treating cancer in combination with a second therapeutic agent, wherein the protein comprises: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16; and wherein the second therapeutic agent is a checkpoint blocker that is an anti-PD1 antibody.

[0007a] In another aspect, the present disclosure refers to the use of a protein or a formulation comprising the protein in the preparation of a medicament for treating cancer in combination with a second therapeutic agent, wherein the protein comprises: a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16; and wherein the second therapeutic agent is selected from: a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer-targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence.

[0007b] In one example, the disclosure provides a method of enhancing tumor cell death directly or indirectly, the method includes exposing a tumor and natural killer cells to a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-

binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16; in combination with a second therapeutic agent selected from: a checkpoint blocker; a cytokine; a TLR agonist; a STING agonist; a chemotherapeutic agent; a cancer target agent that
5 interferes with specific molecules in cancer cells that are involved in cancer cell growth or survival, including, for example, kinase inhibitors such as Ibrutinib, Vemurafenib, or Gleevec; an oncolytic virus; a vaccine; radiation; an adoptive NK therapy which involves infusion of *ex vivo* expanded NK cells, an adoptive T cell therapy which involves infusion of *ex vivo* expanded T cells, including cell that have been modified in vitro to express a chimeric
10 antigen receptor (*e.g.*, CAR-T cells); a stem cell transplant (SCT) therapy, and an agent that induces cellular senescence.

[0008] In one example, the disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a
15 tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16; or a formulation comprising the protein; in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy which involves infusion of
20 *ex vivo* expanded NK cells, an adoptive T cell therapy which involves infusion of *ex vivo* expanded T cells, including cell that have been modified in vitro to express a chimeric antigen receptor (*e.g.*, CAR-T cells), a stem cell transplant (SCT) therapy, and an agent that induces cellular senescence.

[0009] The present disclosure provides a method of enhancing tumor cell death directly
25 or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a checkpoint blocker selected from: an anti-PD1 antibody, an anti-PD-L1 antibody, an anti-CTLA4
30 antibody, an anti-KIR antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody.

[0010] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a cytokine including interferons and interleukins, such as IL-2, IL-15, IL-12, INF α , IL-21, PEG-IL-2 (polyethylene glycol-modified interleukin-2), and IL15/IL15R heterodimers.

[0011] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a TLR agonist selected from a TLR7 agonist, a TLR8 agonist, a TLR7/8 agonist, a TLR9 agonist, a TLR4 agonist, and a TLR3 agonist.

[0012] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a STING agonist ADU-S100.

[0013] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a chemotherapeutic agent including alkylating agents such as cyclophosphamide, mechlorethamine, chlorambucil, melphalan, dacarbazine (DTIC), nitrosoureas, temozolomide (Oral dacarbazine); anthracyclines, such as daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin; cytoskeletal disruptors, such as paclitaxel, nab-paclitaxel, docetaxel, abraxane, and taxotere; epothilones; histone deacetylase inhibitors such as vorinostat and romidepsin; inhibitors of topoisomerase I such as irinotecan and topotecan; inhibitors of topoisomerase II such as etoposide, teniposide and tafluposide; kinase inhibitors

such as bortezomib, erlotinib, gefitinib, imatinib, vemurafenib and vismodegib; nucleotide analogs and precursor analogs such as azacitidine, azathioprine, capecitabine; peptide antibiotics such as bleomycin and actinomycin; platinum-based agents, such as carboplatin, cisplatin and oxaliplatin; retinoids such as tretinoin and alitretinoin; and vinca alkaloids and derivatives such as vinblastine, vincristine, vindesine and vinorelbine.

[0014] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a checkpoint blocker selected from: nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, ipilimumab, tremelimumab, lirilumab, and monalizumab.

[0015] The present disclosure provides a method of enhancing tumor cell death directly or indirectly and/or a method of treating cancer with a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds a tumor-associated antigen; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, in combination with a TLR agonist selected from: R848/resiquimod, VTX-2337, imiquimod, and CpG oligodeoxynucleotide.

[0016] The disclosure provides multi-specific binding proteins that bind to a tumor-associated antigen on a cancer cell and the NKG2D receptor and CD16 receptor on natural killer cells to activate the natural killer cells, pharmaceutical compositions comprising such multi-specific binding proteins, and therapeutic methods using such multi-specific proteins and pharmaceutical compositions, including for the treatment of cancer. Such proteins can engage more than one kind of NK activating receptor, and may block the binding of natural ligands to NKG2D. In certain examples, the protein can agonize NK cells in humans, and in other species such as rodents and/or cynomolgus monkeys. Various aspects and examples of the disclosure are described in further detail below.

[0017] In some examples, the multi-specific binding protein can incorporate a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds a tumor-associated antigen; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

[0018] In some examples, the multi-specific binding protein is trivalent, which includes a first and a second antigen binding site that both bind the same tumor-associated antigen; a third antigen binding site that binds NKG2D; and an antibody Fc domain, a portion thereof sufficient to bind CD16.

