

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 FLT3; and
 - (c) an antibody Fc domain or a portion thereof that binds CD16, or a third antigen-binding site of an immunoglobulin molecule that binds CD16.
2. The protein of claim 1, wherein the first antigen-binding site binds to human and cynomolgus monkey NKG2D.
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. The protein according to claim 3, wherein the heavy chain variable domain of the first antigen binding site 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 of the second antigen-binding site 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 amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:85, and SEQ ID NO:93.

9. 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:42.

10. 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:50.

11. 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:58.

12. 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%~~ 95% 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:61 and a light chain variable domain amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:62.

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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:69 and a light chain variable domain

amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:70.

15. 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:77 and a light chain variable domain amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:78.

16. 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:85 and a light chain variable domain amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:86.

17. 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:93 and a light chain variable domain amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:94.

18. 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:102.

19. 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%~~ 95% 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%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:104.

20. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises:

- (i) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:43, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:44, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:45, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:46, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:47, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:48;
- (ii) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:51, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:52, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:53, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:54, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:55, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:56;
- (iii) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:134, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:135, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:136, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:137, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:138, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:139;
- (iv) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:63, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:64, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:65, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:66, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:67, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:68;
- (v) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:71, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:72, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:73, a light chain CDR1 sequence identical to ~~the~~an

amino acid sequence ~~of~~as set forth in SEQ ID NO:74, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:75, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:76;

(vi) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:79, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:80, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:81, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:82, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:83, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:84;

(vii) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:87, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:88, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:89, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:90, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:91, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:92; or

(viii) a heavy chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:95, a heavy chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:96, a heavy chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:97, a light chain CDR1 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:98, a light chain CDR2 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:99, and a light chain CDR3 sequence identical to ~~the~~an amino acid sequence ~~of~~as set forth in SEQ ID NO:100.

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

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

23. The protein according to any one of claims 1-2 or 21-22, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

24. The protein according to claim 23, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

25. The protein according to any one of claims 1-24, wherein the second antigen-binding site comprises a heavy chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:109 and a light chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:113.

26. The protein according to any one of claims 1-24, wherein the second antigen-binding site comprises a heavy chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:117 and a light chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:121.

27. The protein according to any one of claims 1-24, wherein the second antigen-binding site comprises a heavy chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:125 and a light chain variable domain comprising an amino acid sequence at least ~~90%~~ 95% identical to an amino acid sequence as set forth in SEQ ID NO:129.

28. The protein according to any one of claims 1-24, wherein the second antigen-binding site comprises:

- (i) a heavy chain CDR1 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:110, a heavy chain CDR2 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:111, a heavy chain CDR3 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:112, a light chain CDR1 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:114, a light chain CDR2 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:115, and a light chain CDR3 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:116;
- (ii) a heavy chain CDR1 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:118, a heavy chain CDR2 sequence identical to ~~the~~ an amino acid sequence of ~~as set forth in~~ SEQ ID NO:119, a heavy chain CDR3 sequence identical to ~~the~~ an amino acid

sequence ~~of~~ as set forth in SEQ ID NO:120, a light chain CDR1 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:122, a light chain CDR2 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:123, and a light chain CDR3 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:124; or (iii) a heavy chain CDR1 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:126, a heavy chain CDR2 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:127, a heavy chain CDR3 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:128, a light chain CDR1 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:130, a light chain CDR2 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:131, and a light chain CDR3 sequence identical to ~~the~~ an amino acid sequence ~~of~~ as set forth in SEQ ID NO:132.

29. The protein according to any one of claims 1-4 or 8-22, wherein the second antigen-binding site is a single-domain antibody.

30. The protein of claim 29, wherein the ~~second antigen-binding site~~ single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

31. The protein according to any one of the preceding claims, wherein the protein comprises a portion of an antibody Fc domain that binds CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.

32. The protein according to claim 31, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.

33. The protein according to claim 31 or 32, wherein the antibody Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

34. The protein according to claim 33, 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, ~~E356~~ D356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

35. A formulation comprising the protein according to any one of the preceding claims and a pharmaceutically acceptable carrier.
36. A cell comprising one or more nucleic acids encoding the protein according to any one of claims 1-34.
37. An *in vitro* or *ex vivo* method of enhancing tumor cell death comprising exposing the tumor cells and a natural killer cell to an effective amount of the protein according to any one of claims 1-34, wherein the tumor cell expresses FLT3.
38. Use of the protein according to any one of claims 1-34 or the formulation according to claim 35 in the manufacture of a medicament for treating cancer.
39. The use of claim 38, wherein the cancer is leukemia.
40. The use of claim 39, wherein the leukemia is selected from the group consisting of acute myeloid leukemia, T-cell leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, and hairy cell leukemia.
41. The protein of claim 33, wherein ~~said the antibody~~ the antibody Fc domain comprises one or more of the substitutions K360E, K409W, Q347R, D399V, and F405T.
42. The protein according to any one of claims 1-34 or the formulation according to claim 35 for use in therapy.