

SUMMARY

[0004a] One embodiment provides a FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.

[0004b] One embodiment provides a FLT3 targeting trispecific protein comprising (A) a first domain that binds a human CD3; (B) a second domain that binds a human serum albumin protein; and (C) a third domain that binds a human FLT3 or a cynomolgus FLT3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID Nos. 195, 173, and 186.

[0004c] One embodiment provides pharmaceutical composition comprising the FLT3 binding domain as described herein or the FLT3 targeting trispecific protein as described herein, and a pharmaceutically acceptable carrier.

[0004d] One embodiment provides a use of an FLT3 binding domain in the manufacture of a medicament for the treatment of cancer, wherein the FLT3 binding domain is to be administered to a subject in need thereof, wherein FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising

one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.

[0004e] One embodiment provides a use of an FLT3 targeting trispecific protein in the manufacture of a medicament for the treatment of cancer, wherein the FLT3 targeting trispecific protein is to be administered to a subject in need thereof; wherein the FLT3 targeting trispecific protein comprises (A) a first domain that binds to a human CD3; (B) a second domain that binds to a human serum albumin protein; and (C) a third domain that binds to a human FLT3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.

[0004f] One embodiment provides an FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or an FLT3 targeting trispecific protein comprising (A) a first domain that binds to a human CD3; (B) a second domain that binds to a human serum albumin protein; and (C) a third domain that binds to a human FLT3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151,

393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, for use in therapy.

[0005] One embodiment provides an FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein

- the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 77-108, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-108;

- the CDR2 comprises a sequence selected from the group consisting of SEQ ID Nos. 109-154, and 393-394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID Nos. 109-154, and 393-394;

- the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 155-195, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID Nos. 155-195. In some embodiments, the FLT3 binding domain of claim 1, comprising at least one of the following set of sequences (the CDR1, the CDR2, and the CDR3):

- SEQ ID Nos. 77, 109, and 155;
- SEQ ID Nos. 78, 109, and 155;
- SEQ ID Nos. 79, 110, and 156;
- SEQ ID Nos. 80, 111, and 155;
- SEQ ID Nos. 81, 112, and 155;
- SEQ ID Nos. 77, 113, and 157;
- SEQ ID Nos. 82, 110, and 156;
- SEQ ID Nos. 77, 114, and 158;
- SEQ ID Nos. 80, 111, and 155;
- SEQ ID Nos. 83, 115, and 155;
- SEQ ID Nos. 84, 111, and 155;
- SEQ ID Nos. 79, 116, and 156;
- SEQ ID Nos. 85, 111, and 155;
- SEQ ID Nos. 86, 110, and 156;
- SEQ ID Nos. 87, 110, and 156;
- SEQ ID Nos. 88, 117, and 159;
- SEQ ID Nos. 88, 118, and 160;
- SEQ ID Nos. 88, 119, and 161;
- SEQ ID Nos. 88, 120, and 162;
- SEQ ID Nos. 88, 121, and 163;
- SEQ ID Nos. 88, 122, and 164;
- SEQ ID Nos. 88, 121, and 165;
- SEQ ID Nos. 88, 121, and 166;
- SEQ ID Nos. 88, 122, and 167;
- SEQ ID Nos. 88, 122, and 168;

SEQ ID Nos. 88, 121, and 169;
SEQ ID Nos. 88, 118, and 165;
SEQ ID Nos. 88, 123, and 170;
SEQ ID Nos. 88, 124, and 171;
SEQ ID Nos. 88, 117, and 172;
SEQ ID Nos. 89, 125, and 173;

CLAIMS

WHAT IS CLAIMED IS:

1. An FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein
the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100;
the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394;
the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.
2. The FLT3 binding domain of claim 1, comprising at least one of the following set of sequences (the CDR1, the CDR2, and the CDR3):
SEQ ID NOs. 91, 149, and 195
SEQ ID NOs. 89, 393, and 173;
SEQ ID NOs. 89, 394, and 173; and
SEQ ID NOs. 100, 151, and 186.
3. The FLT3 binding domain of claim 1 or 2, comprising a sequence that is at least about 80% identical to a sequence selected from the group consisting of SEQ ID NOs. 71, 73, 389, and 390.
4. The FLT3 binding domain of any one of claims 1-3, wherein the FLT3 binding domain is an antibody or an antigen binding fragment thereof.
5. The FLT3 binding domain of claim 4, wherein the antibody or an antigen binding fragment thereof comprises at least one of: a monoclonal antibody (mAb), a bispecific antibody, a trispecific antibody, a monovalent antibody, a multivalent antibody, an hIgG, an Fv, an Fd, an Fab, an F(ab')₂, an F(ab'), an Fab2, an Fab3, an scFab, an scFv, an scFvFc, an scFv-zipper, a di-scFv, a tandem scFv, an sdFv, an sdAb, a VH domain, a VL domain, a VHH domain, a half-antibody, a diabody, a single chain diabody, a tandem diabody, a tandem di-scFv, or a tandem tri-scFv.
6. The FLT3 binding domain of claim 5, wherein the FLT3 binding domain is the sdAb.

