

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 multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a first medicament and use of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat cancer ~~the preparation of a medicament for treating cancer in combination with a second therapeutic agent~~, wherein the multi-specific binding 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; and wherein the second therapeutic agent is a checkpoint blocker selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-KIR antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody. In another aspect, the present disclosure refers to the use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a medicament for use in combinatorial therapy for treating cancer, wherein said medicament is to be administered in combination with a second medicament, wherein the multi-specific 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; and wherein the second medicament comprises a second therapeutic agent comprising a checkpoint blocker selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-KIR antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody.

[0007a] In another aspect, the present disclosure refers to the use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a first medicament and use of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat cancer~~the preparation of a medicament for treating cancer in combination with a second therapeutic agent~~, wherein the multi-specific binding 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; and wherein the second therapeutic agent is selected from: (i) a cytokine selected from: IL-2, IFN α , IL-21, PEG-IL-2, and IL-15/IL-15R heterodimer; (ii) a TLR agonist; (iii) a STING agonist; (iv) a chemotherapeutic agent selected from: paclitaxel, nab-paclitaxel or cyclophosphamide; (v) a cancer-targeted agent selected from: ibrutinib, vemurafenib, and imatinib; (vi) an oncolytic virus; (vii) a vaccine; (viii) an adoptive NK therapy; (ix) a stem cell transplant (SCT) therapy; and (x) a chimeric antigen receptor (CAR) T cell therapy. In yet another aspect, the present disclosure refers to the use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a medicament for use in combinatorial therapy for treating cancer, wherein said medicament is to be administered in combination with a second medicament, wherein the multi-specific 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; and wherein the second medicament comprises a second therapeutic agent selected from: (i) a cytokine selected from: IL-2, IFN α , IL-21, PEG-IL-2, and IL-15/IL-15R heterodimer; (ii) a TLR agonist; (iii) a STING agonist; (iv) a chemotherapeutic agent selected from: paclitaxel, nab-paclitaxel or cyclophosphamide; (v) a cancer-targeted agent selected from: ibrutinib, vemurafenib, and imatinib; (vi) an oncolytic virus; (vii) a vaccine; (viii) an adoptive NK therapy; (ix) a stem cell transplant (SCT) therapy; and (x) a chimeric antigen receptor (CAR) T cell therapy.

[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-

What is Claimed is:

1. Use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a first medicament and use of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat cancer~~the preparation of a medicament for treating cancer in combination with a second therapeutic agent~~, wherein the multi-specific binding 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;

and wherein the second therapeutic agent is a checkpoint blocker selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-KIR antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody.

2. Use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a medicament for use in combinatorial therapy for treating cancer, wherein said medicament is to be administered in combination with a second medicament, wherein the multi-specific 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;

and wherein the second medicament comprises a second therapeutic agent comprising a checkpoint blocker selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-KIR antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody.

32. The use of claim 1 or 2, wherein the second therapeutic agent is an anti-PD1 antibody.

43. The use of claim 1 or 2, wherein the second therapeutic agent is nivolumab or pembrolizumab.

54. The use of claim 1 or 2, wherein the second therapeutic agent is an anti-LAG3 antibody.

65. Use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a first medicament and use of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat cancer~~the preparation of a medicament for treating cancer in combination with a second therapeutic agent~~, wherein the multi-specific binding 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;

and wherein the second therapeutic agent is selected from:

(i) a cytokine selected from: IL-2, IFN α , IL-21, PEG-IL-2, and IL-15/IL-15R heterodimer;

(ii) a TLR agonist;

(iii) a STING agonist;

(iv) a chemotherapeutic agent selected from: paclitaxel, nab-paclitaxel or cyclophosphamide;

(v) a cancer-targeted agent selected from: ibrutinib, vemurafenib, and imatinib;

(vi) an oncolytic virus;

(vii) a vaccine;

(viii) an adoptive NK therapy;

(ix) a stem cell transplant (SCT) therapy; and

(x) a chimeric antigen receptor (CAR) T cell therapy.

7. Use of a multi-specific binding protein or a formulation comprising the multi-specific binding protein in the manufacture of a medicament for use in combinatorial therapy for treating cancer, wherein said medicament is to be administered in combination with a second medicament, wherein the multi-specific 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;

and wherein the second medicament comprises a second therapeutic agent selected from:

(i) a cytokine selected from: IL-2, IFN α , IL-21, PEG-IL-2, and IL-15/IL-15R heterodimer;

(ii) a TLR agonist;

(iii) a STING agonist;

(iv) a chemotherapeutic agent selected from: paclitaxel, nab-paclitaxel or cyclophosphamide;

(v) a cancer-targeted agent selected from: ibrutinib, vemurafenib, and imatinib;

(vi) an oncolytic virus;

(vii) a vaccine;

(viii) an adoptive NK therapy;

(ix) a stem cell transplant (SCT) therapy; and

(x) a chimeric antigen receptor (CAR) T cell therapy.

86. The use of claim 6 or 75, wherein the second therapeutic agent is:

_____ (a) 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;

(b) the STING agonist ADU-S100; or

(c) a TLR agonist selected from R848/resiquimod, VTX-2337, imiquimod, and CpG oligodeoxynucleotide.

97. The use of any one of claims 1-86, 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 a high levels of reactive stroma, and cancers with neoangiogenesis.

108. The use of any one of claims 1-97, wherein the first antigen-binding site of the multi-specific binding protein binds to NKG2D in humans and non-human primates.

119. The use of any one of claims 1-108, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, optionally

~~10. The use of claim 9,~~ wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

~~1211.~~ The use of any one of claims 1-~~1110~~, 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.

1312. The use of claim 1211, wherein the first antigen-binding site comprises:

(~~ha~~-c) 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;

(~~id~~) 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;

(~~je~~) 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;

(~~kf~~) 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;

(~~lg~~) 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.

14. The use of any one of claims 1-10, wherein the first antigen-binding site is a single-domain antibody, optionally wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

~~1513.~~ The use of any one of claims 1-~~1412~~, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

~~1614.~~ The use of claim ~~1513~~, wherein the first and second antigen-binding sites each comprises a heavy chain variable domain and a light chain variable domain, 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.

~~15. — The use of any one of claims 1-8, wherein the first antigen-binding site is a single-domain antibody.~~

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

17. The use of any one of claims 1-~~1412 or 15-16~~, wherein the second antigen-binding site is a single-domain antibody-, optionally

~~18. — The use of claim 17,~~ wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.

~~19. — The use of any one of claims 1-18, 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.~~

~~1820.~~ The use of any one of claims 1-~~1719~~, wherein the antibody Fc domain or portion thereof sufficient to bind CD16 comprises hinge and CH2 domains.

~~1921.~~ The use of any one of claims 1-~~1819~~, wherein the antibody Fc domain or portion thereof sufficient to bind CD16 comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

~~2022.~~ The use of any one of claims 1-~~1921~~, wherein the formulation further comprises a pharmaceutically acceptable carrier.