

CD33-BINDING POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 62/843,408, filed May 4, 2019, and US Provisional Application No. 62/844,359, filed May 7, 2019, each of which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present invention relates to CD33-binding polypeptides, and methods of using CD33-binding polypeptides to modulate the biological activity of CD33. Such methods include, but are not limited to, methods of treating cancer.

BACKGROUND

[0003] CD33, also known as p67, SIGLEC3, or SIGLEC-3, binds sialic acids and regulates inflammatory and immune responses of hematopoietic cells. CD33 also can influence initiation, proliferation and progression in a variety of hematologic cancers. For example, over 85% of acute myeloid leukemia (AML) cases express the CD33 antigen. Thus, CD33 is a suitable tumor-associated antigen for targeted therapies for hematologic cancers, such as leukemia. Therefore, there exists a therapeutic need for more potent antagonists of CD33.

SUMMARY

[0004] Provided herein are CD33-binding polypeptides and methods of using CD33-binding polypeptides to treat, for example, leukemia. In some examples, a CD33-binding polypeptide comprises at least one VHH domain.

[0004a] In one aspect, the present disclosure provides a polypeptide comprising at least one VHH domain that binds CD33 and that comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, 3, 7, 11, 15, 19, 23, 27, 31, 35, 50, 53, 56, 59, 62, 65, 68, or 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, 4, 8, 12, 16, 20, 24, 28, 32, 36, 51, 54, 57, 60, 63, 66, 69, or 72; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49, 5, 9, 13, 17, 21, 25, 29, 33, 37, 52, 55, 58, 61, 64, 67, 70, or 73.

[0004b] In another aspect, the present disclosure provides an immunoconjugate comprising the polypeptide disclosed herein and a cytotoxic agent.

[0004c] In another aspect, the present disclosure provides a pharmaceutical composition comprising the polypeptide disclosed herein or the immunoconjugate disclosed herein, and a pharmaceutically acceptable carrier.

[0004d] In yet another aspect, the present disclosure provides an isolated nucleic acid that encodes the polypeptide disclosed herein.

[0004e] In one aspect, the present disclosure provides a vector comprising the nucleic acid disclosed herein.

[0004f] In another aspect, the present disclosure provides a host cell comprising the nucleic acid disclosed herein or the vector disclosed herein.

[0004g] In yet another aspect, the present disclosure provides a host cell that expresses the polypeptide disclosed herein.

[0004h] In one aspect, the present disclosure provides a method of producing the polypeptide disclosed herein, comprising incubating the host cell disclosed herein under conditions suitable for expression of the polypeptide.

[0004i] In one aspect, the present disclosure provides use of the polypeptide disclosed herein, the immunoconjugate disclosed herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating cancer.

[0004j] Some examples are provided below.

Example 1. A polypeptide comprising at least one VHH domain that binds CD33 and that comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, 3, 7, 11, 15, 19, 23, 27, 31, 35, 50, 53, 56, 59, 62, 65, 68, or 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, 4, 8, 12, 16, 20, 24, 28, 32, 36, 51, 54, 57, 60, 63, 66, 69, or 72; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49, 5, 9, 13, 17, 21, 25, 29, 33, 37, 52, 55, 58, 61, 64, 67, 70, or 73.

Example 2. The polypeptide of example 1, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 47 or 3; a CDR2 comprising the amino acid sequence of SEQ ID NO: 48 or 4; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49 or 5.

Example 3. Example 3. The polypeptide of example 1 or example 2, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:

7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 9.

Example 4. The polypeptide of any one of examples 1-3, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 11 or 50; a CDR2 comprising the amino acid sequence of SEQ ID NO: 12 or 51; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 13 or 52.

Example 5. The polypeptide of any one of examples 1-4, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 15, 53, or 56; a CDR2 comprising the amino acid sequence of SEQ ID NO: 16, 54, or 57; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 17, 55, or 58.

Example 6. The polypeptide of any one of examples 1-5, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 19 or 59; a CDR2 comprising the amino acid sequence of SEQ ID NO: 20 or 60; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 21 or 61.

Example 7. The polypeptide of any one of examples 1-6, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 23 or 62; a CDR2 comprising the amino acid sequence of SEQ ID NO: 24 or 63; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25 or 64.

Example 8. The polypeptide of any one of examples 1-7, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 27 or 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 28 or 66; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29 or 67.

Example 9. The polypeptide of any one of examples 1-8, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 31 or 68; a CDR2 comprising the amino acid sequence of SEQ ID NO: 32 or 69; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 33 or 70.

