

NO:103), 07 (SEQ ID NO:104), 08 (SEQ ID NO:105), 09 (SEQ ID NO:106), 10 (SEQ ID NO:107), 11 (SEQ ID NO:108), 12 (SEQ ID NO:109), 13 (SEQ ID NO:110), 14 (SEQ ID NO:111), 15 (SEQ ID NO:112), 16 (SEQ ID NO:113), 17 (SEQ ID NO:114), 18 (SEQ ID NO:115), 19 (SEQ ID NO:116), 20 (SEQ ID NO:117), 21 (SEQ ID NO:118), 22 (SEQ ID NO:119), 23 (SEQ ID NO:120), or 24 (SEQ ID NO:121).

[0050] In any of the above embodiments, are provided methods, wherein the tandem diabody has a sequence selected from the group consisting of SEQ ID NOs:123-146. In any of the above embodiments, are provided methods, wherein the tandem diabody is tandem diabody 01 (SEQ ID NO:123), 02 (SEQ ID NO:124), 03 (SEQ ID NO:125), 04 (SEQ ID NO:126), 05 (SEQ ID NO:127), 06 (SEQ ID NO:128), 07 (SEQ ID NO:129), 08 (SEQ ID NO:130), 09 (SEQ ID NO:131), 10 (SEQ ID NO:132), 11 (SEQ ID NO:133), 12 (SEQ ID NO:134), 13 (SEQ ID NO:135), 14 (SEQ ID NO:136), 15 (SEQ ID NO:137), 16 (SEQ ID NO:138), 17 (SEQ ID NO:139), 18 (SEQ ID NO:140), 19 (SEQ ID NO:141), 20 (SEQ ID NO:142), 21 (SEQ ID NO:143), 22 (SEQ ID NO:144), 23 (SEQ ID NO:145), or 24 (SEQ ID NO:146).

[0050a] In another aspect, provided herein is a bispecific, antigen-binding tandem diabody specific to human CD33 and human CD3, wherein the tandem diabody comprises a sequence selected from SEQ ID NOs:123-146.

[0051] In any of the above embodiments, are provided methods, wherein the method further comprises administering cytarabine, azacitidine, decitabine, anthracycline, fludarabine, clofarabine, cladribine, nelarabine, methotrexate, bortezomib, carfilzomib, melphalan, ibrutinib, thalidomide, lenalidomide, pomalidomide, apremilast, an epipodophyllotoxin, an anthracenedione, an anti-CD20 agent or combinations thereof. In any of the above embodiments, are provided methods, wherein the AML is AML with Recurrent Genetic Abnormalities, AML with myelodysplasia-related changes, Therapy-related myeloid neoplasms, Myeloid sarcoma, Myeloid proliferations related to Down syndrome, Blastic plasmacytoid dendritic cell neoplasm, or AML not otherwise categorized. In any of the above embodiments, are provided methods, wherein the AML is AML-M0, AML-M1, AML-M2, AML-M3, AML-M4, AML-M5, AML-M6, or AML-M7. In any of the above embodiments, are provided methods, wherein administering the protein, in a patient having received allogeneic hematopoietic stem cell transplant, after receiving a myeloablative conditioning regimen, results in improved overall response rate to therapy with the protein. In any of the above embodiments, are provided methods, wherein administering the protein, in a patient having received allogeneic hematopoietic stem cell transplant, after receiving a non-myeloablative conditioning regimen, results in improved overall response rate to therapy with the protein.

