

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 FLT3; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16,wherein the second antigen-binding site that binds FLT3 comprises:
 - (i) a heavy chain variable domain (VH) comprising complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NOs: 11, 4, and 55, respectively; and a light chain variable domain (VL) comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 6, 7, and 8, respectively;
 - (ii) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 59, 63, and 54, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 86, 66, and 67, respectively;
 - (iii) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 88, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 91, 92, and 93, respectively;
 - (iv) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 97, 99, and 100, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 101, 102, and 103, respectively;
 - (v) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 98, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 106, 92, and 93, respectively;
 - (vi) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 109, 110, and 111, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 112, 113, and 114, respectively;
 - (vii) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 117, 118, and 119, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 120, 121, and 122,

respectively;

(viii) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 98, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 106, 92, and 93, respectively;

(ix) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 62, 33, and 127, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 128, 129, and 130, respectively; or

(x) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 132, 133, and 134, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 65, 66, and 46, respectively.

2. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 11, 4, and 55, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 6, 7, and 8, respectively.

3. The protein of claim 2, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 11, 4, and 5, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 6, 7, and 8, respectively.

4. The protein of claim 2, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 11, 4, and 50, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 6, 7, and 8, respectively.

5. The protein of any one of claims 2-4, wherein the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:37, and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:38.

6. The protein of any one of claims 2-5, wherein the VH comprises the amino acid sequence of SEQ ID NO:53, and the VL comprises the amino acid sequence of SEQ ID NO:42.

7. The protein of claim 6, wherein the VH and the VL comprise the amino acid sequences of SEQ ID NOs: 9 and 10; 13 and 10; 17 and 10; 9 and 22; 9 and 26; 9 and 30; 9 and 34; 37 and 38; 41 and 42; 45 and 42; or 49 and 42, respectively.
8. The protein of any one of claims 2-7, wherein the second antigen-binding site is present as a single-chain fragment variable (scFv), and wherein the scFv comprises an amino acid sequence selected from SEQ ID NOs: 3, 12, 15, 16, 19, 20, 23, 24, 27, 28, 31, 32, 35, 36, 39, 40, 43, 44, 47, 48, 51, and 52.
9. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 59, 63, and 54, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 86, 66, and 67, respectively.
10. The protein of claim 9, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 78, 63, 79, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 80, 66, 67, respectively.
11. The protein of claim 9, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 62, 63, 64, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 65, 66, 67, respectively.
12. The protein of any one of claims 9-11, wherein the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:76, and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:77.
13. The protein of any one of claims 9-12, wherein the VH comprises the amino acid sequence of SEQ ID NO:29, and the VL comprises the amino acid sequence of SEQ ID NO:84.
14. The protein of claim 13, wherein the VH and the VL comprise the amino acid sequences of SEQ ID NOs: 68 and 69; 72 and 73; or 76 and 77, respectively.

15. The protein of any one of claims 9-14, wherein second the antigen-binding site is present as an scFv, and wherein the scFv comprises an amino acid sequence selected from SEQ ID NOs: 70, 71, 74, 75, 81, and 82.
16. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 88, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 91, 92, and 93, respectively.
17. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 97, 99, and 100, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 101, 102, and 103, respectively.
18. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 98, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 106, 92, and 93, respectively.
19. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 109, 110, and 111, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 112, 113, and 114, respectively.
20. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 117, 118, and 119, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 120, 121, and 122, respectively.
21. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 87, 98, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 106, 92, and 93, respectively.

22. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 62, 33, and 127, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 128, 129, and 130, respectively.
23. The protein of claim 1, wherein the second antigen-binding site that binds FLT3 comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 132, 133, and 134, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 65, 66, and 46, respectively.
24. The protein of any one of the preceding claims, wherein the second antigen-binding site binds human FLT3 with a dissociation constant (K_D) smaller than or equal to 20 nM as measured by surface plasmon resonance (SPR).
25. The protein of any one of claims 2-8, 16, 17, and 21, wherein the second antigen-binding site binds human FLT3 with a K_D smaller than or equal to 10 nM as measured by SPR.
26. The antigen-binding site of any one of claims 2-8, wherein the antigen-binding site binds a human FLT3 variant comprising the amino acid sequence of SEQ ID NO:318.
27. The antigen-binding site of any one of claims 2-8, wherein the antigen-binding site binds a human FLT3 variant comprising the amino acid sequence of SEQ ID NO:319.
28. The protein of any one of claims 2-21 and 23-27, wherein the antigen-binding site binds cynomolgus FLT3.
29. The protein of any one of claims 2-20 and 22-28, wherein the antigen-binding site does not compete with FLT3L for binding FLT3.
30. The protein of any one of the preceding claims, wherein the protein comprises an antibody Fc domain or a portion thereof sufficient to bind CD16.
31. The protein of any one of claims 1-30, wherein the first antigen-binding site that binds NKG2D is an Fab fragment, and the second antigen-binding site that binds FLT3 is an scFv.