Example 10. The polypeptide of any one of examples 1-9, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 35 or 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 36 or 72; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 37 or 73.

Example 11. The polypeptide of any one of examples 1-10, wherein at least one VHH domain comprises a CDR1, a CDR2, and a CDR3, respectively comprising the amino acid sequences of SEQ ID NOs: 47, 48, and 49; SEQ ID NOs: 3, 4, and 5; SEQ ID NOs: 7, 8, and 9; SEQ ID NOs: 11, 12, and 13; SEQ ID NOs: 15, 16, and 17; SEQ ID NOs: 19, 20, and 21; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 27, 28, and 29; SEQ ID NOs: 31, 32, and 33; SEQ

ID NOs: 35, 36, and 37; SEQ ID NOs: 50, 51, and 52; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 56, 57, and 58; SEQ ID NOs: 59, 60, and 61; SEQ ID NOs: 62, 63, and 64; SEQ ID NOs: 65, 66, and 67; SEQ ID NOs: 68, 69, and 70; or SEQ ID NOs: 71, 72, and 73.

Example 12. The polypeptide of any one of examples 1-11, wherein at least one VHH domain is humanized.

Example 13. The polypeptide of any one of examples 1-12, wherein at least one VHH domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 38, 114, 39, 40, 41, 42, 43, 44, 45, 46, 115, 116, 117, 118, 119, 120, 121, or 122.

Example 14. The polypeptide of any one of examples 1-12, wherein at least one VHH domain comprises the amino acid sequence of SEQ ID NO: 38, 114, 39, 40, 41, 42, 43, 44, 45, 46, 115, 116, 117, 118, 119, 120, 121, or 122.

Example 15. The polypeptide of any one of example 1-12, wherein at least one VHH domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 123, 124, 125, 126, 127, 128, 129, 130, or 131.

Example 16. The polypeptide of any one of examples 1-12, wherein at least one VHH domain comprises the amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 123, 124, 125, 126, 127, 128, 129, 130, or 131.

Example 17. The polypeptide of any one of examples 1-16, comprising two VHH domains.

Example 18. The polypeptide of any one of examples 1-16, comprising three VHH domains.

Example 19. The polypeptide of any one of examples 1-18, wherein the polypeptide comprises at least one binding domain that binds an antigen other than CD33.

Example 20. The polypeptide of example 19, wherein the polypeptide comprises at least one binding domain that binds CD3, T-cell receptor (TCR) α , TCR β , CD28, CD16, CD32A, CD64, CD89, NKp46, or NKG2D.

Example 21. The polypeptide of example 17 or 18, wherein each VHH domain binds CD33.

Example 22. The polypeptide of example 21, wherein each VHH domain comprises the same CDR1, CDR2, and CDR3 amino acid sequences.

Example 23. The polypeptide of example 21, wherein each VHH domain comprises the same VHH sequence.

Example 24. The polypeptide of any one of examples 1-16, comprising one VHH domain.

Example 25. The polypeptide of any one of examples 1-24, wherein the polypeptide comprises an Fc region.

Example 26. The polypeptide of example 25, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 74-109.

Example 27. The polypeptide of example 25 or example 26, which forms a dimer under physiological conditions.

Example 28. The polypeptide of any one of examples 1-27, wherein the CD33 is human CD33.

Example 29. The polypeptide of example 28, wherein the human CD33 comprises the sequence of SEQ ID NO: 1.

Example 30. An immunoconjugate comprising the polypeptide of any one of examples 1-29 and a cytotoxic agent.

Example 31. The immunoconjugate of example 30, wherein the cytotoxic agent is selected from a calicheamicin, an auristatin, a dolastatin, a tubulicin, a maytansinoid, a cryptophycin, a duocarmycin, an esperamicin, a pyrrolobenzodiazepine, and an enediyne antibiotic.

Example 32. A pharmaceutical composition comprising the polypeptide of any one of examples 1-29 or the immunoconjugate of example 30 or example 31, and a pharmaceutically acceptable carrier.

Example 33. An isolated nucleic acid that encodes the polypeptide of any one of examples 1-29.

Example 34. A vector comprising the nucleic acid of example 33.

Example 35. A host cell comprising the nucleic acid of example 33 or the vector of example 34.

Example 36. A host cell that expresses the polypeptide of any one of examples 1-29.

Example 37. A method of producing the polypeptide of any one of examples 1-29, comprising incubating the host cell of example 35 or example 36 under conditions suitable for expression of the polypeptide.

Example 38. The method of example 37, further comprising isolating the polypeptide.