7. The FLT3 binding domain of any one of claims 1-3, wherein the FLT3 binding domain is part of an antibody or an antigen binding fragment thereof.
8. The FLT3 binding domain of claim 7, wherein the antibody or an antigen binding fragment thereof comprises at least one of: a monoclonal antibody (mAb), a bispecific antibody, a multispecific antibody, a monovalent antibody, a multivalent antibody, an hIgG, an Fv, an Fd, an Fab, an F(ab')₂, an F(ab'), an Fab2, an Fab3, an scFab, an scFv, an scFvFc, an scFv-zipper, a di-scFv, a tandem scFv, a half-antibody, a diabody, a single chain diabody, a tandem diabody, a tandem di-scFv, or a tandem tri-scFv.
9. The FLT3 binding domain of claim 8, wherein the FLT3 binding domain is part of the antibody or an antigen binding fragment thereof and wherein the antibody or an antigen binding fragment thereof comprises the bispecific antibody.
10. The FLT3 binding domain of claim 9, wherein the bispecific antibody comprises (i) the FLT3 binding domain (anti-FLT3) and (ii) a CD3 binding domain.
11. The FLT3 binding domain of claim 10, wherein the CD3 comprises a human CD3.
12. The FLT3 binding domain of claim 10 or 11, wherein the anti-FLT3 domain is an sdAb.
13. The FLT3 binding domain of any one of claims 10-12, wherein the anti-CD3 domain is an scFv.
14. The FLT3 binding domain of claim 8, wherein the FLT3 binding domain is part of the antibody or an antigen binding fragment thereof and wherein the antibody or an antigen binding fragment thereof comprises the multispecific antibody.
15. The FLT3 binding domain of claim 14, wherein the multispecific antibody comprises (i) the FLT3 binding domain (anti-FLT3); (ii) a CD3 binding domain (anti-CD3); and (iii) an albumin binding domain (anti-ALB).
16. The FLT3 binding domain of any one of claims 15-17, wherein the CD3 comprises a human CD3.
17. The FLT3 binding domain of any one of claims 15-20, wherein the albumin comprises a human serum albumin.
18. The FLT3 binding domain of any one of claims 15-17, wherein the FLT3 binding domain (anti-FLT3), the CD3 binding domain (anti-CD3), and the albumin binding domain (anti-ALB) are in an at least one of the following formats, from N-terminus to C-terminus:

anti-ALB: anti-FLT3: anti-CD3;
anti-CD3: anti-FLT3: anti-ALB;
anti-FLT3: anti-CD3: anti-ALB;
anti-ALB: anti-CD3: anti-FLT3;
anti-FLT3: anti-ALB: anti-CD3; and
anti-CD3: anti-ALB: anti-FLT3.

19. The FLT3 binding domain of any one of claims 15-18, wherein the anti-FLT3 domain is an sdAb.
20. The FLT3 binding domain of any one of claims 15-19, wherein the anti-CD3 domain is an scFv.
21. The FLT3 binding domain of any one of claims 15-20, wherein the anti-ALB domain is an sdAb.
22. The FLT3 binding domain of any one of claims 15-21, wherein the anti-CD3 domain comprises an amino acid sequence that is at least about 90% identical to the sequence set forth in SEQ ID NO. 373.
23. The FLT3 binding domain of any one of claims 15-22, wherein the anti-ALB domain comprises an amino acid sequence that is at least about 90% identical to the sequence set forth in SEQ ID NO. 372.
24. The FLT3 binding domain of any one of claims 15-23, wherein the FLT3 binding domain is part of the multispecific antibody, wherein the multispecific antibody comprises a sequence that is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOs. 267, 269, 391, and 392.
25. The FLT3 binding domain of any one of claims 1-27, wherein the FLT3 binding domain binds to human FLT3.
26. An FLT3 targeting trispecific protein comprising
 - (A) a first domain that binds a human CD3;
 - (B) a second domain that binds a human serum albumin protein; and
 - (C) a third domain that binds a human FLT3 or a cynomolgus FLT3,wherein

the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100;

the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394;

the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID Nos. 195, 173, and 186.

