

WHAT IS CLAIMED IS:

1. An antigen-binding site comprising a heavy chain variable domain (VH) complementarity-determining region 1 (CDR1) sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH complementarity-determining region 2 (CDR2) sequence identical to the amino acid sequence of SEQ ID NO:46, a VH complementarity-determining region 3 (CDR3) sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50.
2. The antigen-binding site according to claim 1, wherein the heavy chain variable domain comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:9.
3. The antigen-binding site according to claim 1 or 2, wherein the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:10.
4. A protein comprising the antigen-binding site according to any one of claims 1 to 3, wherein the antigen-binding site binds to human CD33.
5. The protein according to claim 4, wherein the protein further comprises a second antigen binding site the same or different from the antigen-binding site that binds to human CD33.
6. A protein capable of specifically binding to CD33, wherein the protein comprises an antigen-binding site comprising a heavy chain variable domain (VH) complementarity-determining region 1 (CDR1) sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH complementarity-determining region 2 (CDR2) sequence identical to the amino acid sequence of SEQ ID NO:46, a VH complementarity-determining region 3 (CDR3) sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50.
7. The protein of any one of claims 4 to 6, further comprising an antibody constant region, wherein the antibody constant region is capable of binding to CD16 and comprises two

polypeptide chains, each of which comprises a hinge, CH2 and CH3 domain.

8. The protein of claim 7, wherein each of the two polypeptide chains of the antibody constant region comprises amino acid sequence at least 90% identical to human IgG1 constant region, and optionally wherein the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, Y349, L351, S354, D356, E357, K360, Q362, S364, T366, L368, K370, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and K439; and the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, Y349, L351, S354, D356, E357, S364, T366, L368, K370, N390, K392, T394, D399, D401, F405, Y407, K409, T411 and K439.

9. The protein of claim 7, wherein

- (i) the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution;
- (ii) the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V and F405T substitutions;
- (iii) the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions; or
- (iv) the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions.

10. A formulation comprising a protein of any one of claims 4-9, and a pharmaceutically

acceptable carrier.

11. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the nucleic acid comprises a nucleic acid sequence that encodes a CD33-binding scFv comprising a VH CDR1 sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH CDR2 sequence identical to the amino acid sequence of SEQ ID NO:46, a VH CDR3 sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50; a nucleic acid sequence encoding a transmembrane domain; and a nucleic acid sequence encoding an intracellular signaling domain.

12. An expression vector comprising the isolated nucleic acid of claim 11.

13. A chimeric antigen receptor (CAR), wherein the CAR comprises a CD33-binding scFv comprising a VH CDR1 sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH CDR2 sequence identical to the amino acid sequence of SEQ ID NO:46, a VH CDR3 sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50; a transmembrane domain; and an intracellular signaling domain.

14. An immune effector cell comprising the isolated nucleic acid of claim 11.

15. An immune effector cell expressing the CAR of claim 13.

16. The immune effector cell of claim 14 or 15, where the immune effector cell is a T cell or a NK cell.

17. A CD33/CD3-directed bispecific T-cell engager comprising a protein comprising a VH CDR1 sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH CDR2 sequence identical to the amino acid sequence of SEQ ID NO:46, a VH CDR3 sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID

NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50.

18. An isolated nucleic acid encoding the CD33/CD3-directed bispecific T-cell engager of claim 17.

19. An antibody-drug conjugate comprising a protein comprising a VH CDR1 sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH CDR2 sequence identical to the amino acid sequence of SEQ ID NO:46, a VH CDR3 sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50.

20. An immunocytokine comprising a CD33-binding scFv connected to a cytokine, wherein the CD33-binding scFv comprises a VH CDR1 sequence identical to the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:181, a VH CDR2 sequence identical to the amino acid sequence of SEQ ID NO:46, a VH CDR3 sequence identical to the amino acid sequence of SEQ ID NO:47 or SEQ ID NO:182, a light chain variable domain (VL) CDR1 sequence identical to the amino acid sequence of SEQ ID NO:48, a VL CDR2 sequence identical to the amino acid sequence of SEQ ID NO:49, and a VL CDR3 sequence identical to the amino acid sequence of SEQ ID NO:50.

21. An isolated nucleic acid encoding an immunocytokine of claim 20.

22. Use of the protein according to any one of claims 4-9, the formulation according to claim 10, the immune effector cell according to claim 16, the CD33/CD3-directed bispecific T-cell engager according to claim 17, the antibody-drug conjugate according to claim 19, or the immunocytokine according to claim 20 in the manufacture of a medicament for treating a CD33-expressing cancer.

23. The use of claim 22, wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), myeloid blast crisis of CML, acute lymphoblastic leukemia (ALL), acute lymphoblastic lymphoma, myeloproliferative neoplasms (MPNs), lymphoma, non-Hodgkin lymphomas, and classical Hodgkin lymphoma.

24. The use of claim 23, wherein the AML is selected from undifferentiated acute myeloblastic leukemia, acute myeloblastic leukemia with minimal maturation, acute myeloblastic leukemia with maturation, acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, acute myelomonocytic leukemia with eosinophilia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia (AMKL), acute basophilic leukemia, acute panmyelosis with fibrosis, and blastic plasmacytoid dendritic cell neoplasm (BPDCN).
25. The use of claim 23 or 24, wherein the AML is characterized by expression of CLL-1 on the AML leukemia stem cells (LSCs).
26. The use of claim 25, wherein the LSCs further express a membrane marker selected from CD34, CD38, CD123, TIM3, CD25, CD32, and CD96.
27. The use of any one of claims 23-26, wherein the AML is a minimal residual disease (MRD).
28. The use of claim 27, wherein the MRD is characterized by the presence or absence of a mutation selected from FLT3-ITD ((Fms-like tyrosine kinase 3)-internal tandem duplications (ITD)), NPM1 (Nucleophosmin 1), DNMT3A (DNA methyltransferase gene DNMT3A), and IDH (Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2)).
29. The use of claim 23, wherein the MDS is selected from MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD), MDS with ring sideroblasts (MDS-RS), MDS with excess blasts (MDS-EB), MDS with isolated del(5q), and MDS, unclassified (MDS-U).
30. The use of claim 23 or 29, wherein the MDS is a primary MDS or a secondary MDS.
31. The use of claim 23, wherein the ALL is selected from B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL).
32. The use of claim 23, wherein the MPN is selected from polycythaemia vera, essential thrombocythemia (ET), and myelofibrosis.
33. The use of claim 23, wherein the non-Hodgkin lymphoma is selected from B-cell lymphoma and T-cell lymphoma.
34. The use of claim 23, wherein the lymphoma is selected from chronic lymphocytic

leukemia (CLL), lymphoblastic lymphoma (LPL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), follicular lymphoma, mantle cell lymphoma, hairy cell leukemia, plasma cell myeloma (PCM) or multiple myeloma (MM), mature T/NK neoplasms, and histiocytic neoplasms.

35. The protein according to any one of claims 4-9, the formulation according to claim 10, the immune effector cell according to claim 16, the CD33/CD3-directed bispecific T-cell engager according to claim 17, the antibody-drug conjugate according to claim 19, or the immunocytokine according to claim 20 for use in therapy.