Example 39. A method of treating cancer comprising administering to a subject with cancer a pharmaceutically effective amount of the polypeptide of any one of examples

1-29, the immunoconjugate of example 30 or example 31, or the pharmaceutical composition of example 32.

Example 40. The method of example 39, wherein the cancer is selected from lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML); Hairy cell leukemia; and chronic myeloblastic leukemia.

Example 41. The method of example 39 or 40, wherein the cancer is acute myeloid leukemia (AML).

Example 42. The method of any one of examples 39-41, further comprising administering an additional therapeutic agent.

Example 43. The method of example 42, wherein the additional therapeutic agent is an anti-cancer agent.

Example 44. The method of example 43, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus.

Example 45. The method of any one of examples 39-44, wherein the cancer is a CD33-expressing cancer.

BRIEF DESCRIPTION OF THE FIGURES

[0005] FIG. 1 shows Bio-Layer Interferometry data for hz1E4v2 compared to other CD33-binding sdAbs described herein.

[0006] FIG. 2A-2M show binding of certain single-domain antibodies (sdAbs) to CD33 expressed on MOLM-13 or HEK 293 cells. "MOLM-13" indicates MOL-13 cells that have been transfected with a plasmid encoding CD33, as described in Example 2. "CD33m" indicates a truncated CD33 protein (SEQ ID NO: 113). "CD33M" indicates a full length CD33 protein (SEQ ID NO: 112). "HEK 293" or "Parental HEK 293" indicates untransfected HEK 293 cells. FIG. 2A shows binding of 1E4-IgG1 to CD33. FIG. 2B shows binding of hz1E4v2-IgG1 to CD33. FIG. 2C shows binding of 1H9-IgG1 to CD33. FIG. 2D shows binding of hz1H9v2-IgG1 to CD33. FIG. 2E shows binding of hz1G3v3-IgG1 to CD33. FIG. 2F shows binding of 1C7-IgG1 to CD33. FIG. 2G shows binding of hz1C7v1-IgG1 to CD33. FIG. 2H shows binding of hz1C7v11-IgG1 to CD33. FIG. 2I shows binding of hzA07v4-IgG1 to CD33. FIG. 2J shows

What is claimed is:

1. A polypeptide comprising at least one VHH domain that binds CD33 and that comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, 3, 7, 11, 15, 19, 23, 27, 31, 35, 50, 53, 56, 59, 62, 65, 68, or 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, 4, 8, 12, 16, 20, 24, 28, 32, 36, 51, 54, 57, 60, 63, 66, 69, or 72; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49, 5, 9, 13, 17, 21, 25, 29, 33, 37, 52, 55, 58, 61, 64, 67, 70, or 73.

2. The polypeptide of claim 1, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 47 or 3; a CDR2 comprising the amino acid sequence of SEQ ID NO: 48 or 4; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49 or 5.

3. The polypeptide of claim 1 or claim 2, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 8; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 9.

4. The polypeptide of any one of claims 1-3, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 11 or 50; a CDR2 comprising the amino acid sequence of SEQ ID NO: 12 or 51; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 13 or 52.

5. The polypeptide of any one of claims 1-4, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 15, 53, or 56; a CDR2 comprising the amino acid sequence of SEQ ID NO: 16, 54, or 57; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 17, 55, or 58.

6. The polypeptide of any one of claims 1-5, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 19 or 59; a CDR2 comprising the amino acid sequence of SEQ ID NO: 20 or 60; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 21 or 61.

7. The polypeptide of any one of claims 1-6, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 23 or 62; a CDR2 comprising the amino acid sequence of SEQ ID NO: 24 or 63; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25 or 64.

8. The polypeptide of any one of claims 1-7, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 27 or 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 28 or 66; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29 or 67.

