

CLAIMS

WHAT IS CLAIMED IS:

1. A protein comprising:
 - (a) a first antigen-binding site that binds NKG2D;
 - (b) a second antigen-binding site that binds CD33; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.
2. The protein of claim 1, wherein the first antigen-binding site binds to NKG2D in humans, non-human primates, and rodents.
3. The protein of 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 the preceding claims, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:1.
9. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:41 and a light chain variable domain at least 90% identical to SEQ ID NO:42.

10. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:43 and a light chain variable domain at least 90% identical to SEQ ID NO:44.
11. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:45 and a light chain variable domain at least 90% identical to SEQ ID NO:46.
12. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:47 and a light chain variable domain at least 90% identical to SEQ ID NO:48.
13. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:69 and a light chain variable domain at least 90% identical to SEQ ID NO:70.
14. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:77 and a light chain variable domain at least 90% identical to SEQ ID NO:78.
15. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:85 and a light chain variable domain at least 90% identical to SEQ ID NO:86.
16. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:133 and a light chain variable domain at least 90% identical to SEQ ID NO:134.
17. The protein of claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.
18. The protein of claim 17, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.
19. The protein according to any one of claims 1-2 or 17-18, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

20. The protein according to claim 19, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.
21. A protein according to any of the preceding claims, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:93 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:94.
22. A protein according to any one of claims 1-20, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:101 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:102.
23. A protein according to any one of claims 1-20, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:109 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:110.
24. A protein according to any one of claims 1-20, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:117 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:118.
25. A protein according to any one of claims 1-20, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:125 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:126.
26. The protein according to any one of claims 1-4 or 8-16, 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. A 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 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, K439.
32. A formulation comprising a protein according to any one of the preceding claims and a pharmaceutically acceptable carrier.
33. A cell comprising one or more nucleic acids expressing a protein according to any one of claims 1-31.
34. A method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to a protein according to any one of claims 1-31.
35. A method of treating cancer, wherein the method comprises administering a protein according to any one of claims 1-31 or a formulation according to claim 32 to a patient.
36. The method of claim 35, wherein the cancer is selected from the group consisting of AML, myelodysplastic syndromes, chronic myelomonocytic leukemia, myeloid blast crisis of chronic myeloid leukemia, and ALLs.