

CD33 ANTIBODIES, CD33/V $\delta$ 2 MULTISPECIFIC ANTIBODIES AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/608,030  
5 filed December 8, 2023, the disclosure of which is incorporated herein by reference in its  
entirety.

## TECHNICAL FIELD

[0002] Provided herein are CD33 antibodies, multispecific CD33/V $\delta$ 2 antibodies, nucleic acids  
and expression vectors encoding the antibodies, recombinant cells containing the vectors, and  
10 compositions comprising the antibodies. Methods of making the antibodies, and methods of  
using the antibodies to treat diseases, including hematological cancers, are also provided.

## REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically. The  
15 information contained in the electronic sequence listing (Sequence Listing JBI6837PCT1  
Sequence Listing.xml; size: 132KB; and date of creation: 5 December, 2024) is incorporated  
herein by reference in its entirety.

## BACKGROUND

20 [0004] Immunotherapy aimed at specific targets in Acute Myeloid Leukemia (AML) poses a  
significant clinical hurdle owing to the diverse characteristics of AML cancer cells (blasts) and  
the absence of AML-specific antigens. CD33 is a cell surface protein that has been detected on  
blasts and leukemic stem cells of 85–90% of patients presenting with AML. Interestingly,  
expression of CD33 is restricted to hematopoietic cells (Paul, Taylor, Stansbury, & McVicar,  
25 2000; Ulyanova, Blasioli, Woodford-Thomas, & Thomas, 1999) but is absent on normal  
hematopoietic stem cells (Andrews, Torok-Storb, & Bernstein, 1983; Griffin, Linch, Sabbath,  
Larcom, & Schlossman, 1984; Jilani et al., 2002).

[0005] Despite ongoing clinical evaluations of various immunotherapies directed at CD33, such  
as chimeric antigen receptor (CAR)-T and CAR-NK cell therapies, challenges have emerged.  
30 Specifically, therapies involving CD3 T-cell engagers targeting CD33 have encountered issues

related to efficacy, tolerability, and an undesirable safety profile. Therefore, addressing the unmet medical needs of AML and MDS patients whose condition does not respond to traditional chemotherapy is imperative.

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## SUMMARY

[0006] In one general aspect, provided herein are antibodies, or antigen-binding fragments thereof, that bind CD33 of cancer cells, in particular of AML or Myelodysplastic Syndrome (MDS) cells. In certain embodiments, the antibodies or antigen-binding fragments thereof, bind membrane-bound CD33 (mCD33).

10 [0007] In another general aspect, provided herein are multispecific antibodies, or antigen-binding fragments thereof, that bind to CD33 of cancer cells and to V $\delta$ 2 chain of a T cell receptor (TCR) expressed on a V $\gamma$ 9V $\delta$ 2 T cell. In certain embodiments, the antibodies, multispecific antibodies, or antigen-binding fragments thereof, bind mCD33. In certain embodiments, the CD33 antibodies, CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof, bind the C2  
15 domain of CD33. In certain embodiments, the CD33 antibodies, CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof, bind specifically to CD33, preferably human CD33. In certain embodiments, the CD33 antibodies, CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof, do not significantly bind to soluble human CD33 (sCD33).

[0008] Provided are antibodies, or antigen-binding fragments thereof, that specifically bind  
20 mCD33. In certain embodiments, the antibody or antigen-binding fragment thereof comprises:  
a. a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 12, 13, and 14, respectively; and  
25 a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;  
b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising  
30 the amino acid sequences of SEQ ID NO: 18, 19, and 14, respectively; and

- a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;
- 5 c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 20, 21, and 14, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively; or
- 10 d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 22, 23, and 24, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 25, 26, and 17, respectively.
- e. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 27, 28, and 29, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- 15 f. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 33, 34, and 29, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- 20 g. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 35, 36, and 29, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively; or
- 25 h. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 37, 38, and 39, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 40, 41, and 32, respectively.

**[0009]** In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42 or 44, or a light

chain variable region (VL), comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 43 or 45.

[0010] In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a VH comprising SEQ ID NO: 42, and a VL comprising SEQ ID NO: 43.

5 [0011] In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a VH comprising SEQ ID NO: 44, and a VL comprising SEQ ID NO: 45.

[0012] In certain embodiments, the antigen-binding fragment of a CD33 antibody is a single heavy chain variable region (VHH).

[0013] In certain embodiments, the antibody or antigen-binding fragment thereof, comprises:

- 10 a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 46, 47, and 48, respectively;
- b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 49, 50, and 48, respectively;
- c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 51, 52, and 48, respectively;
- 15 d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 76, respectively;
- e. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 111, respectively;
- 20 f. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 55, 56, and 57, respectively;
- g. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 58, 59, and 57, respectively;
- h. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 60, 61, and 57, respectively; or
- 25 i. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 62, 63, and 64, respectively.

[0014] In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a VVH comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%,  
30 97%, 98%, or 99% identical to SEQ ID NO: 65 or 66.



- [0015] In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a VHH comprising SEQ ID NO: 65.
- [0016] In certain embodiments, the antibody, or antigen-binding fragment thereof, comprises a VHH comprising SEQ ID NO: 66.
- 5 [0017] In certain embodiments, the antibody, or the antigen-binding fragment thereof, comprises an IgG Fc domain, preferably a human IgG1 Fc domain.
- [0018] In certain embodiments, the antibody or the antigen-binding fragment, comprises a human IgG1 Fc domain comprising one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.
- 10 [0019] In certain embodiments, the antibody or the antigen-binding fragment, comprises a first human IgG1 Fc domain comprising the mutations T366S, L368A and Y407V per the EU numbering system and a second human IgG1 Fc domain comprising the mutation T366W per the EU numbering system.
- [0020] In certain embodiments, the antibody, or the antigen-binding fragment thereof, comprises
- 15 a human IgG1 Fc domain comprising one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.
- [0021] In certain embodiments, the antibody, or the antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising the triple mutation L234A/L235A/ D265S per the EU numbering system.
- 20 [0022] In certain embodiments, the antibody or the antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising mutations H435R and/or Y436F per the EU numbering system.
- [0023] In certain embodiments, the antibody, or the antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising the triple mutation M252Y/S254T/T256E per the EU
- 25 numbering system.
- [0024] In certain embodiments, the antibody, or antigen-binding fragment thereof is chimeric, partially humanized, or fully humanized.
- [0025] Also provided herein are CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof.

[0026] Suitably, the CD33/V $\delta$ 2 multispecific antibodies, comprise a CD33 antibody, or an antigen-binding fragment thereof, specifically binding human mCD33, and a V $\delta$ 2 antibody, or an antigen-binding fragment thereof, specifically binding the V $\delta$ 2 chain of the human V $\gamma$ 9V $\delta$ 2 T cell receptor. Suitably, the CD33 antibody or antigen-binding fragment thereof in the CD33/V $\delta$ 2 multispecific antibody is as described herein for CD33 antibodies or antigen-binding fragments thereof, and the V $\delta$ 2 antibody or antigen-binding fragment thereof in the CD33/V $\delta$ 2 multispecific antibody is as described herein for V $\delta$ 2 antibodies or antigen-binding fragments thereof.

[0027] In certain embodiments the CD33/V $\delta$ 2 multispecific antibody, comprises a CD33 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- a. SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively;
- b. SEQ ID NOs: 18, 19, 14, 15, 16, and 17, respectively;
- c. SEQ ID NOs: 20, 21, 14, 15, 16, and 17, respectively; or
- d. SEQ ID NOs: 22, 23, 24, 25, 26, and 17, respectively;

and a V $\delta$ 2 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- e. SEQ ID NOs: 77, 78, and 79, respectively;
- f. SEQ ID NOs: 80, 81, and 79, respectively;
- g. SEQ ID NOs: 82, 83, and 79, respectively; or
- h. SEQ ID NOs: 84, 85, and 86, respectively.

[0028] In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises a CD33 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- a. SEQ ID NOs: 27, 28, 29, 30, 31, and 32, respectively;
- b. SEQ ID NOs: 33, 34, 29, 30, 31, and 32, respectively;
- c. SEQ ID NOs: 35, 36, 29, 30, 31, and 32, respectively; or

d. SEQ ID NOs: 37, 38, 39, 40, 41, and 32, respectively;  
and a V $\delta$ 2 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- 5 e. SEQ ID NOs: 77, 78, and 79, respectively;  
f. SEQ ID NOs: 80, 81, and 79, respectively;  
g. SEQ ID NOs: 82, 83, and 79, respectively; or  
h. SEQ ID NOs: 84, 85, and 86, respectively.

[0029] In certain embodiment the CD33/V $\delta$ 2 multispecific antibody, comprises a CD33 antibody or, an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

- 10 a. SEQ ID NOs: 46, 47, and 48, respectively;  
b. SEQ ID NOs: 49, 50, and 48, respectively;  
c. SEQ ID NOs: 51, 52, and 48, respectively;  
d. SEQ ID NOs: 53, 54, and 76, respectively; or  
15 e. SEQ ID NOs: 53, 54, and 111, respectively;

and a V $\delta$ 2 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- f. SEQ ID NOs: 77, 78, and 79, respectively;  
g. SEQ ID NOs: 80, 81, and 79, respectively;  
20 h. SEQ ID NOs: 82, 83, and 79, respectively; or  
i. SEQ ID NOs: 84, 85, and 86, respectively.

[0030] In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises a CD33 antibody, or an antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

- 25 a. SEQ ID NOs: 55, 56, and 57, respectively;  
b. SEQ ID NOs: 58, 59, and 57, respectively;  
c. SEQ ID NOs: 60, 61, and 57, respectively; or  
d. SEQ ID NOs: 62, 63, and 64, respectively;

and a V $\delta$ 2 antibody, or the antigen-binding fragment thereof, comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

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- e. SEQ ID NOs: 77, 78, and 79, respectively;
- f. SEQ ID NOs: 80, 81, and 79, respectively;
- g. SEQ ID NOs: 82, 83, and 79, respectively; or
- h. SEQ ID NOs: 84, 85, and 86, respectively.

5   **[0031]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises a heavy chain variable region (VH) having an amino acid sequence at least 95% identical to SEQ ID NO:42 or 44, and a light chain variable region (VL) having an amino acid sequence at least 95% identical to SEQ ID NO: 43 or 45; and a single heavy chain only variable region (VHH) having an amino acid sequence at least 95% identical to SEQ ID NO:87.

10   **[0032]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises:

- a. a VH comprising the amino acid sequence of SEQ ID NO:42, and a VL comprising the amino acid sequence of SEQ ID NO: 43; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
- b. a VH comprising the amino acid sequence of SEQ ID NO:44, and a VL comprising the amino acid sequence of SEQ ID NO: 45; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

**[0033]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises a VHH having an amino acid sequence at least 95% identical to SEQ ID NO:65 or 66, and a VHH having an amino acid sequence at least 95% identical to SEQ ID NO: 87.

20   **[0034]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises:

- a. a VHH comprising the amino acid sequence of SEQ ID NO:65; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
- b. a VHH comprising the amino acid sequence of SEQ ID NO:66; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

25   **[0035]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises:

- a. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:70, and a light chain comprising the amino acid sequence of SEQ ID NO:71;

- b. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:72, and a light chain comprising the amino acid sequence of SEQ ID NO:73;
- c. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:68;
- d. a heavy chain comprising the amino acid sequence of SEQ ID NO:74; and a heavy chain comprising the amino acid sequence of SEQ ID NO:75 or
- e. a heavy chain comprising the amino acid sequence of SEQ ID NO:91; and a heavy chain comprising the amino acid sequence of SEQ ID NO:92.
- 10 **[0036]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody is a bispecific antibody.
- [0037]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, comprises an IgG Fc domain, preferably a human IgG1 Fc domain.
- [0038]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising one or more mutations
- 15 selected from T366S, L368A, T366W and Y407V per the EU numbering system.
- [0039]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, comprises a first human IgG1 Fc domain comprising the triple mutations T366S/L368A/Y407V per the EU numbering system, and a second human IgG1 Fc domain comprising the mutation T366W per the EU numbering system.
- 20 **[0040]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.
- [0041]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or the antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising the triple mutations L234A/
- 25 L235A/ D265S per the EU numbering system.
- [0042]** In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising mutations H435R and/or Y436F per the EU numbering system.

[0043] In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, comprises a human IgG1 Fc domain comprising the triple mutation M252Y/S254T/T256E per the EU numbering system.

[0044] In certain embodiments, CD33/V $\delta$ 2 multispecific the antibody, or an antigen-binding fragment thereof, is chimeric, partially humanized, or fully humanized.

[0045] Also provided are one or more synthetic polynucleotides encoding the CD33 and/or CD33/V $\delta$ 2 multispecific antibodies or, antigen-binding fragments thereof, described herein.

[0046] Also provided are one or more vectors comprising the polynucleotides encoding the CD33 and/or CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof,

described herein.

[0047] Also provided are host cells comprising (i) one or more polynucleotides encoding the CD33 and/or CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof, described herein, or (ii) the one or more vectors comprising the polynucleotides encoding the CD33 and/or CD33/V $\delta$ 2 multispecific antibodies, or antigen-binding fragments thereof,

described herein.

[0048] In certain embodiments, provided is a pharmaceutical composition comprising the CD33 antibody, or antigen-binding fragment thereof, described herein and a pharmaceutically acceptable carrier. In certain embodiments, provided is a pharmaceutical composition, comprising the CD33/V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof,

described herein and a pharmaceutically acceptable carrier.

[0049] Also provided are methods of treating a hematological cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the present disclosure. In certain embodiments, the hematologic cancer can be acute myeloid leukemia (AML), myelodysplastic syndrome (MDS, low or high risk), acute lymphocytic leukemia (ALL, including all subtypes), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML), or blastic plasmacytoid dendritic cell neoplasm (BPDCN). In particular embodiments, the hematological cancer is AML or MDS.

[0050] Also provided are methods of producing the CD33 or CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, described herein. The methods comprise culturing a cell

comprising one or more polynucleotides encoding the CD33 or CD33/V $\delta$ 2 multispecific antibody, or antigen-binding fragment(s) thereof, under conditions to produce the CD33 or CD33/V $\delta$ 2 multispecific antibody, or antigen-binding fragment(s) thereof, and recovering the CD33 or CD33/V $\delta$ 2 multispecific antibody, or antigen-binding fragment(s) thereof, from the cell  
5 or culture.

[0051] Also provided are methods of producing a pharmaceutical composition comprising the CD33 and/or CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, described herein, comprising combining CD33 and/or CD33/V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, with a pharmaceutically acceptable carrier to obtain the  
10 pharmaceutical composition.

#### BRIEF DESCRIPTION OF THE FIGURES

[0052] FIG. 1 shows a CD33xV $\delta$ 2 bispecific antibody (GD33B273) binding to V $\gamma$ 9V $\delta$ 2 T cells. V $\gamma$ 9V $\delta$ 2 T cells isolated and expanded from healthy donors were incubated with a dose range of the CD33xV $\delta$ 2 bispecific antibody (GD33B273) or nullxV $\delta$ 2 bispecific antibody (GD33B73) negative control. NullxV $\delta$ 2 bispecific antibody (GD33B73) has no CD33 binding arm. Binding was detected by flow cytometry. Data is shown from 5 different T-cell donors. MFI: Mean Fluorescence Intensity.

[0053] FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D, and FIG. 2E: A LC-MS assay was developed to measure the amount of free soluble CD33 (sCD33) in AML patients' sera (AML1-AML30) after spike-in with the indicated antibody. CD33xV $\delta$ 2 bispecific antibodies GD33B112, GD33B116, GD33B139, and GD33B134 show minimal binding to sCD33. For reference, the binding results of JL5 to sCD33 are also shown using sera from different AML patients (Indiv 517-Indiv 532)  
25 (FIG. 2E).

[0054] FIG. 3 shows the results of an experiment demonstrating that the tested CD33xV $\delta$ 2 bispecific antibodies (GD33B112, GD33B116, and GD33B134) induce T-cell mediated cytotoxicity against THP1 cancer cells in vitro. THP1 cells are a human monocytic cell line derived from an acute monocytic leukemia patient. In the experiment, pan T cells (Effectors)  
30 were co-cultured with CFSE labelled THP1 cells (Targets) at absolute E:T ratio of 10:1 in the

presence of various concentrations of the bispecific antibody for 72 hours. Cytotoxicity values represented here were corrected for basal cytotoxicity observed in the absence of bispecific antibody. CFSE is carboxyfluorescein succinimidyl ester, a fluorescent dye.

[0055] FIG. 4A, FIG. 4B, FIG. 4C, and FIG. 4D depict the cell binding of CD33xVδ2 bispecific antibodies GD33B273 (FIG. 4A), GD33B112 (FIG. 4B), GD33B116 (FIG. 4C), and GD33B139 (FIG. 4D), to CD33 positive THP1 cancer cells, and to CD33 negative THP1 CD33 knock-out cell lines. FIG. 4E depicts the cell binding of CD33xVδ2 bispecific antibody GD33B273 to an isogenic THP1\_C2 cell line that specifically expresses the IgC2 domain of CD33. The IgC2 domain of CD33 is a conserved region of the CD33 cell surface receptor protein.

[0056] FIG. 5A, FIG. 5B, FIG. 5C, and FIG. 5D present the efficacy of representative bispecific CD33xδ2 antibodies, GD33B134 (FIG. 5A and FIG. 5B) and GD33B273 (FIG. 5C and FIG. 5D) in the presence of recombinant CD33 (rCD33). The presence of rCD33 does not impact the cytotoxicity and T cell activation effects of GD33B134 and GD33B273. Increasing concentrations of rCD33 were added to cytotoxicity assays with pan-T cells as effector cells (n=3 different healthy donors) and THP1 cells as target cells. Cytotoxicity and T-cell activation were evaluated by flow cytometry at 72 hours at absolute E:T ratio of 10:1 (relative E:T ratio ~0.13-0.4 : 1, donor dependent). Increasing concentrations of rCD33 were added to T-cell activation assays. T-cell activation was assessed by determination of CD25 expression on Vδ2 T cells.

[0057] FIG. 6 presents the efficacy of bispecific antibody GD33B273 using AML derived Vδ2+ T cells. Vδ2+ T cells were expanded from AML patient-derived PBMCs and tested in a T cell cytotoxicity assay with GD33B273 and THP-1 target cells at an E:T ratio of 1:1. Cytotoxicity of THP-1 cells was evaluated after 24 hours.

[0058] FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, and FIG. 7E present the preferential cancer cell cytotoxicity of representative bispecific CD33xδ2 antibodies GD33B134, GD33B273, and NullxVδ2 antibody (GD33B73), showing activity against MOLM-13 AML model cells (FIG. 7A) and no activity against monocyte (FIG. 7B) and NK immune cells (FIG. 7C). Preferential cancer cell cytotoxicity was compared between representative bispecific CD33xδ2 antibody GD33B134 and representative CD33xδ2 antibody GD33B273 in flow-cytometry based assays at 96 hours with MOLM-13 as target cancer cells (FIG. 7A) and healthy PBMCs freshly isolated from whole blood as effector cells (n=7 different healthy donors). Absolute E:T ratio shown is



20:1 (relative E:T ratio 0.16-3.6:1, donor dependent). Cytotoxicity to monocyte (FIG. 7B) and NK cells (FIG. 7C) was evaluated in the presence of cancer cell coculture. T-cell activation was also assessed by evaluating the expression of CD25 on either total CD3+ T cells (FIG. 7D) or Vδ2 T cells (FIG. 7E). The results show specific activation of Vδ2+ T cells when compared to CD3+ T cells, which are not activated. CD, cluster of differentiation, E:T, effector to target ratio; NK, natural killer; PBMC, peripheral blood mononuclear cell.

[0059] FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D show how bispecific CD33xδ2 antibody GD33B273 mediates selective cytotoxicity of CD33+ cancer cells (MOLM-13 and THP-1) and selective activation of Vδ2 T cells when compared to the antibody lacking the CD33 arm (NullxVδ2, aka GD33B73). T cells from healthy donors were evaluated in T-cell cytotoxicity and activation assays with GD33B273 and NullxVδ2 antibodies and target cell lines. Cancer cells cytotoxicity was determined for GD33B273 in flow-cytometry based assays at 72 hours with MOLM-13 and THP-1 as target cancer cells (FIG. 8A and 8C). The assay was conducted at a relative E:T ratio of 0.5:1. T-cell activation was assessed by evaluating the expression of CD25 on Vδ2 T cells (FIG. 8B and 8D) and was measured after 72 hours. FIG. 8E and 8F show how bispecific CD33xδ2 antibody GD33B273 mediates selective cytotoxicity of THP-1\_C2 cells expressing CD33 IgC2 domain (FIG. 8E) and selective activation of Vδ2 T cells (FIG. 8F) when compared to the antibody lacking the CD33 arm (NullxVδ2, aka GD33B73). FIG. 8G and 8H show how bispecific CD33xδ2 antibody GD33B273 does not mediate selective cytotoxicity of OCI-Ly10 / CD33negative cells, i.e., not expressing CD33 (FIG. 8G), and does not mediate or shows minimal selective activation of Vδ2 T cells (FIG. 8H) when compared to the antibody lacking the CD33 arm (NullxVδ2, aka GD33B73). OCI-Ly10 cells are a human-derived cell line and are used as target cancer cells.

[0060] FIGs. 9A and 9B: the bispecific CD33xδ2 antibody GD33B273 shows no on-target off-tumor toxicities. In FIGs. 9A healthy cells like monocytes, B cells, and NK cells, when in the presence of the bispecific CD33xδ2 antibody GD33B273, show no antibody effect on cytotoxicity of these healthy cells. In FIGs. 9B, the presence of the bispecific CD33xδ2 antibody GD33B273 results in no or minimal cytokine production (IFN-γ, TNF-α, IL1-β). Whole blood from healthy donors was tested in a T-cell cytotoxicity assay with GD33B273 for 24 or 96 hours in the absence of cancer cell cocultures. The percentage cytotoxicity of monocytes, B cells and

NK cells (FIG. 9A) and secreted cytokines IFN- $\gamma$ , TNF- $\alpha$ , and IL1- $\beta$  (FIG. 9B) were determined and shown.

[0061] FIG. 10 shows how bispecific CD33x $\delta$ 2 antibody GD33B273 induces potent cytotoxicity of AML bone marrow (BM) blast cells from 3 different human patients. T cells from healthy donors were tested in T-cell cytotoxicity assays with GD33B273 and AML patient-derived BM cells, performed at a relative E:T ratio of 2:1. Cytotoxicity of CD33+ blast was assessed 24 hours. AML, acute myeloid leukemia, BM, bone marrow, CD, cluster of differentiation, DN, donor, E:T, effector to target.

[0062] FIG. 11 shows how bispecific CD33x $\delta$ 2 antibody GD33B273 represents low risk of hematopoietic toxicity while showing cytotoxicity for the cancer cells THP-1 in a dose response fashion. CD34+ HSPC are stem cells from healthy donors, and the presence of surviving robust colonies (light grey bars) shows that the antibody does not impact them. In contrast, THP-1 cancer cells colonies (black bars) decrease survival in a dose-dependent relation to the antibody concentration. Naïve V $\delta$ 2+ T cells were isolated from healthy donor PBMCs and used as effectors at 1:1 relative E:T ratio with CD34+ HSPC cells or THP-1 cells as target cells in a CFU assay. HSPC: hematopoietic stem and progenitor cells; CD, cluster of differentiation; CFU, colony forming unit; E:T, effector to target; PBMC, peripheral blood mononuclear cell.

[0063] FIG. 12 presents the effect of treatment with a representative bispecific CD33x $\delta$ 2 antibody GD33B134 on growth of MOLM-13 xenografts admixed with pan-T cells in NSG mice. The size of the tumor increases in absence of antibody (with just buffer DPBS) and the size of tumor decreases in the presence of the antibody at different doses (1, 3, and 10 mg/Kg). T-cell-humanized NSG mice were injected SC with MOLM-13 tumors admixed with pan-T cells and dosed IP with indicated doses on Days 1, 4, 7, 10, 13, 16, 20, 23, 27, 30, and 35 (the dosing period is indicated by the solid bar under the X-axis, i.e., from 0 to 35 days). Tumor volume was measured twice weekly, and results are presented as the mean tumor volume  $\pm$  SEM for each group. Data are only displayed for when two thirds of animals were still alive in a group. DPBS, Dulbecco's phosphate-buffered saline; IP, intraperitoneal; NSG, non-obese diabetic (NOD) severe combined immunodeficiency (scid) gamma; SC, subcutaneous; SEM, standard error of the mean.

[0064] FIG. 13A and FIG. 13B presents the efficacy of a representative bispecific CD33x $\delta$ 2 antibody GD33B134 in a MOLM-13 regression mice model with enriched V $\delta$ 2 T cells as effectors. hIL-15 NOG mice were injected IV with MOLM-13 cells, humanized with enriched V $\delta$ 2 T cells (IV) on Day 3 and 16, and dosed IP with DPBS or antibody at the indicated doses (1, 3, and 10 mg/Kg) on Days 4, 7, 10, 14, 17, 21, 24, and 28 (the dosing period is indicated by the solid bar under the X-axis, i.e. from day 5 to day 28 for both FIGs. 13A and 13B). From Day 7 onward, body weight and disease-related clinical signs were observed daily, and results are presented as (FIG. 9A) percent survival or (FIG. 9B) mean body weight change  $\pm$  SEM for each group. Data are only displayed for the period during which two thirds of animals were still alive in a group. hIL-15, human interleukin-15; IP, intraperitoneal; IV, intravenous; NOG, non-obese diabetic (NOD)/Shi-severe combined immunodeficiency (scid) IL2rgamma(null); SEM, standard error of the mean.

[0065] FIG. 14A and FIG. 14B presents the efficacy of the bispecific CD33x $\delta$ 2 antibody GD33B273 in a MOLM-13 regression mice model. In this test, the MOLM-13 cancer cells were labelled with luciferase. This labeled MOLM-13 cancer cells were implanted on Day 0 in the hIL-15 NOG mice. The same mice were injected with the T cells on Day 3 and 16, and dosed with antibody at the indicated doses (0, 0.3, and 3 mg/Kg) on Days 4, 7, 10, 14, 17, 21, and 24 (the dosing period is indicated by the solid bar under the X-axis, i.e. from day 4 to day 24 for both FIGs. 14A and 14B). Results are presented as average tumor radiance (FIG. 14A) and as percent survival (FIG. 14B). Data are only displayed for the period during which two thirds of animals were still alive in a group. FIG. 14A shows an increase in average radiance for the untreated mice (0 mg/Kg) when compared to the treated mice with 0.3 and 3 mg/Kg antibody. FIG. 14A also shows 61% and 54.9% of tumor growth inhibition at day 17 for treated mice with antibody at 0.3 and 3 mg/Kg, respectively, when compared to untreated mice (0 mg/Kg). FIG. 14B shows an increase in the survival of the mice treated with 0.3 and 3 mg/Kg of antibody when compared to untreated mice (0 mg/Kg). ILS, increased life span; TGI, tumor growth inhibition.

## DETAILED DESCRIPTION

[0066] The present disclosure relates to antibodies, multispecific antibodies or antigen-binding fragments thereof that specifically target CD33, a critical target for hematological cancers. The multispecific antibodies or antigen binding fragments thereof may additionally bind V $\delta$ 2.

5 [0067] *DEFINITIONS*

[0068] Techniques and procedures described or referenced herein include those that are generally well understood and/or commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3d ed. 2001); *Current Protocols in Molecular*  
10 *Biology* (Ausubel et al. eds., 2003); *Therapeutic Monoclonal Antibodies: From Bench to Clinic* (An ed. 2009); *Monoclonal Antibodies: Methods and Protocols* (Albitar ed. 2010); and *Antibody Engineering* Vols 1 and 2 (Kontermann and Dübel eds., 2d ed. 2010).

[0069] Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art.  
15 For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0070] In an attempt to help the reader of the present application, the description has been  
20 separated in various paragraphs or sections. These separations should not be considered as disconnecting the substance of a paragraph or section from the substance of another paragraph or section. To the contrary, the present description encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated.

[0071] The terms “polypeptide” and “peptide” and “protein” are used interchangeably herein and  
25 refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example,  
30 polypeptides containing one or more analogs of an amino acid, including but not limited to,

unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies or other members of the immunoglobulin superfamily, in certain embodiments, a “polypeptide” can occur as a single chain or as two or more associated chains.

5 [0072] An “antigen” is a structure to which an antibody can specifically and/or selectively bind. A target antigen may be a polypeptide, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen is a polypeptide. In certain embodiments, an antigen is associated with a cell, for example, is present on or in a cell.