5 **[0019]** In some examples, the multi-specific binding protein is tetravalent, which includes a first and a second antigen binding site that both bind the same tumor-associated antigen; a third and fourth antigen binding site that both bind NKG2D; and an antibody Fc domain, a portion thereof sufficient to bind CD16.

[0020] 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. In some instances, the tumor-associated antigen can be selected from the group consisting of HER2, CD20, CD33, B-cell maturation antigen (BCMA),
10 EpCAM, CD2, CD19, CD25, CD30, CD38, CD40, CD52, CD70, CLL1/CLEC12A, FLT3, EGFR/ERBB1, IGF1R, HER3/ERBB3, HER4/ERBB4, MUC1, cMET, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, HLA-E, and PD-L1.

[0021] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain and the light chain variable domain each comprising an amino acid sequence at least 90%
20 (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequences of the heavy chain variable region and the light chain variable region of an antigen binding site disclosed in Table 1. In some examples, the heavy chain variable domain comprises the heavy chain complementarity-determining region 1 (CDR1),
25 complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences and the light chain CDR1, CDR2, and CDR3 sequences as disclosed in Table 1 of the antigen binding site.

[0022] For example, in some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain
30 variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:47 and the light chain variable domain comprising an amino acid sequence at least 90%

(e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:48. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:92, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:113; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

[0023] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:47 and the light chain variable domain comprising an amino acid sequence at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:48. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:92, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:93; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:57, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:59; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

[0024] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:47 and the light chain variable domain comprising an amino acid sequence at least 90% (e.g., 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:48. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:92, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:104; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:57, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:103; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

[0025] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:45 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:46. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:90, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:52, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:91; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:54, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:55, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:56. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:51, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:52, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:53; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:54, a CDR2 sequence represented by

the amino acid sequence of SEQ ID NO:55, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:56.

[0026] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:49 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:50. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:94, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:64, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:95; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:66, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:67, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:68. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:63, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:64, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:65; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:66, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:67, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:68.

[0027] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:114 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:115. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:122, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:123; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID

NO:119, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:120, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:121. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:116, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:118; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:119, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:120, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:121.

[0028] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:124 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:125. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:122, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:130; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:127, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:128, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:129. In some examples, the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:116, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:126; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:127, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:128, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:129.

[0029] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:1.

[0030] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:41 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:42.

[0031] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:43 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:44.

[0032] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:69 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:70.

[0033] In some examples, the antigen binding site that binds NKG2D comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:71 and the light chain variable domain comprising an amino acid sequence at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO:72.

[0034] Another example of the disclosure provides a method of treating cancer in a patient. The method comprises administering to a patient in need thereof a therapeutically effective amount of a multi-specific binding protein described herein to treat the cancer.

Exemplary cancers for treatment using the multi-specific binding proteins include, for example, a carcinoma that expresses HER2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] 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.

[0036] 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.

[0037] FIG. 3 is a representation of a TriNKET in the Triomab form, which is a trifunctional, bispecific antibody that maintains an IgG-like shape. This chimera consists of two half antibodies, each with one light and one heavy chain, that originate from two parental antibodies. Triomab form is an heterodimeric construct containing ½ of rat antibody and ½ of mouse antibody.

[0038] FIG. 4 is a representation of a TriNKET in the KiH Common Light Chain (LC) form, which involves the knobs-into-holes (KIHS) technology. KiH is a heterodimer containing 2 Fabs binding to target 1 and 2, and an F_C stabilized by heterodimerization mutations. TriNKET in the KiH format may be an heterodimeric construct with 2 fabs binding to target 1 and target 2, containing 2 different heavy chains and a common light chain that pairs with both HC.

WHAT IS CLAIMED IS:

1. Use of a protein or a formulation comprising the protein in the preparation of a medicament for treating cancer in combination with a second therapeutic agent, wherein the protein comprises:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

and wherein the second therapeutic agent is a checkpoint blocker that is an anti-PD1 antibody.

2. The use of claim 1, wherein the checkpoint blocker is selected from nivolumab and pembrolizumab.

3. Use of a protein or a formulation comprising the protein in the preparation of a medicament for treating cancer in combination with a second therapeutic agent, wherein the protein comprises:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

and wherein the second therapeutic agent is selected from: a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer-targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence.

4. The use of claim 3, wherein the second therapeutic agent is:

- (a) a cytokine selected from IL-2, IL-15, IL-12, $\text{INF}\alpha$, IL-21, PEG-IL-2 and IL15/IL15R heterodimers;
- (b) a TLR agonist selected from a TLR7 agonist, a TLR8 agonist, a TLR7/8 agonist, a TLR9 agonist, a TLR4 agonist, and a TLR3 agonist;
- (c) the STING agonist ADU-S100;
- (d) a chemotherapeutic agent selected from paclitaxel, nab-paclitaxel, or cyclophosphamide; or
- (e) a TLR agonist selected from R848/resiquimod, VTX-2337, imiquimod, and CpG oligodeoxynucleotide.

