

WHAT IS CLAIMED IS:

1. An antigen-binding site that binds FLT3, comprising:
  - (a) 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
  - (b) 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 antigen-binding site of claim 1, wherein the VH comprises an amino acid sequence at least 95% identical to SEQ ID NO:37, and the VL comprises an amino acid sequence at least 95% identical to SEQ ID NO:38.
3. The antigen-binding site of claim 1 or 2, wherein the 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).
4. The antigen-binding site of claim 1 or 2, wherein the antigen-binding site binds human FLT3 with a  $K_D$  smaller than or equal to 10 nM as measured by SPR.
5. The antigen-binding site of claim 1 or 2, wherein the antigen-binding site binds a human FLT3 variant comprising the amino acid sequence of SEQ ID NO:25 or SEQ ID NO:18.
6. The antigen-binding site of any one of claims 1-5, wherein the antigen-binding site
  - (i) binds cynomolgus FLT3; or
  - (ii) does not compete with FLT3L for binding FLT3.
7. The antigen-binding site of any one of claims 1-6, wherein the antigen-binding site is present as a single-chain fragment variable (scFv).
8. The antigen-binding site of claim 7, wherein the scFv comprises an amino acid sequence selected from SEQ ID NOs: 39 and 40.

9. A protein comprising the antigen-binding site of any one of claims 1-8.
10. The protein of claim 9, further comprising an antibody heavy chain constant region.
11. The protein of claim 10, wherein the antibody heavy chain constant region is a human IgG heavy chain constant region.
12. The protein of claim 11, wherein each polypeptide chain of the antibody heavy chain constant region comprises an amino acid sequence at least 90% identical to SEQ ID NO:21.
13. The protein of claim 12, wherein:
- (i) at least one polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:21, 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;
  - (ii) at least one polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:21, 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; or
  - (iii) one polypeptide chain of the antibody heavy chain constant region comprises one or more mutations, relative to SEQ ID NO:21, 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:21, 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.

14. The protein of claim 13, wherein one polypeptide chain of the antibody heavy chain constant region comprises K360E and K409W substitutions relative to SEQ ID NO:21; and the other polypeptide chain of the antibody heavy chain constant region comprises Q347R, D399V and F405T substitutions relative to SEQ ID NO:21, numbered according to the EU numbering system.

15. The protein of claim 13 or 14, wherein one polypeptide chain of the antibody heavy chain constant region comprises a Y349C substitution relative to SEQ ID NO:21; and the other polypeptide chain of the antibody heavy chain constant region comprises an S354C substitution relative to SEQ ID NO:21, numbered according to the EU numbering system.

16. An antibody-drug conjugate comprising the protein of any one of claims 9-15 and a drug moiety.

17. The antibody-drug conjugate of claim 16, wherein the drug moiety is selected from the group consisting of auristatin, N-acetyl- $\gamma$  calicheamicin, maytansinoid, pyrrolobenzodiazepine, and SN-38.

18. An immunocytokine comprising the antigen-binding site of any one of claims 1-8 and a cytokine.

19. The immunocytokine of claim 18, wherein the cytokine is selected from the group consisting of IL-2, IL-4, IL-10, IL-12, IL-15, TNF, and IFN $\alpha$ .

20. A bispecific T-cell engager comprising the antigen-binding site of any one of claims 1-8 and an antigen-binding site that binds CD3.

21. A chimeric antigen receptor (CAR) comprising:

(a) the antigen-binding site of any one of claims 1-8;

(b) a transmembrane domain; and

(c) an intracellular signaling domain.

22. The CAR of claim 21, wherein
- (i) the transmembrane domain is selected from the transmembrane regions of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, FLT3, CD37, CD64, CD80, CD86, CD134, CD137, CD152, and CD154;
  - (ii) the intracellular signaling domain comprises a primary signaling domain comprising a functional signaling domain of CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12; or
  - (iii) the intracellular signaling domain further comprises a costimulatory signaling domain comprising a functional signaling domain of a costimulatory receptor.
23. The CAR of claim 22, wherein the costimulatory receptor is selected from the group consisting of OX40, CD27, CD28, CD30, CD40, PD-1, CD2, CD7, CD258, NKG2C, B7-H3, a ligand that binds to CD83, ICAM-1, LFA-1 (CD11a/CD18), ICOS and 4-1BB (CD137), or any combination thereof.
24. An isolated nucleic acid encoding the CAR of any one of claims 21-23.
25. An expression vector comprising the isolated nucleic acid of claim 24.
26. An immune effector cell
- (i) comprising the nucleic acid of claim 24 or the expression vector of claim 25; or
  - (ii) expressing the CAR of any one of claims 21-23.
27. The immune effector cell of claim 26, wherein the immune effector cell is a T cell or an NK cell.
28. A pharmaceutical composition comprising the protein of any one of claims 9-15, the antibody-drug conjugate of claim 17, the immunocytokine of claim 19, the bispecific T-cell engager of claim 20, or the immune effector cell of claim 27 ; and a pharmaceutically acceptable carrier.

29. Use of the protein of any one of claims 9-15, the antibody-drug conjugate of claim 17, the immunocytokine of claim 19, the bispecific T-cell engager of claim 20, the immune effector cell of any one of claim 27, or the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a cancer.
30. The use of claim 29, wherein the cancer is a hematologic malignancy.
31. The use of claim 30, wherein the hematologic malignancy is leukemia.
32. The use of claim 30 or 31, 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.
33. The use of any one of claims 29-32, wherein the cancer expresses FLT3.
34. The protein of any one of claims 9-15, the antibody-drug conjugate of claim 17, the immunocytokine of claim 19, the bispecific T-cell engager of claim 20, the immune effector cell of claim 27, or the pharmaceutical composition of claim 28 for use in therapy.