10 [0073] The term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence,  
15 after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, or MEGALIGN (DNASTar, Inc.)  
20 software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0074] The meaning of “substantially the same” can differ depending on the context in which the term is used. Because of the natural sequence variation likely to exist among heavy and light  
25 chains and the genes encoding them, one would expect to find some level of variation within the amino acid sequences or the genes encoding the antibodies or antigen-binding fragments described herein, with little or no impact on their unique binding properties (e.g., specificity and affinity). Such an expectation is due in part to the degeneracy of the genetic code, as well as to the evolutionary success of conservative amino acid sequence variations, which do not  
30 appreciably alter the nature of the encoded protein. Accordingly, in the context of nucleic acid

sequences, “substantially the same” means at least 65% identity between two or more sequences. Preferably, the term refers to at least 70% identity between two or more sequences, more preferably at least 75% identity, more preferably at least 80% identity, more preferably at least 85% identity, more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, and more preferably at least 99% or greater identity. The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions × 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, *Comput. Appl. Biosci* 4, 11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch, *J. Mol. Biol.* 48, 444-453 (1970) algorithm.

[0075] The degree of variation that may occur within the amino acid sequence of a protein without having a substantial effect on protein function is much lower than that of a nucleic acid sequence, since the same degeneracy principles do not apply to amino acid sequences. Accordingly, in the context of an antibody or antigen-binding fragment, “substantially the same” means antibodies or antigen-binding fragments having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the antibodies or antigen-binding fragments described. Other embodiments include CD33 specific antibodies, or antigen-binding fragments, that have framework, scaffold, or other non-binding regions that do not share significant identity with the antibodies and antigen-binding fragments described herein, but do incorporate one or more CDRs or other sequences needed to confer binding that are 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to such sequences described herein. A “vector” is a replicon, such as plasmid, phage, cosmid, or virus in which another nucleic acid segment may be operably inserted so as to bring about the replication or expression of the segment.

[0076] The terms “subject” and “patient” may be used interchangeably. As used herein, in certain embodiments, a subject is a mammal, such as a non-primate or a primate (*e.g.*, human). In specific embodiments, the subject is a human. In one embodiment, the subject is a mammal, *e.g.*, a human, diagnosed with a disease or disorder. In another embodiment, the subject is a

5 mammal, *e.g.*, a human, at risk of developing a disease or disorder.

[0077] “Administer” or “administration” refers to the act of injecting or otherwise physically delivering a substance as it exists outside the body into a patient, such as by mucosal, intradermal, intravenous, intramuscular delivery, and/or any other method of physical delivery described herein or known in the art.

10 [0078] The terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less of a given value or range.

[0079] As used in the present disclosure and claims, the singular forms “a”, “an” and “the” include plural forms unless the context clearly dictates otherwise.

15 [0080] It is understood that wherever embodiments are described herein with the term “comprising” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided. It is also understood that wherever embodiments are described herein with the phrase “consisting essentially of” otherwise analogous embodiments described in terms of “consisting of” are also provided.

20 [0081] The term “between” as used in a phrase such as “between A and B” or “between A-B” refers to a range including both A and B.

[0082] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A and B

25 and C; A and B or C; B and A or C; C and A or B; A and B; A and C; B and C; A or B or C; A or B; A or C; B or C; A (alone); B (alone); and C (alone).

[0083] CD33

[0084] The term “CD33” refers to a 67 kD single pass transmembrane (*i.e.* membrane-bound)

30 glycoprotein, a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) family.

Also recognized as Siglec-3, gp67, or p67, CD33 comprises an amino-terminal V-set Ig-like domain (exon 2 of CD33), responsible for sialic acid binding, and a C2-set Ig-like domain (exon 4) in its extracellular portion (Laszlo GS et al. Expression and functional characterization of CD33 transcript variants in human acute myeloid leukemia. *Oncotarget*. 2016;7(28):43281-43294). Alternative splicing of CD33 RNA generates a shorter isoform lacking the V-set but retaining the C2-set Ig-like domain (Laszlo GS, Estey EH, Walter RB. The past and future of CD33 as therapeutic target in acute myeloid leukemia. *Blood Rev*. 2014;28(4):143-153.; Laszlo et al., 2016, *ibid*). This splicing process gained significance with recent studies highlighting the presence of a single nucleotide polymorphism (SNP) rs12459419 in approximately 50% of AML patients. This SNP leads to the exclusion of exon 2 in CD33, resulting in the deletion of the V domain (Lamba JK, Chauhan L, Shin M, et al. CD33 splicing polymorphism determines gemtuzumab ozogamicin response in de novo acute myeloid leukemia: report from randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol*. 2017;35(23):2674-2682). The full-length reference human CD33 sequence is available on Uniprot P20138 (SEQ ID NO: 88).

SEQ ID NO: 88

MPLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLVPCTFFHPIPIYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEVQEETQGRFRLGDPNRNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLEPGHKNLTCSVSWACEQGTPIFSWLSAAPTSLGPRTHSSVLIITPRPDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNP TTGIFPGDGSQKQETRAGVVHGAIGGAGVTALLALCLCLIFFIVKTHRRKAARTAVGRN DTHPTTGSASPKHQKSKLHGPTETSSCSGAAPTVMDEELHYASLNFHGMNPSKDTST EYSEVRTQ

[0085] CD33, is primarily considered a myeloid differentiation antigen. It exhibits low expression in myeloid progenitors, neutrophils, and macrophages, yet is highly expressed in circulating monocytes and dendritic cells. Notably, CD33 is detected on blasts and leukemic stem cells in 85–90% of AML patients. Remarkably, its expression is confined to hematopoietic cells but absent on normal hematopoietic stem cells (Paul SP, Taylor LS, Stansbury EK, McVicar DW. Myeloid specific human CD33 is an inhibitory receptor with differential ITIM function in recruiting the phosphatases SHP-1 and SHP-2. *Blood*. 2000;96(2):483-490; Ulyanova T, Blasioli J, Woodford-Thomas TA, Thomas ML. The sialoadhesin CD33 is a myeloid-specific



inhibitory receptor. Eur J Immunol. 1999;29(11):3440-3449). This distinctive expression pattern suggests CD33 as a promising target for antibody-based therapy in AML and MDS.

[0086] As noted above, recent studies highlighting the SNP rs12459419's presence in ~50% of AML patients revealed its association with the exclusion of exon 2 in CD33, resulting in the V domain's deletion (Lamba et al., 2017 *supra*). Interestingly, several CD33 antibody-based therapies, including Mylotarg® (INN: gemtuzumab ozogamicin) (the sole approved antibody for AML), bind and recognize the V domain of CD33. This study indicated that Mylotarg® lacks efficacy in patients expressing the SNP, thus being effective in only ~50% of the AML population (Lamba et al., 2017 *supra*). Considering Mylotarg®'s data, it is reasonable to expect that other V-binding CD33 antibodies will also be efficacious solely in a subset of AML patients, specifically those without the SNP rs12459419 mutation.

[0087] The terms “soluble CD33 protein” or “sCD33” refer to a form of a CD33 protein, e.g., a human CD33 protein, that is not associated or bound to a cell membrane. “Soluble CD33 protein” or “sCD33” encompass the extracellular domain (ECD) of full length CD33 proteins, and variants thereof, that are shed from cells, i.e., not associated or bound to a cell membrane. “Soluble CD33 protein” or “sCD33” also encompass the ECD of any fragment of a CD33 protein, or variants thereof, that are shed from cells; i.e., not associated or bound to a cell membrane. “Soluble CD33 protein” or “sCD33” also encompass the ECD of CD33 proteins that are shed from cells, i.e., not associated or bound to a cell membrane and that lack one or more domains, such as a transmembrane domain and/or a cytoplasmic domain, for example, as a result of proteolysis of the CD33 protein (e.g., in an endosome in a cell). In some cases, a “soluble CD33 protein” or “sCD33” comprises the IgV domain of a CD33 protein, or a fragment thereof. In some cases, a “soluble CD33 protein” or “sCD33” comprises the C2 domain of a CD33 protein, or a fragment thereof. In some cases, a “soluble CD33 protein” or “sCD33” comprises the IgV domain of a CD33 protein, or a fragment thereof, and the IgC2 domain of a CD33 protein, or a fragment thereof. The extracellular domain (ECD) of CD33 is shed from cells; therefore, normal and AML patient samples contain a soluble form of CD33 (i.e., sCD33). sCD33 could compete for the bispecific antibody in the blood and impact its efficacy due to sink effects. The inventors have recognized that antibodies which bind sCD33 may suffer from “antigen sink effect”, whereby the antibody bound to sCD33 in the blood is

removed from the system diminishing the amount of antibody that can reach the target cells, decreasing its efficacy.

#### *CD33 ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS*

5 [0088] Described herein are antibodies or antigen-binding fragments capable of specifically binding membrane-bound CD33.

[0089] The term “antibody,” “immunoglobulin,” or “Ig” is used interchangeably herein, and is used in the broadest sense and specifically covers, for example, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal  
10 antibodies), antibody compositions with polyeptopic or monoepitopic specificity, polyclonal or monovalent antibodies, multivalent antibodies, multispecific antibodies (*e.g.*, bispecific antibodies so long as they exhibit the desired biological activity) formed from at least two intact antibodies, single chain antibodies, and fragments thereof (*e.g.*, domain antibodies), as described below. An antibody can be human, humanized, chimeric and/or affinity matured, as well as an  
15 antibody from other species, for example, mouse, rabbit, llama, *etc.* The term “antibody” is intended to include a polypeptide product of B cells within the immunoglobulin class of polypeptides that is able to bind to a specific molecular antigen and is composed of two identical pairs of polypeptide chains, wherein each pair has one heavy chain (about 50-70 kDa) and one light chain (about 25 kDa), each amino-terminal portion of each chain includes a variable region  
20 of about 100 to about 130 or more amino acids, and each carboxy-terminal portion of each chain includes a constant region. *See, e.g.*, Antibody Engineering (Borrebaeck ed., 2d ed. 1995); and Kuby, Immunology (3d ed. 1997). Antibodies also include, but are not limited to, synthetic antibodies, recombinantly produced antibodies, antibodies including from Camelidae species (*e.g.*, llama or alpaca), including VHH or nanobodies, or their humanized variants, intrabodies,  
25 anti-idiotypic (anti-Id) antibodies, and functional fragments (*e.g.*, antigen binding fragments) of any of the above, which refers to a portion of an antibody heavy or light chain polypeptide that retains some or all of the binding activity of the antibody from which the fragment was derived. Non-limiting examples of functional fragments (*e.g.*, antigen binding fragments) include single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, *etc.*), Fab fragments, F(ab') fragments,  
30 F(ab)<sub>2</sub> fragments, F(ab')<sub>2</sub> fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fv fragments,

VHH, diabody, triabody, tetrabody, and minibody. In particular, antibodies provided herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules. The antibodies provided herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule. Antibodies may be agonistic antibodies or antagonistic antibodies. Antibodies may be neither agonistic nor antagonistic.

**[0090]** “Antigen-binding fragment” refers to a portion of the protein that binds an antigen.

Antigen binding fragments may be synthetic, enzymatically obtainable or genetically engineered polypeptides and include portions of an immunoglobulin that bind an antigen, such as VH, the VL, the VH and the VL, Fab, Fab', F(ab')<sub>2</sub>, Fd and Fv fragments, domain antibodies (dAb) consisting of one VH domain or one VL domain, shark variable IgNAR domains, camelized VH domains, VHH domains, minimal recognition units consisting of the amino acid residues that mimic the CDRs of an antibody, such as FR3-CDR3-FR4 portions, the HCDR1, the HCDR2 and/or the HCDR3 and the LCDR1, the LCDR2 and/or the LCDR3, alternative scaffolds that bind an antigen, and multispecific proteins comprising the antigen binding fragments. Antigen binding fragments (such as VH and VL) may be linked together via a synthetic linker to form various types of single antibody designs where the VH/VL domains may pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate single chains, to form a monovalent antigen binding domain, such as single chain Fv (scFv), stapled single chain Fv (spFv), or diabody. Antigen binding fragments may also be conjugated to other antibodies, proteins, antigen-binding fragments or alternative scaffolds which may be monospecific or multispecific to engineer bispecific and multispecific proteins.

**[0091]** The terms “binds” or “binding” refer to an interaction between molecules including, for example, to form a complex. Interactions can be, for example, non-covalent interactions including hydrogen bonds, ionic bonds, hydrophobic interactions, and/or van der Waals interactions. A complex can also include the binding of two or more molecules held together by covalent or non-covalent bonds, interactions, or forces. The strength of the total non-covalent interactions between a single antigen-binding site on an antibody and a single epitope of a target molecule, such as an antigen, is the affinity of the antibody or functional fragment for that epitope. The ratio of dissociation rate ( $k_{\text{off}}$ ) to association rate ( $k_{\text{on}}$ ) of a binding molecule (*e.g.*,

an antibody) to a monovalent antigen ( $k_{\text{off}}/k_{\text{on}}$ ) is the dissociation constant  $K_D$ , which is inversely related to affinity. The lower the  $K_D$  value, the higher the affinity of the antibody. The value of  $K_D$  varies for different complexes of antibody and antigen and depends on both  $k_{\text{on}}$  and  $k_{\text{off}}$ . The dissociation constant  $K_D$  for an antibody provided herein can be determined using any method  
5 provided herein or any other method well known to those skilled in the art. The affinity at one binding site does not always reflect the true strength of the interaction between an antibody and an antigen. When complex antigens containing multiple, repeating antigenic determinants, such as a polyvalent antigen, come in contact with antibodies containing multiple binding sites, the interaction of antibody with antigen at one site will increase the probability of a reaction at a  
10 second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity.

[0092] In connection with the antibodies or antigen-binding fragments described herein terms such as “specifically binding to,” and analogous terms are also used interchangeably herein and refer to antibodies or antigen-binding fragments that can specifically bind to an antigen, such as a  
15 polypeptide. Antibodies or antigen-binding fragments that bind to or specifically bind to an antigen can be identified, for example, by immunoassays, Octet<sup>®</sup>, Biacore<sup>®</sup>, or other techniques known to those of skill in the art. In some embodiments, antibodies or antigen-binding fragments binds to or specifically binds to an antigen when it binds to an antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as  
20 radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA). Typically, a specific or selective reaction will be at least twice background signal or noise and may be more than 10 times background. See, *e.g.*, Fundamental Immunology 332-36 (Paul ed., 2d ed. 1989) for a discussion regarding binding specificity. In certain embodiments, the extent of binding of an antibody or antigen-binding fragment to a “non-target” protein is less than about 10% of the  
25 binding of the antibody or antigen-binding fragment to its particular target antigen, for example, as determined by fluorescence activated cell sorting (FACS) analysis or RIA. An antibody or antigen-binding fragment that binds to an antigen includes one that is capable of binding the antigen with sufficient affinity such that the antibody or antigen-binding fragment is useful, for example, as a therapeutic and/or diagnostic agent in targeting the antigen. In certain  
30 embodiments, an antibody or antigen-binding fragment that binds to an antigen has a dissociation

constant ( $K_D$ ) of less than or equal to 1  $\mu$ M, 800 nM, 600 nM, 550 nM, 500 nM, 300 nM, 250 nM, 100 nM, 50 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, or 0.1 nM. In certain embodiments, an antibody or antigen-binding fragment binds to an epitope of an antigen that is conserved among the antigen from different species.

[0093] In certain embodiments, the antibodies or antigen-binding fragments can comprise portions of a “fully human antibody” or “human antibody,” which terms are used interchangeably herein and refer to an antibody that comprises a human variable region and, for example, a human constant region. In specific embodiments, the terms refer to an antibody that comprises a variable region and constant region of human origin. “Fully human” antibodies, in certain embodiments, can also encompass antibodies which bind polypeptides and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid sequence. The term “fully human antibody” includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat *et al.* (See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). This Kabat reference defines a numbering scheme for the variable regions of immunoglobulins (antibodies), often referred to as the Kabat numbering system or Kabat index. The EU numbering system, also called the EU index, classifies and numbers amino acid residues in Ig-like domains is explained in Edelman GM *et al.*, Proc Natl Acad Sci USA 63(1):78-85, 1969. A “human antibody” is one that possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, J. Mol. Biol. 227:381 (1991); Marks *et al.*, J. Mol. Biol. 222:581 (1991)) and yeast display libraries (Chao *et al.*, Nature Protocols 1: 755-68 (2006)). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, Monoclonal Antibodies and Cancer Therapy 77 (1985); Boerner *et al.*, J. Immunol. 147(1):86-95 (1991); and van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001).

Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, mice (*see, e.g.*, Jakobovits, Curr. Opin. Biotechnol. 6(5):561-66 (1995); Brüggemann and Taussing, Curr. Opin. Biotechnol. 8(4):455-58 (1997); and U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, Proc. Natl. Acad. Sci. USA 103:3557-62 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0094] In certain embodiments, the antibodies or antigen-binding fragments can comprise a portion of a “monoclonal antibody,” wherein the term as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts or well-known post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation, each monoclonal antibody will typically recognize a single epitope on the antigen. In specific embodiments, a “monoclonal antibody,” as used herein, is an antibody produced by a single hybridoma or other cell. The term “monoclonal” is not limited to any particular method for making the antibody. For example, the monoclonal antibodies useful in the present disclosure may be prepared by the hybridoma methodology first described by Kohler *et al.*, Nature 256:495 (1975), or may be made using recombinant DNA methods in bacterial or eukaryotic animal or plant cells (*see, e.g.*, U.S. Pat. No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature 352:624-28 (1991) and Marks *et al.*, J. Mol. Biol. 222:581-97 (1991), for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art. *See, e.g.*, Short Protocols in Molecular Biology (Ausubel *et al.* eds., 5th ed. 2002).

[0095] A typical 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain

disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the  $\alpha$  and  $\gamma$  chains and four CH domains for  $\mu$  and  $\epsilon$  isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end. The VL is aligned with the VH, and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, for example, Basic and Clinical Immunology 71 (Stites *et al.* eds., 8th ed. 1994); and Immunobiology (Janeway *et al.* eds., 5<sup>th</sup> ed. 2001).

10 [0096] The term “Fab” or “Fab region” refers to an antibody region that binds to antigens. A conventional IgG usually comprises two Fab regions, each residing on one of the two arms of the Y-shaped IgG structure. Each Fab region is typically composed of one variable region and one constant region of each of the heavy and the light chain. More specifically, the variable region and the constant region of the heavy chain in a Fab region are VH and CH1 regions, and the variable region and the constant region of the light chain in a Fab region are VL and CL regions. The VH, CH1, VL, and CL in a Fab region can be arranged in various ways to confer an antigen binding capability according to the present disclosure. For example, VH and CH1 regions can be on one polypeptide, and VL and CL regions can be on a separate polypeptide, similarly to a Fab region of a conventional IgG. Alternatively, VH, CH1, VL and CL regions can all be on the same polypeptide and oriented in different orders as described in more detail the sections below.

20 [0097] The term “variable region,” “variable domain,” “V region,” or “V domain” refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable region of the heavy chain may be referred to as “VH.” The variable region of the light chain may be referred to as “VL.” The term “variable” refers to the fact that certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of less

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variable (*e.g.*, relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (*e.g.*, extreme variability) called “hypervariable regions” that are each about 9-12 amino acids long. The variable regions of heavy and light chains each comprise four FRs, largely adopting a  $\beta$  sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases form part of, the  $\beta$  sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see, e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest (5th ed. 1991)). The constant regions are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The variable regions differ extensively in sequence between different antibodies. In specific embodiments, the variable region is a human variable region.

[0098] The term “heavy chain” when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy-terminal portion includes a constant region. The constant region can be one of five distinct types, (*e.g.*, isotypes) referred to as alpha ( $\alpha$ ), delta ( $\delta$ ), epsilon ( $\epsilon$ ), gamma ( $\gamma$ ), and mu ( $\mu$ ), based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size:  $\alpha$ ,  $\delta$ , and  $\gamma$  contain approximately 450 amino acids, while  $\mu$  and  $\epsilon$  contain approximately 550 amino acids. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes (*e.g.*, isotypes) of antibodies, IgA, IgD, IgE, IgG, and IgM, respectively, including four subclasses of IgG, namely IgG1, IgG2, IgG3, and IgG4.

[0099] The term “light chain” when used in reference to an antibody refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy-terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa ( $\kappa$ ) or lambda ( $\lambda$ ) based on the amino acid sequence of the constant domains.



- [0100] As used herein, the terms “hypervariable region,” “HVR,” “Complementarity Determining Region,” and “CDR” are used interchangeably. A “CDR” refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH  $\beta$ -sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non-framework region of the antibody VL  $\beta$ -sheet framework. CDR1, CDR2 and CDR3 in VH domain are also referred to as HCDR1, HCDR2 and HCDR3, respectively. CDR1, CDR2 and CDR3 in VL domain are also referred to as LCDR1, LCDR2 and LCDR3, respectively. Accordingly, CDRs are variable region sequences interspersed within the framework region sequences.
- [0101] CDR regions are well known to those skilled in the art and have been defined by well-known numbering systems. For example, the Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (*see, e.g., Kabat et al., supra*; Nick Deschacht et al., J Immunol 2010; 184:5696-5704). Chothia refers instead to the location of the structural loops (*see, e.g., Chothia and Lesk, J. Mol. Biol. 196:901-17 (1987)*). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular’s AbM antibody modeling software (*see, e.g., Antibody Engineering* Vol. 2 (Kontermann and Dübel eds., 2d ed. 2010)). The “contact” hypervariable regions are based on an analysis of the available complex crystal structures. Another universal numbering system that has been developed and widely adopted is ImMunoGeneTics (IMGT) Information System<sup>®</sup> (Lafranc *et al.*, Dev. Comp. Immunol. 27(1):55-77 (2003)). IMGT is an integrated information system specializing in immunoglobulins (IG), T-cell receptors (TCR), and major histocompatibility complex (MHC) of human and other vertebrates. Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain. As the “location” of the CDRs within the structure of the immunoglobulin variable domain is conserved between species and present in structures called loops, by using numbering systems that align variable domain sequences

according to structural features, CDR and framework residues are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody. An additional numbering system (AHon) has been developed by Honegger and Plückthun, J. Mol. Biol. 309: 657-70 (2001). Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT unique numbering system, is well known to one skilled in the art (*see, e.g., Kabat, supra; Chothia and Lesk, supra; Martin, supra; Lefranc et al., supra*). The residues from each of these hypervariable regions or CDRs are exemplified in Table 1 below.

10 Table 1. Exemplary CDRs According to Various Numbering Systems

| Loop   | Kabat                              | AbM       | Chothia                   | Contact   | IMGT      |
|--------|------------------------------------|-----------|---------------------------|-----------|-----------|
| CDR L1 | L24--L34                           | L24--L34  | L26--L32 or<br>L24--L34   | L30--L36  | L27--L38  |
| CDR L2 | L50--L56                           | L50--L56  | L50--L52 or<br>L50--L56   | L46--L55  | L56--L65  |
| CDR L3 | L89--L97                           | L89--L97  | L91--L96 or<br>L89--L97   | L89--L96  | L105-L117 |
| CDR H1 | H31--H35B<br>(Kabat<br>Numbering)  | H26--H35B | H26--H32..34              | H30--H35B | H27--H38  |
| CDR H1 | H31--H35<br>(Chothia<br>Numbering) | H26--H35  | H26--H32                  | H30--H35  |           |
| CDR H2 | H50--H65                           | H50--H58  | H53--H55 or<br>H52--H56   | H47--H58  | H56--H65  |
| CDR H3 | H95--H102                          | H95--H102 | H96--H101 or<br>H95--H102 | H93--H101 | H105-H117 |

[0102] The boundaries of a given CDR may vary depending on the scheme used for identification. Thus, unless otherwise specified, the terms “CDR” and “complementary determining region” of a given antibody or region thereof, such as a variable region, as well as individual CDRs (*e.g., CDR-H1, CDR-H2*) of the antibody or region thereof, should be

understood to encompass the complementary determining region as defined by any of the known schemes described herein above. In some instances, the scheme for identification of a particular CDR or CDRs is specified, such as the CDR as defined by the AbM, IMGT, Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR is given. It should  
5 be noted CDR regions may also be defined by a combination of various numbering systems, *e.g.*, a combination of Kabat and Chothia numbering systems, or a combination of Kabat and IMGT numbering systems. Therefore, the term such as “a CDR1 as set forth in a specific VH” includes any CDR1 as defined by the exemplary CDR numbering systems described above, but is not limited thereby. Once a variable region (*e.g.*, a VH or VL) is given, those skilled in the art would  
10 understand that CDRs within the region can be defined by different numbering systems or combinations thereof.

**[0103]** Hypervariable regions may comprise “extended hypervariable regions” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2), and 93-102, 94-102, or 95-102 (H3) in the VH.

**[0104]** The term “constant region” or “constant domain” refers to a carboxy terminal portion of the light and heavy chain which is not directly involved in binding of the antibody to antigen but exhibits various effector function, such as interaction with the Fc receptor. The term refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable region, which contains the antigen  
20 binding site. The constant region may contain the CH1, CH2, and CH3 regions of the heavy chain and the CL region of the light chain. Amino acid numbering for constant heavy domain regions typically use the EU numbering system of Kabat.

**[0105]** The term “framework” or “FR” refers to those variable region residues flanking the CDRs. FR residues are present, for example, in chimeric, humanized, human, domain  
25 antibodies, diabodies, linear antibodies, and bispecific antibodies. FR residues are those variable domain residues other than the hypervariable region residues or CDR residues.

**[0106]** The term “Fc domain” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain  
30 might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid

residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact

5 antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Functional Fc region is typically formed by coming together of two Fc domains (either homodimer or heterodimer) as described herein. Exemplary

10 “effector functions” include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; downregulation of cell surface receptors (*e.g.*, B cell receptor), *etc.* Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.*, an antibody variable region or domain) and can be assessed using various assays known to those skilled in the art. A “variant Fc region” comprises an amino acid sequence which differs from

15 that of a native sequence Fc region by virtue of at least one amino acid modification (*e.g.*, substituting, addition, or deletion). In certain embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, or from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of a

20 parent polypeptide. The variant Fc region herein can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith.

[0107] The term “variant” when used in relation to an antigen or an antibody may refer to a peptide or polypeptide comprising one or more (such as, for example, about 1 to about 25, about

25 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) amino acid sequence substitutions, deletions, and/or additions as compared to a native or unmodified sequence. For example, a variant of a CD33 antibody may result from one or more (such as, for example, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) changes to an amino acid sequence of a native or previously unmodified a

30 CD33 antibody. Variants may be naturally occurring, such as allelic or splice variants, or may

be artificially constructed. Polypeptide variants may be prepared from the corresponding nucleic acid molecules encoding the variants. In specific embodiments, the CD33 antibody variant at least retains CD33 antibody functional activity, respectively. In specific embodiments, a CD33 antibody variant binds CD33. In certain embodiments, the variant is encoded by a single  
5 nucleotide polymorphism (SNP) variant of a nucleic acid molecule that encodes anti-CD33 antibody VH or VL regions or subregions, such as one or more CDRs.

**[0108]** The term “valent” as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms “monovalent,” “trivalent,” “tetravalent,”  
10 “pentavalent” and “hexavalent” denote the presence of one binding site, two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antibody)

**[0109]** The described CD33-specific antibodies or antigen-binding fragments include all isotypes, IgA, IgD, IgE, IgG and IgM, and synthetic multimers of the four-chain  
15 immunoglobulin structure. The described antibodies or antigen-binding fragments also include the IgY isotype generally found in hen or turkey serum and hen or turkey egg yolk.

**[0110]** The CD33-specific antibodies and antigen-binding fragments may be derived from any species by recombinant means. For example, the antibodies or antigen-binding fragments may be mouse, rat, goat, horse, swine, bovine, chicken, rabbit, camelid, donkey, human, or chimeric  
20 versions thereof. For use in administration to humans, non-human derived antibodies or antigen-binding fragments may be genetically or structurally altered to be less antigenic upon administration to a human patient.

**[0111]** In some embodiments, the antibodies or antigen-binding fragments are chimeric. As used herein, the term “chimeric” refers to an antibody, or antigen-binding fragment thereof, having at  
25 least some portion of at least one variable domain derived from the antibody amino acid sequence of a non-human mammal, a rodent, or a reptile, while the remaining portions of the antibody, or antigen-binding fragment thereof, are derived from a human.