27. The FLT3 targeting trispecific protein of claim 26, wherein the first domain is an scFv that comprises a heavy chain comprising HC CDR1, HC CDR2, HC CDR3, and a light chain comprising LC CDR1, LC CDR2, or LC CDR3, wherein the HC CDR1 comprises the amino acid sequence of SEQ ID NO: 397 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 397; the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 398 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 398; the HC CDR3 comprises the amino acid sequence of SEQ ID NO: 399 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 399; the LC CDR1 comprises the amino acid sequence of SEQ ID NO: 400 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 400; the LC CDR2 comprises the amino acid sequence of SEQ ID NO: 401 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 401; and the LC CDR3 comprises the amino acid sequence of SEQ ID NO: 402 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 402.

28. The FLT3 targeting trispecific protein of claim 26 or 27, wherein the CD3 binding domain is an scFv that comprises an amino acid sequence that is at least about 90% identical to the sequence set forth in SEQ ID No. 373.

29. The FLT3 targeting trispecific protein of any one of claims 26-28, wherein the second domain is an sdAb that comprises a CDR1, a CDR2, and a CDR3, wherein the CDR1 comprises the amino acid sequence of SEQ ID NO: 403 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 403; the CDR2 comprises the amino acid sequence of SEQ ID NO: 404 or an amino acid sequence comprising one or more substitutions in the sequence of

SEQ ID NO: 404; and the CDR3 comprises the amino acid sequence of SEQ ID NO: 405 or an amino acid sequence comprising one or more substitutions in the sequence of SEQ ID NO: 405.

30. The FLT3 targeting trispecific protein of any one of claims 26-29, wherein the second domain is an sdAb that comprises an amino acid sequence that is at least about 90% identical to the sequence set forth in SEQ ID No. 372.

31. A pharmaceutical composition comprising the FLT3 binding domain according to any one of claims 1-25 or the FLT3 targeting trispecific protein according to any one of claims 26-30, and a pharmaceutically acceptable carrier.

32. Use of an FLT3 binding domain in the manufacture of a medicament for the treatment of cancer, wherein the FLT3 binding domain is to be administered to a subject in need thereof, wherein FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein

the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100;

the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394;

the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.

33. The use of claim 32, wherein the FLT3 binding domain comprises a sequence that is at least about 80% identical to a sequence selected from the group consisting of SEQ ID NOs. 71, 73, 389, and 390.

34. Use of an FLT3 targeting trispecific protein in the manufacture of a medicament for the treatment of cancer, wherein the FLT3 targeting trispecific protein is to be administered to a subject in need thereof;

wherein the FLT3 targeting trispecific protein comprises

(A) a first domain that binds to a human CD3;

(B) a second domain that binds to a human serum albumin protein; and

(C) a third domain that binds to a human FLT3,

wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186.

35. The use of any one of claims 32-34, wherein the cancer comprises a hematologic malignancy.

36. The use of claim 34 or 35, wherein the third domain comprises an amino acid sequence that is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOs. 71, 73, 389, and 390.

37. The use of any one of claims 34-36, wherein the trispecific protein comprises an amino acid sequence that is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOs. 267, 269, 391, and 392.

38. The use of any one of claims 35-37, wherein hematologic malignancy comprises an acute myeloid leukemia (AML), a myelodysplastic syndrome, or a chronic myelomonocytic leukemia.

39. An FLT3 binding domain comprising at least three complementarity determining regions (a CDR1, a CDR2, and a CDR3), wherein

the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100;

the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394;

the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or

an FLT3 targeting trispecific protein comprising

(A) a first domain that binds to a human CD3;

(B) a second domain that binds to a human serum albumin protein; and

(C) a third domain that binds to a human FLT3,

wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 91, 89, and 100; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 149, 151, 393, and 394; the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, or a sequence comprising one or more amino acid substitutions in a sequence selected from the group consisting of SEQ ID NOs. 195, 173, and 186, for use in therapy.