

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

HER2 on a cancer cell and bind NKG2D on a natural killer (NK) cell to activate the NK cell and bind CD16 on the NK cell to activate the NK cell. In certain examples, the proteins can agonize NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various examples and examples of the disclosure are described in further detail below.

- 5 **[0010]** Accordingly, one example of the disclosure provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds to HER2; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. The antigen-binding sites may each incorporate an

CLAIMS

WHAT IS CLAIMED IS:

1. A multi-specific binding protein comprising:
 - (a) a first antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds NKG2D;
 - (b) a second antigen-binding site, comprising a heavy chain variable domain and a light chain variable domain that binds HER2, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16,wherein the multi-specific binding protein is configured to bind HER2 on a cancer cell and bind NKG2D on a natural killer (NK) cell to activate the NK cell and bind CD16 on the NK cell to activate the NK cell.
2. The multi-specific binding protein of claim 1, wherein the first antigen-binding site binds to NKG2D in humans and non-human primates.
3. The multi-specific binding protein of claim 1 or 2, wherein the heavy chain variable domain and the light chain variable domain of the first antigen-binding site are present on the same polypeptide.
4. The multi-specific binding protein according to claim 1, consisting essentially of:
 - (i) a first immunoglobulin heavy chain consisting essentially of a single-chain variable fragment (scFv) fused via a linker or an antibody hinge to the N-terminus of a first Fc domain;
 - (ii) a second immunoglobulin heavy chain consisting essentially of a heavy chain variable domain and a CH1 heavy chain domain, linked to the N-terminus of a second Fc domain; and
 - (iii) an immunoglobulin light chain consisting essentially of a light chain variable domain and a constant light chain domain,

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

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

5. The multi-specific binding protein according to any one of claims 1-4, wherein the heavy chain variable domain and the light chain variable domain that pair to bind NKG2D block a natural ligand from binding to and activating NKG2D.

6. The multi-specific binding protein according to claim 5, wherein the natural ligand is ULBP6 or MICA.

7. The multi-specific binding protein according to any one of claims 1-4, wherein the heavy chain variable domain and the light chain variable domain that pair to bind NKG2D comprise:

(a) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 62, 63, and 64, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:1, and a light chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 90;

(b) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 65, 66, and 67, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:41, and a light chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 68, 69, and 70, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:42;

(c) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 71, 72, and 73, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:43, and a light chain variable domain comprising

CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 74, 75, and 76, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:44;

(d) a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:45 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:46;

(e) a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:47 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:48;

(f) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 96, 97, and 98, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:94, and a light chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 99, 100, and 101, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:95; or

(g) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 104, 105, and 106, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:102, and a light chain variable domain comprising CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 107, 108, and 109, respectively, and comprising an amino acid sequence at least 90% identical to SEQ ID NO:103.

8. The multi-specific binding protein according to any one of claims 1-7, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 50, 51, and 52, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 54, 55, and 56, respectively,

optionally wherein the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:49 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:53.

9. The multi-specific binding protein according to any one of claims 1-7, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 77, 78, and 79, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 80, 81, and 82, respectively,

optionally wherein the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:57 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:58.

10. The multi-specific binding protein according to any one of claims 1-7, wherein the heavy chain variable domain and the light chain variable domain that pair to bind HER2 comprise heavy chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 83, 84, and 85, respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOs: 86, 87, and 88, respectively,

optionally wherein the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:59 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:60.

11. The multi-specific binding protein according to any one of claims 1-10, comprising hinge and CH2 domains of an antibody Fc domain, optionally hinge and CH2 domains of a human IgG1 antibody.

12. The multi-specific binding protein according to claim 11, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, optionally wherein the Fc domain comprises an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, T350, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439, numbered according to the EU index as in Kabat.

13. A formulation comprising the multi-specific binding protein according to any one of claims 1-12 and a pharmaceutically acceptable carrier.
14. A cell comprising one or more nucleic acids encoding the multi-specific binding protein according to any one of claims 1-12.
15. Use of the multi-specific binding protein according to any one of claims 1-12 or the formulation according to claim 13 in the manufacture of a medicament for treating cancer.
16. The use of claim 15, wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer, esophageal cancer, bladder cancer, gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung, and an aggressive form of uterine cancer, optionally wherein the aggressive form of uterine cancer is uterine serous endometrial carcinoma.