**[0112]** In some embodiments, the antibodies are humanized antibodies. In certain embodiments, the antibodies or antigen-binding fragments can comprise portions of “humanized” forms of  
30 nonhuman (*e.g.*, camelid, murine, non-human primate) antibodies that include sequences from

human immunoglobulins (*e.g.*, recipient antibody) in which the native CDR residues are replaced by residues from the corresponding CDR of a nonhuman species (*e.g.*, donor antibody) such as camelid, mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, one or more FR region residues of the human immunoglobulin sequences are replaced by corresponding nonhuman residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. A humanized antibody heavy or light chain can comprise substantially all of at least one or more variable regions, in which all or substantially all of the CDRs correspond to those of a nonhuman immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. In certain embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, Jones *et al.*, Nature 321:522-25 (1986); Riechmann *et al.*, Nature 332:323-29 (1988); Presta, Curr. Op. Struct. Biol. 2:593-96 (1992); Carter *et al.*, Proc. Natl. Acad. Sci. USA 89:4285-89 (1992); U.S. Pat. Nos: 6,800,738; 6,719,971; 6,639,055; 6,407,213; and 6,054,297.

[0113] Humanized antibodies may be chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody may include at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0114] The antibodies or antigen-binding fragments described herein can occur in a variety of forms, but will include one or more of the antibody CDRs shown in Table 2.

Table 2. List of CDR Regions for representative CD33 antibodies.

|                                     | CDR1 (SEQ ID NO:)      | CDR2 (SEQ ID NO:)      | CDR3 (SEQ ID NO:) |
|-------------------------------------|------------------------|------------------------|-------------------|
| CD33 antibody name:<br>C33SB1SC1494 |                        |                        |                   |
| Heavy (AbM)                         | GFTFDDYTMH (12)        | LVSWSGGSAY (13)        | ESAYNAYFDY (14)   |
| Light (AbM)                         | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (KABAT)                       | DYTMH (18)             | LVSWSGGSAYYVDSVKG (19) | ESAYNAYFDY (14)   |
| Light (CHOTHIA)                     | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (CHOTHIA)                     | GFTFDDY (20)           | SWSGGS (21)            | ESAYNAYFDY (14)   |
| Light (CHOTHIA)                     | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (IMGT)                        | GFTFDDYT (22)          | VSWSGGSA (23)          | AKESAYNAYFDY (24) |
| Light (IMGT)                        | QSVLYSSNNKNY (25)      | WAS (26)               | HQFYITPYT (17)    |
| CD33 antibody name:<br>C33F1318     |                        |                        |                   |
| Heavy (AbM)                         | GFTFSRYWMS (27)        | NIKRDGSEKY (28)        | NSGTWEFDQ (29)    |
| Light (AbM)                         | TRSSGSIASNDVQ (30)     | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (KABAT)                       | RYWMS (33)             | NIKRDGSEKYYVDAVRG (34) | NSGTWEFDQ (29)    |
| Light (KABAT)                       | TRSSGSIASNDVQ (30)     | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (CHOTHIA)                     | GFTFSRY (35)           | KRDGSE (36)            | NSGTWEFDQ (29)    |
| Light (CHOTHIA)                     | TRSSGSIASNDVQ (30)     | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (IMGT)                        | GFTFSRYW (37)          | IKRDGSEK (38)          | ARNSGTWEFDQ (39)  |
| Light (IMGT)                        | SGSIASND (40)          | EDK (41)               | QSYDNNNQV (32)    |

[0115] Described herein are antibodies and antigen-binding fragments specifically binding to membrane-bound CD33. In some embodiments, the CD33-specific antibodies or antigen-binding fragments are human, humanized IgG, or derivatives thereof. While the CD33-specific antibodies or antigen-binding fragments exemplified herein are human or humanized, the antibodies or antigen-binding fragments exemplified may be chimerized.

[0116] In some embodiments are provided a CD33-specific antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR1, a CDR2, and a CDR3 of any

one of the antibodies described in Table 2. In some embodiments are provided a CD33-specific antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR1, a CDR2, and a CDR3 of any one of the antibodies described in Table 2 and a light chain comprising a CDR1, a CDR2, and a CDR3 of any one of the antibodies described in Table 2.

5 [0117] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 12, 13, and 14, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain  
10 complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 42 and a light chain variable domain substantially the same as, or identical to, SEQ  
15 ID NO: 43. The CDRs, the heavy chain variable domain, and the light chain variable domain, discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0118] In some embodiments, the CD33-specific antibodies and antigen-binding fragments  
20 comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 18, 19, and 14, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining  
25 region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 42 and a light chain variable domain substantially the same as, or identical to, SEQ  
30 ID NO: 43. The CDRs, the heavy chain variable domain, and the light chain variable domain,



discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0119] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 20, 21, and 14, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 42 and a light chain variable domain substantially the same as, or identical to, SEQ ID NO: 43. The CDRs, the heavy chain variable domain, and the light chain variable domain, discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0120] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 22, 23, and 24, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 25, 26, and 17, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 42 and a light chain variable domain substantially the same as, or identical to, SEQ ID NO: 43. The CDRs, the heavy chain variable domain, and the light chain variable domain, discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

- [0121] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 27, 28, and 29, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively. This CD33 -specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 44 and a light chain variable domain substantially the same as, or identical to, SEQ ID NO: 45. The CDRs, the heavy chain variable domain, and the light chain variable domain, discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.
- [0122] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 33, 34, and 29, respectively, and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively. This CD33 -specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain variable domain substantially the same as, or identical to, SEQ ID NO: 44 and a light chain variable domain substantially the same as, or identical to, SEQ ID NO: 45. The CDRs, the heavy chain variable domain, and the light chain variable domain, discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0123] Also described herein are antibodies or antigen-binding fragments specifically binding membrane-bound CD33 wherein the antigen binding fragment thereof is a single heavy chain variable region (VHH).

[0124] "Single heavy chain variable region", "VHH" or "dAb fragment" refers to an antibody fragment composed of a VH domain (Ward et al., Nature 341 :544 546 (1989)). The antigen-binding fragment of the present disclosure may comprise a VHH. VHHs are well known to the skilled person, see e.g., Hamers-Casterman et al. (1993) Nature 363:446, Roovers et al. (2007) Curr Opin Mol Ther 9:327 and Krah et al. (2016) Immunopharmacol Immunotoxicol 38:21. VHHs comprise a single heavy chain CDR1, a single heavy chain CDR2 and a single heavy chain CDR3. VHHs may be derived from any species including mouse, human, camel, llama, shark, goat, rabbit, and cow. For example, naturally occurring VHH molecules can be derived from antibodies raised in Camelidae species, for example in camel, dromedary, llama, alpaca and guanaco. Like a whole antibody, a VHH is able to bind selectively to a single specific antigen. VHHs may contain only the variable domain of an immunoglobulin chain, i.e., CDR1, CDR2 and CDR3 and framework regions.

[0125] The antibodies or antigen-binding fragments comprising a VHH described herein can occur in a variety of forms, but will include one or more of the antibody CDRs shown in Table 3.

Table 3. List of CDR Regions for representative CD33 antibodies comprising a VHH.

|                                                  | CDR1 (SEQ ID NO:) | CDR2 (SEQ ID NO:)     | CDR3 (SEQ ID NO:)                                |
|--------------------------------------------------|-------------------|-----------------------|--------------------------------------------------|
| CD33 antibody name:<br>JL45-VHH                  |                   |                       |                                                  |
| Heavy (AbM)                                      | GSIFSIFDMG (46)   | RITNGGITN (47)        | DIAANIGSNILYDDY (48)                             |
| Heavy (KABAT)                                    | IFDMG (49)        | RITNGGITNYLDSVKG (50) | DIAANIGSNILYDDY (48)                             |
| Heavy (CHOTHIA)                                  | GSIFSIF (51)      | TNGGI (52)            | DIAANIGSNILYDDY (48)                             |
| Heavy (IMGT)                                     | GSIFSIFD (53)     | ITNGGIT (54)          | YADIAANIGSNILYDDY (76) or<br>YADIAANIGSNIL (111) |
| CD33 antibody name:<br>AB266_1_CD33_ B12_P09.VR1 |                   |                       |                                                  |
| Heavy (AbM)                                      | GSIFSINAMG (55)   | RITGGGATD (56)        | DLVTRVGSDLLYDDY (57)                             |
| Heavy (KABAT)                                    | INAMG (58)        | RITGGGATDYVDSVKG (59) | DLVTRVGSDLLYDDY (57)                             |
| Heavy (CHOTHIA)                                  | GSIFSIN (60)      | TGGGA (61)            | DLVTRVGSDLLYDDY (57)                             |
| Heavy (IMGT)                                     | GSIFSINA (62)     | ITGGGAT (63)          | YADLVTRVGSDLLYDDY (64)                           |

[0126] In some embodiments are provided a CD33-specific antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR1, a CDR2, and a CDR3 of any one of the antibodies described in Table 3. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 46, 47, and 48, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 65. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:65. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0127] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 49, 50, and 48, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 65. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:65. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0128] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining

region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 51, 52, and 48, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or  
5 identical to, SEQ ID NO: 65. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:65. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

10 **[0129]** In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 53, 54, and 76, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human  
15 framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 65. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:65. The  
20 CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

**[0130]** In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining  
25 region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 53, 54, and 97, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 65. In some embodiments, the CD33-specific antibodies and antigen-  
30 binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%,

92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:65. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0131] In some embodiments, the CD33-specific antibodies and antigen-binding fragments  
5 comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 55, 56, and 57, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding  
10 fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 66. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:66. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific  
15 constructs in which one arm is an anti-CD33 arm.

[0132] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 58, 59, and 57,  
20 respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 66. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%,  
25 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:66. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

[0133] In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain  
30 complementarity determining region 2 (CDR2), and a heavy chain complementarity determining

region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 60, 61, and 57, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or  
5 identical to, SEQ ID NO: 66. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:66. The CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

10 **[0134]** In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 62, 63, and 64, respectively. This CD33-specific antibody or antigen-binding fragment may comprise human  
15 framework sequences. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a single heavy chain variable domain (VHH) substantially the same as, or identical to, SEQ ID NO: 66. In some embodiments, the CD33-specific antibodies and antigen-binding fragments comprise a VHH having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:66. The  
20 CDRs and the VHH discussed in this paragraph are suitable for inclusion in multispecific constructs in which one arm is an anti-CD33 arm.

**[0135]** In some embodiments, the antibodies or antigen-binding fragments are IgG, or derivatives thereof, e.g., IgG1, IgG2, IgG3, and IgG4 isotypes. In some embodiments wherein the antibody is of IgG1 isotype, the antibody comprises an IgG1 Fc region or IgG Fc domain.

25 **[0136]** In certain embodiments, the CD33 antibodies or antigen-binding fragments thereof bind the C2 domain of CD33.

**[0137]** In certain embodiments, the CD33 antibodies or antigen-binding fragments thereof bind the V domain of CD33.

**[0138]** In certain embodiments, the CD33 antibodies or antigen-binding fragments thereof bind  
30 mCD33.

[0139] In certain embodiments, the CD33 antibodies or antigen-binding fragments thereof do not significantly bind sCD33.

*CD33/V $\delta$ 2 MULTISPECIFIC ANTIBODIES*

- 5 [0140] T cells are the most abundant (~75% of blood lymphocytes) and potent immune killer cells. The role of effector T cells in the anti-cancer immune response is strongly supported by *in vitro* studies and the observation that a high infiltration of CD8<sup>+</sup> T cells in several types of cancers correlates with a favorable clinical prognostic.
- 10 [0141] Recently, substantial progress has been made to harness the therapeutic potential of T cells for the treatment of cancers. Two different strategies to redirect T cells to lyse cancer cells are currently explored in clinical trials: 1) Donor T cells engineered *ex vivo* with chimeric antigen receptor (CAR) by using antibody fragments that bind to cancer cells and 2) recombinant bispecific protein therapeutics consisting of one arm binding to CD3 on T cells while the second arm binding to a cancer-associated antigen. Focusing on the latter, bispecific proteins allow
- 15 efficient engagement of T cells with cancer cells. This results in CD3 co-receptor stimulation induced by cancer-bound bispecific molecules, which elicits an MHC-independent polyclonal T cell activation and potent cancer cell lysis. This approach bypasses some of the cancer specific tolerance mechanisms and allows the recruited T cells to kill the cancer cells.
- 20 [0142] Indeed, one of the CD3 bispecific proteins, blinatumomab, a CD3/CD19 Bispecific T Cell Engager (BiTE) has been approved by the FDA for the treatment of refractory B-acute lymphoblastic leukemia (ALL). Although, the mechanism of action of how BiTE like molecules work is still not fully understood, it does provide evidence that bi-specific reagents can induce formation of an artificial lytic synapse between the two cells, which mimics the naturally occurring lytic mediated killing of cancer cells by T cells. Preclinical experiments with chimeric
- 25 antigen receptor (CAR) T cells and blinatumomab has validated the concept and provided strong rationale for this approach, and clinical trials have now provided proof of concept (POC) in human patients. Due to the clinical success of this approach, the field of CD3-directed bispecifics is rapidly growing, and a variety of antibody formats are being used to generate therapeutics to target a large number of cancer antigens. Some of the formats hold the promise of mitigation of
- 30 key issues seen with blinatumomab, for example, blinatumomab is rapidly cleared from



circulation and requires a continuous i.v. infusion during 4-week treatment cycle. New formats are designed for a longer serum half-life, thereby circumventing the continuous infusion.

[0143] Since T cell mediated responses are extremely potent, severe side effects can arise by inducing cytokine storm or directing T cells towards healthy tissues that express low levels of target antigen. Most CD3 bispecific proteins currently in clinical trials are targeting receptors where expression is confined to the hematopoietic lineage (CD19, CD20, CD123, *etc.*), or highly specific cancer antigens, such as CEA, PSMA, and MHCII-gp100. Thus, the applicability of CD3-based redirection could be limited to antigens with cancer specificity or hematological cancers, which impedes the application to many solid cancer types. In addition, CD3-directed T cell redirection with currently available technologies has not shown much efficacy in solid cancers due to various reasons (*e.g.*, recruiting all types of CD3+ T cells, including immature, CD4+, Tregs, pan CD8 (no CTL), exhausted T cells, *etc.*, that could lead to inefficient cancer removal; premature T cell activation that could result in a narrow therapeutic index; suboptimal T cell activation; T cell exhaustion or activation induced death of T cells; induction of cytokine release syndrome that could limit optimal dosing level; inhibition of cancer cell apoptosis; less activation of anti-cancer adaptive immune response; limited ability to combine with other immunotherapies, *etc.*).

[0144] Although, redirecting T cells via CD3 is attractive, as it results in a polyclonal cytotoxic response bypassing the classical antigen-specific T cell response, it raises two key concerns: 1) CD3+ T cells can be indiscriminately stimulated including various immunoregulatory and immunosuppressive T cells, which are described as playing an active role in immune evasion, and 2) Pan T cell activation that can result in severe side effects can arise by inducing cytokine storm. Thus, redirection via CD3 could potentially result in suboptimal efficacy and a narrow therapeutic index. To alleviate some of the CD3-redirection limitations, alternative strategies to re-direct T cells to cancer cells must be sought. One approach would be to select re-direction of only cytotoxic cells (a subset) that are capable of lysing cancer cells rather than indiscriminately stimulating and recruiting pan-T cells.

[0145] Another way to recruit T cells is to target specific subset of T cells. Recently,  $\gamma\delta$  T cells have provided a great interest in the cancer immunotherapy field. These unconventional T cells,

well known for their innate immunity, represent only a minor proportion of the peripheral CD3+ T cells (1%–5%), but constitute a major subset (20%–50%) of T cells in epithelial tissues.

[0146] Circulating  $\gamma\delta$  T cells mainly express heterodimers of V $\gamma$ 9 (TRGV9) and V $\delta$ 2 (TRDV2) chains whereas tissue  $\gamma\delta$  T cells preferentially express V $\delta$ 1 chains associated with different V $\gamma$  chains.

[0147] In humans,  $\gamma\delta$  T cells are endowed with potent anti-cancer functions (high cytotoxicity and interferon  $\gamma$  secretion). Moreover,  $\gamma\delta$  T cells are capable of phagocytosis, a function previously exclusive to innate myeloid lineage cells, and behave as efficient antigen-presenting cells for  $\alpha\beta$  T cells and induce adaptive immune response.  $\gamma\delta$  T cells have been shown to infiltrate cancers, but the clinical relevance of their presence is still debated. Up to now, all the research efforts have been focused on V $\gamma$ 9V $\delta$ 2 T cells, and mainly aimed at activating  $\gamma\delta$  T cells *in vivo* or *ex vivo* for adoptive transfer. Although clinical studies are not yet abundant, preliminary data highlight the importance of considering the  $\gamma\delta$  T cells subset in T cell-based immunotherapy.

[0148] Thus, against this backdrop, approaches that help to overcome the limitations of CD3-based redirection, avoid pan activation of T cells and induce potent cancer lysis by selectively recruiting  $\gamma\delta$  T cells are sought. Specifically, strategies centered on bispecific antibody therapies with one arm binding to a cancer-associated antigen and the other arm binding to  $\gamma\delta$  T cell expressed V $\delta$ 2 T cell receptor to recruit and activate  $\gamma\delta$  T cells, could address this unmet medical need by having a bispecific antibody that binds to an antigen on a bona fide cytotoxic T cell and an antigen expressed on a cancer cell in the treatment of cancers.

[0149] As used herein, the terms “V $\delta$ 2”, “ $\delta$ 2”, or “TRDV2” refer to a polypeptide capable of forming a T cell receptor together with a V $\gamma$ 9 chain when expressed on the surface of  $\gamma\delta$  T cells.  $\delta$ 2-expressing  $\gamma\delta$  T cells are among the first T cells to develop in the human fetus and are the predominant  $\gamma\delta$  T cell subset in healthy adult peripheral blood cells. The term “ $\delta$ 2” includes any  $\delta$ 2 variant, isoform, and species homolog, which is naturally expressed by cells (including T cells) or can be expressed on cells transfected with genes or cDNA encoding the polypeptide. In specific embodiments, the  $\delta$ 2 is a human  $\delta$ 2. An exemplary human  $\delta$ 2 amino acid sequence is provided with UniProtKB entry A0JD36 with GenBank Accession Number AAB69040.1.

[0150] Also provided herein are CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof comprising a CD33 antibody or an antigen-binding fragment thereof and a V $\delta$ 2 antibody or antigen-binding fragment thereof, wherein the CD33 antibody or the antigen-binding fragment thereof, specifically binds to mCD33, and wherein the V $\delta$ 2 antibody, or the antigen-binding fragment thereof, specifically binds to the V $\delta$ 2 chain of the human V $\gamma$ 9V $\delta$ 2 T cell receptor.

[0151] As used herein, the term “multispecific antibody” refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule.

[0152] As used herein, the term “bispecific antibody” refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope (*e.g.*, an epitope on a CD33 antigen) and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope (*e.g.*, an epitope on a V $\delta$ 2 antigen). In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a single heavy chain variable domain sequence which has binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a single heavy chain variable domain sequence which has binding specificity for a first epitope and a single heavy chain variable domain sequence which has binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a

bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first epitope is located on CD33 and the second epitope is located on V $\delta$ 2 chain of the human V $\gamma$ 9V $\delta$ 2 T cell receptor. The bispecific antibody may comprise a third, fourth, or fifth immunoglobulin variable domain that binds one or other of the first and second epitope (e.g. it may have two immunoglobulin variable domains that bind the first epitope and two immunoglobulin variable domains that bind the second epitope, or it may have two immunoglobulin variable domains that bind the first epitope and one immunoglobulin variable domain that bind the second epitope).

- 10 **[0153]** In certain embodiments, the CD33 antibody or antigen-binding fragment thereof is an CD33 antibody or antigen-binding fragment thereof described herein and the V $\delta$ 2 antibody or antigen-binding fragment thereof comprises a heavy chain HCDR1, HCDR2, and HCDR3, having the amino acid sequences of (1) SEQ ID NOs: 77, 78, and 79, respectively, (2) SEQ ID NOs: 80, 81, 82, respectively, (3) SEQ ID NOs: 82, 83, and 79, respectively, or (4) SEQ ID NOs: 84, 85, and 86, respectively.

**[0154]** Different formats of multispecific antibodies have been described and were reviewed by Chames and Baty (2009) Curr Opin Drug Disc Dev 12: 276.

- 20 **[0155]** In some embodiments, the multispecific antibody of the present disclosure is a diabody, a cross-body, or a multispecific antibody obtained via a controlled Fab arm exchange as those described in the present disclosure.

- 25 **[0156]** In some embodiments, the multispecific antibodies include IgG-like molecules with complementary CH3 domains to force heterodimerisation; recombinant IgG-like dual targeting molecules, wherein the multiple sides of the molecule each contain the binding fragment or part of the binding fragment of at least two different antibodies; IgG fusion molecules, wherein full length IgG antibodies are fused to an extra Fab fragment or parts of Fab fragment; Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc-regions or parts thereof; Fab fusion molecules, wherein different Fab-fragments are fused together; ScFv- and diabody-based and heavy chain antibodies (e.g., domain antibodies, nanobodies) wherein different single chain Fv molecules or different diabodies or

different heavy-chain antibodies (e.g. domain antibodies, nanobodies) are fused to each other or to another protein or carrier molecule.

[0157] In some embodiments, IgG-like molecules with complementary CH3 domains molecules include the Triomab/Quadroma (Trion Pharma/Fresenius Biotech), the Knobs-into-Holes

5 (Genentech), CrossMAbs (Roche) and the electrostatically-matched (Amgen), the LUZ-Y (Genentech), the Strand Exchange Engineered Domain body (SEEDbody)(EMD Serono), the Biclonic (Merus) and the DuoBody (Genmab A/S).

[0158] In some embodiments, recombinant IgG-like dual targeting molecules include Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech), Cross-linked Mabs

10 (Karmanos Cancer Center), mAb2 (F-Star) and CovX-body (CovX/Pfizer).

[0159] In some embodiments, IgG fusion molecules include Dual Variable Domain (DVD)-Ig (Abbott), IgG-like Bispecific (InnClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idec) and TvAb (Roche).

[0160] In some embodiments, Fc fusion molecules include to ScFv/Fc Fusions (Academic

15 Institution), SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual(ScFv).sub.2-Fab (National Research Center for Antibody Medicine--China).

[0161] In some embodiments, Fab fusion bispecific antibodies include F(ab)<sub>2</sub>

(Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL)

20 (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech). ScFv-, diabody-based and domain antibodies include but are not limited to Bispecific T Cell Engager (BiTE)

(Micromet), Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology

(DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT,

ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack) and COMBODY (Epigen

25 Biotech), dual targeting nanobodies (Ablynx), dual targeting heavy chain only domain antibodies.

[0162] Multispecific antibodies disclosed herein may be generated for example using binding

arm exchange (or half molecule exchange) between antibodies by introducing substitutions at the heavy chain CH3 interface in each half molecule to favor multimer formation of antibody half

30 molecules having distinct specificity either in vitro in cell-free environment or using co-

expression. The Fab arm exchange reaction is the result of a disulfide-bond isomerization reaction and dissociation-association of CH3 domains. The heavy-chain disulfide bonds in the hinge regions of the parent mono specific antibodies are reduced. The resulting free cysteines of one of the parent monospecific antibodies form an inter heavy-chain disulfide bond with cysteine residues of a second parent mono specific antibody molecule and simultaneously CH3 domains of the parent antibodies release and reform by dissociation-association. The CH3 domains of the Fab arms may be engineered to favor heterodimerization over homodimerization. The resulting product is a bispecific antibody having two Fab arms or half molecules which each bind a distinct epitope, i.e. an epitope on CD33 and an epitope on CD3.

**[0163]** "Homodimerization" as used herein refers to an interaction of two heavy chains having identical CH3 amino acid sequences. "Homodimer" as used herein refers to an antibody having two heavy chains with identical CH3 amino acid sequences.

**[0164]** "Heterodimerization" as used herein refers to an interaction of two heavy chains having non-identical CH3 amino acid sequences. "Heterodimer" as used herein refers to an antibody having two heavy chains with non-identical CH3 amino acid sequences.

**[0165]** The "knob-into-hole" strategy (see, e.g., PCT Int. Publ. No. WO 2006/028936) may be used to generate full length multispecific antibodies. Briefly, selected amino acids forming the interface of the CH3 domains in human IgG can be mutated at positions affecting CH3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically binding a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically binding a second antigen. After co-expression of the antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a "hole" with the heavy chain with a "knob". Exemplary CH3 substitution pairs forming a knob and a hole are (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain): T366Y/F405A, T366W/ F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S\_L368A\_Y407V. Numbering here is EU numbering.

**[0166]** Other strategies such as promoting heavy chain heterodimerization using electrostatic interactions by substituting positively charged residues at one CH3 surface and negatively

charged residues at a second CH3 surface may be used, as described in US Pat. Publ. No. US2010/0015133; US Pat. Publ. No. US2009/0182127; US Pat. Publ. No. US2010/028637 or US Pat. Publ. No. US2011/0123532. In other strategies, heterodimerization may be promoted by the following substitutions (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain):

5 L351Y\_F405AY407V/T394W, T366I\_K392M\_T394W/F405A\_Y407V, T366L\_K392M\_T394W/F405A\_Y407V, L351Y\_Y407A/T366A\_K409F, L351Y\_Y407A/T366V\_K409F Y407A/T366A\_K409F, or T350V\_L351Y\_F405A Y407V/T350V\_T366L\_K392L\_T394W as described in U.S. Pat. Publ. No. US2012/0149876 or

10 U.S. Pat. Publ. No. US2013/0195849. Numbering here is EU numbering.

**[0167]** In addition to methods described above, multispecific antibodies provided herein may be generated in vitro in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of at least two mono specific homodimeric antibodies and forming the multispecific heterodimeric antibody from at least two parent monospecific homodimeric antibodies in

15 reducing conditions to allow disulfide bond isomerization according to methods described in Intl. Pat. Publ. No. W02011/131746. In the methods, the first antibody (e.g., CD33 antibody) and the second antibody (e.g., V $\delta$ 2 antibody) are engineered to have certain substitutions at the CH3 domain that promotes heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond

20 isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing conditions. Exemplary reducing agents that may be used are 2-mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris (2-carboxyethyl) phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2-

25 mercaptoethylamine, dithiothreitol and tris (2-carboxyethyl) phosphine. For example, incubation for at least 90 min at a temperature of at least 20° C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

[0168] In certain embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- a. SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively;
- 5       b. SEQ ID NOs: 18, 19, 14, 15, 16, and 17, respectively;
- c. SEQ ID NOs: 20, 21, 14, 15, 16, and 17, respectively;
- d. SEQ ID NOs: 22, 23, 24, 25, 26, and 17, respectively;
- e. SEQ ID NOs: 27, 28, 29, 30, 31, and 32, respectively;
- f. SEQ ID NOs: 33, 34, 29, 30, 31, and 32, respectively;
- 10       g. SEQ ID NOs: 35, 36, 29, 30, 31, and 32, respectively; or
- h. SEQ ID NOs: 37, 38, 39, 40, 41, and 32, respectively;

and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- a. SEQ ID NOs: 77, 78, and 79, respectively;
- 15       b. SEQ ID NOs: 80, 81, and 79, respectively;
- c. SEQ ID NOs: 82, 83, and 79, respectively; or
- d. SEQ ID NOs: 84, 85, and 86, respectively.

[0169] In certain embodiments, the CD33 antibody or antigen-binding fragment thereof comprise a VH comprising an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 42 or 44 and a VL comprising an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 43 or 45; and the V $\delta$ 2 antibody or antigen-binding fragment thereof comprises a VHH comprising an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 87.

[0170] In certain embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

- a. SEQ ID NOs: 46, 47, and 48, respectively;
- b. SEQ ID NOs: 49, 50, and 48, respectively;
- c. SEQ ID NOs: 51, 52, and 48, respectively;
- 30       d. SEQ ID NOs: 53, 54, and 76, respectively;



- e. SEQ ID NOs: 53, 54, and 111, respectively;  
f. SEQ ID NOs: 55, 56, and 57, respectively;  
g. SEQ ID NOs: 58, 59, and 57, respectively;  
h. SEQ ID NOs: 60, 61, and 57, respectively; or  
5 i. SEQ ID NOs: 62, 63, and 64, respectively;
- and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:
- a. SEQ ID NOs: 77, 78, and 79, respectively;  
b. SEQ ID NOs: 80, 81, and 79, respectively;  
10 c. SEQ ID NOs: 82, 83, and 79, respectively; or  
d. SEQ ID NOs: 84, 85, and 86, respectively.
- [0171] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 46, 47, and 48, respectively;
- 15 and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 77, 78, and 79, respectively.
- [0172] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 49, 50, and 48, respectively;
- 20 and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 80, 81, and 79, respectively.
- [0173] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 51, 52, and 48, respectively;
- 25 and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 82, 83, and 79, respectively.