5. The use of any one of claims 1-4, wherein the cancer is selected from the group consisting of acute myeloid leukemia, acute myelomonocytic leukemia, B cell lymphoma, bladder cancer, breast cancer, colorectal cancer, diffuse large B cell lymphoma esophageal cancer, Ewing's sarcoma, follicular lymphoma, gastric cancer, gastrointestinal cancer, gastrointestinal stromal tumors, glioblastoma, head and neck cancer, melanoma, mesothelioma, multiple myeloma, myelodysplastic syndrome, renal cell carcinoma, neuroblastoma, non-small cell lung cancer, neuroendocrine tumors, ovarian cancer, and pancreatic cancer, prostate cancer, sarcomas, small cell lung cancer, T cell lymphoma, testis cancer, thymic carcinoma, thyroid cancer, urothelial cancer, cancers infiltrated by myeloid-derived suppressor cells, cancers with extracellular matrix deposition, cancers with high levels of reactive stroma, and cancers with neoangiogenesis.
6. The use of any one of claims 1-5, wherein the first antigen-binding site of the protein binds to NKG2D in humans and non-human primates.
7. The use of any one of claims 1-6, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
8. The use of any one of claims 1-7, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

9. The use of any one of claims 1-8, wherein the first antigen binding site comprises:

(a) a heavy chain variable domain comprising a complementarity-determining region (CDR) 1 amino acid sequence of SEQ ID NO:92, a CDR2 amino acid sequence of SEQ ID NO:58, and a CDR3 amino acid sequence of SEQ ID NO:113; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:60, a CDR2 amino acid sequence of SEQ ID NO:61, and a CDR3 amino acid sequence of SEQ ID NO:62;

(b) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:92, a CDR2 amino acid sequence of SEQ ID NO:58, and a CDR3 amino acid sequence of SEQ ID NO:93; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:60, a CDR2 amino acid sequence of SEQ ID NO:61, and a CDR3 amino acid sequence of SEQ ID NO:62;

(c) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:92, a CDR2 amino acid sequence of SEQ ID NO:58, and a CDR3 amino acid sequence of SEQ ID NO:104; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:60, a CDR2 amino acid sequence of SEQ ID NO:61, and a CDR3 amino acid sequence of SEQ ID NO:62;

(d) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:90, a CDR2 amino acid sequence of SEQ ID NO:52, and a CDR3 amino acid sequence of SEQ ID NO:91; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:54, a CDR2 amino acid sequence of SEQ ID NO:55, and a CDR3 amino acid sequence of SEQ ID NO:56;

(e) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:94, a CDR2 amino acid sequence of SEQ ID NO:64, and a CDR3 amino acid sequence of SEQ ID NO:95; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:66, a CDR2 amino acid sequence of SEQ ID NO:67, and a CDR3 amino acid sequence of SEQ ID NO:68;

(f) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:122, a CDR2 amino acid sequence of SEQ ID NO:117, and a CDR3 amino acid sequence

of SEQ ID NO:123; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:119, a CDR2 amino acid sequence of SEQ ID NO:120, and a CDR3 acid sequence of SEQ ID NO:121; or

(g) a heavy chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:122, a CDR2 amino acid sequence of SEQ ID NO:117, and a CDR3 amino acid sequence of SEQ ID NO:130; and a light chain variable domain comprising a CDR1 amino acid sequence of SEQ ID NO:127, a CDR2 amino acid sequence of SEQ ID NO:128, and a CDR3 amino acid sequence of SEQ ID NO:129.

10. The use of any one of claims 1-9, wherein the first antigen-binding site comprises:

(a) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:47 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:48;

(b) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:45 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:46;

(c) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:49 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:50;

(d) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:114 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:115;

(e) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:124 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:125

11. The use of any one of claims 1-10, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

12. The use of claim 11, 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.
13. The use of any one of claims 1-6, wherein the first antigen-binding site is a single-domain antibody.
14. The use of claim 13, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.
15. The use of any one of claims 1-10 and 13-14, wherein the second antigen-binding site is a single-domain antibody.
16. The use of claim 15, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.
17. The use of any one of claims 1-16, wherein the tumor-associated antigen is selected from the group consisting of EpCAM, CD2, CD20, CD30, CD38, CD40, CD52, CD70, EGFR/ERBB1, IGF1R, HER3/ERBB3, HER4/ERBB4, MUC1, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, and PD-L1.
18. The use of any one of claims 1-17, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.
19. The use of any one of claims 1-17, wherein the protein comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.
20. The use of any one of claims 1-19, wherein the formulation further comprises a pharmaceutically acceptable carrier.