

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,

wherein the first antigen-binding site that binds NKG2D comprises:

a heavy chain variable domain (VH) CDR1 sequence comprising the amino acid sequence of SEQ ID NO: 241, a VH CDR2 sequence comprising the amino acid sequence of SEQ ID NO:242, a VH CDR3 sequence comprising the amino acid sequence of SEQ ID NO: 256, a VL CDR1 sequence comprising the amino acid sequence of SEQ ID NO:276, a light chain variable domain (VL) CDR2 sequence comprising the amino acid sequence of SEQ ID NO:236, and a VL CDR3 sequence comprising the amino acid sequence of SEQ ID NO:245; and

wherein the second antigen-binding site that binds FLT3 comprises:

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 5, 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.

2. The protein of claim 1, wherein:

(i) the VH and the VL of the second antigen-binding site that binds FLT3 comprises an amino acid sequence at least 95% identical to SEQ ID NO:37 and an amino acid sequence at least 95% identical to SEQ ID NO:38, respectively; and

(ii) the VH and the VL of the first antigen-binding site that binds NKG2D comprises the amino acid sequence of SEQ ID NO:254 and the amino acid sequence of SEQ ID NO:239, respectively.

3. The protein of claim 2, wherein the VH and the VL of the second antigen-binding site that binds FLT3 comprise the amino acid sequences of SEQ ID NOs: 37 and 38, respectively; and the VH and the VL of the first antigen-binding site that binds NKG2D comprise the amino acid sequences of SEQ ID NOs: 254 and 239, respectively.

4. The protein of any one of claims 1-3, wherein the second antigen-binding site that binds FLT3 is present as a single-chain fragment variable (scFv), and wherein the scFv comprises an amino acid sequence of SEQ ID NO: 39 or 40.
5. The protein of any one of claims 1-4, 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).
6. The protein of any one of claims 1-5, wherein the second antigen-binding site binds a human FLT3 variant comprising the amino acid sequence of SEQ ID NO:318 or SEQ ID NO:319.
7. The protein of any one of claims 1-6, wherein the second antigen-binding site:
 - (i) binds cynomolgus FLT3; or
 - (ii) does not compete with FLT3L for binding FLT3.
8. The protein of any one of claims 1-7, wherein the protein comprises an antibody Fc domain or a portion thereof sufficient to bind CD16.
9. The protein of any one of claims 1-8, wherein
 - (i) the first antigen-binding site that binds NKG2D is an Fab fragment, and the second antigen-binding site that binds FLT3 is an scFv; or
 - (ii) the first antigen-binding site that binds NKG2D is an scFv, and the second antigen-binding site that binds FLT3 is an Fab fragment.
10. The protein of any one claims 1-9, further comprising an additional antigen-binding site that binds FLT3.
11. The protein of claim 10, 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 or an scFv.
12. The protein of any one of claims 9-11, 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.

13. The protein of claim 12, wherein the scFv that binds FLT3 is linked to an antibody constant domain or a portion thereof sufficient to bind CD16, via a hinge comprising Ala-Ser or Gly-Ser.
14. The protein of claim 13, wherein the hinge further comprises amino acid sequence Thr-Lys-Gly.
15. The protein of any one of claims 12-14, wherein the heavy chain variable domain of the scFv that binds FLT3 forms a disulfide bridge with the light chain variable domain of the scFv.
16. The protein of claim 15, 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.
17. The protein of any one of claims 12-16, wherein the heavy chain variable domain of the scFv that binds FLT3 is linked to the light chain variable domain of the scFv that binds FLT3 via a flexible linker.
18. The protein of claim 17, wherein the flexible linker comprises (G₄S)₄.
19. The protein of any one of claims 1-18 wherein the antibody Fc domain is a human IgG1 antibody Fc domain, and wherein the human IgG1 antibody Fc domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:136.
20. The protein of claim 19, 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.

21. The protein of claim 20, wherein
- (i) 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; or
 - (ii) 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.
22. A protein comprising:
- (a) a first polypeptide comprising the amino acid sequence of SEQ ID NO:278, comprising a heavy chain comprising a heavy chain variable domain of an antigen-binding site that binds NKG2D and CH1 domain connected to a human IgG1 Fc domain;
 - (b) a second polypeptide comprising the amino acid sequence of SEQ ID NO:279, comprising a light chain comprising a light chain variable domain of the antigen-binding site that binds NKG2D and a light chain constant domain; and
 - (c) a third polypeptide comprising an amino acid sequence selected from SEQ ID NO: 277, 285, 286, 287, 288, and 289, comprising an scFv that binds to FLT3 linked to a human IgG1 Fc domain via a hinge comprising Gly-Ser.
23. A pharmaceutical composition comprising the protein of any one claims 1-22 and a pharmaceutically acceptable carrier.
24. An isolated cell comprising one or more nucleic acids encoding the protein of any one of claims 1-22.
25. Use of the protein of any one of claims 1-22 or the pharmaceutical composition of claim 23 in the manufacture of a medicament for enhancing tumor cell death, by exposing the tumor cell and a natural killer cell to an effective amount of the protein or the pharmaceutical composition.

26. Use of the protein of any one of claims 1-22 or the pharmaceutical composition of claim 23 in the manufacture of a medicament for treating a cancer.
27. The use of claim 26, wherein the cancer is a hematologic malignancy.
28. The use of claim 27, wherein the hematologic malignancy is leukemia.
29. The use of claim 27 or 28, wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplasia, acute T-lymphoblastic leukemia, and acute promyelocytic leukemia.
30. The use of any one of claims 26-29, wherein the cancer expresses FLT3.