NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspects and examples of the disclosure are described in further detail below.

[0010] In one aspect, the present disclosure refers to a protein comprising: (a) a first antigen-binding site of an immunoglobulin molecule that binds NKG2D; (b) a second antigen-binding site of an immunoglobulin molecule that binds CLL-1/CLEC12A; and (c) an antibody Fc domain or a portion thereof that binds CD16. 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 CLEC12A; 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 antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.* arranged as in an antibody, or fused together to from 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.

[0010a] In another aspect, the present disclosure refers to a formulation comprising the protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a cell comprising one or more nucleic acids encoding the protein as described herein. In a further aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of directly and/or indirectly enhancing tumor cell death comprising exposing the tumor cell and a natural killer cell to an effective amount of the protein as described herein.

[0010b] In another aspect, the present disclosure refers to the use of the protein as described herein or the formulation as described herein in the manufacture of a medicament for treating cancer. In a further aspect, the present disclosure refers to protein as described herein or the formulation as described herein for use in therapy.

[0011] The first antigen-binding site, which binds to NKG2D, in some examples, can incorporate a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:105), CDR2 (SEQ ID NO:106), and CDR3 (SEQ ID NO:107) sequences of SEQ ID NO:1. The heavy chain variable domain related to SEQ ID NO:1 can be coupled with a variety of light chain variable domains to form an NKG2D binding site. For example, the first antigen-binding site that incorporates a heavy chain variable domain related to SEQ ID NO:1 can further incorporate a light chain variable domain selected from any one of the sequences related to SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40. For example, the first antigen-binding site incorporates a heavy chain variable domain with amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID

Claims

1. A protein comprising:

(a) a first antigen-binding site of an immunoglobulin molecule that binds NKG2D;

(b) a second antigen-binding site of an immunoglobulin molecule that binds CLL-1/CLEC12A; and

(c) an antibody Fc domain or a portion thereof that binds CD16.

2. The protein according to claim 1, wherein the first antigen-binding site binds to human and non-human primate NKG2D.

3. The protein according to claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

4. The protein according to claim 3, wherein the heavy chain variable domain and the light chain variable domain of the first antigen-binding site are present on the same polypeptide.

5. The protein according to claims 3 or 4, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

6. The protein according to claim 5, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

7. The protein according to claim 5 or 6, wherein the light chain variable domain of the first antigen-binding site comprises an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.

8. The protein according to any one of the preceding claims, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at

least 90% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, and SEQ ID NO:59.

9. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:41 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:42.

10. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:49 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:50.

11. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:57 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:58.

12. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:59 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:60.

13. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:101 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:102.

14. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:103 and a light chain variable domain amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:104.

15. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises:

(i) a heavy chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:43, a heavy chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:44, a heavy chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:45, a light chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:46, a light chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:47, and a light chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:48;

(ii) a heavy chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:51, a heavy chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:52, a heavy chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:53, a light chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:54, a light chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:55, and a light chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:56;

(iii) a heavy chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:109, a heavy chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:110, a heavy chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:111, a light chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:112, a light chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:113, and a light chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:114.

16. The protein according to claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.

17. The protein according to claim 16, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

18. The protein according to any one of claims 1-2 or 16-17, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

19. The protein according to claim 18, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

20. The protein according to any one of claims 1-4 or 8-15, wherein the second antigen-binding site is a single-domain antibody.

21. The protein according to claim 20, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

22. The protein according to any one of claims 1-21, wherein the second antigen-binding site comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:115 and a light chain variable domain comprising an amino acid sequence at least 90% identical to an amino acid sequence as set forth in SEQ ID NO:119.

23. The protein according to claim 22, wherein the second antigen-binding site comprises:

a heavy chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:116;

a heavy chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:117;

a heavy chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:118;
a light chain CDR1 sequence identical to an amino acid sequence as set forth in SEQ ID NO:120;
a light chain CDR2 sequence identical to an amino acid sequence as set forth in SEQ ID NO:121; and
a light chain CDR3 sequence identical to an amino acid sequence as set forth in SEQ ID NO:122.

24. The protein according to any one of claims 1-23, wherein the antibody Fc domain comprises hinge and CH2 domains.

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

26. The protein according to any one of claims 1-25, wherein the antibody Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, wherein the amino acids are numbered according to EU index as in Kabat.

27. The protein according to any one of claims 1-29, wherein the antibody 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, L351, S354, D356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439, numbered according to EU index as in Kabat.

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

29. A cell comprising one or more nucleic acids encoding the protein according to any one of claims 1-27.

30. An *in vitro* or *ex vivo* method of directly and/or indirectly enhancing tumor cell death comprising exposing a tumor cell and a natural killer cell to an effective amount of the protein according to any one of claims 1-27.

31. Use of the protein according to any one of claims 1-27 or the formulation according to claim 28 in the manufacture of a medicament for treating cancer.

32. The use according to claim 31, wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), myeloproliferative neoplasm (MPN), lymphoma, non-Hodgkin lymphoma, and classical Hodgkin lymphoma.

33. The use according to claim 32, wherein the AML is selected from undifferentiated acute myeloblastic leukemia, acute myeloblastic leukemia with minimal maturation, acute myeloblastic leukemia with maturation, acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, acute myelomonocytic leukemia with eosinophilia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia (AMKL), acute basophilic leukemia, acute panmyelosis with fibrosis, and blastic plasmacytoid dendritic cell neoplasm (BPDCN).

34. The use according to claim 32 or 33, wherein the AML is characterized by expression of CLL-1 on AML leukemia stem cells (LSCs).

35. The use according to claim 34, wherein the LSCs further express a membrane marker selected from CD34, CD38, CD123, TIM3, CD25, CD32, and CD96.

36. The use according to any one of claims 32-35, wherein the AML is a minimal residual disease (MRD).

37. The use according to claim 36, wherein the MRD is characterized by the presence or absence of a mutation selected from *FLT3-ITD* ((Fms-like tyrosine kinase 3)-internal tandem duplications (ITD)), *NPM1* (Nucleophosmin 1), *DNMT3A* (DNA

methyltransferase gene DNMT3A), and *IDH* (Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2)).

38. The use according to claim 36, wherein the MDS is selected from MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD), MDS with ring sideroblasts (MDS-RS), MDS with excess blasts (MDS-EB), MDS with isolated del(5q), and MDS, unclassified (MDS-U).

39. The use according to claim 36, wherein the MDS is a primary MDS or a secondary MDS.

40. The use according to claim 36, wherein the ALL is selected from B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL).

41. The use according to claim 36, wherein the MPN is selected from polycythaemia vera, essential thrombocythemia (ET), and myelofibrosis.

42. The use according to claim 36, wherein the non-Hodgkin lymphoma is selected from B-cell lymphoma and T-cell lymphoma.

43. The use according to claim 36, wherein the lymphoma is selected from chronic lymphocytic leukemia (CLL), lymphoblastic lymphoma (LPL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), follicular lymphoma, mantle cell lymphoma, hairy cell leukemia, plasma cell myeloma (PCM) or multiple myeloma (MM), mature T/NK neoplasms, and histiocytic neoplasms.

44. The protein according to any one of claims 1-27 or the formulation according to claim 28 for use in therapy.