[0174] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 53, 54, and 76, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 84, 85, and 86, respectively.

[0175] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 53, 54, and 111, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 84, 85, and 86, respectively.

[0176] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 55, 56, and 57, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 77, 78, and 79, respectively.

[0177] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 58, 59, and 57, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 80, 81, and 79, respectively.

[0178] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 60, 61, and 57, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 82, 83, and 79, respectively.

[0179] In particular embodiments, the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, comprising the amino sequences of: SEQ ID NOs: 62, 63, and 64, respectively; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of: SEQ ID NOs: 84, 85, and 86, respectively.

[0180] In certain embodiments, the CD33 antibody or antigen-binding fragment thereof comprise a VHH comprising an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 65 or 66; and the V $\delta$ 2 antibody or antigen-binding fragment thereof comprises a VHH comprising an amino acid sequence substantially the same as, or identical to, SEQ ID NO:87.

[0181] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO:42, and a VL comprising the amino acid sequence of SEQ ID NO: 43; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

[0182] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO:44, and a VL comprising the amino acid sequence of SEQ ID NO: 45; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

[0183] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a VHH comprising the amino acid sequence of SEQ ID NO:65; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

[0184] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a VHH comprising the amino acid sequence of SEQ ID NO:66; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

[0185] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:70, and a light chain comprising the amino acid sequence of SEQ ID NO:71.

- [0186] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:72, and a light chain comprising the amino acid sequence of SEQ ID NO:73
- 5 [0187] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:68.
- [0188] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:74; and a heavy chain comprising the amino acid sequence of SEQ ID NO:75.
- 10 [0189] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:68.
- [0190] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof bind the C2 domain of CD33.
- 15 [0191] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof bind the V domain of CD33.
- [0192] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof bind the mCD33.
- 20 [0193] In certain embodiments, the CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof do not or do not substantially bind sCD33.
- In certain embodiments, the CD33/V $\delta$ 2 multispecific antibody comprises:
- (A) a CD33 antibody or the antigen-binding fragment thereof comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:
- 25       a. SEQ ID NOs: 46, 47, and 48, respectively;
- b. SEQ ID NOs: 49, 50, and 48, respectively;
- c. SEQ ID NOs: 51, 52, and 48, respectively;
- d. SEQ ID NOs: 53, 54, and 76, respectively; or
- e. SEQ ID NOs: 53, 54, and 111, respectively;

and a V $\delta$ 2 antibody or the antigen-binding fragment thereof comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- f. SEQ ID NOs: 77, 78, and 79, respectively;
- g. SEQ ID NOs: 80, 81, and 79, respectively;
- 5 h. SEQ ID NOs: 82, 83, and 79, respectively; or
- i. SEQ ID NOs: 84, 85, and 86, respectively; and/or

(B) a VHH comprising the amino acid sequence of SEQ ID NO:65 and a VHH comprising the amino acid sequence of SEQ ID NO:87; and/or

10 (C) a heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a heavy chain comprising the amino acid sequence of SEQ ID NO:68.

#### *MUTATIONS, DELETIONS, OR INSERTIONS*

[0194] Variants of the CD33 antibodies and/or the CD33/ V $\delta$ 2 multispecific antibodies are intended to be encompassed by the present disclosure. Variations may be a  
15 mutation/substitution, deletion, or insertion of one or more codons encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the original antibody or polypeptide. Sites of interest for substitutional mutagenesis include the CDRs and FRs.

[0195] Amino acid mutations can be the result of replacing one amino acid with another amino  
20 acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule provided herein, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid mutations. Insertions or deletions may optionally be in  
25 the range of about 1 to 5 amino acids. In certain embodiments, the mutation, deletion, or insertion (collectively modifications) includes fewer than 25 amino acid modifications, fewer than 20 amino acid modifications, fewer than 15 amino acid modifications, fewer than 10 amino acid modifications, fewer than 5 amino acid modifications, fewer than 4 amino acid modifications, fewer than 3 amino acid modifications, or fewer than 2 amino acid modifications  
30 relative to the original molecule. Thus, as used herein reference to “an amino acid sequence

substantially the same as” includes molecules having the amino acid sequence (original molecule) with fewer than 25 amino acid modifications, fewer than 20 amino acid modifications, fewer than 15 amino acid modifications, fewer than 10 amino acid modifications, fewer than 5 amino acid modifications, fewer than 4 amino acid modifications, fewer than 3 amino acid modifications, or fewer than 2 amino acid modifications relative to the original molecule. In particular embodiments, a molecule (e.g. polypeptide) that has “an amino acid sequence substantially the same as” a reference amino acid sequence has 5, 4, 3, 2 or 1 amino acid substitutions relative to the reference amino acid sequence. In a specific embodiment, the mutation/substitution is a conservative amino acid mutation/substitution made at one or more predicted non-essential amino acid residues. The variation allowed may be determined by systematically making insertions, deletions, or mutations/substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the parental antibodies.

[0196] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing multiple residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue.

[0197] Antibodies generated by conservative amino acid substitutions are included in the present disclosure. In a conservative amino acid substitution, an amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. As described above, families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, substitution mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined. Conservative (e.g., within an amino acid group with similar properties and/or side

chains) substitutions may be made, so as to maintain or not significantly change the properties. Exemplary mutations are shown in Table 4 below.

[0198]

Table 4. Conservative Amino Acid Substitution Mutations

5

| Original Residue | Exemplary Mutations                 | Original Residue | Exemplary Mutations                 |
|------------------|-------------------------------------|------------------|-------------------------------------|
| Ala (A)          | Val; Leu; Ile                       | Leu (L)          | Norleucine; Ile; Val; Met; Ala; Phe |
| Arg (R)          | Lys; Gln; Asn                       | Lys (K)          | Arg; Gln; Asn                       |
| Asn (N)          | Gln; His; Asp, Lys; Arg             | Met (M)          | Leu; Phe; Ile                       |
| Asp (D)          | Glu; Asn                            | Phe (F)          | Trp; Leu; Val; Ile; Ala; Tyr        |
| Cys (C)          | Ser; Ala                            | Pro (P)          | Ala                                 |
| Gln (Q)          | Asn; Glu                            | Ser (S)          | Thr                                 |
| Glu (E)          | Asp; Gln                            | Thr (T)          | Val; Ser                            |
| Gly (G)          | Ala                                 | Trp (W)          | Tyr; Phe                            |
| His (H)          | Asn; Gln; Lys; Arg                  | Tyr (Y)          | Trp; Phe; Thr; Ser                  |
| Ile (I)          | Leu; Val; Met; Ala; Phe; Norleucine | Val (V)          | Ile; Leu; Met; Phe; Ala; Norleucine |

[0199] Amino acids may be grouped according to similarities in the properties of their side chains (*see, e.g.*, Lehninger, Biochemistry 73-75 (2d ed. 1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); and (4) basic: Lys (K), Arg (R), His(H). Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. For example, any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, for example, with another amino acid, such as alanine or serine, to improve the

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oxidative stability of the molecule and to prevent aberrant crosslinking. Non-conservative mutations will entail exchanging a member of one of these classes for another class.

[0200] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0201] Alterations (*e.g.*, mutations) may be made in CDRs, *e.g.*, to improve antibody affinity. Such alterations may be made in CDR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant antibody or fragment thereof being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (*e.g.*, 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. More detailed description regarding affinity maturation is provided in the section below.

[0202] In some embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided



herein) that do not substantially reduce binding affinity may be made in CDRs. In some embodiments of the variant antibody sequences provided herein, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0203] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells, *Science*, 244:1081-1085 (1989). In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0204] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0205] The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (see, e.g., Carter, *Biochem J.* 237:1-7 (1986); and Zoller *et al.*, *Nucl. Acids Res.* 10:6487-500 (1982)), cassette mutagenesis (see, e.g., Wells *et al.*, *Gene* 34:315-23 (1985)), or other known techniques can be performed on the cloned DNA to produce the antibody variant DNA.

[0206] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or the antigen-binding fragment thereof, is an IgG. In some embodiments, the antibody, or antigen-binding fragment thereof, is an IgG1, IgG2, IgG3, or IgG4 isotype. In some

embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, is an IgG1 isotype, or comprises a human IgG1 Fc domain.

[0207] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises knob-into-hole (KiH) substitution in the human  
5 IgG1 Fc domain. One human IgG1 Fc domain comprises an T366W substitution (the “knob” Fc domain), while the other human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, and Y407V per the EU numbering (the “hole” Fc domain). Suitably, one human IgG1 Fc domain comprises an T366W substitution (the “knob” Fc domain), while the other human IgG1 Fc domain comprises the triple mutations T366S/L368A/Y407V per the EU  
10 numbering (the “hole” Fc domain).

[0208] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises one or more mutations selected from L234A, L235A, and D265S, per the EU numbering, in the human IgG1 Fc domain. Suitably, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof,  
15 comprises the triple mutations L234A/L235A/D265S in the Fc domain or Fc region. In some embodiments, these mutations, known as AAS mutations, in the human IgG1 Fc domain are known to limit or abolish antibody interactions with Fc receptors.

[0209] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises mutations H435R and/or Y436F in the human  
20 IgG1 Fc domain. In some embodiments, these substitutions in the Fc region are known to disrupt protein A binding of monomeric and homodimerized of “hole” human IgG1 Fc domains.

[0210] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises the triple mutation M252Y/S254T/T256E in the human IgG1 Fc domain. In some embodiments, these mutations, known as YTE mutations, in  
25 the human IgG1 Fc domain have been shown to increase the serum half-life of antibodies compared to wild-type versions of the same antibody.

[0211] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises one or more mutations selected from L234A, L235A, and D265S, per the EU numbering, in the human IgG1 Fc domain.

[0212] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises H435R and Y436F substitution, per the EU numbering, in at least one Fc domain. In some embodiment, these mutations, known as RF mutations, facilitate purification of the desired multispecific molecule.

- 5 [0213] The 435R and 436F amino acids found in IgG3 CH3 domains are known to prevent binding to protein A resin (Jedberg et al. J Immunol Methods. 201(1):25-34, 1997). Thus, by incorporating the H435R and Y436F substitutions into just one of the heavy chains of a heterodimeric molecule (e.g. creating substitutions in IgG1 Fcs that equate with the 435R and 436F amino acids found in IgG3) it means that three species are produced after expression, two  
10 homodimers and one heterodimer. From a statistical point of view, 25% of the expressed products will carry the double mutation in both heavy chains and will not interact with the Protein-A column during purification. Consequently, this unwanted side product can be found in the flow-through. Another species with approximately 25% of the total expression product carries no mutations in their heavy chains and thus, will strongly bind to Protein-A. The main  
15 species being about 50% is the desired bispecific comprising the RF double mutation in just one chain, which displays medium affinity to the resin.

- [0214] In some embodiments the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, comprises the knob and hole mutations, the AAS mutations and the YTE mutations. In particular embodiments the CD33 antibody and/or the CD33/ V $\delta$ 2  
20 multispecific antibody, or antigen-binding fragment thereof, further comprises the RF mutation in at least one Fc domain.

#### *METHODS FOR DETERMINING sCD33 LEVELS*

- [0215] Any suitable method known in the art may be used to determine the levels of sCD33 in a  
25 sample. For example, sCD33 levels in a sample (e.g., a cerebrospinal fluid sample, or a blood sample such as a whole blood, serum or plasma sample) may be determined using immunoblots (e.g., Western blots), mass spectrometry, flow cytometry, an immunoassay (e.g., a SIMOA assay from Quanterix, see, e.g., the website: [www.quanterix.com/simoa-technology/](http://www.quanterix.com/simoa-technology/)), a proximity  
extension assay (e.g., an assay from Olink, see, e.g., the website: [www.olink.com/our-](http://www.olink.com/our-platform/our-pea-technology)  
30 platform/our-pea- technology), an electrochemiluminescence-based assay (e.g., an assay from

Meso Scale Diagnostics, see, e.g., the website:

[www.mesoscale.com/en/technical\\_resources/our\\_technology/ecl](http://www.mesoscale.com/en/technical_resources/our_technology/ecl)), and/or aptamer-based methods such as SOMASCAN assay (see, e.g., Candia et al. (2017) Sci Rep 7, 14248).

- [0216] In some embodiments, sCD33 levels in a sample (e.g., a cerebrospinal fluid sample, or a blood sample such as a whole blood, serum or plasma sample) are determined using a mass spectrometry-based method. For example, sCD33 levels in a sample (e.g., a cerebrospinal fluid sample, or a blood sample such as a whole blood, serum or plasma sample) may be determined using a Quantitative Liquid Chromatography Multiple-Reaction Monitoring Mass Spectrometry (LCMRM/MS) assay. Determination of sCD33 levels using a Quantitative Liquid Chromatography Multiple-Reaction Monitoring Mass Spectrometry (LCMRM/MS) assay may comprise one or more, or all, of the following steps, as described in Example 5 herein: (a) preparation of calibration standards (e.g., calibration standards may be prepared at levels, e.g., eight levels, covering the assay range by spiking [i.e., adding] CD33 protein, e.g., recombinant CD33 protein, in surrogate matrix, e.g., surrogate matrix for the sample treated with the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody [e.g., a surrogate matrix for cerebrospinal fluid, whole blood, serum or plasma, such as a suitable buffer solution]); (b) preparation of quality control (QC) samples (e.g., QC samples may be prepared at levels, e.g., three levels, covering the assay range by spiking CD33 protein, e.g., recombinant CD33 protein, in pooled samples from reference individuals [e.g., in pooled samples of cerebrospinal fluid, whole blood, serum or plasma from healthy individuals]), and/or QC samples at endogenous levels (non-spiked QC samples, e.g., non-spiked QC samples may be prepared from pooled samples from reference individuals [e.g., pooled samples of cerebrospinal fluid, whole blood, serum or plasma from healthy individuals] not spiked with CD33 protein), and/or blank samples; (c) providing samples from one or more individuals treated with anti-CD33 antibody, e.g., cerebrospinal fluid samples, or blood samples such as a whole blood, serum or plasma samples; (d) digesting, e.g., with trypsin or another suitable enzyme, the calibration standards, QC samples, non-spiked QC samples, blank samples, and/or samples from one or more individuals treated with anti-CD33 antibody; (e) spiking (i.e., adding to) the calibration standards, QC samples, non-spiked QC samples, blank samples, and/or samples from one or more individuals treated with anti-CD33 antibody a Stable Isotope Labeled (SIL) internal standard peptide for

sCD33; (f) desalting the calibration standards, QC samples, non-spiked QC samples, blank samples, and/or samples from one or more individuals treated with anti-CD33 antibody using Solid-Phase Extraction (SPE) sorbent matrix; (g) analyzing the calibration standards, QC samples, non-spiked QC samples, blank samples, and/or samples treated with the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody using an LC-MRM/MS assay, wherein transitions from endogenous target peptide and its SIL counterpart are monitored; (h) determining the concentration of sCD33 in the samples treated with the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody by calculating the ratio of endogenous target peptide and SIL peptide and back-calculating the value onto a calibration curve.

10 [0217] In some embodiments, the CD33 antibody and/or the CD33/ V $\delta$ 2 multispecific antibody or the antigen binding fragment thereof, does not bind to sCD33 (or to its surrogate, rCD33). As described herein the meaning of the expression “does not bind to sCD33” encompasses absence of significant binding to sCD33 (or to its surrogate, rCD33). Absence of significant binding to sCD33, (e.g. “does not significantly bind sCD33” as used herein) is understood in accordance  
15 with its ordinary meaning in the field, and can be assessed by any means, which the skilled person finds appropriate.

[0218] For example, sCD33 as a free form in human serum is not significantly decreased, or not decreased by more than 50% (e.g., not decreased by more than 40%) [or is decreased by less than 50% (e.g., is decreased by less than 40%)] once placed in contact with 0.2 nM of the CD33  
20 antibody and/or the CD33/ V  $\delta$  2 multispecific antibody or the antigen-binding fragment thereof.

[0219] For example, when placed in contact with a biological sample containing sCD33 (such as a serum sample of an AML human patient), or in a biological sample, which has been spiked with 10 ng/ml of rCD33, the antibody and/or the CD33/ V  $\delta$  2 multispecific antibody or antigen-binding fragment thereof (e.g., at a concentration of 0.2 nM) does not significantly decrease the  
25 level of free form of sCD33, or at least does not decrease it by more than 50% (e.g., does not decrease it by more than 40%)

[0220] For example, the T-cell cytotoxicity EC50 value of the antibody and/or the CD33/ V  $\delta$  2 multispecific antibody or antigen-binding fragment thereof on tumor cells such as the THP-1 cell line is not significantly impacted by the presence of 10 ng/mL of rCD33 (i.e., the same or

substantially the same EC<sub>50</sub> value in the absence or in the presence of 10 ng/mL rCD33; cf. T cell cytotoxicity assay of example 9).

[0221] For example, the CD33 antibody and/or the CD33/ V δ 2 multispecific antibody or antigen-binding fragment thereof has an affinity for sCD33 or rCD33 lower than an antibody that is not mCD33-specific (i.e., which binds sCD33 or rCD33).

[0222] In particular embodiments, the CD33 antibody and/or antibody and/or the CD33/ V δ 2 multispecific antibody or antigen-binding fragment thereof, has greater affinity for binding mCD33 than sCD33.

## 10 HUMANIZED ANTIBODIES

[0223] The CD33 antibody and/or the CD33/ Vδ2 multispecific antibodies described herein include humanized antibodies. Humanized antibodies, such as the humanized antibodies disclosed herein can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka *et al.*, *Protein Engineering* 7(6):805-814 (1994); and Roguska *et al.*, *PNAS* 91:969-973 (1994)), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, e.g., U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 9317105, Tan *et al.*, *J. Immunol.* 169:1119-1125 (2002), Caldas *et al.*, *Protein Eng.* 13(5):353-60 (2000), Morea *et al.*, *Methods* 20(3):267-79 (2000), Baca *et al.*, *J. Biol. Chem.* 272(16):10678-84 (1997), Roguska *et al.*, *Protein Eng.* 9(10):895-904 (1996), Couto *et al.*, *Cancer Res.* 55 (23 Supp):5973s-5977s (1995), Couto *et al.*, *Cancer Res.* 55(8):1717-22 (1995), Sandhu JS, *Gene* 150(2):409-10 (1994), and Pedersen *et al.*, *J. Mol. Biol.* 235(3):959-73 (1994). See also U.S. Patent Pub. No. US 2005/0042664 A1 (Feb. 24, 2005), each of which is incorporated by reference herein in its entirety.

[0224] In some embodiments, antibodies provided herein can be humanized antibodies that bind to CD33 or CD33 and Vδ2, including human CD33 and human Vδ2. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can

have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization may be performed, for example, following the method of Jones *et al.*, Nature 321:522-25 (1986); Riechmann *et al.*, Nature 332:323-27 (1988); and Verhoeven *et al.*, Science 239:1534-36 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody.

[0225] In some cases, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the CDRs of the parent non-human antibody are grafted onto a human antibody framework. For example, Padlan *et al.* determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the “specificity determining residues,” or SDRs (Padlan *et al.*, FASEB J. 9:133-39 (1995)). In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (*see, e.g.*, Kashmiri *et al.*, Methods 36:25-34 (2005)).

[0226] The choice of human variable domains to be used in making the humanized antibodies can be important to reduce antigenicity. For example, according to the so-called “best-fit” method, the sequence of the variable domain of a non-human antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the non-human antibody may be selected as the human framework for the humanized antibody (Sims *et al.*, J. Immunol. 151:2296-308 (1993); and Chothia *et al.*, J. Mol. Biol. 196:901-17 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, Proc. Natl. Acad. Sci. USA 89:4285-89 (1992); and Presta *et al.*, J. Immunol. 151:2623-32 (1993)). In some cases, the framework is derived from the consensus sequences of the most abundant human subclasses, V<sub>L</sub>6 subgroup I (V<sub>L</sub>6I) and V<sub>H</sub> subgroup III (V<sub>H</sub>III). In another method, human germline genes are used as the source of the framework regions.

[0227] In an alternative paradigm based on comparison of CDRs, called superhumanization, FR homology is irrelevant. The method consists of comparison of the non-human sequence with the functional human germline gene repertoire. Those genes encoding the same or closely related canonical structures to the murine sequences are then selected. Next, within the genes sharing

the canonical structures with the non-human antibody, those with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these FRs (*see, e.g., Tan et al., J. Immunol. 169:1119-25 (2002)*).

- [0228] It is further generally desirable that antibodies be humanized with retention of their
- 5 affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which
- 10 illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. These include, for example, WAM (Whitelegg and Rees, *Protein Eng. 13:819-24 (2002)*), Modeller (Sali and Blundell, *J. Mol. Biol. 234:779-815 (1993)*), and Swiss PDB Viewer (Guex and Peitsch, *Electrophoresis 18:2714-23 (1997)*). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate
- 15 immunoglobulin sequence, *e.g.,* the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.
- 20 [0229] Another method for antibody humanization is based on a metric of antibody humanness termed Human String Content (HSC). This method compares the mouse sequence with the repertoire of human germline genes, and the differences are scored as HSC. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants (Lazar *et al., Mol. Immunol. 44:1986-98 (2007)*).
- 25 [0230] In addition to the methods described above, empirical methods may be used to generate and select humanized antibodies. These methods include those that are based upon the generation of large libraries of humanized variants and selection of the best clones using enrichment technologies or high throughput screening techniques. Antibody variants may be isolated from phage, ribosome, and yeast display libraries as well as by bacterial colony
- 30 screening (*see, e.g., Hoogenboom, Nat. Biotechnol. 23:1105-16 (2005); Dufner et al., Trends*



Biotechnol. 24:523-29 (2006); Feldhaus *et al.*, Nat. Biotechnol. 21:163-70 (2003); and Schlapschy *et al.*, Protein Eng. Des. Sel. 17:847-60 (2004)).

[0231] In the FR library approach, a collection of residue variants are introduced at specific positions in the FR followed by screening of the library to select the FR that best supports the grafted CDR. The residues to be substituted may include some or all of the “Vernier” residues identified as potentially contributing to CDR structure (*see, e.g.*, Foote and Winter, J. Mol. Biol. 224:487-99 (1992)), or from the more limited set of target residues identified by Baca *et al.* J. Biol. Chem. 272:10678-84 (1997).

[0232] In FR shuffling, whole FRs are combined with the non-human CDRs instead of creating combinatorial libraries of selected residue variants (*see, e.g.*, Dall’Acqua *et al.*, Methods 36:43-60 (2005)). A one-step FR shuffling process may be used. Such a process has been shown to be efficient, as the resulting antibodies exhibited improved biochemical and physicochemical properties including enhanced expression, increased affinity, and thermal stability (*see, e.g.*, Damschroder *et al.*, Mol. Immunol. 44:3049-60 (2007)).

[0233] The “humaneering” method is based on experimental identification of essential minimum specificity determinants (MSDs) and is based on sequential replacement of non-human fragments into libraries of human FRs and assessment of binding. This methodology typically results in epitope retention and identification of antibodies from multiple subclasses with distinct human V-segment CDRs.

[0234] The “human engineering” method involves altering a non-human antibody or antibody fragment by making specific changes to the amino acid sequence of the antibody so as to produce a modified antibody with reduced immunogenicity in a human that nonetheless retains the desirable binding properties of the original non-human antibodies. Generally, the technique involves classifying amino acid residues of a non-human antibody as “low risk,” “moderate risk,” or “high risk” residues. The classification is performed using a global risk/reward calculation that evaluates the predicted benefits of making particular substitution (*e.g.*, for immunogenicity in humans) against the risk that the substitution will affect the resulting antibody’s folding. The particular human amino acid residue to be substituted at a given position (*e.g.*, low or moderate risk) of a non-human antibody sequence can be selected by aligning an amino acid sequence from the non-human antibody’s variable regions with the corresponding

region of a specific or consensus human antibody sequence. The amino acid residues at low or moderate risk positions in the non-human sequence can be substituted for the corresponding residues in the human antibody sequence according to the alignment. Techniques for making human engineered proteins are described in greater detail in Studnicka *et al.*, Protein Engineering 7:805-14 (1994); U.S. Pat. Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619; and PCT Publication WO 93/11794.

[0235] A composite human antibody can be generated using, for example, Composite Human Antibody™ technology (Antitope Ltd., Cambridge, United Kingdom). To generate composite human antibodies, variable region sequences are designed from fragments of multiple human antibody variable region sequences in a manner that avoids T cell epitopes, thereby minimizing the immunogenicity of the resulting antibody.

[0236] A deimmunized antibody is an antibody in which T-cell epitopes have been removed. Methods for making deimmunized antibodies have been described. *See, e.g.*, Jones *et al.*, Methods Mol Biol. 525:405-23 (2009), xiv, and De Groot *et al.*, Cell. Immunol. 244:148-153(2006)). Deimmunized antibodies comprise T-cell epitope-depleted variable regions and human constant regions. Briefly, variable regions of an antibody are cloned and T-cell epitopes are subsequently identified by testing overlapping peptides derived from the variable regions of the antibody in a T cell proliferation assay. T cell epitopes are identified via *in silico* methods to identify peptide binding to human MHC class II. Mutations are introduced in the variable regions to abrogate binding to human MHC class II. Mutated variable regions are then utilized to generate the deimmunized antibody.

#### POLYNUCLEOTIDES, VECTORS AND HOST CELLS

[0237] In another general aspect, the present disclosure relates to synthetic polynucleotides encoding a CD33 antibody or antigen-binding fragment thereof described herein. In another general aspect, the present disclosure relates to one or more isolated nucleic acid encoding a CD33 antibody or antigen-binding fragment thereof, or a CD33/ Vδ2 multispecific antibody or antigen-binding fragment thereof described herein.

[0238] “Polynucleotide” or “nucleic acid,” as used interchangeably herein, refers to polymers of nucleotides of any length and includes DNA and RNA. The nucleotides can be

deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs.