[0051a] In another aspect, provided herein is a use of a therapeutically effective amount of a protein that binds to human CD33 and human CD3 in the manufacture of a medicament for the treatment of acute myelogenous leukemia (AML) in a patient having received hematopoietic stem cell transplantation, wherein the medicament is to be administered to the patient.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of a therapeutically effective amount of a protein that binds to human CD33 and human CD3 in the manufacture of a medicament for the treatment of acute myelogenous leukemia (AML) in a patient having received hematopoietic stem cell transplantation, wherein the medicament is to be administered to the patient.
2. The use according to claim 1, wherein the transplantation is allogenic.
3. The use according to claim 1, wherein the transplantation is autologous.
4. The use according to any of claims 1-3, wherein the patient does not receive a conditioning regimen.
5. The use according to any of claims 1-3, wherein a conditioning regimen is to be further administered to the patient.
6. The use according to claim 5, wherein the conditioning regimen is myeloablative.
7. The use according to claim 5, wherein the conditioning regimen is non-myeloablative.
8. The use according to any of claims 5-7, wherein the protein is to be administered after the conditioning regimen.
9. The use according to any of claims 1-8, wherein the patient is in complete remission.
10. The use according to any of claims 1-8, wherein the patient has minimal residual disease.
11. The use according to any of claims 1-8, wherein the patient has failed chemotherapy or radiation.
12. The use according to any of claims 1-8, wherein the patient has failed inductive chemotherapy.
13. The use according to any of claims 1-8, wherein the patient has failed consolidation or maintenance (postremission) chemotherapy.
14. The use according to any of claims 1-13, wherein the AML is relapsed.
15. The use according to any of claims 1-13, wherein the AML is refractory.
16. The use according to any of claims 1-13, wherein the AML is high risk and in remission.
17. The use according to any of claims 1-16, wherein the medicament is to be administered immediately after the hematopoietic stem cell transplantation.
18. The use according to any of claims 1-16, wherein the medicament is to be administered three days post-hematopoietic stem cell transplantation.

19. The use according to any of claims 1-16, wherein the medicament is to be administered seven days post-hematopoietic stem cell transplantation.

20. The use according to any of claims 1-16, wherein the medicament is to be administered two weeks post-hematopoietic stem cell transplantation.

21. The use according to any of claims 1-16, wherein the medicament is to be administered four weeks post-hematopoietic stem cell transplantation.

22. The use according to any of claims 1-21, wherein the medicament is to be administered for a period of time selected from a group consisting of four weeks, eight weeks, three months, four months, six months, eight months, ten months, twelve months, eighteen months, and twenty-four months.

23. The use according to any of claims 1-2, wherein the medicament is to be administered at time of progression in allogeneic setting, with donor lymphocytes.

24. The use according to any of claims 1-2, wherein the medicament is to be administered at time of progression in allogeneic setting, without donor lymphocytes.

25. The use according to any of claims 1-24, wherein the protein comprises heavy and light chain domains specific for human CD33 and human CD3.

26. The use according to any of claims 1-25, wherein the protein is an antibody or antibody derivative.

27. The use according to any of claims 1-25, wherein the protein comprises Fab, Fab', or F(ab')₂ fragments.

28. The use according to any of claims 1-25, wherein the protein is a single-chain Fv, tandem single-chain Fv, bi-specific T-cell engager, dual affinity retargeting antibody, diabody, or bispecific tandem diabody.

29. The use according to any of claims 1-25, wherein the protein is a bispecific tandem diabody.

30. The use according to claim 29, wherein the tandem diabody comprises a first polypeptide and a second polypeptide, each polypeptide having at least four variable chain domains linked one after another, wherein each polypeptide comprises

- (i) a variable heavy chain (VH) domain specific to human CD33;
- (ii) a variable light chain (VL) domain specific to human CD33;
- (iii) a VH domain specific for human CD3, and
- (iv) a VL domain specific for human CD3.

31. The use according to claim 30, wherein in each polypeptide, the four variable chain domains are linked with one after another by peptide linkers L1, L2 and L3 in the order of:

VL(CD3)-L1-VH(CD33)-L2-VL(CD33)-L3-VH(CD3);
VH(CD3)-L1-VL(CD33)-L2-VH(CD33)-L3-VL(CD3);
VL(CD33)-L1-VH(CD3)-L2-VL(CD3)-L3-VH(CD33); or
VH(CD33)-L1-VL(CD3)-L2-VH(CD3)-L3-VL(CD33).

32. The use according to any of claims 29-31, wherein the VL domain specific to human CD33 comprises a CDR1 consisting of the sequence selected from the group consisting of SEQ ID NOs:21-27, a CDR2 consisting of the sequence selected from the group consisting of SEQ ID NOs:28-34 and a CDR3 consisting of the sequence of the group consisting of SEQ ID NOs:35-41.

