

CLAIMS

WHAT IS CLAIMED IS:

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 CD33; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind 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. A protein according to claim 3, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.
5. The protein according to any one of claims 3-4, 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 has 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 claims 1-7, wherein the first antigen-binding site comprises
 - (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:54, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:55, and a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:56;
 - (b) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:57, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:58, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:59, a light chain

CDR1 sequence identical to the amino acid sequence of SEQ ID NO:60, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:61, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:62;

(c) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:63, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:64, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:65, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:66, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:67, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:68;

(d) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:71, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:72, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:73, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:74, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:75, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:76;

(e) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:87, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:88, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:89, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:90, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:91, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:92; or

(f) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:135, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:136, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:137, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:138, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:139, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:140.

9. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:1.

10. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid

sequence of SEQ ID NO:41 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:42.

11. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:43 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:44.

12. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:45 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:46.

13. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:69 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:70.

14. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:85 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:86.

15. The protein according to claim 8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:133 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:134.

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

17. The protein of 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 and 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 1-19, wherein the second antigen-binding site comprises

(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:95, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:96, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:97, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:98, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:99, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:100;

(b) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:103, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:104, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:105, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:106, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:107, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:108;

(c) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:111, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:112, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:113, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:114, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:115, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:116;

(d) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:119, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:120, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:121, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:122, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:123, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:124; or

(e) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:127, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:128, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:129, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:130, a light chain

CDR2 sequence identical to the amino acid sequence of SEQ ID NO:131, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:132.

21. The protein according claim 20, wherein the second antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:93 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:94.

22. The protein according claim 20, wherein the second antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:101 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:102.

23. The protein according claim 20, wherein the second antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:109 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:110.

24. The protein according claim 20, wherein the second antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:117 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:118.

25. The protein according claim 20, wherein the second antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:125 and a light chain variable domain amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:126.

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

27. The protein of claim 26, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.

28. The protein according to any one of the preceding claims, 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.

29. The protein according to claim 28, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.
30. The protein according to claim 28 or 29, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.
31. The protein according to any one of claims 28-30, 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.
32. A formulation comprising the protein according to any one of the preceding claims and a pharmaceutically acceptable carrier.
33. A host or *in vitro* cell comprising one or more nucleic acids encoding the protein according to any one of claims 1-31.
34. Use of the protein according to any one of claims 1-31 in the manufacture of a medicament for directly and/or indirectly enhancing CD33-expressing tumor cell death by exposing the tumor cell and a natural killer cell to an effective amount of the protein wherein the first antigen-binding domain activates NKG2D.
35. Use of the protein according to any one of claims 1-31 or the formulation according to claim 32 in the manufacture of a medicament for treating CD33-expressing cancer wherein the first antigen-binding domain activates NKG2D.
36. The use of claim 35, wherein the CD33-expressing cancer is AML, myelodysplastic syndromes, chronic myelomonocytic leukemia, myeloid blast crisis of chronic myeloid leukemia, or ALLs.