

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, wherein said protein 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:

- a variable heavy chain (VH) domain specific to human CD33;
- a variable light chain (VL) domain specific to human CD33;

a VH domain specific for human CD3, and
a VL domain specific for human CD3,

and 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),

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, and

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,

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, wherein said protein 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:

a variable heavy chain (VH) domain specific to human CD33;
a variable light chain (VL) domain specific to human CD33;
a VH domain specific for human CD3, and
a VL domain specific for human CD3,

and 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),

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, and

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,

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 1-2, wherein the medicament is to be administered at time of progression in allogeneic setting, with donor lymphocytes.

9. 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.

10. 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.

11. The bispecific antigen-binding tandem diabody according to claim 10, wherein the tandem diabody consists of a sequence as recited in SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, or SEQ ID NO:146.

12. The bispecific antigen-binding tandem diabody according to claim 11, 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.

13. The bispecific antigen-binding tandem diabody according to claim 12, wherein the tandem diabody possesses binding K_D of 15 nM or less to CD33 on CD33⁺ HL-60 tumor cells.

14. The use according to claim 1, 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.

15. The use according to claim 1, wherein the CDR1, CDR2 and CDR3 of the VH domain specific to CD33 are sequences selected from the group consisting of:

- (viii) SEQ ID NOs:42, 49 and 56;
- (ix) SEQ ID NOs:43, 50 and 57;
- (x) SEQ ID NOs:43, 50 and 58;
- (xi) SEQ ID NOs:43, 50 and 59;
- (xii) SEQ ID NOs:43, 50 and 60;
- (xiii) SEQ ID NOs:44, 51 and 61;
- (xiv) SEQ ID NOs:45, 52 and 62;
- (xv) SEQ ID NOs:46, 53 and 63;
- (xvi) SEQ ID NOs:47, 54 and 63;.and
- (xvii) SEQ ID NOs:48, 55 and 63.

16. The use according to claim 1, wherein the VL and VH domains specific to CD33 are sequences selected from the group consisting of:

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| (xviii) | SEQ ID NO:1 and SEQ ID NO:11; |
| (xix) | SEQ ID NO:2 and SEQ ID NO:12; |
| (xx) | SEQ ID NO:3 and SEQ ID NO:13; |
| (xxi) | SEQ ID NO:4 and SEQ ID NO:14; |
| (xxii) | SEQ ID NO:5 and SEQ ID NO:15; |
| (xxiii) | SEQ ID NO:6 and SEQ ID NO:16; |
| (xxiv) | SEQ ID NO:7 and SEQ ID NO:17; |
| (xxv) | SEQ ID NO:8 and SEQ ID NO:18; |
| (xxvi) | SEQ ID NO:9 and SEQ ID NO:19; and |
| (xxvii) | SEQ ID NO:10 and SEQ ID NO:20. |

17. The use according to claim 1, 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).

18. The use according to claim 1, 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).

19. The use according to claim 1, wherein the VL and VH domains specific to CD3 are sequences selected from the group consisting of:

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|----------|------------------------------------|
| (xxviii) | SEQ ID NO:64 and SEQ ID NO:68; |
| (xxix) | SEQ ID NO:65 and SEQ ID NO:69; |
| (xxx) | SEQ ID NO:66 and SEQ ID NO:70; and |
| (xxxi) | SEQ ID NO:67 and SEQ ID NO:71. |

20. The use according to claim 1, wherein each polypeptide comprises four variable chain domains selected from the group consisting of:

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|-----------|------------------------------|
| (xxxii) | SEQ ID NOs:2, 12, 65 and 69; |
| (xxxiii) | SEQ ID NOs:3, 13, 65 and 69; |
| (xxxiv) | SEQ ID NOs:4, 14, 65 and 69; |
| (xxxv) | SEQ ID NOs:5, 15, 65 and 69; |
| (xxxvi) | SEQ ID NOs:1, 11, 64 and 68; |
| (xxxvii) | SEQ ID NOs:2, 12, 64 and 68; |
| (xxxviii) | SEQ ID NOs:2, 12, 66 and 70; |
| (xxxix) | SEQ ID NOs:4, 14, 66 and 70; |
| (xl) | SEQ ID NOs:5, 15, 66 and 70; |
| (xli) | SEQ ID NOs:3, 13, 64 and 68; |
| (xlii) | SEQ ID NOs:3, 13, 67 and 71; |

(xliii)	SEQ ID NOs:4, 14, 64 and 68;
(xliv)	SEQ ID NOs:5, 15, 64 and 68;
(xlv)	SEQ ID NOs:7, 17, 64 and 68;
(xlvi)	SEQ ID NOs:6, 16, 64 and 68;
(xlvii)	SEQ ID NOs:6, 16, 67 and 71;
(xlviii)	SEQ ID NOs:8, 18, 64 and 68;
(xlix)	SEQ ID NOs:9, 19, 64 and 68;
(l)	SEQ ID NOs:9, 19, 67 and 71; and
(li)	SEQ ID NOs:10, 20, 64 and 68.

21. The use according to claim 1, wherein linkers L1, L2 and L3 consist of about 12 or less amino acid residues.

22. The use according to claim 1, 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).

23. The use according to any of claims 1-9, wherein the protein comprises a sequence selected from the group consisting of SEQ ID NOs:123-146.

24. The use according to 1, wherein the tandem diabody is selected from: 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).