- 5 [0239] Unless otherwise specified, a “polynucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase polynucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).
- 10 [0240] It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that synthetic polynucleotides encoding CD33 antibodies and/or CD33/ V $\delta$ 2 multispecific antibodies of the disclosure can be altered without changing the amino acid sequences of the proteins.
- 15 [0241] In some embodiments, the nucleotide sequence encoding for the anti-CD33 heavy chain of GD33B273 comprises:

SEQ ID NO: 89:

CAAGTGCAACTTGTGGAAAGTGGTGGCGGACTTGTGCAAGCTGGTGGCTCATTGAGGCTGTCTTGTGA  
AGCATCTGGCTCAATCTTCAGCATCTTGATATGGGTTGGTATAGAAGACCTCCCGGGGCTCAGAGAG  
20 AACTGGTGGCTAGAATCACAATGGTGGGATTACCAATTACCTCGATTGAGTTAAGGGAAGGTCTCT  
ATTTCCAGAGACAATGCCAAGAATACTGTGTACTTGCAAATGAACAGCTTGAATGCCGAAGATACAGC  
CGTGTACTATTGTTACGCAGATATTGCAGCAAACATTGGCTCAAACATTCTGTACGATGACTATTGGGG  
TCAAGGAACACAAGTTACAGTCAGTTCTGAGCCCAAATCTAGCGACAAAACCTCACACATGCCCCCGT  
25 GCCCAGCACCTGAAGCCGCGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTC  
TACATCACCCGGGAGCCTGAGGTCACATGCGTGGTGGTGGAGCGTGAGCCACGAAGACCCTGAGGTCA  
AGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA  
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCCAGGACTGGCTGAATGGCAAGGAGT  
ACAAGTGCAAGGTGTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG  
GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC  
30 AGCCTGTCTGCGCCGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA  
GCCGGAGAACAATAACAAGACCACGCTCCCGTGCTGGACTCCGACGGCTCCTTCTCTCTCGTGAGCA  
AGCTCACCGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT  
CTGCACAACCGGTTACGCAGAAGTCTCTCCCTGTCTCCGGGAAAA

- 35 [0242] In some embodiments, the nucleotide sequence encoding for the anti-V $\delta$ 2 heavy chain of GD33B273 comprises:

SEQ ID NO: 90:

GAAGTTCAGCTGGTTGAAAGTGGTGGTGGCCTTGTGCAAGCCGGTGGCTCATTACGCTTGTCATGTGC  
 AGCCTCTGGAAGACCTTTTCAGCAATTATGGGATGGGGTGGTTTAGACAAGCACCCGAAAGAAACGC  
 GAGTTTGTGCTGGCATTTCATGGAGTGGCGGAAGTACAGATTATGCAGATTCTGTTAAAGGACGCTTT  
 ACAATTAGTCGGGACAACGCAAGAATAACCGTGATCTCCAGATGAATAGCCTTAAGCCAGAAGATA  
 5 CAGCCGTTTATTATTGTGCCGCTGTTTTCTCTGGTGCTGAAACTGCATACTATCCATCAGACGACTACG  
 ATTATTGGGGTCAAGGAACTCAAGTGACAGTTTCTAGTGAGCCCAAATCTAGCGACAAAACCTCACACA  
 TGCCACCGTGCCAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAA  
 GGACACCCTCTACATCACCCGGGAGCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAAGAC  
 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG  
 10 AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT  
 GGCAAGGAGTACAAGTGCAAGGTGTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCA  
 AAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCGTGCCCCCATCCCGGGAGGAGATGACCAA  
 GAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA  
 GCAATGGGCAGCCGAGAACAACTACAAGACCACGCCTCCCGTGGTGGACTCCGACGGCTCCTTCTTC  
 15 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGATGGCAGAGGGGAACGTCTTCTCATGCTCCGTGAT  
 GCATGAGGCTCTGCACAACCACTACACGCAGAAGTCTCTCCCTGTCTCCGGGAAAA

[0243] In another general aspect, the present disclosure relates to one or more vectors  
 comprising one or more synthetic polynucleotide sequences encoding a CD33 antibody, or  
 20 antigen-binding fragment thereof of, disclosed herein. In another general aspect, the present  
 disclosure relates to one or more vectors comprising one or more synthetic polynucleotide  
 sequences encoding a CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof  
 of, disclosed herein. The term “vector” refers to a substance that is used to carry or include a  
 polynucleotide sequence, including for example, a polynucleotide sequence encoding an  
 25 antibody or antigen-binding fragment as described herein, in order to introduce a polynucleotide  
 sequence into a host cell. Vectors applicable for use include, for example, expression vectors,  
 plasmids, phage vectors, viral vectors, episomes, and artificial chromosomes, which can include  
 selection sequences or markers operable for stable integration into a host cell’s chromosome.  
 Additionally, the vectors can include one or more selectable marker genes and appropriate  
 30 expression control sequences. Selectable marker genes that can be included, for example,  
 provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply  
 critical nutrients not in the culture media. Expression control sequences can include constitutive  
 and inducible promoters, transcription enhancers, transcription terminators, and the like, which  
 are well known in the art. When two or more polynucleotide molecules are to be co-expressed  
 35 (e.g., both an antibody heavy and light chain or an antibody VH and VL), both polynucleotide  
 molecules can be inserted, for example, into a single expression vector or in separate expression  
 vectors. For single vector expression, the encoding polynucleotides can be operationally linked  
 to one common expression control sequence or linked to different expression control sequences,

such as one inducible promoter and one constitutive promoter. The introduction of polynucleotide molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced polynucleotide sequence or its corresponding gene product. It is understood by those skilled in the art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

10 [0244] In another general aspect, the present disclosure relates to a host cell comprising a synthetic polynucleotide encoding a CD33 antibody and/or a CD33/ V $\delta$ 2 multispecific antibody, or an antigen-binding fragment thereof, described herein. The term “host cell” as used herein refers to a particular subject cell that may be transfected with a polynucleotide molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the polynucleotide molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the polynucleotide molecule into the host cell genome.

[0245] The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0246] Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of antibodies or antigen-binding fragments thereof, disclosed herein. Mammalian, fungal, bacterial or plant cells are particularly suitable. Mammalian cells, including human, monkey, rat, mouse, guinea pig are routinely used and suitable. Fungal cells including yeast (*Saccharomyces cerevisiae*), *Pichia pastoris*, *Neurospora crassa* could also be used. Bacterial, such as *E. coli* can also be used. However, mammalian cell lines, such as CHO, HEK and NS0 are the preferred and most commonly used expression system for recombinant antibody production (e.g., for clinical use) are most conveniently expressed in the often because

they easily allow post-translational modifications and can produce antibodies with human-like glycosylation patterns. In some embodiments, the host cells are *E. coli* TG1 or BL21 cells (for expression of, e.g., an scFv or Fab antibody), CHO-DG44 or CHO-K1 cells or HEK293 cells or NS0 cells (for expression of, e.g., a full-length IgG antibody). According to particular

5   embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

[0247] In another general aspect, the present disclosure relates to a method of producing a CD33

10   antibody, or antigen-binding fragment thereof, described herein, comprising culturing a cell comprising one or more polynucleotide sequences (molecules) encoding the CD33 antibody or antigen-binding fragment thereof under conditions to produce a CD33 antibody, or antigen-binding fragment thereof, disclosed herein, and recovering the CD33 antibody, or antigen-binding fragment thereof, from the cell or cell culture (e.g., from the supernatant). In another

15   general aspect, the present disclosure relates to a method of producing a CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, disclosed herein, comprising culturing a cell comprising one or more polynucleotide sequences (molecules) encoding the bispecific antibody or, antigen-binding fragment thereof, under conditions to produce a CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, disclosed herein, and recovering

20   the antibody or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed antibodies or antigen-binding fragments thereof can be harvested from the cells and purified according to conventional techniques known in the art and as described herein.

## 25   PHARMACEUTICAL COMPOSITIONS

[0248] In another general aspect, the present disclosure relates to a pharmaceutical composition comprising a CD33 antibody, or antigen-binding fragment thereof, described herein and a pharmaceutically acceptable carrier. In another general aspect, the present disclosure relates to a

30   pharmaceutical composition comprising a CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding

fragment thereof, described herein and a pharmaceutically acceptable carrier. The term “pharmaceutical composition” as used herein means a product comprising an antibody described herein together with a pharmaceutically acceptable carrier. Antibodies of the present disclosure and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[0249] As used herein, the term “carrier” refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term “pharmaceutically acceptable carrier” refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the present disclosure or the biological activity of a composition according to the present disclosure. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody pharmaceutical composition can be used.

[0250] In another general aspect, the present disclosure relates to a method of producing a pharmaceutical composition comprising a CD33 antibody or antigen-binding fragment thereof, described herein, comprising combining a CD33 antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition. In another general aspect, the present disclosure relates to a method of producing a pharmaceutical composition comprising a CD33/ V $\delta$ 2 multispecific antibody, or antigen-binding fragment thereof, described herein, comprising combining a CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

#### *METHODS OF USE*

[0251] In another general aspect, the present disclosure relates to a method of targeting CD33 on a cancer cell surface in a subject, the method comprising administering to the subject a CD33 antibody or antigen binding fragment thereof that specifically binds CD33 or a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof or a pharmaceutical composition

comprising either, as described herein. In particular embodiments, the CD33 antibody, or antigen binding fragment thereof, or CD33/ V $\delta$ 2 antibody, or antigen binding fragments thereof or a pharmaceutical composition comprising either are administered to the subject (e.g. human) in need thereof in a therapeutically effective amount.

5 [0252] The functional activity of antibodies and antigen-binding fragments thereof that bind CD33 can be characterized by methods known in the art and as described herein. Methods for characterizing antibodies and antigen-binding fragments thereof that bind CD33 include, but are not limited to, affinity and specificity assays including Biacore, ELISA, and OctetRed analysis; binding assays to detect the binding of antibodies to CD33 on cancer cells by FACS. According  
10 to particular embodiments, the methods for characterizing antibodies and antigen-binding fragments thereof that bind CD33 include those described below.

[0253] In another general aspect, the present disclosure relates to a method of treating a hematological cancer in a subject in need thereof, comprising administering to the subject a CD33 antibody, a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof that  
15 specifically binds CD33 or a pharmaceutical composition of the present disclosure. The hematologic cancer can, for example, be a leukemia, a lymphoma, and a myeloma. In certain embodiments, the hematologic cancer can be acute myeloid leukemia (AML), myelodysplastic syndrome (MDS, low or high risk), acute lymphocytic leukemia (ALL, including all subtypes), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML), or blastic  
20 plasmacytoid dendritic cell neoplasm (BPDCN).

[0254] In another general aspect, the present disclosure relates to a CD33 antibody, a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof or a pharmaceutical composition comprising either, as described herein, for use in therapy.

[0255] In another general aspect, the present disclosure relates to a CD33 antibody, a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof or a pharmaceutical composition  
25 comprising either, as described herein, for use in the treatment of a hematological cancer.

[0256] In another general aspect, the present disclosure relates to use of a CD33 antibody, a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof or a pharmaceutical composition comprising either, as described herein, in the manufacture of a medicament for  
30 treating a hematological cancer.



[0257] The hematologic cancer can, for example, be a leukemia, a lymphoma, and a myeloma. In certain embodiments, the hematologic cancer can be acute myeloid leukemia (AML), myelodysplastic syndrome (MDS, low or high risk), acute lymphocytic leukemia (ALL, including all subtypes), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML), or blastic plasmacytoid dendritic cell neoplasm (BPDCN).

[0258] In particular embodiments, the subject with the hematologic cancer, e.g. AML or MDS, lacks the single nucleotide polymorphism (SNP) rs12459419 in CD33 gene.

[0259] In particular embodiments, the subject with the hematologic cancer, e.g. AML or MDS, has the single nucleotide polymorphism (SNP) rs12459419 in CD33 gene.

10 [0260] According to embodiments herein, the pharmaceutical composition comprises a therapeutically effective amount of a CD33 antibody, a CD33/ V $\delta$ 2 multispecific antibody or antigen-binding fragment(s) thereof. As used herein, the term “therapeutically effective amount” refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject.

15 [0261] As used herein with reference to CD33 antibodies, CD33/ V $\delta$ 2 multispecific antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the CD33 antibody, a CD33/ V $\delta$ 2 multispecific, or antigen-binding fragment thereof, that modulates an immune response in a subject in need thereof.

[0262] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a

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symptom associated therewith; (ix) increase the survival (e.g. overall survival [OS] or progression free survival [PFS]) of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[0263] The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[0264] According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

[0265] As used herein, the terms “treat,” “treating,” and “treatment” are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms “treat,” “treating,” and “treatment,” can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, “treat,” “treating,” and “treatment” refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to elimination of the disease, disorder, or condition in the subject.

[0266] The invention is generally disclosed herein using affirmative language to describe the numerous embodiments. The invention also specifically includes embodiments in which

particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. Thus, even though the invention is generally not expressed herein in terms of what the invention does not include, aspects that are not expressly included in the invention are nevertheless disclosed herein.

5

#### ENUMERATED EMBODIMENTS

[0267] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the following examples are intended to illustrate but not limit the scope of inventions described in the claims.

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[0268] Embodiment 1 is an antibody or an antigen-binding fragment specifically binding to membrane-bound human CD33 (mCD33).

[0269] Embodiment 2 is the antibody or the antigen-binding fragment thereof of embodiment 1 comprising:

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- a. a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 12, 13, and 14, respectively; and  
a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;
- b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 18, 19, and 14, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;
- c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 20, 21, and 14, respectively; and  
a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively; or

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- d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 22, 23, and 24, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 25, 26, and 17, respectively.

5 [0270] Embodiment 3 is the antibody or antigen-binding fragment thereof of embodiment 1 comprising:

- a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 27, 28, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- 10 b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 33, 34, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- 15 c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 35, 36, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively; or
- 20 d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 37, 38, and 39, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 40, 41, and 32, respectively.

[0271] Embodiment 4 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 3, comprising a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42 or 44.

[0272] Embodiment 5 is the antibody or the antigen-binding fragment thereof of embodiment 1 to 4, comprising a VH comprising SEQ ID NO: 42 or 44.

[0273] Embodiment 6 is the antibody or the antigen-binding fragment thereof of any one of 30 embodiments 1 to 5, comprising a light chain variable region (VL) comprising an amino acid

sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 43 or 45.

[0274] Embodiment 7 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 6, comprising a VL comprising SEQ ID NO: 43 or 45.

- 5 [0275] Embodiment 8 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 7, comprising a VH comprising SEQ ID NO: 42, and a VL comprising SEQ ID NO: 43.

- [0276] Embodiment 9 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 8, comprising a VH comprising SEQ ID NO: 44, and a VL comprising SEQ  
10 ID NO: 45.

[0277] Embodiment 10 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 9, wherein the antibody or antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.

- [0278] Embodiment 11 is the antibody or the antigen-binding fragment thereof of any one of  
15 embodiments 1 to 10, wherein the IgG Fc domain is a human IgG1 Fc domain.

[0279] Embodiment 12 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 11, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.

- [0280] Embodiment 13 is the antibody or the antigen-binding fragment thereof of any one of  
20 embodiments 1 to 12, wherein the human IgG1 Fc domain further comprises one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.

[0281] Embodiment 14 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 13, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.

- 25 [0282] Embodiment 15 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 14, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.

[0283] Embodiment 16 is the antibody or the antigen-binding fragment thereof of embodiment 1, wherein the antibody is a heavy chain only antibody.

[0284] Embodiment 17 is the antibody or the antigen-binding fragment thereof of embodiment 1, wherein the antigen-binding fragment thereof is a single heavy chain variable region (VHH).

[0285] Embodiment 18 is the antibody or the antigen-binding fragment thereof of embodiment 16 or embodiment 17 comprising:

- 5           a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 46, 47, and 48, respectively;
- b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 49, 50, and 48, respectively;
- c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising  
10           the amino acid sequences of SEQ ID NO: 51, 52, and 48, respectively;
- d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 76, respectively; or
- e. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 111, respectively.

15   [0286] Embodiment 19 is the antibody or the antigen-binding fragment thereof of embodiment 16 or embodiment 17 comprising:

- a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 55, 56, and 57, respectively;
- b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising  
20           the amino acid sequences of SEQ ID NO: 58, 59, and 57, respectively;
- c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 60, 61, and 57, respectively; or
- d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 62, 63, and 64, respectively.

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[0287] Embodiment 20 is the antibody or the antigen-binding fragment thereof of any one of embodiments 16 to 19, particularly embodiment 18, comprising a VHH comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 65

[0288] Embodiment 21 is the antibody or the antigen-binding fragment thereof of embodiment 20, comprising a VHH comprising SEQ ID NO: 65.

[0289] Embodiment 22 is the antibody or the antigen-binding fragment thereof of any one of embodiments 16 to 21, particularly embodiment 19, comprising a VHH comprising an amino  
5 acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 66.

[0290] Embodiment 23 is the antibody or the antigen-binding fragment thereof of embodiment 22, comprising a VHH comprising SEQ ID NO: 66.

[0291] Embodiment 24 is the antibody or the antigen-binding fragment thereof of any one of  
10 embodiments 16 to 23, wherein the antibody or antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.

[0292] Embodiment 25 is the antibody or the antigen-binding fragment thereof of any one of embodiments 16 to 24, wherein the IgG Fc domain is a human IgG1 Fc domain.

[0293] Embodiment 26 is the antibody or the antigen-binding fragment thereof of any one of  
15 embodiments 16 to 25, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.

[0294] Embodiment 27 is the antibody or the antigen-binding fragment thereof of any one of embodiments 16 to 26, wherein the human IgG1 Fc domain further comprises one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.

[0295] Embodiment 28 is the antibody or the antigen-binding fragment thereof of any one of  
20 embodiments 16 to 27, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.

[0296] Embodiment 29 is the antibody or the antigen-binding fragment thereof of any one of  
25 embodiments 16 to 28, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.

[0297] Embodiment 30 is the antibody or the antigen-binding fragment of any one of embodiments 1 to 29, wherein the antibody or antigen-binding fragment binds the C2 domain of human CD33.

[0298] Embodiment 31 is the antibody or the antigen-binding fragment of any one of embodiments 1 to 30, wherein the antibody or the antigen-binding fragment thereof does not bind to soluble human CD33 (sCD33).

5 [0299] Embodiment 32 is the antibody or antigen-binding fragment thereof of any one of embodiments 1 to 31, wherein the antibody or the antigen-binding fragment thereof is chimeric.

[0300] Embodiment 33 is the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 32, wherein the antibody or the antigen-binding fragment thereof is human or humanized.

10 [0301] Embodiment 34 is a synthetic polynucleotide encoding the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 33.

[0302] Embodiment 35 is a vector comprising the synthetic polynucleotide of embodiment 34.

[0303] Embodiment 36 is a host cell comprising the vector of embodiment 35.

15 [0304] Embodiment 37 is a pharmaceutical composition, comprising the antibody or the antigen-binding fragment thereof of any one of embodiments 1 to 33 and a pharmaceutically acceptable carrier.

[0305] Embodiment 38 is a method of treating a hematological cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 36.

20 [0306] Embodiment 39 is the method of embodiment 38, wherein the hematological cancer is acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN).

25 [0307] Embodiment 40 is a method of producing the antibody or the antigen-binding fragment thereof of any one of embodiments 1-33, comprising culturing a cell comprising a polynucleotide encoding the antibody or the antigen-binding fragment thereof under conditions to produce the antibody or the antigen-binding fragment, and recovering the antibody or the antigen-binding fragment thereof from the cell or culture.

[0308] Embodiment 41 is a method of producing a pharmaceutical composition comprising the antibody or the antigen-binding fragment thereof of any one of embodiments 1-33, comprising



combining the antibody or the antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[0309] Embodiment 42 is a CD33/V $\delta$ 2 multispecific antibody comprising a CD33 antibody or an antigen-binding fragment thereof and a V $\delta$ 2 antibody or an antigen-binding fragment thereof,

5 wherein the CD33 antibody or the antigen-binding fragment thereof specifically binds to mCD33, and

wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof specifically binds to the V $\delta$ 2 chain of the human V $\gamma$ 9V $\delta$ 2 T cell receptor.

[0310] Embodiment 43 the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of embodiment 42, wherein the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- 15
- a. SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively;
  - b. SEQ ID NOs: 18, 19, 14, 15, 16, and 17, respectively;
  - c. SEQ ID NOs: 20, 21, 14, 15, 16, and 17, respectively; or
  - d. SEQ ID NOs: 22, 23, 24, 25, 26, and 17, respectively;

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- 20
- e. SEQ ID NOs: 77, 78, and 79, respectively;
  - f. SEQ ID NOs: 80, 81, and 79, respectively;
  - g. SEQ ID NOs: 82, 83, and 79, respectively; or
  - h. SEQ ID NOs: 84, 85, and 86, respectively.

[0311] Embodiment 44 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of embodiment 42, wherein the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- 25
- a. SEQ ID NOs: 27, 28, 29, 30, 31, and 32, respectively;
  - b. SEQ ID NOs: 33, 34, 29, 30, 31, and 32, respectively;
  - c. SEQ ID NOs: 35, 36, 29, 30, 31, and 32, respectively; or

d. SEQ ID NOs: 37, 38, 39, 40, 41, and 32, respectively;

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

e. SEQ ID NOs: 77, 78, and 79, respectively;

5 f. SEQ ID NOs: 80, 81, and 79, respectively;

g. SEQ ID NOs: 82, 83, and 79, respectively; or

h. SEQ ID NOs: 84, 85, and 86, respectively.

[0312] Embodiment 45 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of embodiment 42, wherein the CD33 antibody or the antigen-binding fragment thereof  
10 comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

a. SEQ ID NOs: 46, 47, and 48, respectively;

b. SEQ ID NOs: 49, 50, and 48, respectively;

c. SEQ ID NOs: 51, 52, and 48, respectively;

15 d. SEQ ID NOs: 53, 54, and 76, respectively; or

e. SEQ ID NOs: 53, 54, and 111, respectively.

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

f. SEQ ID NOs: 77, 78, and 79, respectively;

20 g. SEQ ID NOs: 80, 81, and 79, respectively;

h. SEQ ID NOs: 82, 83, and 79, respectively; or

i. SEQ ID NOs: 84, 85, and 86, respectively.

[0313] Embodiment 46 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of embodiment 42, wherein the CD33 antibody or the antigen-binding fragment  
25 thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

a. SEQ ID NOs: 55, 56, and 57, respectively;

b. SEQ ID NOs: 58, 59, and 57, respectively;

c. SEQ ID NOs: 60, 61, and 57, respectively; or

30 d. SEQ ID NOs: 62, 63, and 64, respectively;

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- e. SEQ ID NOs: 77, 78, and 79, respectively;
- f. SEQ ID NOs: 80, 81, and 79, respectively;
- 5 g. SEQ ID NOs: 82, 83, and 79, respectively; or
- h. SEQ ID NOs: 84, 85, and 86, respectively.

[0314] Embodiment 47 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 44, wherein the CD33 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising an amino acid  
10 sequence at least 95% identical to SEQ ID NO: 42 or 44, and a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO:43 or 45; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain only variable region (VHH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 87.

[0315] Embodiment 48 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment  
15 thereof of embodiment 47 comprising:

- a. a VH comprising the amino acid sequence of SEQ ID NO:42, and a VL comprising the amino acid sequence of SEQ ID NO: 43; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
- b. a VH comprising the amino acid sequence of SEQ ID NO:44, and a VL  
20 comprising the amino acid sequence of SEQ ID NO: 45; and a VHH comprising the amino acid sequence of SEQ ID NO:87.

[0316] Embodiment 49 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42, 45 and 46,  
wherein the CD33 antibody or the antigen-binding fragment thereof comprises a VHH  
25 comprising an amino acid sequence at least 95% identical to SEQ ID NO: 65 or 66; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a VHH comprising an amino acid sequence at least 95% identical to SEQ ID NO: 87.

[0317] Embodiment 50 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of embodiment 49 comprising:

- a. a VHH comprising the amino acid sequence of SEQ ID NO:65; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
  - b. a VHH comprising the amino acid sequence of SEQ ID NO:66; and a VHH comprising the amino acid sequence of SEQ ID NO:87.
- 5   **[0318]** Embodiment 51 is the CD33/Vδ2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42, 44, 47 and 48, comprising:
- a. a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:67; and a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:70, and a light chain comprising an amino acid sequence at least  
10   90% identical to SEQ ID NO:71; or
  - b. a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:67; and a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:72, and a light chain comprising an amino acid sequence at least  
15   90% identical to SEQ ID NO:73; or
- the CD33/Vδ2 multispecific antibody or the antigen-binding fragment thereof of any one of  
embodiments 42, 45, 46, 49 and 50, comprising
- c. a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:67; and a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:68; or
  - d. a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:74; and a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:75.
- Embodiment 51b is the CD33/Vδ2 multispecific antibody or the antigen-binding  
fragment thereof of embodiment 51, comprising:
- a. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy  
25   chain comprising the amino acid sequence of SEQ ID NO:70, and a light chain  
comprising the amino acid sequence of SEQ ID NO:71;
  - b. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy  
chain comprising the amino acid sequence of SEQ ID NO:72, and a light chain  
30   comprising the amino acid sequence of SEQ ID NO:73;

- c. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:68;
- d. a heavy chain comprising the amino acid sequence of SEQ ID NO:74; and a heavy chain comprising the amino acid sequence of SEQ ID NO:75 or
- 5 e. a heavy chain comprising the amino acid sequence of SEQ ID NO:91; and a heavy chain comprising the amino acid sequence of SEQ ID NO:92.

[0319] Embodiment 52 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 51, wherein the multispecific antibody is a bispecific antibody.

- 10 [0320] Embodiment 53 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 52, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.

- [0321] Embodiment 54 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 53, wherein the IgG Fc domain is a human IgG1 Fc domain.
- 15

[0322] Embodiment 55 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 54, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.

- 20 [0323] Embodiment 56 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 55, wherein the human IgG1 Fc domain further comprises one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.

- [0324] Embodiment 57 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 56, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.
- 25

[0325] Embodiment 58 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 57, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.

[0326] Embodiment 59 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 58, wherein CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof binds the C2 domain of human CD33.

[0327] Embodiment 60 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 59, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof does not bind to sCD33.

[0328] Embodiment 61 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 60, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof is chimeric.

10 [0329] Embodiment 62 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 61, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof is human or humanized.

[0330] Embodiment 63 is a synthetic polynucleotide encoding the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 62.

15 [0331] Embodiment 64 is a vector comprising the synthetic polynucleotide of embodiment 63.

[0332] Embodiment 65 is a host cell comprising the vector of embodiment 64.

[0333] Embodiment 66 is a pharmaceutical composition, comprising the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42 to 62 and a pharmaceutically acceptable carrier.

20 [0334] Embodiment 67 is a method of treating a hematological cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 66.

[0335] Embodiment 68 is the method of embodiment 67, wherein the hematological cancer is acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN).

25 [0336] Embodiment 69 is a method of producing the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42-62, comprising culturing a cell

comprising a polynucleotide encoding the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof under conditions to produce the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof, and recovering the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof from the cell or culture.

- 5 [0337] Embodiment 70 is a method of producing a pharmaceutical composition comprising the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42-62, comprising combining the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.
- 10 [0338] Embodiment 71 is the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of embodiments 42-62 or the pharmaceutical composition of embodiment 66 for use in treating a hematological cancer in a subject in need thereof, such as a hematological cancer selected from acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid
- 15 leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN).

## EXAMPLES

### EXAMPLE 1. CD33 ANTIGEN GENERATION

- 20 [0339] Expression constructs encoding the human CD33 extracellular domain (ECD) or its sub-domains were designed based on the sequence of myeloid cell surface antigen CD33 (Uniprot accession # P20138) and its domain annotation with either 6X His-tag sequence (SEQ ID NO: 246) or as a fusion protein to a C34S variant of human serum albumin (HSA) with a 6X His-tag sequence at the C-terminus. Similar expression constructs encoding CD33 (ECD) or its sub-
- 25 domains from cynomolgus monkey (*Macaca fascicularis*) were designed based on NCBI Accession # XP\_005590138.1. The amino acid sequences of the generated antigens are shown in Table 5.
- [0340] For transient expression, the human and cyno CD33 full-length ECD or sub-domain expression constructs were transfected into Expi293 cells (HEK293 derived), using
- 30 Expifectamine as per the manufacturer's guidelines. Following incubation for 5 days at 37 °C

with 8% CO2 on an orbital shaker, cells expressing the target protein were separated via centrifugation. The soluble CD33 proteins bearing his-tags were then purified from the culture media using immobilized metal-ion affinity chromatography (IMAC) facilitated by Ni NTA Sepharose 6 Fast Flow resin (GE Healthcare). Subsequently, purification included buffer exchange into 1X Dubelcco’s Phosphate Saline buffer at pH 7.2 devoid of calcium or magnesium, accomplished using Zeba™ Spin Desalting Columns with a 7K MWCO, 10 mL capacity, in accordance with the manufacturer’s specifications (ThermoScientific Catalog number: 89893).