33. The use according to any of claims 29-32, wherein the VH domain specific to human CD33 comprises a CDR1 consisting of the sequence selected from the group consisting of SEQ ID NOs:42-48, a CDR2 consisting of the sequence selected from the group consisting of SEQ ID NOs:49-55 and a CDR3 consisting of a sequences selected from the group consisting of SEQ ID NOs:56-63.

34. The use according to any of claims 31-33, wherein the CDR1, CDR2 and CDR3 of the VL domain specific to human CD33 are sequences selected from the group consisting of:

- (i) SEQ ID NOs:21, 28 and 35;
- (ii) SEQ ID NOs:22, 29 and 36;
- (iii) SEQ ID NOs:23, 30 and 37;
- (iv) SEQ ID NOs:24, 31 and 38;
- (v) SEQ ID NOs:25, 32 and 39;
- (vi) SEQ ID NOs:26, 33 and 40; and
- (vii) SEQ ID NOs:27, 34 and 41.

35. The use according to any of claims 29-34, wherein the CDR1, CDR2 and CDR3 of the VH domain specific to CD33 are sequences selected from the group consisting of:

- (i) SEQ ID NOs:42, 49 and 56;
- (ii) SEQ ID NOs:43, 50 and 57;
- (iii) SEQ ID NOs:43, 50 and 58;
- (iv) SEQ ID NOs:43, 50 and 59;
- (v) SEQ ID NOs:43, 50 and 60;
- (vi) SEQ ID NOs:44, 51 and 61;
- (vii) SEQ ID NOs:45, 52 and 62;
- (viii) SEQ ID NOs:46, 53 and 63;
- (ix) SEQ ID NOs:47, 54 and 63; and
- (x) SEQ ID NOs:48, 55 and 63.

36. The use according to any of claims 29-35, wherein the VL and VH domains specific to CD3 are sequences selected from the group consisting of:

- (i) SEQ ID NO:1 and SEQ ID NO:11;
- (ii) SEQ ID NO:2 and SEQ ID NO:12;
- (iii) SEQ ID NO:3 and SEQ ID NO:13;
- (iv) SEQ ID NO:4 and SEQ ID NO:14;
- (v) SEQ ID NO:5 and SEQ ID NO:15;
- (vi) SEQ ID NO:6 and SEQ ID NO:16;
- (vii) SEQ ID NO:7 and SEQ ID NO:17;
- (viii) SEQ ID NO:8 and SEQ ID NO:18;
- (ix) SEQ ID NO:9 and SEQ ID NO:19; and
- (x) SEQ ID NO:10 and SEQ ID NO:20.

37. The use according to any of claims 29-36, wherein the VH domain specific for human CD3 comprises a CDR1 sequence of STYAMN (SEQ ID NO:72), a CDR2 sequence of RIRSKYNNYATYYADSVKD (SEQ ID NO:73) and a CDR3 sequence of HGNFGNSYVSWFAY (SEQ ID NO:74) or HGNFGNSYVSYFAY (SEQ ID NO:75).

38. The use according to any of claims 29-37, wherein the VL domain specific for human CD3 comprises a CDR1 sequence of RSSTGAVTTSNYAN (SEQ ID NO:90), a CDR2 sequence of GTNKRAP (SEQ ID NO:91), and a CDR3 sequence of ALWYSNL (SEQ ID NO:92).

39. The use according to any of claims 27-36, wherein the VL and VH domains specific to CD3 are sequences selected from the group consisting of:

- (i) SEQ ID NO:64 and SEQ ID NO:68;
- (ii) SEQ ID NO:65 and SEQ ID NO:69;
- (iii) SEQ ID NO:66 and SEQ ID NO:70; and
- (iv) SEQ ID NO:67 and SEQ ID NO:71.