32. The protein of any one of claims 1-30, wherein the first antigen-binding site that binds NKG2D is an scFv, and the second antigen-binding site that binds FLT3 is an Fab fragment.
33. The protein of any one of the preceding claims, further comprising an additional antigen-binding site that binds FLT3.
34. The protein of any one of claims 1-30 and 32-33, wherein the first antigen-binding site that binds NKG2D is an scFv, and the second and the additional antigen-binding sites that bind FLT3 are each an Fab fragment.
35. The protein of any one of claims 1-30 and 33, wherein the first antigen-binding site that binds NKG2D is an scFv, and the second and the additional antigen-binding sites that bind FLT3 are each an scFv.
36. The protein of claim 31, 32, 34, or 35, wherein the scFv that binds FLT3 and/or the scFv that binds NKG2D comprise a heavy chain variable domain and a light chain variable domain.
37. The protein of claim 36, wherein the scFv is linked to an antibody constant domain or a portion thereof sufficient to bind CD16, via a hinge comprising Ala-Ser or Gly-Ser.
38. The protein of claim 37, wherein the hinge further comprises amino acid sequence Thr-Lys-Gly.
39. The protein of any one of claims 36-38, wherein the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv.
40. The protein of claim 39, wherein the disulfide bridge is formed between C44 of the heavy chain variable domain and C100 of the light chain variable domain, numbered under the Kabat numbering scheme.
41. The protein of any one of claims 36-40, wherein the heavy chain variable domain of the scFv is linked to the light chain variable domain of the scFv via a flexible linker.
42. The protein of claim 41, wherein the flexible linker comprises (G₄S)₄.
43. The protein of any one of claims 36-42, wherein within the scFv the heavy chain variable domain is positioned at the C-terminus of the light chain variable domain.

44. The protein of any one of claims 36-42, wherein within the scFv the heavy chain variable domain is positioned at the N-terminus of the light chain variable domain.
45. The protein of any one of claims 31-34 and 36-44, wherein the Fab is not positioned between an antigen-binding site and the Fc or the portion thereof.
46. The protein according any one of the preceding claims, wherein the first antigen-binding site that binds NKG2D comprises a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 240 or 241, 242, and 270 or 271, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 276, 236, and 245, respectively.
47. The protein of claim 46, wherein the first antigen-binding site that binds NKG2D comprises:
- (i) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 240 or 241, 242, and 255 or 256, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 276, 236, and 245, respectively; or
 - (ii) a VH comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 240 or 241, 242, and 243 or 244, respectively; and a VL comprising CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 276, 236, and 245, respectively.
48. The protein of claim 46 or 47, wherein the VH of the first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:254, and the VL of the first antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:239.
49. The protein of any one of claims 46-48, wherein the VH of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:254, and the VL of the first antigen-binding site comprises the amino acid sequence of SEQ ID NO:239.
50. The protein of any one of the preceding claims, wherein the antibody Fc domain is a human IgG1 antibody Fc domain.

51. The protein of claim 50, wherein the antibody Fc domain or the portion thereof comprises an amino acid sequence at least 90% identical to SEQ ID NO:136.
52. The protein of claim 50 or 51, wherein at least one polypeptide chain of the antibody Fc domain comprises one or more mutations, relative to SEQ ID NO:136, at one or more positions selected from Q347, Y349, 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 numbering system.
53. The protein of any one of claims 50-52, wherein at least one polypeptide chain of the antibody Fc domain comprises one or more mutations, relative to SEQ ID NO:136, selected from Q347E, Q347R, Y349S, Y349K, Y349T, Y349D, Y349E, Y349C, L351K, L351D, L351Y, S354C, E356K, E357Q, E357L, E357W, K360E, K360W, Q362E, S364K, S364E, S364H, S364D, T366V, T366I, T366L, T366M, T366K, T366W, T366S, L368E, L368A, L368D, K370S, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, T394F, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I, Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E, numbered according to the EU numbering system.
54. The protein of any one of claims 50-53, wherein one polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:136, at one or more positions selected from Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and K439; and the other polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:136, at one or more positions selected from Q347, Y349, L351, S354, E356, E357, S364, T366, L368, K370, N390, K392, T394, D399, D401, F405, Y407, K409, T411, and K439, numbered according to the EU numbering system.
55. The protein of claim 54, wherein one polypeptide chain of the antibody heavy chain constant region comprises K360E and K409W substitutions relative to SEQ ID NO:136; and the other polypeptide chain of the antibody heavy chain constant region comprises Q347R, D399V and F405T substitutions relative to SEQ ID NO:136, numbered according to the EU numbering system.

56. The protein of claim 54 or 55, wherein one polypeptide chain of the antibody heavy chain constant region comprises a Y349C substitution relative to SEQ ID NO:136; and the other polypeptide chain of the antibody heavy chain constant region comprises an S354C substitution relative to SEQ ID NO:136, numbered according to the EU numbering system.
57. A protein comprising:
- (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:278;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:279;
- and
- (c) a third polypeptide comprising an amino acid sequence selected from SEQ ID NO: 277, 283, 284, 285, 286, 287, 288, and 289.
58. A protein comprising:
- (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:280;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:281;
- and
- (c) a third polypeptide comprising the amino acid sequence of SEQ ID NO:282.
59. A pharmaceutical composition comprising a protein of any one of the preceding claims and a pharmaceutically acceptable carrier.
60. A cell comprising one or more nucleic acids encoding a protein of any one of claims 1-58.
61. A method of enhancing tumor cell death, the method comprising exposing the tumor cell and a natural killer cell to an effective amount of the protein of any one of claims 1-58 or the pharmaceutical composition of claim 59.
62. A method of treating cancer, the method comprising administering an effective amount of the protein of any one of claims 1-58 or the pharmaceutical composition of claim 59 to a patient in need thereof.
63. The method of claim 62, wherein the cancer is a hematologic malignancy.
64. The method of claim 63, wherein the hematologic malignancy is leukemia.

65. The method of claim 63 or 64, wherein selected from the group consisting of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplasia, acute T-lymphoblastic leukemia, and acute promyelocytic leukemia.
66. The method of any one of claims 62-65, wherein the cancer expresses FLT3.