Table 5. Amino acid sequences of the CD33 antigens.

| Protein AA ID | Description                                           | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------|-------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C33W1         | HSA N-terminal Fusion;<br>CynoCD33ECD-HSA(C34S)-6xHis | DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFR<br>EGAIVSLDSPVATNKLDQEVQEETQGRFRLLGDP SRNNCSLSIVDA<br>RRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRPQILIPGALD<br>PDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTHSSVLIITP<br>RPQDHGTNLTQVKFPGAGVT TERTIQLNVSYASQNPRTDIFLGDG<br>SGKQGVVQGS GSGSENLYFQGVRS SDAHKSEVAHRFKDLGEENF<br>KALVLIAFAQYLQQSPFEDHVKL VNEVTEFAKTCVADESAENCDK<br>SLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDD<br>NPNLPRLVRPEVDMCTAFHDNEETFLKKYL YEIARRHPYFYAPE<br>LLFFAKRYKAAFT ECCQAADKAA CLLPKLDEL RDEGKASSAKQRL<br>KCASLQKFGERA FKAWAVARLSQRFPKAEFAEVSKL VTDLTKVH<br>TECCHGDLLECADD RADLAKYICENQDSISSKLKECCEKPLEKSH<br>CIAEVENDEMPADLPSLAADFVESKD VCKNYAEAKD VFLGMFLY<br>EYARRHPDYSV VLLLRLAKTYETTLEKCCAAADPHECYAKVFDEF<br>KPLVEEPQNLIKQNC ELFELGEYKFQNAL LVRYTKKVPQVSTPTL<br>VEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSVVLNQLCVLHEKT<br>PVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADI<br>CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEK<br>CCKADDKETCFAEEGKKLVAASQAALGLGGGSHHHHHH<br><br>(SEQ ID NO: 1) |



| Protein AA ID | Description                                                                                                             | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------|-------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C33W2         | HSA N-terminal Fusion; Human CD33ECD-Tev-HSA(C34S)-6xHis                                                                | DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYDKNSPVHGYWFR<br>EGAIISRDSPVATNKLDQEVQEETQGRFRLLDGPSRNNSLSIVDA<br>RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE<br>PGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTTHSSVLIITP<br>RPQDHGTNLTQVKFAGAGVTERTIQLNVTVYPQNPTTGIFPGD<br>GSGKQETRAGVVHSGSGSENLYFQGVRSDDAHKSEVAHRFKD<br>LGEENFKALVLIAFAQYLQQSPFEDHVKL VNEVTEFAKTCVADES<br>AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL<br>QHKDDNP NL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY<br>FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDEGKASS<br>AKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTD<br>LTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL<br>LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG<br>MFLYEYARRHPDYSVLLLLRLAKTYETTLEKCCAAADPHECYAK<br>VFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQ<br>VSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC<br>VLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETF<br>TFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFA<br>AFVEKCKADDKETCFAEEGKKLVAASQAALGLGGGSHHHHHH<br>(SEQ ID NO: 2) |
| C33W3         | HSA N-terminal Fusion; hCD33 Ig-like V-type domain (Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus | DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYDKNSPVHGYWFR<br>EGAIISRDSPVATNKLDQEVQEETQGRFRLLDGPSRNNSLSIVDA<br>RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRDAHKSEVA<br>HRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKL VNEVTEFAKTC<br>VADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPE<br>RNECFLQHKDDNP NL PRLVRPEVDVMCTAFHDNEETFLKKYLYEI<br>ARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDE<br>EGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAEFAEV<br>SKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLK<br>ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAE<br>AKDVFLGMFLYEYARRHPDYSVLLLLRLAKTYETTLEKCCAAAD                                                                                                                                                                                                                                                                                                                                                                                                                        |

| Protein AA ID | Description                                                                                                              | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------|--------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|               |                                                                                                                          | <p>PHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR<br/>YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLS<br/>VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVP<br/>KEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLK<br/>AVMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLHHH<br/>HHH</p> <p>(SEQ ID NO: 3)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| C33W4         | HSA N-terminal Fusion; hCD33 Ig-like C2-type domain (Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus | <p>VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPIFSWLSA<br/>APTSLGPRTHSSVLIITPRPQDHGTNLTQVKFAGAGVTTERTIQL<br/>NVTYVPQNPTTGIFPGDGSQKQETRAGVVHDAHKSEVAHRFKDL<br/>GEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESA<br/>ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLO<br/>HKDDNPNLPRPVRPEVDMCTAFHDNEETFLKKYLYEIARRHPYF<br/>YAPELLFFAKRYKAAFTGCCAADKAAACLLPKLDELDEGKASSA<br/>KQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDL<br/>TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECKEPL<br/>EKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG<br/>MFLYEYARRHPDYSVLLLLRLAKTYETTLEKCCAAADPHECYAK<br/>VFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQ<br/>VSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC<br/>VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAET<br/>TFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFA<br/>AFVEKCKKADDKETCFAEEGKKLVAASQAALGLHHHHHHH</p> <p>(SEQ ID NO: 4)</p> |
| C33W8         | hCD33_Ig-like V-Type domain and 6xHis Tag                                                                                | <p>DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYDKNSPVHGYWFR<br/>EGAIISRDSPVATNKLDQEVQEETQGRFRLGDPNRNCSLSIVDA<br/>RRRDNGSYFFRMERGSKYSYKSPQLSVHVTDLTHRGSHHHHHH</p> <p>(SEQ ID NO: 5)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

| Protein AA ID | Description                                                             | Amino Acid Sequence                                                                                                                                                                                                                                                                              |
|---------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C33W9         | hCD33 Ig-like C2-type domain and 6xHis Tag                              | VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPIFSWLSA<br>APTSLGPRTTTHSSVLIITPRPQDHGTNLTQVKFAGAGVTTERTIQL<br>NVTYVPQNPTTGIFPGDGSQKQETRAGVVHGSHHHHHH<br><br>(SEQ ID NO: 6)                                                                                                                                |
| C33W10        | MuIgV_Hu IgC2 chimera CD33-6xHis_v1                                     | QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLKRGVSLHEDSPVATSDPRQLVQKATQGRFQLLGDPQKHDCSLFIRDA<br>QKNDTGMYYFRVVRPEFVRYSYKKSQSLHVTLSRTPKILIPGTL<br>EPGHSKNLTCSVSWACEQGTPIFSWLSAAPTSLGPRTTTHSSVLIITP<br>RPQDHGTNLTQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGD<br>GSGKQETRAGVVHGSHHHHHH<br><br>(SEQ ID NO: 7)   |
| C33W12        | MuIgV_cyno IgC2 chimera CD33-6xHis_v1                                   | QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLKRGVSLHEDSPVATSDPRQLVQKATQGRFQLLGDPQKHDCSLFIRDA<br>QKNDTGMYYFRVVRPEFVRYSYKKSQSLHVTLSRTPQILIPGAL<br>DPDHSKNLTCSVPWACEQGTPIFSWMSAAPTSLGLRTHSSVLIIT<br>PRPQDHGTNLTQVKFPGAGVTTERTIQLNVSYSQNPRTDIFLGD<br>GSGRKARKQGVVQGSHHHHHH<br><br>(SEQ ID NO: 8)      |
| C33W49        | Human CD33 ECD (D18-H259) from Uniprot P20138 with C-terminal 6xHis tag | DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYDKNSPVHGYWFR<br>EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNCSLSIVDA<br>RRRDNGSYFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE<br>PGHSKNLTCSVSWACEQGTPIFSWLSAAPTSLGPRTTTHSSVLIITP<br>RPQDHGTNLTQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGD<br>GSGKQETRAGVVHGSHHHHHH<br><br>(SEQ ID NO: 9) |

| Protein AA ID | Description                                                            | Amino Acid Sequence                                                                                                                                                                                                                                                                               |
|---------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C33W50        | Cyno CD33 ECD (D37-Q274) from XP_005590138.1 with C-terminal 6xHis tag | DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFR<br>EGAIVSLDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA<br>RRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRPQILIPGALD<br>PDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTHSSVLIITP<br>RPQDHGTNLTQCQVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDG<br>SGKQGVVQGSHHHHHH<br><br>(SEQ ID NO: 10) |
| C33W51        | Human CD33 ECD (D18-G241) from Uniprot P20138 with 6xHis tag           | DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYDKNSPVHGYWFR<br>EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA<br>RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE<br>PGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTHSSVLIITP<br>RPQDHGTNLTQCQVKFAGAGVTTERTIQLNVTYVPQNPTTGHHHH<br>HH<br><br>(SEQ ID NO: 11)                  |

EXAMPLE 2: GENERATION OF ANTI-CD33 ANTIBODIES

[0341] The production process of anti-CD33 antibodies involved the use of Ablexis transgenic mice technologies. Initially, AlivaMab mice engineered to yield human/mouse immunoglobulins were immunized with recombinant human CD33 protein. Lymphocytes were obtained from secondary lymphoid organs and either fused with FO mouse myeloma cells for hybridoma generation or individually sorted via FACS. Hybridoma supernatants underwent screening using MSD electrochemiluminescence to detect binding to human CD33 ECD-overexpressing HEK cells. Positive samples were further evaluated by FACS for confirmation of binding to the overexpressed CD33 ECD, and confirmed cell binders underwent light chain isotyping through ELISA.

[0342] The single cell sorting supernatants were screened for binding to recombinant human CD33 protein via MSD electrochemiluminescence. Hits exhibiting the desired binding profile were selected and sequenced for further analysis. The process of V region cloning ensued,

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involving cDNA synthesis from RNA using the Smarter cDNA synthesis kit, followed by amplification of VH and VL fragments via PCR. Subsequently, the VH and VL genes were cloned directionally into Lonza mother vectors (VH and VL) using the In-Fusion® HD Cloning Kit. Confirmation of complete V-gene fragments was done through Sanger sequencing of mini-prepped DNAs.

[0343] The subsequent step involved the expression of anti-CD33 antibodies in ExpiCHO-S™ cells through transient transfection using purified plasmid DNA encoding the proteins. The cells were maintained in suspension using ExpiCHO™ expression medium under specific environmental conditions. Transfection was conducted using the ExpiFectamine™ CHO transfection kit, and culture supernatants were harvested after seven days.

[0344] Protein purification was carried out by loading the filtered cell culture supernatant onto a MabSelect Sure Protein A column, followed by washing and elution steps. Eluted protein fractions were neutralized and pooled, followed by filtration. The quality of the purified protein was assessed using analytical size exclusion HPLC via an Agilent HPLC system.

[0345] The amino acid sequences of the CDRs and the heavy and light chain variable regions of exemplary antibodies generated through this method are outlined in Table 6 and Table 7, respectively. The polynucleotide sequences are outlined in Table 8.

Table 6. List of CDR Regions for CD33 Antibodies Generated from Ablexis Mice.

|                                     | CDR1 (SEQ ID NO:)      | CDR2 (SEQ ID NO:)      | CDR3 (SEQ ID NO:) |
|-------------------------------------|------------------------|------------------------|-------------------|
| CD33 antibody name:<br>C33SB1SC1494 |                        |                        |                   |
| Heavy (AbM)                         | GFTFDDYTMH (12)        | LVSWSGGSAY (13)        | ESAYNAYFDY (14)   |
| Light (AbM)                         | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (KABAT)                       | DYTMH (18)             | LVSWSGGSAYYVDSVKG (19) | ESAYNAYFDY (14)   |
| Light (CHOTHIA)                     | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (CHOTHIA)                     | GFTFDDY (20)           | SWSGGS (21)            | ESAYNAYFDY (14)   |
| Light (CHOTHIA)                     | KSSQSVLYSSNNKNYLA (15) | WASTRES (16)           | HQFYITPYT (17)    |
| Heavy (IMGT)                        | GFTFDDYT (22)          | VSWSGGSA (23)          | AKESAYNAYFDY (24) |
| Light (IMGT)                        | QSVLYSSNNKNY (25)      | WAS (26)               | HQFYITPYT (17)    |
| CD33 antibody name:<br>C33F1318     |                        |                        |                   |

|                 |                    |                        |                   |
|-----------------|--------------------|------------------------|-------------------|
|                 | CDR1 (SEQ ID NO:)  | CDR2 (SEQ ID NO:)      | CDR3 (SEQ ID NO:) |
| Heavy (AbM)     | GFTFSRYWMS (27)    | NIKRDGSEKY (28)        | NSGTWEFDQ (29)    |
| Light (AbM)     | TRSSGSIASNDVQ (30) | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (KABAT)   | RYWMS (33)         | NIKRDGSEKYYVDAVRG (34) | NSGTWEFDQ (29)    |
| Light (KABAT)   | TRSSGSIASNDVQ (30) | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (CHOTHIA) | GFTFSRY (35)       | KRDGSE (36)            | NSGTWEFDQ (29)    |
| Light (CHOTHIA) | TRSSGSIASNDVQ (30) | EDKQRPS (31)           | QSYDNNNQV (32)    |
| Heavy (IMGT)    | GFTFSRYW (37)      | IKRDGSEK (38)          | ARNSGTWEFDQ (39)  |
| Light (IMGT)    | SGSIASND (40)      | EDK (41)               | QSYDNNNQV (32)    |

Table 7. List of Variable Regions for CD33 Antibodies Generated from Ablexis Mice.

|                       |                                                                                                                                                                  |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CD33<br>Antibody Name | Amino acid sequence of Variable Regions                                                                                                                          |
| C33SB1SC1494          | VH:<br><br>EVQLVESGGDVVQPGGSLRLSCAASGFTFDDYTMHWVRQAPGKGLEWVSLVSW<br>SGGSAYYVDSVKGRFTISRDN SKNSLYLQMNSLKSEDTALYYCAKESAYNAYFD<br>YWQGTLTVSS<br><br>(SEQ ID NO: 42) |
| C33SB1SC1494          | VL:<br><br>DIVMTQSPDSLAVSLGERATIIYCKSSQSVLYSSNNKNYLAWYQQKAGQPPELLIY<br>WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVALYYCHQFYITPYTFGGGTKVEI<br>K<br><br>(SEQ ID NO: 43)     |
| C33F1318              | VH:<br><br>EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWMANIKR<br>DGSEKYYVDAVRGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCARNSGTWEFDQ<br>WGQGTLTVSS<br><br>(SEQ ID NO: 44) |
| C33F1318              | VL:<br><br>NFMLTQPHSVSESPGKTVIISCTRSSGSIASNDVQWYQQRPGSSPTIVIEDKQRPSG<br>VPDRFSGSIDSSNSASLTISGLKTEDEADYYCQSYDNNNQVFGGGTKLTVL<br><br>(SEQ ID NO: 45)               |

Table 8. Full chain polynucleotide sequences for the following CD33 antibodies:

C33SB1SC1494\_041E121SV7\_1.VR1  
C33F1318 heavy and light

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|                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C33SB1S<br>C1494_0<br>41E121S<br>V7_1.VR<br>1 –<br>Heavy<br>(INS1593<br>46)<br><br>(SEQ ID<br>NO: 97) | GAGGTGCAGCTGGTGGAGTCTGGGGGAGACGTGGTACAGCCTGGGGGGTCCCTGAGAC<br>TCTCCTGTGCAGCCTCTGGATTACCTTTGATGATTATACCATGCACTGGGTCCGTCAAG<br>CTCCGGGGAAGGGTCTGGAGTGGGTCTCTTGTAGTTGGAGTGGTGGTAGCGCATAC<br>TATGTAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACAGCAAAAACCTCCCT<br>GTATCTGCAAATGAACAGTCTGAAATCTGAGGACACCGCCCTGTATTACTGTGCAAAAG<br>AAAGTGCATATAATGCCTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCC<br>TCA |
| C33SB1S<br>C1494_0<br>41E121S<br>V7_1.VR<br>1 – Light<br>(INS1593<br>45)<br><br>(SEQ ID<br>NO: 98)    | GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCAC<br>CATCTACTGCAAGTCCAGCCAGAGTGTGTTTATACAGCTCCAACAATAAGAACTACTTAG<br>CTTGGTACCAACAGAAGGCAGGACAGCCTCCTGAACTGCTCATTACTGGGCATCTACC<br>CGGGAGTCCGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTGGGACAGATTTCACTCT<br>CACCATCAGCAGCCTGCAGGCTGAGGATGTGGCACTTTATTACTGTCACCAATTTTATAT<br>TACTCCGTACACTTTTGGCCAGGGGACCAAGGTGGAGATCAAA                  |
| C33F131<br>8 –<br>Heavy<br>(INS1719<br>43)<br>(SEQ ID<br>NO: 99)                                      | GAGGTCCAACCTTGTGGAGTCAGGCGGCGGCCTCGTGCAACCAGGGGGTAGTTTGCGCCT<br>GAGTTGCGCTGCCAGCGGGTTTACTTTCTCACGATATGGATGAGTTGGGTTCGACAGG<br>CACCTGGGAAGGGACTCGAGTGGATGGCTAATATCAAGAGGGATGGATCTGAAAAATA<br>CTATGTGCGATGCCGTCAGGGGTCGATTTACCATTCTCGTGACAATGCTAAAAACTCCCT<br>TTACCTGCAAATGAACAGTTTGAGAGCTGAAGATACAGCAGTCTATTATTGTGCTCGCA<br>ATAGCGGCACTTGGGAATTTGACCAATGGGGACAAGGTACACTTGTAACAGTCTCCTCA    |
| C33F131<br>8 – Light<br>(INS1719<br>44)<br>(SEQ ID<br>NO: 100)                                        | AATTTTATGCTTACACAGCCCCATTCTGTTTCCGAAAAGCCCAGGCAAGACCGTTATCATC<br>TCTTGACCCCGATCAAGCGGGTCTATAGCTTCCAACGACGTACAATGGTACCAACAGAG<br>GCCAGGCAGCAGTCCCACCATTTGTCAATTTATGAGGATAAACAGCGTCCATCTGGAGTAC<br>CCGACCGTTTTAGTGGATCTATTGATTCCAGCTCAAACCTCCGCATCTCTGACTATAAGTG<br>GGCTGAAGACTGAGGATGAAGCCGACTACTATTGTCAATCATACGACAACAACAATCA<br>GGTATTGGTGGCGGCACCAAGCTCACCGTGCTT                         |

Single heavy chain CD33 antibodies were derived from a VHH phage display campaign. The amino acid sequences of the CDRs and the heavy chain variable regions (VHHs) of exemplary antibodies generated through this method are outlined in Table 9 and Table 10, respectively. The polynucleotide sequences are outlined in Table 11.

10

Table 9. List of CDR Regions for CD33 antibodies generated from llamas.

|                                                     | CDR1 (SEQ ID NO:) | CDR2 (SEQ ID NO:)     | CDR3 (SEQ ID NO:)                                |
|-----------------------------------------------------|-------------------|-----------------------|--------------------------------------------------|
| CD33 antibody name:<br>JL45-VHH                     |                   |                       |                                                  |
| Heavy (AbM)                                         | GSIFSIFDMG (46)   | RITNGGITN (47)        | DIAANIGSNILYDDY (48)                             |
| Heavy (KABAT)                                       | IFDMG (49)        | RITNGGITNYLDSVKG (50) | DIAANIGSNILYDDY (48)                             |
| Heavy (CHOTHIA)                                     | GSIFSIF (51)      | TNGGI (52)            | DIAANIGSNILYDDY (48)                             |
| Heavy (IMGT)                                        | GSIFSIFD (53)     | ITNGGIT (54)          | YADIAANIGSNILYDDY (76) or<br>YADIAANIGSNIL (111) |
| CD33 antibody name:<br>AB266_1_CD33_<br>B12_P09.VR1 |                   |                       |                                                  |
| Heavy (AbM)                                         | GSIFSINAMG (55)   | RITGGGATD (56)        | DLVTRVGSDLLYDDY (57)                             |
| Heavy (KABAT)                                       | INAMG (58)        | RITGGGATDYVDSVKG (59) | DLVTRVGSDLLYDDY (57)                             |
| Heavy (CHOTHIA)                                     | GSIFSIN (60)      | TGGGA (61)            | DLVTRVGSDLLYDDY (57)                             |
| Heavy (IMGT)                                        | GSIFSINA (62)     | ITGGGAT (63)          | YADLVTRVGSDLLYDDY (64)                           |

Table 10. Heavy Chain Variable Regions for CD33 antibodies generated from llamas.

| CD33 Antibody Name           | Amino acid sequence of Variable Regions                                                                                                                                |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| JL45                         | VHH:<br><br>QVQLVESGGGLVQAGGSLRLSCEASGSIFSIFDMGWYRRPPGAQRELVARITNGGI<br>TNYLDSVKGRFSISRDNANTVYLQMNSLNAEDTAVYYCYADIAANIGSNILYDD<br>YWGQGTQVTSS<br><br>(SEQ ID NO: 65)   |
| AB266_1_CD33_<br>B12_P09.VR1 | VHH:<br><br>EVQVVESGGGLVQGGGSLRLSCAASGSIFSINAMGWYHQAPGKQRELVARITGGG<br>ATDYVDSVKGRFTISLDSAKSTVYLQMNSLKPEDTAVYHICYADLVTRVGSDLLY<br>DDYWGQGTQVTSS<br><br>(SEQ ID NO: 66) |

5 Table 11. Full chain polynucleotide sequences for the CD33 antibodies:

B266\_1\_CD33\_B12\_P09.VR1  
JL45



| ID                                                           | Polynucleotide sequence                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AB266_1_CD33_B12_P09.VR1 (INS24 1328)<br><br>(SEQ ID NO: 95) | GAAGTTCAAGTTGTCGAATCTGGCGGCGGGCTTGTGCAAGGTGGTGGGTCATTGAGGCTG<br>TCTTGTGCTGCATCTGGTTCCATCTTCAGCATTAAACGCCATGGGTTGGTATCATCAAGCAC<br>CCGGCAAACAGAGGGAAGTGGTGGCAAGAATTACTGGAGGAGGAGCTACCGATTATGTG<br>GATTCGGTTAAGGGTCGCTTCACAATCAGTCTGGATTGAGCTAAGTCTACAGTCTATCTGC<br>AAATGAACTCACTCAAACCAGAAGATACAGCAGTCTACCATTGTTATGCAGATCTTGTGA<br>CAAGAGTGGGATCTGATCTTCTGTACGACGATTATTGGGGTCAAGGAACTCAAGTTACTG<br>TGTC AAGT |
| JL45 (INS24 2239)<br><br>(SEQ ID NO: 96)                     | CAAGTGCAACTTGTGGAAAGTGGTGGCGGACTTGTGCAAGCTGGTGGCTCATTGAGGCTG<br>TCTTGTGAAGCATCTGGCTCAATCTTCAGCATCTTTGATATGGGTTGGTATAGAAGACCTC<br>CCGGGGCTCAGAGAGAACTGGTGGCTAGAATCACAAATGGTGGGATTACCAATTACCTC<br>GATTCAGTTAAGGGAAGGTTCTCTATTTCCAGAGACAATGCCAAGAATACTGTGTACTTG<br>CAAATGAACAGCTTGAATGCCGAAGATACAGCCGTGTACTATTGTTACGCAGATATTGCA<br>GCAAACATTGGCTCAAACATTCTGTACGATGACTATTGGGGTCAAGGAACACAAGTTACA<br>GTCAGTTCT   |

EXAMPLE 3. GENERATION OF BISPECIFIC CD33 X Vδ2 ANTIBODIES.

[0346] Bispecific molecules were developed through the implementation of knob-into-hole mutations, creating heterodimers consisting of a CD33 binder fused to Fc and a Vδ2 binder fused to Fc In these constructs, the CD33 binder is located on the Hole Fc, while the Vδ2 binder is on the Knob Fc. To achieve this, in an embodiment, the CD33 VH and human CH1 constant region are combined with a hinge on Fc, featuring several mutations: L234A/L235A/D265S\_M252Y/S254T/T256E\_T366S/L368A/Y407V\_H435R/Y436F; and the Vδ2 binder is fused to the hinge and Knob Fc, incorporating the following mutations: C220S\_L234A/L235A/D265S\_M252Y/S254T/T256E\_T366W. Regarding the CD33xVδ2 bispecific antibody, the Vδ2 arm may be prepared as described in the Examples section of patent application WO 2015/156673A1 and WO 2023/037333 A1. The Vδ2 binding sequences utilized were derived from WO2023/037333 (see Table 10 and Table 11 below). The art already provides the necessary teachings for the person skilled in the art to prepare the CD33xVδ2 bispecific antibody of the disclosure, having knowledge of the actual sequences of the two arms, as provided herein.

[0347] The AAS mutations (L234A/L235A/D265S) are deliberately introduced into the Fc portion of both heavy chains to reduce the Fc receptor binding. Additionally, the YTE mutations

(M252Y/S254T/T256E) are incorporated into both heavy chains of to extend the bispecific antibodies' half-life.

[0348] The RF mutations are introduced on the hole heavy chain to aid in purification. These RF mutations may be introduced on the hole heavy chain of the CD33 arm, as disclosed  
5 hereinabove or as in SEQ ID NO. 34, or on the hole heavy chain of the Vδ2 arm as in SEQ ID NO. 35. Alternatively, these RF mutations may be introduced on both heavy chains.

[0349] The bispecific antibody CD33xVδ2 may be produced by cultivation of recombinant Chinese hamster ovary cells, followed by isolation, chromatographic purification, and formulation.

10 [0350] In particular, the protein molecules—each CD33 and Vδ2 arm— may be co-produced in a CHO cell line by co-transfection of each of the encoding expression plasmids and may be purified using a two-step process involving ProA capture followed by CH1 affinity capture. Initially, the antibodies may undergo purification via a Mab Select SuRe Protein A column from GE Healthcare. The column may be prepped with PBS at pH 7.2 and then loaded  
15 with fermentation supernatant at a flow rate of 2 mL/min. Following loading, the column may undergo a wash with 4 column volumes of PBS, succeeded by elution using 30 mM sodium acetate at pH 3.5. The fractions containing protein peaks, detected by absorbance at 280 nm, may be combined and their pH neutralized to 5.0 using a 1% solution of 3 M sodium acetate at pH 9.0.

20 [0351] Variants of the sequences disclosed herein preferably comprise conservative modifications of the disclosed sequences. “Conservative modifications” refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid modifications. Conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are those in which  
25 the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine,  
30 threonine, tyrosine, tryptophan), aromatic side chains (e.g., phenylalanine, tryptophan, histidine,

tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan et al., (1988) Acta Physiol Scand Suppl 643:55-67; Sasaki et al., (1988) Adv Biophys 35:1-24). Amino acid substitutions to the antibodies of the disclosure may be made by known methods for example by PCR mutagenesis (U.S. Patent No. 4,683,195). Alternatively, libraries of variants may be generated for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting variants may be tested for their characteristics using assays described herein.

Table 12. List of CDR Regions for Vδ2 antibody generated from llamas

|                               |                   |                        |                          |
|-------------------------------|-------------------|------------------------|--------------------------|
| Vδ2 antibody name:<br>LAVA177 | CDR1 (SEQ ID NO:) | CDR1 (SEQ ID NO:)      | CDR1 (SEQ ID NO:)        |
| Heavy (AbM)                   | GRPFSNYGMG (77)   | GISWSGGSTD (78)        | VFSGAETAYYPSDDYDY (79)   |
| Heavy (KABAT)                 | NYGMG (80)        | GISWSGGSTDYADSVKG (81) | VFSGAETAYYPSDDYDY (79)   |
| Heavy (CHOTHIA)               | GRPFSNY (82)      | SWSGGS (83)            | VFSGAETAYYPSDDYDY (79)   |
| Heavy (IMGT)                  | GRPFSNYG (84)     | ISWSGGST (85)          | AAVFSGAETAYYPSDDYDY (86) |

Table 13. Heavy Chain Variable Region for Vδ2 antibody generated from llamas

|                   |                                                                                                                                                                          |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vδ2 antibody name | Amino acid sequence of Variable Regions                                                                                                                                  |
| LAVA177           | VHH:<br><br>EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPGKKREFVAGISWSGGSTD<br>YADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQG<br>TQVTVSS<br><br>(SEQ ID NO:87) |

[0352] In these constructs, the CD33 binder was located on the Hole Fc, while the Vδ2 binder was on the Knob Fc. To achieve this, the CD33 VH and human CH1 constant region were combined with a hinge on Fc, featuring several mutations—

L234A/L235A/D265S\_M252Y/S254T/T256E\_T366S/L368A/Y407V\_H435R/Y436F. These mutations were deliberately introduced into the Fc portion of both heavy chains to reduce the Fc receptor binding. Additionally, the YTE mutations (M252Y/S254T/T256E) were incorporated into both heavy chains of to extend the bispecific antibodies’ half-life.

5 [0353] In the case of CD33 VHH binders, the VHH was combined with a hinge on Fc, featuring several mutations —

L234A/L235A/D265S\_M252Y/S254T/T256E\_T366S/L368A/Y407V\_H435R/Y436F.

[0354] The Vδ2 binder was fused to the hinge and Knob Fc, incorporating the following mutations: C220S\_L234A/L235A/D265S\_M252Y/S254T/T256E\_T366W.

10 [0355] The RF mutations were introduced on the Fc region of the heavy chain to aid in purification.

[0356] The molecules were produced in a CHO cell line by co-transfection of the encoding expression plasmids and purified using a two-step process involving ProA capture followed by CH1 affinity capture. Initially, the antibodies underwent purification via a Mab Select SuRe

15 Protein A column from GE Healthcare. The column was prepped with PBS at pH 7.2 and then loaded with fermentation supernatant at a flow rate of 2 mL/min. Following loading, the column underwent a wash with 4 column volumes of PBS, succeeded by elution using 30 mM sodium acetate at pH 3.5. The fractions containing protein peaks, detected by absorbance at 280 nm, were combined and their pH neutralized to 5.0 using a 1% solution of 3 M sodium acetate at pH  
20 9.0. Subsequently, the antibodies underwent further purification through CH1 capture in case the anti-CD33 arm was an Fab and were eluted using a histidine buffer.

[0357] Table 14 presents the heavy and/or light chains for exemplary CD33 X Vδ2 bispecific antibodies.