40. The use according to any of claims 29-39, wherein each polypeptide comprises four variable chain domains selected from the group consisting of:

- (i) SEQ ID NOs:2, 12, 65 and 69;
- (ii) SEQ ID NOs:3, 13, 65 and 69;
- (iii) SEQ ID NOs:4, 14, 65 and 69;
- (iv) SEQ ID NOs:5, 15, 65 and 69;
- (v) SEQ ID NOs:1, 11, 64 and 68;
- (vi) SEQ ID NOs:2, 12, 64 and 68;
- (vii) SEQ ID NOs:2, 12, 66 and 70;

- (viii) SEQ ID NOs:4, 14, 66 and 70;
- (ix) SEQ ID NOs:5, 15, 66 and 70;
- (x) SEQ ID NOs:3, 13, 64 and 68;
- (xi) SEQ ID NOs:3, 13, 67 and 71;
- (xii) SEQ ID NOs:4, 14, 64 and 68;
- (xiii) SEQ ID NOs:5, 15, 64 and 68;
- (xiv) SEQ ID NOs:7, 17, 64 and 68;
- (xv) SEQ ID NOs:6, 16, 64 and 68;
- (xvi) SEQ ID NOs:6, 16, 67 and 71;
- (xvii) SEQ ID NOs:8, 18, 64 and 68;
- (xviii) SEQ ID NOs:9, 19, 64 and 68;
- (xix) SEQ ID NOs:9, 19, 67 and 71; and
- (xx) SEQ ID NOs:10, 20, 64 and 68.

41. The use according to any of claims 29-40, wherein linkers L1, L2 and L3 consist of about 12 or less amino acid residues.

42. The use according to any of claims 29-41, wherein linkers L1, L2 and L3 are each independently selected from GGSGGS (SEQ ID NO:95), GGSG (SEQ ID NO:96) or GGSGG (SEQ ID NO:97).

43. The use according to any of claims 29-42, wherein the tandem diabody comprises a sequence selected from the group consisting of SEQ ID NOs:98-121.

44. The use according to any of claims 29-43, wherein the tandem diabody is tandem diabody 01 (SEQ ID NO:98), 02 (SEQ ID NO:99), 03 (SEQ ID NO:100), 04 (SEQ ID NO:101), 05 (SEQ ID NO:102), 06 (SEQ ID NO:103), 07 (SEQ ID NO:104), 08 (SEQ ID NO:105), 09 (SEQ ID NO:106), 10 (SEQ ID NO:107), 11 (SEQ ID NO:108), 12 (SEQ ID NO:109), 13 (SEQ ID NO:110), 14 (SEQ ID NO:111), 15 (SEQ ID NO:112), 16 (SEQ ID NO:113), 17 (SEQ ID NO:114), 18 (SEQ ID NO:115), 19 (SEQ ID NO:116), 20 (SEQ ID NO:117), 21 (SEQ ID NO:118), 22 (SEQ ID NO:119), 23 (SEQ ID NO:120), or 24 (SEQ ID NO:121).

45. The use according to any of claims 29-42, wherein the tandem diabody comprises a sequence selected from the group consisting of SEQ ID NOs:123-146.

46. The use according to any of claims 29-42 and 45, wherein the tandem diabody is tandem diabody 01 (SEQ ID NO:123), 02 (SEQ ID NO:124), 03 (SEQ ID NO:125), 04 (SEQ ID NO:126), 05 (SEQ ID NO:127), 06 (SEQ ID NO:128), 07 (SEQ ID NO:129), 08 (SEQ ID NO:130), 09 (SEQ ID NO:131), 10 (SEQ ID NO:132), 11 (SEQ ID NO:133), 12 (SEQ ID NO:134), 13 (SEQ ID NO:135), 14 (SEQ ID NO:136), 15 (SEQ ID NO:137), 16 (SEQ ID NO:138), 17 (SEQ ID

NO:139), 18 (SEQ ID NO:140), 19 (SEQ ID NO:141), 20 (SEQ ID NO:142), 21 (SEQ ID NO:143), 22 (SEQ ID NO:144), 23 (SEQ ID NO:145), or 24 (SEQ ID NO:146).

47. The use according to any of claims 1-46, wherein cytarabine, azacitidine, decitabine, anthracycline, fludarabine, clofarabine, cladribine, nelarabine, methotrexate, bortezomib, carfilzomib, melphalan, ibrutinib, thalidomide, lenalidomide, pomalidomide, apremilast, an epipodophyllotoxin, an anthracenedione, an anti-CD20 agent or combinations thereof is to be further administered.