9. The polypeptide of any one of claims 1-8, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 31 or 68; a CDR2 comprising the amino acid sequence of SEQ ID NO: 32 or 69; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 33 or 70.
10. The polypeptide of any one of claims 1-9, wherein at least one VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 35 or 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 36 or 72; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 37 or 73.
11. The polypeptide of any one of claims 1-10, wherein at least one VHH domain comprises a CDR1, a CDR2, and a CDR3, respectively comprising the amino acid sequences of SEQ ID NOs: 47, 48, and 49; SEQ ID NOs: 3, 4, and 5; SEQ ID NOs: 7, 8, and 9; SEQ ID NOs: 11, 12, and 13; SEQ ID NOs: 15, 16, and 17; SEQ ID NOs: 19, 20, and 21; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 27, 28, and 29; SEQ ID NOs: 31, 32, and 33; SEQ ID NOs: 35, 36, and 37; SEQ ID NOs: 50, 51, and 52; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 56, 57, and 58; SEQ ID NOs: 59, 60, and 61; SEQ ID NOs: 62, 63, and 64; SEQ ID NOs: 65, 66, and 67; SEQ ID NOs: 68, 69, and 70; or SEQ ID NOs: 71, 72, and 73.
12. The polypeptide of any one of claims 1-11, wherein at least one VHH domain is humanized.
13. The polypeptide of any one of claims 1-12, wherein at least one VHH domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 38, 114, 39, 40, 41, 42, 43, 44, 45, 46, 115, 116, 117, 118, 119, 120, 121, or 122.
14. The polypeptide of any one of claims 1-12, wherein at least one VHH domain comprises the amino acid sequence of SEQ ID NO: 38, 114, 39, 40, 41, 42, 43, 44, 45, 46, 115, 116, 117, 118, 119, 120, 121, or 122.
15. The polypeptide of any one of claim 1-12, wherein at least one VHH domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 123, 124, 125, 126, 127, 128, 129, 130, 131.
16. The polypeptide of any one of claims 1-12, wherein at least one VHH domain comprises the amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 123, 124, 125, 126, 127, 128, 129, 130, or 131.
17. The polypeptide of any one of claims 1-16, comprising two VHH domains.
18. The polypeptide of any one of claims 1-16, comprising three VHH domains.

19. The polypeptide of any one of claims 1-18, wherein the polypeptide comprises at least one binding domain that binds an antigen other than CD33.

20. The polypeptide of claim 19, wherein the polypeptide comprises at least one binding domain that binds CD3, T-cell receptor (TCR) α , TCR β , CD28, CD16, CD32A, CD64, CD89, NKp46, or NKG2D.

21. The polypeptide of claim 17 or 18, wherein each VHH domain binds CD33.

22. The polypeptide of claim 21, wherein each VHH domain comprises the same CDR1, CDR2, and CDR3 amino acid sequences.

23. The polypeptide of claim 21, wherein each VHH domain comprises the same VHH sequence.

24. The polypeptide of any one of claims 1-16, comprising one VHH domain.

25. The polypeptide of any one of claims 1-24, wherein the polypeptide comprises an Fc region.

26. The polypeptide of claim 25, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 74-109.

27. The polypeptide of claim 25 or claim 26, which forms a dimer under physiological conditions.

28. The polypeptide of any one of claims 1-27, wherein the CD33 is human CD33.

29. The polypeptide of claim 28, wherein the human CD33 comprises the sequence of SEQ ID NO: 1.

30. An immunoconjugate comprising the polypeptide of any one of claims 1-29 and a cytotoxic agent.

31. The immunoconjugate of claim 30, wherein the cytotoxic agent is selected from a calicheamicin, an auristatin, a dolastatin, a tubulicin, a maytansinoid, a cryptophycin, a duocarmycin, an esperamicin, a pyrrolobenzodiazepine, and an enediyne antibiotic.

32. A pharmaceutical composition comprising the polypeptide of any one of claims 1-29 or the immunoconjugate of claim 30 or claim 31, and a pharmaceutically acceptable carrier.

33. An isolated nucleic acid that encodes the polypeptide of any one of claims 1-29.

34. A vector comprising the nucleic acid of claim 33.

35. A host cell comprising the nucleic acid of claim 33 or the vector of claim 34.

36. A host cell that expresses the polypeptide of any one of claims 1-29.

37. A method of producing the polypeptide of any one of claims 1-29, comprising incubating the host cell of claim 35 or claim 36 under conditions suitable for expression of the polypeptide.

38. The method of claim 37, further comprising isolating the polypeptide.
39. Use of the polypeptide of any one of claims 1-29, the immunoconjugate of claim 30 or claim 31, or the pharmaceutical composition of claim 32 in the manufacture of a medicament for treating cancer.
40. The use of claim 39, wherein the cancer is selected from lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML); Hairy cell leukemia; and chronic myeloblastic leukemia.
41. The use of claim 39 or 40, wherein the cancer is acute myeloid leukemia (AML).
42. The use of any one of claims 39-41, wherein an additional therapeutic agent is to be administered.
43. The use of claim 42, wherein the additional therapeutic agent is an anti-cancer agent.
44. The use of claim 43, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus.
45. The use of any one of claims 39-44, wherein the cancer is a CD33-expressing cancer.