Table 14: CD33 X Vδ2 bispecific antibodies

| Bispecific ID: |                                                       | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                    |
|----------------|-------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GD33B273       | Heavy chain 1<br>LAVA177 (Vδ2 arm)<br>(SEQ ID NO: 67) | EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSE<br>PKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE<br>PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE<br>ALHNHYTQKSLSLSPGK |

| Bispecific ID: |                                                           | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------|-----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                | Heavy Chain 2<br>JL45 (CD33 arm)<br>(SEQ ID NO: 68)       | QVQLVESGGGLVQAGGSLRLSCEASGSIFSIFDMGWYRRPPGA<br>QRELVARITNGGITNYLDSVKGRFSISRDNANTVYLQMNSLN<br>AEDTAVYYCYADIAANIGSNILYDDYWGGQTQVTVSSEPKSSD<br>KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVS<br>VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT<br>VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY<br>LPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT<br>TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNR<br>FTQKSLSLSPGK                                                                                                        |
| GD33B112       | Heavy chain 1<br>LAVA177 (Vδ2 arm)<br><br>(SEQ ID NO: 67) | EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNANTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSE<br>PKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE<br>PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVME<br>ALHNHYTQKSLSLSPGK                                                                                                    |
|                | Heavy chain 2<br>C33SB1SC1494<br>(SEQ ID NO: 70)          | EVQLVESGGDVVQPGGSLRLSCAASGFTFDDYTMHWVRQAPG<br>KGLEWVSLVSWSGGSAYYVDSVKGRFTISRDNKNSLYLQMN<br>SLKSEDTALYYCAKESAYNAYFDYWGQGTQVTVSSASTKGPS<br>VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV<br>HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV<br>DKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITR<br>EPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN<br>STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK<br>GQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWES<br>NGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCS<br>VMHEALHNRFTQKSLSLSPGK |
|                | Light chain 2<br>C33SB1SC1494<br>(SEQ ID NO: 71 )         | DIVMTQSPDSLAVSLGERATYICKSSQSVLYSSNNKNYLAWYQ<br>QKAGQPPELLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE<br>DVALYYCHQFYITPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL<br>KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD<br>SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN<br>RGEC                                                                                                                                                                                                                                                         |
| GD33B116       | Heavy chain 1<br>LAVA177 (Vδ2 arm)<br>(SEQ ID NO: 67)     | EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNANTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSE<br>PKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE<br>PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVME<br>ALHNHYTQKSLSLSPGK                                                                                                    |
|                | Heavy chain 2<br>C33F1318<br>(SEQ ID NO: 72)              | EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPG<br>KGLEWMANIKRDGSEKYYVDAVRGRFTISRDNKNSLYLQMN<br>SLRAEDTAVYYCARNSGTWEFDQWGQGTQVTVSSASTKGPSV<br>FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH<br>TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD<br>KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITRE<br>PEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST<br>YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG                                                                                                                        |

| Bispecific ID: |                                                              | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------|--------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                |                                                              | QPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESN<br>GQPENNYKTTTPVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSV<br>MHEALHNRFTQKSLSLSPGK                                                                                                                                                                                                                                                                                      |
|                | Light chain 2<br>C33F1318<br>(SEQ ID NO: 73)                 | NFMLTQPHSVSESPGKTVIISCTRSSGSIASNDVQWYQQRPGSSP<br>TIVYEDKQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYY<br>CQSYDNNNQVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK<br>ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY<br>AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS                                                                                                                                                                 |
| GD33B139       | Heavy chain 1 (Vδ2<br>arm) (SEQ ID NO:<br>74 )               | EVQLVESGGGLVQAGGSLRLSCAASGRPFSSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSE<br>PKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE<br>PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHE<br>ALHNRFTQKSLSLSPGK |
|                | Heavy chain 2<br>AB266_1_CD33_B1<br>2_P09<br>(SEQ ID NO: 75) | EVQVVESGGGLVQGGGSLRLSCAASGSIFSINAMGWYHQAPG<br>KQRELVARITGGGATDYVDSVKGRFTISLDSAKSTVYLQMNSL<br>KPEDTAVYHCYADLVTRVGSDDLDDYWGQGTQVTVSSEPKS<br>SDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVV<br>VSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV<br>LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV<br>YTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY<br>KTTTPVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN<br>HYTQKSLSLSPGK      |

Table 15. Full chain polynucleotide sequences for the following CD33 antibodies:

GD33B273

- 5
- GD33B112 heavy and light  
GD33B116 heavy and light  
GD33B139

|                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GD33B273 –<br>Heavy Chain<br>1<br>(PBD203084)<br><br>(SEQ ID NO:<br>101) | GAAGTTCAGCTGGTTGAAAAGTGGTGGTGGCCTTGTGCAAGCCGGTGGCTCA<br>TTACGCTTGTGTCATGTGCAGCCTCTGGAAGACCTTTCAGCAATTATGGGATG<br>GGGTGGTTTAGACAAGCACCCGAAAGAAACGCGAGTTTGTGCTGGCATT<br>TCATGGAGTGGCGGAAGTACAGATTATGCAGATTCTGTTAAAGGACGCTTT<br>ACAATTAGTCGGGACAACGCAAAGAATACCGTGTATCTCCAGATGAATAG<br>CCTTAAGCCAGAAGATACAGCCGTTTATTATTGTGCCGCTGTTTTCTCTGGT<br>GCTGAAACTGCATACTATCCATCAGACGACTACGATTATTGGGGTCAAGGA<br>ACTCAAGTGACAGTTTCTAGTGAGCCCAAATCTAGCGACAAAACCTCACACA<br>TGCCCAACCGTGCCCAAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCTC<br>TTCCCCCAAAACCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGTC<br>ACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAA<br>CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG<br>AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCCTGC<br>ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTGTCCAACAAA<br>GCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCC |
|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                 | CCGAGAACCACAGGTGTACACCCTGCCCCATCCCCGGGAGGAGATGACCA<br>AGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACA<br>TCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACC<br>ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCA<br>CCGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG<br>ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGTCTCTCTCCCTGTCT<br>CCGGGAAAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| GD33B273 – Heavy<br>Chain 2 (PBD203084)<br><br>(SEQ ID NO: 102) | CAAGTGCAACTTGTGGAAAGTGGTGGCGGACTTGTGCAAGCTGGTGGCTCA<br>TTGAGGCTGTCTTGTGAAGCATCTGGCTCAATCTTCAGCATCTTTGATATGG<br>GTTGGTATAGAAGACCTCCCGGGGCTCAGAGAGAACTGGTGGCTAGAATC<br>ACAAATGGTGGGATTACCAATTACCTCGATTCAAGTTAAGGGAAGGTTCTCT<br>ATTTCCAGAGACAATGCCAAGAATACTGTGTACTTGCAAATGAACAGCTTG<br>AATGCCGAAGATACAGCCGTGTACTATTGTTACGCAGATATTGCAGCAAAC<br>ATTGGCTCAAACATTCTGTACGATGACTATTGGGGTCAAGGAACACAAGTT<br>ACAGTCAGTTCTGAGCCCAAATCTAGCGACAAAACACTACACATGCCACCG<br>TGCCAGCACCTGAAGCCGCCGGGGACCGTCAGTCTTCTCTTCCCCCA<br>AAACCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGTCACATGCGTG<br>GTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAAGTTCAAAGTGGTACGT<br>GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGAGGAGCAGT<br>ACAACAGCACGTACCGTGTGGTCAAGCTCCTCACCGTCTGCACCAAGACT<br>GGCTGAATGGCAAGGAGTACAAGTGCAAGGTGTCCAACAAAAGCCCTCCA<br>GCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC<br>ACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGG<br>TCAGCCTGTCTGCGCCGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG<br>AGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCC<br>GTGCTGGACTCCGACGGCTCCTTCTTCTCTCGTGAGCAAGCTCACCGTGAC<br>AAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA<br>GGCTCTGCACAACCGTTACGCAGAAGTCTCTCTCCCTGTCTCCGGGAAA<br>A          |
| GD33B112 – Heavy<br>Chain 1 (PBD142624)<br><br>(SEQ ID NO: 103) | GAAGTGCAATTAGTGGAAAGTGGTGGTGGTCTGGTGCAGGCTGGCGGAAG<br>CCTGAGACTGTCTTGTGCTGCCTTGGCCGGCCATTTTCCAACACTACGGCATG<br>GGCTGGTTCAGGCAGGCCCTGGCAAGAAAAGAGAGTTTCGTGGCCGGCAT<br>CTCCTGGTCCGGCGGCTCTACAGATTACGCCGACTCCGTGAAGGGCAGATT<br>CACCATCTCTCGGATAATGCCAAGAACACCGTCTACCTGCAGATGAATC<br>CCTGAAGCCTGAGGACACCGCTGTGTACTACTGCGCCGCCGTGTTACGCGG<br>AGCTGAAACCGCTACTATCCTTCCGACGACTACGACTACTGGGGCCAGGG<br>CACCAAGTGACCGTGTCTCTGAGCCCAAATCTAGCGACAAAACACTACAC<br>ATGCCACCGTGCCAGCACCTGAAGCCGCCGGGGACCGTCAGTCTTCTCT<br>CTTCCCCCAAAAACCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGT<br>CACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAAGTTCA<br>ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG<br>GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTTACCCTGCTG<br>CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTGTGGAACAA<br>AGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC<br>CCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACC<br>AAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGAC<br>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGAC<br>CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTC<br>ACCGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGT<br>GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGTCTCTCTCCCTGTC<br>TCCGGGAAAA |
| GD33B112 – Heavy<br>Chain 2 (PBD167215)                         | GAAGTGCACTGGTGGAGAGCGCGGGGACGTAGTGCAACCTGGCGGCAG<br>CCTGAGACTGAGCTGCGCCGCTAGCGGCTTACCTTCGACGACTACACCAT<br>GCACTGGGTGAGACAAGCCCCCTGGCAAGGGCCTGGAGTGGGTGAGCCTGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

|                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (SEQ ID NO: 104)                                                    | <p>TGAGCTGGAGCGGCGGCAGCGCCTACTACGTGGACAGCGTGAAGGGCAGATTACCATCAGCAGAGACAACAGCAAGAACAGCCTGTACCTGCAGATGAACAGCCTCAAGAGCGAGGACACCGCCCTGTACTACTGCGCCAAGGAGAGCGCCTACAACGCCTACTTCGACTACTGGGGCCAAGGCACCCTGGTGACCGTGAAGCAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA</p> <p>CGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTTGTGACAAAACCTCACACATGTCCACCGTGCCAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGTACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCA</p> <p>TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC</p> <p>CCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGTCTGCGCCGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTCGTGAGCAAGCTACCGTGGAACAAGTCTAGATGGCAGCAGGGAAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGGTTCACGCAGAAGAGCCTCTCCCTGTCTCCGGGAAAA</p> |
| <p>GD33B112 – Light Chain 2 (PBD167214)</p> <p>(SEQ ID NO: 105)</p> | <p>GACATCGTGATGACACAGAGCCCTGACAGCCTGGCCGTGAGCCTGGGCGAGAGAGCCACCATCTACTGCAAGAGCTCTCAGAGCGTGCTGTACAGCAGCAACAACAAGAACCTACCTGGCCTGGTATCAGCAGAAGGCCGGACAGCCTCCTGAGCTGCTGATCTACTGGGCTAGCACAAGAGAGAGCGGCGTGCTGACAGATTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAAGCCGAGGACGTGGCCCTGTACTACTGCCATCAGTTCTACATCAGCCCC</p> <p>TTACACCTTTCGGCCAAGGCACCAAGGTGGAGATCAAGCGTACCGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC</p> <p>GCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT</p> <p>CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAAGCAGACTACGAGAAACACAAAGTCTACGCTGCGAAGTCA</p> <p>ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <p>GD33B116 - Heavy Chain 1 (PBD142624)</p> <p>(SEQ ID NO: 106)</p> | <p>GAAGTGCAATTAGTGAAAAGTGGTGGTGGTCTGGTGCAGGCTGGCGGAAGCCTGAGACTGTCTTGTGCTGCCTCTGGCCGGCCATTTTCCAACCTACGGCATGGCTGGTTCAGGCAGGCCCTGGCAAGAAAAGAGAGTTCTGTGGCCGGCAT</p> <p>CTCCTGGTCCGGCGGCTCTACAGATTACGCCGACTCCGTGAAGGGCAGATTACCATCTCTCGGGATAATGCCAAGAACACCGTCTACCTGCAGATGAACCTCCTGAAGCCTGAGGACACCGCTGTGTACTACTGCGCCGCCGTGTTCAGCGG</p> <p>AGCTGAAACCGCTACTATCCTTCCGACGACTACGACTACTGGGGCCAGGACACCAAGTGACCGTGTCTCTGAGCCCAAATCTAGCGACAAAACCTACACATGCCCCACCGTGCCAGCACCTGAAGCCGCCGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGT</p> <p>CACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGG</p> <p>GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCTGCACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTGTGCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC</p>                                                                                                                                                                                                                                                                                                                                                                                           |



|                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                 | AAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGAC<br>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC<br>CACGCCTCCCCTGCTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTC<br>ACCGTGAGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGT<br>GATGCATGAGGCTCTGCACAACCACTACACGCAGAAAGTCTCTCTCCCTGTC<br>TCCGGGAAAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| GD33B116 - Heavy<br>Chain 2 (PBD156894)<br><br>(SEQ ID NO: 107) | GAGGTCCAACCTGTGGAGTCAGGCGGCGGCTCGTGCAACCAGGGGGTAG<br>TTTGCGCCTGAGTTGCGCTGCCAGCGGGTTTACTTTCTCACGATATTGGATG<br>AGTTGGGTTCGACAGGCACCTGGGAAGGGACTCGAGTGGATGGCTAATAT<br>CAAGAGGGATGGATCTGAAAAATACTATGTGCGATGCCGTGAGGGGTGCGATT<br>TACCATTTCTCGTGACAATGCTAAAAACTCCCTTTACCTGCAATGAACAG<br>TTTGAGAGCTGAAGATACAGCAGTCTATTATTGTGCTCGCAATAGCGGCAC<br>TTGGGAATTTGACCAATGGGGACAAGGTACACTTGTAAACAGTCTCCTCAGC<br>CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCAC<br>CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGA<br>ACCGGTGACGGTGTCTGTGAACTCAGGCGCCCTGACCAGCGGCGTGCACA<br>CCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGT<br>GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA<br>TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTT<br>GTGACAAAACCTACACATGTCCACCGTGCCAGCACCTGAAGCCCGGG<br>GGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTCTACATC<br>ACCCGGGAGCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAAGA<br>CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGC<br>CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA<br>GCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT<br>GCAAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCTCTCCA<br>AAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCC<br>CGGGAGGAGATGACCAAGAACCAGGTCAGCCTGTCTGCGCCGTCAAAGG<br>CTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG<br>AGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT<br>TCCTCGTGAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAAC<br>GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGGTTACGCAG<br>AAGAGCCTCTCCCTGTCTCCGGGAAAA |
| GD33B116 – Light<br>Chain 2 (PBD156378)<br><br>(SEQ ID NO: 108) | AATTTTATGCTTACACAGCCCCATTCTGTTCCGAAAGCCCAGGCAAGACC<br>GTTATCATCTCTTGACCCCGATCAAGCGGGTCTATAGCTTCCAACGACGTA<br>CAATGGTACCAACAGAGGCCAGGCAGCAGTCCCACCATTGTCAATTTATGAG<br>GATAAACAGCGTCCATCTGGAGTACCCGACCGTTTTAGTGGATCTATTGAT<br>TCCAGCTCAAACTCCGCATCTCTGACTATAAGTGGGCTGAAGACTGAGGAT<br>GAAGCCGACTACTATTGTCAATCATAACGACAACAACAATCAGGTATTTGGT<br>GGCGGCACCAAGCTCACCGTGCTTGGTCAGCCCAAGGCTGCACCCAGTGT<br>ACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTG<br>GTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG<br>GCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCTCCAA<br>ACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTG<br>AGCAGTGGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGG<br>AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAAATGTTCA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| GD33B139 – Heavy<br>Chain 1 (PBD171082)<br><br>(SEQ ID NO: 109) | GAAGTGCAATTAGTGAAAAGTGGTGGTGGTCTGGTGCAGGCTGGCGGAAG<br>CCTGAGACTGTCTTGTGCTGCCTCTGGCCGGCCATTTTCCAACCTACGGCATG<br>GGCTGGTTCAGGCAGGCCCTGGCAAGAAAAGAGAGTTCTGTGGCCGGCAT<br>CTCCTGGTCCGGCGGCTCTACAGATTACGCCGACTCCGTGAAGGGCAGATT<br>CACCATCTCTCGGGATAATGCCAAGAACACCGTCTACCTGCAGATGAATC<br>CCTGAAGCCTGAGGACACCGCTGTGTACTACTGCGCCGCCGTGTTACAGCGG<br>AGCTGAAACCGCTACTATCCTTCCGACGACTACGACTACTGGGGCCAGGG<br>CACCCAAGTGACCGTGTCTCTGAGCCCAATCTAGCGACAAAACCTCACAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

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|-----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                 | ATGCCCCACCGTGCCAGCACCTGAAGCCGCCGGGGACCGTCAGTCTTCCT<br>CTTCCCCCAAAACCCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGT<br>CACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCA<br>ACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGG<br>GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG<br>CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTGTCCGAACAA<br>AGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC<br>CCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACC<br>AAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGAC<br>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAATAACAAGAC<br>CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTC<br>ACCGTGGACAAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGT<br>GATGCATGAGGCTCTGCACAACCGGTTACGCAGAAGTCTCTCTCCCTGTC<br>TCCGGGAAAA                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| GD33B139 – Heavy<br>Chain 2 (PBD171087)<br><br>(SEQ ID NO: 110) | GAAAGTGCAAGTGGTGGAAGTGGTGGTGGTCTCGTGAGGGCGCGGAAG<br>CCTGAGACTGTCTTGCTGCTTCTGGCTCTATCTTCTCCATCAACGCTATG<br>GGCTGGTATCACCAGGCTCCTGGCAAGCAGCGGGAAGTGGTGGCCCGGAT<br>CACCGGCGGAGGCGCTACCGACTACGTGGACTCCGTGAAGGGCAGATTCA<br>CCATCTCTCTGGATTCTGCCAAGTCCACAGTGACCTGCAGATGAACTCCCT<br>GAAACCCGAGGACACCGCCGTGTACCACTGCTACGCCGATCTGGTCACAG<br>AGTGGGCTCCGACCTGTGTACGACGACTACTGGGGCCAAGGCACCCAGGT<br>GACCGTGTCCAGCGAGCCCAAATCTAGCGACAAAACCTACACATGTCCACC<br>GTGCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCTCTTCCCCC<br>AAAACCCAAGGACACCCTCTACATCACCCGGGAGCCTGAGGTACATGCGT<br>GGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG<br>TGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG<br>TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC<br>TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC<br>AGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC<br>CACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAG<br>GTCAGCCTGTCTGCGCCGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG<br>GAGTGGGAGAGCAATGGGCAGCCGGAGAACAATAACAAGACCACGCCTCC<br>CGTGCTGGACTCCGACGGCTCCTTCTTCTCTCGTGAGCAAGCTCACCGTGA<br>CAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA<br>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAA<br>A |

A further molecule was synthesized and tested. GD33B134 is identical to GD33B273 except the H435R and Y436F substitutions (RF substitutions) are on heavy chain 1 instead of heavy chain 2.

Table 16.

| Bispecific ID: |                                                       | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                   |
|----------------|-------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GD33B134       | Heavy chain 1<br>LAVA177 (Vδ2 arm)<br>(SEQ ID NO: 91) | EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSE<br>PKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE<br>PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHE<br>ALHNRTQKSLSLSPGK |
|                | Heavy Chain 2<br>JL45 (CD33 arm)<br>(SEQ ID NO: 92)   | QVQLVESGGGLVQAGGSLRLSCEASGSIFSIFDMGWYRRPPGA<br>QRELVARITNGGITNYLDSVKGRFSISRDNAKNTVYLQMNSLN<br>AEDTAVYYCYADIAANIGSNILYDDYWGQGTQVTVSSEPKSSD<br>KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVS<br>VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT<br>VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT<br>LPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT<br>TPPVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNH<br>YTQKSLSLSPGK   |

Table 17. The NullxVδ2 antibody, which lacks a CD33 binding arm, and is used for comparison against the bispecific CD33xVδ2 antibodies disclosed herein, has the following sequences:

| Bispecific ID:          |                                                       | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-------------------------|-------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GD33B73<br><br>NullxVδ2 | Heavy chain 1<br>(SEQ ID NO: 95)                      | QITLKESGPTLVKPTQTLTLCTFSGFSLSTSGMGVSWIRQPPGK<br>ALEWL AHYIWDDDKRYNPSLKSRLTITKDTSKNQVVLTMNM<br>DPVDTATYYCARLYGFTYGFAYWGQGTLVTVSSASTKGPSVF<br>LAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTF<br>AVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV<br>EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTC<br>VVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ<br>VYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY<br>KTTTPVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN<br>RFTQKSLSLSPGK |
|                         | Light chain 1<br>(SEQ ID NO: 96)                      | DIVMTQSPDSLAVSLGERATINCRASQSVDYNGISYMHWYQQK<br>PGQPPKLLIYAASNPESGVPDRFSGSGSGTDFTLTISSLQAEDVA<br>VYYCQQIIEDPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT<br>ASVVKLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDT<br>YLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                             |
|                         | Heavy chain 2<br>LAVA177 (Vδ2 arm)<br>(SEQ ID NO: 67) | EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPG<br>KKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNS<br>LKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSSEP<br>KSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCV<br>VSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS<br>VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ<br>VYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN<br>YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGK                                                                                                 |

EXAMPLE 4. BISPECIFIC ANTIBODIES TARGETING CD33 X V $\delta$ 2 EXHIBIT BINDING  
AFFINITY TOWARD V $\gamma$ 9V $\delta$ 2 CELLS.

5 [0358] The following materials and methods were employed:

[0359] To evaluate V $\delta$ 2 binding, V $\gamma$ 9V $\delta$ 2 T cells were incubated at 37°C for 60 minutes with varying concentrations (ranging from 300 to 0.00508 nM) of the bispecific antibody GD33B273 or with a variant lacking the CD33 binding arm NullxV $\delta$ 2, aka GD33B73. Detection of bound V $\delta$ 2 antibody was achieved by incubating with MonoRab™ Anti-Rabbit Camelid-anti-VHH  
10 AF647 secondary detection antibody (GenScript, A02019-200) for 30 minutes at 4°C. FACS Lyric (BD) was used to measure samples, and FlowJo software (FlowJo) was employed for data analysis.

[0360] Results revealed that the CD33 x V $\delta$ 2 bispecific antibody GD33B273 binds to V $\delta$ 2 positive cells, e.g., the V $\gamma$ 9V $\delta$ 2 T cells (see FIG. 1). The nullxV $\delta$ 2 antibody GD33B73 also  
15 exhibited binding to the V $\gamma$ 9V $\delta$ 2 T cells.

EXAMPLE 5. ASSESSING BINDING TO SOLUBLE CD33

[0361] Experiments were conducted to demonstrate binding specificity of CD33 X V $\delta$ 2  
20 bispecific antibodies to membrane-bound CD33 (mCD33).

[0362] Materials and Methods: Soluble CD33 (sCD33) containing serum samples from patients (e.g., AML patients) were mixed with specified concentrations of the test CD33 X V $\delta$ 2 bispecific antibody (test molecule). The test molecule's binding to soluble CD33 was determined by capturing soluble CD33 bound to the test molecule using magnetic beads coated with an anti-  
25 molecule antibody. This process effectively removed the formed complex from the solution. Subsequently, the supernatant serum was subjected to analysis using LC-MS (liquid chromatography-mass spectrometry) to identify soluble CD33 in the serum samples. The LC-MS assay was formulated specifically to quantify the quantity of unbound sCD33 in the sera of AML patients subsequent to the addition of the specified antibody. Comparable levels of soluble  
30 CD33 detected in both the test molecule-treated samples and samples not treated with the test

molecule (0 nM samples) indicated a lack of binding between the test molecule and soluble CD33 in the serum. The antibody JL-5 (anti-CD33 Ab, which is not mCD33-specific; WO2023037333 incorporated by reference in its entirety), was used as a positive control to confirm binding to soluble CD33 in the serum. JL5 sequences are disclosed in Table 18.

5     Table 18: JL5 antibody sequences

|                      |                                                                                                                                                |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| JL5 VH<br>(C33B1517) | QVQLQESGPGLVKPSSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHYSTGNIH<br>YNPSLKSRVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDYWGQGTLLVT<br>VSS (SEQ ID NO: 93) |
| JL5 VL<br>(C33B1517) | QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWWYQQFPGTAPKLLIYSNNQRPSGV<br>PDRVSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGPGTKVTVL (SEQ ID<br>NO: 94 )        |

[0363] Results: FIGs. 2A-2D showed limited binding of the various tested CD33xVδ2 bispecific antibodies to sCD33, demonstrating that these antibodies bind specifically to mCD33. In contrast, the reference antibody JL5 is non-specific and displayed dosage dependent binding to sCD33.

10

EXAMPLE 6: BISPECIFIC CD33 X Vδ2 ANTIBODIES CAN MEDIATE CYTOTOXICITY  
AGAINST CD33-EXPRESSING CELLS

[0364] Cytotoxicity assay was conducted using the following materials and methods: THP1 target cells (human leukemia monocytic cell line, available from SIGMA ALDRICH or from  
15     ATCC) were labeled with the CellTrace CFSE proliferation kit (Thermo Scientific, C34554) and then incubated at 37°C alongside bispecific CD33xVδ2 antibody or a negative control antibody (nullxVδ2, aka GD33B73) in the presence of Pan T cells (E) at a 10:1 (E:T) ratio, comprising 200,000 effector cells and 20,000 target cells. An antibody concentration series, starting at 300 nM and including 12 of 4-fold dilutions, was tested for GD33B112, GD33B116, GD33B134,  
20     GD33B273, as well as the Null x Vδ2 negative control (GD33B73). After 72 hours incubation, dead cells were stained using eBioscience Fixable Viability dye eFluor 780 (Thermo Scientific, 65-0865). Flow cytometry was utilized for sample analysis. The determination of cancer cell killing was based on the calculation of live cancer cells as a percentage relative to untreated wells. Plotting and analysis were conducted using Prism software (GraphPad).

[0365] Results of the Cytotoxicity Assay: The measurement of THP1 cell killing was conducted. No observable killing was detected with negative control antibodies. However, bispecific CD33xVδ2 antibodies exhibited the ability to induce killing of THP-1 tumor cells (FIG. 3 and FIG. 6).

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EXAMPLE 7: EVALUATING BISPECIFIC CD33 X Vδ2 ANTIBODIES VIA SURFACE PLASMON RESONANCE ANALYSIS

[0366] A Biacore 8k instrument (Cytiva, [cytivalifesciences.com](https://www.cytivalifesciences.com)) was utilized for SPR binding experiments by capture of the test antibodies onto a CM4 sensor SPR chip with Goat anti-Fc. CD33 antigen was flowed over at 100, 33, 11, and 3.7 nM in single cycle kinetics mode. Running buffer utilized was HBSP+0.05%BSA+3mM EDTA. All instrument maintenance and liquid supply line priming was performed according to manufacturer operating instructions.

15 Table 19.  
SPR binding results:

| Antibodies (CD33 x δ2) | Affinity K <sub>D</sub> (M) |
|------------------------|-----------------------------|
| GD33B273               | 1.75 x 10 <sup>-8</sup>     |
| GD33B112               | 9.37 x 10 <sup>-10</sup>    |
| GD33B116               | 2.76 x 10 <sup>-10</sup>    |
| GD33B139               | 2.24 x 10 <sup>-9</sup>     |

EXAMPLE 8: CHARACTERIZATION OF CD33 CELL BINDING SPECIFICITY FOR BISPECIFIC CD33 X Vδ2 ANTIBODIES VIA FLUORESCENT STAINING AND FLOW CYTOMETRY ANALYSIS

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[0367] Materials and Methods for CD33 Cell Binding:  
[0368] For the CD33 cell binding experiments, 96-well plates were utilized along with an Intellicyt iQue flow cytometry instrument from Sartorius.com. The cells were cultivated, collected, and stained in staining buffer (BD catalog number 554657) supplemented with a

secondary antibody, either Alexa-Fluor 647 goat anti-human IgG from Jackson Immuno Research (jacksonimmuno.com) or Alexa-Fluor 647 rabbit anti-camelid from Genscript (Genscript.com), to detect the antibody test molecules. To enable gating on live cells, the cells were pre-stained with near-IR live/dead stain (Thermofisher catalog number L10119). The test molecules were incubated with cells at 37°C for 1 hour, following which the cells were washed via centrifugation and then suspended in staining buffer. Subsequently, a detection secondary antibody was introduced, the samples were incubated for 30 minutes, washed, suspended again, and finally, the samples were analyzed on the iQue instrument to quantify the fluorescent staining.

10 [0369] Results of CD33 cell binding analysis. The binding of GD33B273, GD33B112, GD33B116, and GD33B139 to CD33 positive THP-1 cancer cells, as well as to the CD33 negative THP-1 CD33 knock-out cell line, is detailed in FIGs 4A-4D. All the molecules exhibited specific binding to CD33 positive THP-1 WT cells while demonstrating no binding to the CD33 knock-out cells. FIG. 4E depicts the cell binding of GD33B273 to an isogenic THP-1\_C2 cell line that specifically express the IgC2 domain of CD33.