48. The use according to any of claims 1-47, wherein the AML is AML with Recurrent Genetic Abnormalities, AML with myelodysplasia-related changes, Therapy-related myeloid neoplasms, Myeloid sarcoma, Myeloid proliferations related to Down syndrome, Blastic plasmacytoid dendritic cell neoplasm, or AML not otherwise categorized.

49. The use according to any of claims 1-48, wherein the AML is AML-M0, AML-M1, AML-M2, AML-M3, AML-M4, AML-M5, AML-M6, or AML-M7.

50. The use according to any of claims 1-49, wherein administration of the protein, in a patient having received allogenic hematopoietic stem cell transplant, after receiving a myeloablative conditioning regimen, results in improved overall response rate to therapy with the protein.

51. The use according to any of claims 1-49, wherein administration of the protein, in a patient having received allogenic hematopoietic stem cell transplant, after receiving a non-myeloablative conditioning regimen, results in improved overall response rate to therapy with the protein.

52. A bispecific, antigen-binding tandem diabody specific to human CD33 and human CD3, wherein the tandem diabody comprises a sequence selected from SEQ ID NOs:123-146.

53. The bispecific antigen-binding tandem diabody according to claim 52, wherein the tandem diabody is tandem diabody 01 (SEQ ID NO:123), 02 (SEQ ID NO:124), 03 (SEQ ID NO:125), 04 (SEQ ID NO:126), 05 (SEQ ID NO:127), 06 (SEQ ID NO:128), 07 (SEQ ID NO:129), 08 (SEQ ID NO:130), 09 (SEQ ID NO:131), 10 (SEQ ID NO:132), 11 (SEQ ID NO:133), 12 (SEQ ID NO:134), 13 (SEQ ID NO:135), 14 (SEQ ID NO:136), 15 (SEQ ID NO:137), 16 (SEQ ID NO:138), 17 (SEQ ID NO:139), 18 (SEQ ID NO:140), 19 (SEQ ID NO:141), 20 (SEQ ID NO:142), 21 (SEQ ID NO:143), 22 (SEQ ID NO:144), 23 (SEQ ID NO:145), or 24 (SEQ ID NO:146).

54. The bispecific antigen-binding tandem diabody according to claim 53, wherein the tandem diabody possesses binding K_D of 50 nM or less to CD33 on CD33⁺ tumor cells selected from HL-60, KG-1 and U-937.

55. The bispecific antigen-binding tandem diabody according to claim 54, wherein the tandem diabody possesses binding K_D of 15 nM or less to CD33 on CD33⁺ HL-60 tumor cells.

56. The bispecific antigen-binding tandem diabody according to claim 55, wherein tandem diabody specifically binds to an epitope of human CD33 which is within ₆₂DQEVQEETQ₇₀ (SEQ ID NO:94) (amino acid residues 62-70 of SEQ ID NO:93) of human CD33.

57. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 01 (SEQ ID NO: 123).

58. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 02 (SEQ ID NO: 124).

59. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 03 (SEQ ID NO: 125).

60. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 04 (SEQ ID NO: 126).

61. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 05 (SEQ ID NO: 127).

62. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 06 (SEQ ID NO: 128).

63. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 07 (SEQ ID NO: 129).

64. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 08 (SEQ ID NO: 130).

65. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 09 (SEQ ID NO: 131).

66. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 10 (SEQ ID NO: 132).

67. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 11 (SEQ ID NO: 133).

68. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 12 (SEQ ID NO: 134).

69. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 13 (SEQ ID NO: 135).

70. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 14 (SEQ ID NO: 136).

71. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 15 (SEQ ID NO: 137).

72. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 16 (SEQ ID NO: 138).

73. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 17 (SEQ ID NO: 139).

74. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 18 (SEQ ID NO: 140).

75. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 19 (SEQ ID NO: 141).

76. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 20 (SEQ ID NO: 142).

77. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 21 (SEQ ID NO: 143).

78. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 22 (SEQ ID NO: 144).

79. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 23 (SEQ ID NO: 145).

80. The bispecific antigen-binding tandem diabody according to any one of claims 52-56, wherein the tandem diabody is tandem diabody 24 (SEQ ID NO: 146).