#### EXAMPLE 9: EFFICACY OF CD33 X Vδ2 BISPECIFIC ANTIBODIES IN THE PRESENCE OF sCD33

[0370] To further mitigate the potential impact of sCD33 on the efficacy of the bispecific antibody, human recombinant CD33 (rCD33; SEQ ID NO: 9) ECD was utilized as a surrogate for sCD33. A T-cell cytotoxicity assay was used where THP-1 cells and pan-T cells were incubated with increasing amounts of rCD33 protein prior to addition of a dose range of the CD33 X Vδ2 bispecific antibody (*cf. e.g.,* example 3). The average EC50 cell cytotoxicity value in the absence of rCD33 was 0.002 nM (averaged from n=5 T-cell donors). The presence of 5, 10, 25, 50, or 100 ng/mL rCD33 protein resulted in comparable average EC50 values of 0.001, 0.002, 0.001, 0.001, and 0.001 nM, respectively (FIG. 5A and 5C). Similarly, the addition of rCD33 did not affect the specific activation of Vδ2 T cells (FIG. 5B and 5D). In summary, the addition of rCD33 ECD at physiological (~13.04 ng/mL) or high concentrations did not have any impact on the efficacy nor T-cell activation of the bispecific antibody in the T-cell cytotoxicity assay.

EXAMPLE 10: ON-TARGET OFF-TUMOR ASSESSMENT OF CD33 X Vδ2 BISPECIFIC ANTIBODIES

5 [0371] Bispecific CD33 X Vδ2 antibodies (*cf. e.g., example 3*) were further assessed in PBMC cytotoxicity assays with MOLM-13 coculture to evaluate cancer cell cytotoxicity versus healthy monocytes and NK cells. Similar preferential cancer cell cytotoxicity was observed with bispecific GD33B134 and bispecific GD33B273 with comparable average cytotoxicity EC<sub>50</sub> values of 0.004 nM (95% CI = 0.003-0.02 nM) and 0.008 nM (95% CI = 0.0004-0.02 nM),  
10 respectively (FIG. 7A). No significant effects on healthy monocytes and NK cells were observed with both antibodies (FIG. 7B and FIG. 7C). While overall T-cell activation was not observed (FIG. 7D), similar activation of Vδ2 T cells was detected with bispecific GD33B134 and bispecific GD33B273 (FIG. 7E).

15 EXAMPLE 11: CD33 X Vδ2 BISPECIFIC ANTIBODY MEDIATES SELECTIVE CYTOTOXICITY OF CD33+ CANCER CELLS

[0372] FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D show how bispecific CD33xδ2 antibody GD33B273 mediates selective cytotoxicity of CD33+ cancer cells (MOLM-13 and THP-1) and selective  
20 activation of Vδ2 T cells when compared to the antibody lacking the CD33 arm (NullxVδ2, aka GD33B73). T cells from healthy donors were evaluated in T-cell cytotoxicity and activation assays with GD33B273 and NullxVδ2 antibodies and target cell lines. Cancer cells cytotoxicity was determined for GD33B273 in flow-cytometry based assays at 72 hours with MOLM-13 and THP-1 as target cancer cells (FIG. 8A and 8C). The assay was conducted at a relative E:T ratio  
25 of 0.5:1. T-cell activation was assessed by evaluating the expression of CD25 on Vδ2 T cells (FIG. 8B and 8D) and was measured after 72 hours.

FIG. 8E and 8F show how bispecific CD33xδ2 antibody GD33B273 mediates selective cytotoxicity of THP-1\_C2 cells expressing CD33 IgC2 domain (FIG. 8E) and selective activation of Vδ2 T cells (FIG. 8F) when compared to the antibody lacking the CD33 arm  
30 (NullxVδ2, aka GD33B73). FIG. 8G and 8H show how bispecific CD33xδ2 antibody



GD33B273 does not mediate selective cytotoxicity of OCI-Ly10 / CD33negative cells, i.e., not expressing CD33 (FIG. 8G), and does not mediate or shows minimal selective activation of Vδ2 T cells (FIG. 8H) when compared to the antibody lacking the CD33 arm (NullxVδ2, aka GD33B73). OCI-Ly10 cells are a human-derived cell line and are used as target cancer cells.

5

#### EXAMPLE 12: EFFICACY OF CD33 X Vδ2 ANTIBODY IN AML BM BLAST MODEL

[0373] T cells from healthy donors were tested in T-cell cytotoxicity assays with GD33B273 and AML patient-derived bone marrow (BM) cells, performed at a relative E:T ratio of 2:1. Cytotoxicity of CD33+ blast was assessed 24 hours. DN, donor. CD33xδ2 bispecific antibody GD33B273 was observed to induce potent cytotoxicity of AML BM cells from all tested donors (FIG. 10).

15

#### EXAMPLE 13: EVALUATING BISPECIFIC CD33 X Vδ2 ANTIBODY TOXICITY ON HEMATOPOIETIC CELLS

[0374] Naïve Vδ2+ T cells were isolated from healthy donor PBMCs and used as effectors at 1:1 relative E:T ratio with CD34+ HSPC cells or THP-1 cells as target cells in a CFU assay. HSPC: hematopoietic stem and progenitor cells; CD, cluster of differentiation; CFU, colony forming unit; E:T, effector to target; PBMC, peripheral blood mononuclear cell. GD33B273 showed low risk of hematopoietic toxicity while showing cytotoxicity for the cancer cells THP-1 in a dose response fashion. (FIG. 11). CD34+ HSPC are stem cells from healthy donors, and the presence of surviving robust colonies (light grey bars) shows that the antibody does not impact them. In contrast, THP-1 cancer cells colonies (black bars) decrease survival in a dose-dependent relation to the antibody concentration.

25

#### EXAMPLE 14: EFFICACY OF CD33 X Vδ2 ANTIBODIES IN MOLM-13 PREVENTION MODEL

[0375] The antitumor effect of the CD33 X Vδ2 bispecific antibody GD33B134 (*cf. e.g., example 3*) was evaluated in a prophylactic MOLM-13 admixture tumor model with expanded pan-T cells or enriched Vδ2 T cells as effector cells in T-cell humanized mice. Female NSG (i.e., non-obese diabetic [NOD] severe combined immunodeficiency [scid] gamma or NOD.Cg

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Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ) mice were used to provide a suitable host for reconstituting a human CD3+ T-cell compartment.

[0376] Mice were inoculated subcutaneously (SC) with an admixture of  $1 \times 10^6$  MOLM-13 AML cells and  $5 \times 10^6$  expanded pan-T cells or  $2.5 \times 10^6$  enriched V $\delta$ 2 T cells in Matrigel on Day 0. T-cell humanized mice were given Fc block antibody (a commercial antibody that is used to block the Fc receptor in immune receptor cells) and intravenous immunoglobulin (IVIg) intraperitoneally (IP) at least 30 minutes prior to bispecific antibody dosing to correct for the low Ig environment in NSG mice. In the cohort with expanded pan-T cells as effector cells, NSG mice were randomized into groups of 8 animals and IP treatment was initiated on Day 1 post cell implantation with bispecific antibody GD33B134 at 1, 3, and 10 mg/kg twice weekly for a total of 11 doses. Treatment with bispecific antibody did not affect body weight of the animals. However, some allogeneic responses were observed in MOLM-13 tumors mixed with pan-T cells delaying tumor growth. Percent tumor growth inhibition (TGI) of SC MOLM-13 xenografts was calculated on Day 35 post tumor implantation, when two thirds of animals remained on study in all groups. Statistically significant antitumor efficacy was observed in tumors treated with bispecific antibody at 1, 3, and 10 mg/kg resulting in 99.2%, 99.3%, and 91.2% TGI, respectively, compared to vehicle-treated controls (FIG. 12).

#### EXAMPLE 15: EFFICACY OF A CD33 X V $\delta$ 2 ANTIBODY IN MOLM-13 REGRESSION MODEL

[0377] The antitumor efficacy of the CD33 X V $\delta$ 2 bispecific antibody GD33B134 (*cf. e.g., example 3*) was evaluated in a disseminated MOLM-13 regression model with enriched V $\delta$ 2 T cells as effector cells in humanized mice (female NOG mice). hIL-15 NOG mice were injected intravenously (IV) with  $1 \times 10^6$  MOLM-13 AML cells on Day 0. On Day 3,  $9.4 \times 10^6$  enriched V $\delta$ 2 T cells were injected IV and after 24 hours, T-cell-humanized mice were given Fc block and IVIg at least 30 minutes prior to bispecific antibody dosing. Mice were randomized into groups of 8 animals and IP treatment with 1, 3, and 10 mg/kg bispecific antibody was initiated on Day 4 post cell implantation twice weekly for a total of 8 doses. On Day 16, a second injection of enriched V $\delta$ 2 T cells ( $7.9 \times 10^6$ ) was administered IV. Mice were monitored for survival and

body weight loss. Statistically significant increased life span (ILS) was observed with bispecific antibody GD33B134 at 1, 3, and 10 mg/kg resulting in 117.9%, 112.8%, and 102.6% ILS, respectively, compared with vehicle-treated (DPBS) animals (FIG. 13A). Reduction in body weight (reaching ~23%) was observed in all treated animals following the injection of V $\delta$ 2 T cells. However, no other clinical signs were observed and body weight loss was recovered within a few days from V $\delta$ 2 T-cell administration (FIG. 13B). hIL-15, human interleukin-15; NOG, non-obese diabetic (NOD)/Shi-severe combined immunodeficiency (scid) IL2rgamma(null); DPBS, Dulbecco's phosphate-buffered saline.

10           EXAMPLE 16: A CD33 X V $\Delta$ 2 ANTIBODY MEDIATES ANTI-TUMOR ACTIVITY IN  
DISSEMINATED MOLM-13 IN VIVO MODEL

FIG. 14A and FIG. 14B present the efficacy of the bispecific CD33x $\delta$ 2 antibody GD33B273 in a MOLM-13 regression mice model. In this test, the MOLM-13 cancer cells were labelled with luciferase. This labeled MOLM-13 cancer cells were implanted on Day 0 in the hIL-15 NOG mice. The same mice were injected with the T cells on Day 3 and 16, and dosed with antibody at the indicated doses (0, 0.3, and 3 mg/Kg) on Days 4, 7, 10, 14, 17, 21, and 24 (the dosing period is indicated by the solid bar under the X-axis, i.e. from day 4 to day 24 for both FIGs. 14A and 14B). Results are presented as average tumor radiance (FIG. 14A) and as percent survival (FIG. 14B). Data are only displayed for the period during which two thirds of animals were still alive in a group. FIG. 14A shows an increase in average radiance for the untreated mice (0 mg/Kg) when compared to the treated mice with 0.3 and 3 mg/Kg antibody. FIG. 14A also shows 61% and 54.9% of tumor growth inhibition at day 17 for treated mice with antibody at 0.3 and 3 mg/Kg, respectively, when compared to untreated mice (0 mg/Kg). FIG. 14B shows an increase in the survival of the mice treated with 0.3 and 3 mg/Kg of antibody when compared to untreated mice (0 mg/Kg). ILS, increased life span; TGI, tumor growth inhibition.

## WE CLAIM:

1. An antibody or an antigen-binding fragment capable of specifically binding to membrane-bound human CD33 (mCD33), comprising:
  - 5 a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 46, 47, and 48, respectively;
  - b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 49, 50, and 48, respectively;
  - c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising  
10 the amino acid sequences of SEQ ID NO: 51, 52, and 48, respectively;
  - d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 76, respectively; or
  - e. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 53, 54, and 111, respectively.
- 15 2. A CD33/V $\delta$ 2 multispecific antibody comprising a CD33 antibody or an antigen-binding fragment thereof and a V $\delta$ 2 antibody or an antigen-binding fragment thereof, wherein the CD33 antibody or the antigen-binding fragment thereof specifically binds to mCD33, and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof specifically binds to  
20 the V $\delta$ 2 chain of the human V $\gamma$ 9V $\delta$ 2 T cell receptor.
3. The CD33/V $\delta$ 2 multispecific antibody of claim 2, wherein the CD33/V $\delta$ 2 multispecific antibody comprises:
  - (A) a CD33 antibody or the antigen-binding fragment thereof comprising a heavy chain  
25 CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:
    - a. SEQ ID NOs: 46, 47, and 48, respectively;
    - b. SEQ ID NOs: 49, 50, and 48, respectively;
    - c. SEQ ID NOs: 51, 52, and 48, respectively;
    - 30 d. SEQ ID NOs: 53, 54, and 76, respectively; or

e. SEQ ID NOs: 53, 54, and 111, respectively;  
and a V $\delta$ 2 antibody or the antigen-binding fragment thereof comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- 5           f. SEQ ID NOs: 77, 78, and 79, respectively;  
            g. SEQ ID NOs: 80, 81, and 79, respectively;  
            h. SEQ ID NOs: 82, 83, and 79, respectively; or  
            i. SEQ ID NOs: 84, 85, and 86, respectively; and/or

(B) a CD33 antibody or the antigen-binding fragment thereof comprising a heavy chain CDR1 (33CDR1), a heavy chain CDR2 (33CDR2), and a heavy chain CDR3 (33CDR3)  
10           and a V $\delta$ 2 antibody or the antigen-binding fragment thereof comprising a heavy chain CDR1 (V $\delta$ 2CDR1), a heavy chain CDR2 (V $\delta$ 2CDR2), and a heavy chain CDR3 (V $\delta$ 2CDR3), comprising:

- (a) 33CDR1 SEQ ID NO: 46, 33CDR2 SEQ ID NO: 47, 33CDR3 SEQ ID NO: 48, V  
15            $\delta$ 2CDR1 SEQ ID NO: 77, V $\delta$ 2CDR2 SEQ ID NO: 78 and V $\delta$ 2CDR3 SEQ ID NO: 79;  
(b) 33CDR1 SEQ ID NO: 49, 33CDR2 SEQ ID NO: 50, 33CDR3 SEQ ID NO: 48, V  
             $\delta$ 2CDR1 SEQ ID NO: 80, V $\delta$ 2CDR2 SEQ ID NO: 81 and V $\delta$ 2CDR3 SEQ ID NO: 79;  
(c) 33CDR1 SEQ ID NO: 51, 33CDR2 SEQ ID NO: 52, 33CDR3 SEQ ID NO: 48, V  
             $\delta$ 2CDR1 SEQ ID NO: 82, V $\delta$ 2CDR2 SEQ ID NO: 83 and V $\delta$ 2CDR3 SEQ ID NO: 79;  
20           (d) 33CDR1 SEQ ID NO: 53, 33CDR2 SEQ ID NO: 54, 33CDR3 SEQ ID NO: 76, V  
             $\delta$ 2CDR1 SEQ ID NO: 84, V $\delta$ 2CDR2 SEQ ID NO: 85 and V $\delta$ 2CDR3 SEQ ID NO: 86;  
(e) 33CDR1 SEQ ID NO: 53, 33CDR2 SEQ ID NO: 54, 33CDR3 SEQ ID NO: 111, V  
             $\delta$ 2CDR1 SEQ ID NO: 84, V $\delta$ 2CDR2 SEQ ID NO: 85 and V $\delta$ 2CDR3 SEQ ID NO: 86;  
(f) 33CDR1 SEQ ID NO: 55, 33CDR2 SEQ ID NO: 56, 33CDR3 SEQ ID NO: 57, V  
25            $\delta$ 2CDR1 SEQ ID NO: 77, V $\delta$ 2CDR2 SEQ ID NO: 78 and V $\delta$ 2CDR3 SEQ ID NO: 79;  
(g) 33CDR1 SEQ ID NO: 58, 33CDR2 SEQ ID NO: 59, 33CDR3 SEQ ID NO: 57, V  
             $\delta$ 2CDR1 SEQ ID NO: 80, V $\delta$ 2CDR2 SEQ ID NO: 81 and V $\delta$ 2CDR3 SEQ ID NO: 79;

(h) 33CDR1 SEQ ID NO: 60, 33CDR2 SEQ ID NO: 61, 33CDR3 SEQ ID NO: 57, V  
δ2CDR1 SEQ ID NO: 82, Vδ2CDR2 SEQ ID NO: 83 and Vδ2CDR3 SEQ ID NO: 79;

or

(i) 33CDR1 SEQ ID NO: 62, 33CDR2 SEQ ID NO: 63, 33CDR3 SEQ ID NO: 64, V  
δ2CDR1 SEQ ID NO: 84, Vδ2CDR2 SEQ ID NO: 85 and Vδ2CDR3 SEQ ID NO: 86;

and/or

(C) a VHH comprising the amino acid sequence of SEQ ID NO:65 and a VHH  
comprising the amino acid sequence of SEQ ID NO:87; and/or

(D) a heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a heavy  
chain comprising the amino acid sequence of SEQ ID NO:68.

4. The CD33/Vδ2 multispecific antibody of claim 2, wherein the CD33 antibody or the  
antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2,  
a heavy chain CDR3, a light chain CDR1, a light chain CDR2, and a light chain CDR3,  
comprising the amino sequences of:

- a. SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively;  
b. SEQ ID NOs: 18, 19, 14, 15, 16, and 17, respectively;  
c. SEQ ID NOs: 20, 21, 14, 15, 16, and 17, respectively; or  
d. SEQ ID NOs: 22, 23, 24, 25, 26, and 17, respectively;

and wherein the Vδ2 antibody or the antigen-binding fragment thereof comprises a heavy chain

CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

- e. SEQ ID NOs: 77, 78, and 79, respectively;  
f. SEQ ID NOs: 80, 81, and 79, respectively;  
g. SEQ ID NOs: 82, 83, and 79, respectively; or  
h. SEQ ID NOs: 84, 85, and 86, respectively.

5. The CD33/Vδ2 multispecific antibody or the antigen-binding fragment thereof of claim  
2, wherein the CD33 antibody or the antigen-binding fragment thereof comprises a  
heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, a light chain CDR1, a  
light chain CDR2, and a light chain CDR3, comprising the amino sequences of:

- a. SEQ ID NOs: 27, 28, 29, 30, 31, and 32, respectively;  
b. SEQ ID NOs: 33, 34, 29, 30, 31, and 32, respectively;

c. SEQ ID NOs: 35, 36, 29, 30, 31, and 32, respectively; or

d. SEQ ID NOs: 37, 38, 39, 40, 41, and 32, respectively;

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

5 e. SEQ ID NOs: 77, 78, and 79, respectively;

f. SEQ ID NOs: 80, 81, and 79, respectively;

g. SEQ ID NOs: 82, 83, and 79, respectively; or

h. SEQ ID NOs: 84, 85, and 86, respectively.

6. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of claim  
10 2, wherein the CD33 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino sequences of:

a. SEQ ID NOs: 55, 56, and 57, respectively;

b. SEQ ID NOs: 58, 59, and 57, respectively;

15 c. SEQ ID NOs: 60, 61, and 57, respectively; or

d. SEQ ID NOs: 62, 63, and 64, respectively;

and wherein the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, comprising the amino acid sequences of:

20 e. SEQ ID NOs: 77, 78, and 79, respectively;

f. SEQ ID NOs: 80, 81, and 79, respectively;

g. SEQ ID NOs: 82, 83, and 79, respectively; or

h. SEQ ID NOs: 84, 85, and 86, respectively.

7. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any  
25 one of claims 2 and 4 to 6, wherein the CD33 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 42 or 44, and a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 43  
or 45; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a heavy  
chain only variable region (VHH) comprising an amino acid sequence at least 95%  
30 identical to SEQ ID NO: 87.

8. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 and 4 to 7 comprising:
- 5 a. a VH comprising the amino acid sequence of SEQ ID NO:42, and a VL comprising the amino acid sequence of SEQ ID NO: 43; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
- b. a VH comprising the amino acid sequence of SEQ ID NO:44, and a VL comprising the amino acid sequence of SEQ ID NO: 45; and a VHH comprising the amino acid sequence of SEQ ID NO:87.
- 10 9. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 8, wherein the CD33 antibody or the antigen-binding fragment thereof comprises a VHH comprising an amino acid sequence at least 95% identical to SEQ ID NO: 65 or 66; and the V $\delta$ 2 antibody or the antigen-binding fragment thereof comprises a VHH comprising an amino acid sequence at least 95% identical to SEQ ID NO: 87.
- 15 10. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 9 comprising:
- a. a VHH comprising the amino acid sequence of SEQ ID NO:65; and a VHH comprising the amino acid sequence of SEQ ID NO:87; or
- b. a VHH comprising the amino acid sequence of SEQ ID NO:66; and a VHH comprising the amino acid sequence of SEQ ID NO:87.
- 20 11. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 10, comprising:
- a. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:70, and a light chain comprising the amino acid sequence of SEQ ID NO:71;
- 25 b. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:72, and a light chain comprising the amino acid sequence of SEQ ID NO:73;
- c. a heavy chain comprising the amino acid sequence of SEQ ID NO:67; and a heavy chain comprising the amino acid sequence of SEQ ID NO:68;



- d. a heavy chain comprising the amino acid sequence of SEQ ID NO:74; and a heavy chain comprising the amino acid sequence of SEQ ID NO:75 or
  - e. a heavy chain comprising the amino acid sequence of SEQ ID NO:91; and a heavy chain comprising the amino acid sequence of SEQ ID NO:92.
- 5 12. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 11, wherein the multispecific antibody is a bispecific antibody.
13. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 12, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.
- 10 14. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 13, wherein the IgG Fc domain is a human IgG1 Fc domain.
- 15 15. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 14, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.
16. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 15, wherein the human IgG1 Fc domain further comprises one or more mutations selected from L234A, L235A, and D265S per the EU numbering system.
- 20 17. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 16, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.
18. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 17, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.
- 25 19. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 18, wherein CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof binds the C2 domain of human CD33.

20. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 19, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof does not significantly bind to sCD33.
21. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any  
5 one of claims 2 to 20, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof is chimeric.
22. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 21, wherein the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof is human or humanized.
- 10 23. One or more synthetic polynucleotide(s) encoding the antibody or an antigen-binding fragment of claim 1 or the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 22.
24. One or more vectors comprising the synthetic polynucleotide(s) of claim 23.
25. A host cell comprising the one or more vectors of claim 24 or one or more  
15 polynucleotides of claim 23.
26. A pharmaceutical composition, comprising the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 22 and a pharmaceutically acceptable carrier.
27. A method of treating a hematological cancer in a subject in need thereof, comprising  
20 administering to the subject the pharmaceutical composition of claim 26.
28. The method of claim 27, wherein the hematological cancer is acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN).
- 25 29. The CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 22 for use in the treatment of a hematological cancer.
30. Use of a CD33/ V $\delta$ 2 multispecific antibody or antigen binding fragment thereof of any one of claims 2 to 22 in the manufacture of a medicament for treating a hematological cancer.

31. A method of producing the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 22, comprising culturing a cell comprising polynucleotide(s) encoding the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof under conditions to produce the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof, and recovering the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof from the cell or culture.
32. A method of producing a pharmaceutical composition comprising the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof of any one of claims 2 to 22, comprising combining the CD33/V $\delta$ 2 multispecific antibody or the antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.
33. The antibody or the antigen-binding fragment thereof of claim 1 comprising:
- a. a heavy chain complementarity determining region 1 (CDR1), a heavy chain complementarity determining region 2 (CDR2), and a heavy chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 12, 13, and 14, respectively; and a light chain complementarity determining region 1 (CDR1), a light chain complementarity determining region 2 (CDR2), and a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;
  - b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 18, 19, and 14, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively;
  - c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 20, 21, and 14, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 15, 16, and 17, respectively; or

- d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 22, 23, and 24, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 25, 26, and 17, respectively.
- 5 34. The antibody or antigen-binding fragment thereof of claim 1 comprising:
- a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 27, 28, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- 10 b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 33, 34, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively;
- c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 35, 36, and 29, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 30, 31, and 32, respectively; or
- 15 d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 37, 38, and 39, respectively; and a light chain CDR1, a light chain CDR2, and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 40, 41, and 32, respectively.
- 20
35. The antibody or the antigen-binding fragment thereof of any one of claims 1, 33 or 34, comprising a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ
- 25 ID NO: 42 or 44.
36. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 33 to 35, comprising a VH comprising SEQ ID NO: 42 or 44.
37. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 33 to 36, comprising a light chain variable region (VL) comprising an amino acid sequence

that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 43 or 45.

38. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 33 to 387, comprising a VL comprising SEQ ID NO: 43 or 45.
- 5 39. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 33 to 38, comprising a VH comprising SEQ ID NO: 42, and a VL comprising SEQ ID NO: 43.
40. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 33 to 39, comprising a VH comprising SEQ ID NO: 44, and a VL comprising SEQ ID NO: 45.
41. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 10 40, wherein the antibody or antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.
42. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 41, wherein the IgG Fc domain is a human IgG1 Fc domain.
43. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 15 to 42, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.
44. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 43, wherein the human IgG1 Fc domain further comprises one or more mutations, 20 preferably all three, selected from L234A, L235A, and D265S per the EU numbering system.
45. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 44, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.
46. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 25 45, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.
47. The antibody or the antigen-binding fragment thereof of any one of claim 1, 2 and 33 to 46, wherein the antibody is a heavy chain only antibody.
48. The antibody or the antigen-binding fragment thereof of claim 1, 2 and 33 to 47 wherein 30 the antigen-binding fragment thereof is a single heavy chain variable region (VHH).

49. The antibody or the antigen-binding fragment thereof of claim 47 or claim 48 comprising:
- a. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 55, 56, and 57, respectively;
  - 5 b. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 58, 59, and 57, respectively;
  - c. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 60, 61, and 57, respectively; or
  - 10 d. a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 comprising the amino acid sequences of SEQ ID NO: 62, 63, and 64, respectively.
50. The antibody or the antigen binding fragment of any one of claims 1, 2 and 47 to 49, comprising a VHH comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 65.
- 15 51. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 50, comprising a VHH comprising SEQ ID NO: 65.
52. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 47 to 51, comprising a VHH comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 66.
- 20 53. The antibody or the antigen-binding fragment thereof of any one of claims 1 and 47 to 52, comprising a VHH comprising SEQ ID NO: 66.
54. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 53, wherein the antibody or antigen-binding fragment thereof comprises an immunoglobulin (IgG) Fc domain.
- 25 55. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 54, wherein the IgG Fc domain is a human IgG1 Fc domain.
56. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 55, wherein the human IgG1 Fc domain comprises one or more mutations selected from T366S, L368A, T366W and Y407V per the EU numbering system.
- 30 57. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 56, wherein the human IgG1 Fc domain further comprises one or more mutations,

preferably all three, selected from L234A, L235A, and D265S per the EU numbering system.

58. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 57, wherein the human IgG1 Fc domain further comprises mutations H435R and/or Y436F per the EU numbering system.
59. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 47 to 58, wherein the human IgG1 Fc domain further comprises the triple mutation M252Y/S254T/T256E per the EU numbering system.
60. The antibody or the antigen-binding fragment of any one of claims 1, 2 and 33 to 59, wherein the antibody or antigen-binding fragment binds the C2 domain of human CD33.
61. The antibody or the antigen-binding fragment of any one of claims 1, 2 and 33 to 60, wherein the antibody or the antigen-binding fragment thereof does not significantly bind to soluble human CD33 (sCD33).
62. The antibody or antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 61, wherein the antibody or the antigen-binding fragment thereof is chimeric.
63. The antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 61, wherein the antibody or the antigen-binding fragment thereof is human or humanized.
64. One or more synthetic polynucleotides encoding the antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 63.
65. One or more vectors comprising the synthetic polynucleotide(s) of claim 64.
66. A host cell comprising the vector of claim 655 or the polynucleotide(s) of claim 64.
67. A pharmaceutical composition, comprising the antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 63 and a pharmaceutically acceptable carrier.
68. A method of treating a hematological cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 67.
69. The method of claim 68, wherein the hematological cancer is acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN).

70. The CD33 antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 63 for use in the treatment of a hematological cancer.
71. Use of a CD33 antibody or antigen binding fragment thereof of any one of claims 1, 2 and 33 to 63 in the manufacture of a medicament for treating a hematological cancer.
- 5 72. A method of producing the antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33-63, comprising culturing a cell comprising a polynucleotide encoding the antibody or the antigen-binding fragment thereof under conditions to produce the antibody or the antigen-binding fragment, and recovering the antibody or the antigen-binding fragment thereof from the cell or culture.
- 10 73. A method of producing a pharmaceutical composition comprising the antibody or the antigen-binding fragment thereof of any one of claims 1, 2 and 33 to 63, comprising combining the antibody or the antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.