# ANTI-CD33 ANTIBODIES AND IMMUNOCONJUGATES

## FIELD OF THE INVENTION

**[0001]** The present invention relates to anti-CD33 antibodies and immunoconjugates and methods of using the same.

## BACKGROUND

**[0002]** CD33, a member of the sialic acid binding, immunoglobulin-like lectin family, is a 67-kDa glycosylated transmembrane protein. It is expressed on most myeloid and monocytic leukemia cells in addition to committed myelomonocytic and erythroid progenitor cells. It is not seen on the earliest pluripotent stem cells, mature granulocytes, lymphoid cells, or nonhematopoietic cells. *See* Sabbath et al., *J. Clin. Invest.* 75:756-56 (1985) and Andrews et al., *Blood* 68:1030-5 (1986). CD33 contains two tyrosine residues on its cytoplasmic tail, each of which is followed by hydrophobic residues similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM) seen in many inhibitory receptors.

**[0003]** Monoclonal antibody (mAb)–based therapy has become an important treatment modality for cancer. Leukemia is well suited to this approach because of the accessibility of malignant cells in the blood, bone marrow, spleen, and lymph nodes and the well-defined immunophenotypes of the various lineages and stages of hematopoietic differentiation that permit identification of antigenic targets. Most studies for acute myeloid leukemia (AML) have focused on CD33. Responses with the unconjugated anti-CD33 mAb lintuzumab have had modest single agent and activity against AML and failed to improve patient outcomes in two randomized trials when combined with conventional chemotherapy. The immunoconjugate gemtuzumab ozogamicin (GO; Mylotarg), an anti-CD33 monoclonal antibody conjugated to the antitumor antibiotic calicheamicin, improved survival in a subset of AML patients when combined with standard chemotherapy, but safety concerns led to marketing withdrawal in the US. Additionally, three phase I studies of an anti-CD33-maytansine conjugate (AVE9633; huMy9-6-DM4) in AML patients. The maximum tolerated dose (MTD) was determined only in one of the phase I studies (administration schedule day 1/8) as the other two studies were discontinued before reaching the MTD since no signs of activity were apparent at doses much higher than the saturating dose. The activity of AVE9633 in the phase I administration schedule day 1/8 was modest. Lapusan et al., *Invest. New Drugs* 30:1121-1131 (2012).

**[0004]** There is a need in the art for safe and effective agents that target CD33 for the diagnosis and treatment of CD33-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

## SUMMARY

**[0005]** The ~~invention~~ disclosure provides anti-CD33 antibodies and immunoconjugates and methods of using the same.

**[0006]** In some embodiments, an isolated antibody that binds to CD33 is provided. In some embodiments, the antibody binds to CD33 and has one or more of the following characteristics:

a) binds to recombinant human CD33;
b) binds to recombinant cynomolgus monkey CD33;
c) binds to endogenous CD33 on the surface of human peripheral blood mononucleocytes (PBMCs);
d) binds to endogenous CD33 on the surface of cynomolgus monkey PBMCs;
e) binds to endogenous CD33 on the surface of a cancer cell;
f) binds to endogenous CD33 on the surface of an AML cancer cell;
g) binds to endogenous CD33 on the surface of Molm-13 cells;
h) binds to CD33 comprising a R69G mutation;
i) binds to CD33 Ig V domain;
j) binds to CD33 that is void of N-linked glycosylation at N100;
k) binds to CD33 that is void of N-linked glycosylation at N113;
l) binds to CD33 comprising an S102A mutation;
m) binds to CD33 comprising an S115A mutation;
n) does not bind CD33 Ig C2 domain;
o) competes for human CD33 binding with My9.6 antibody;
p) competes for human CD33 binding with antibody 33H4;
q) competes for human CD33 binding with antibody 23E4;
r) binds to endogenous human CD33 with a Kd of less than 15 nM, less than 10 nM, less than 7 nM, less than 5 nM, or less than 3 nM;
s) binds to recombinant human CD33 with a Kd of less than 10 nM, less than 7 nM, less than 5 nM, or less than 3 nM; and/or
t) binds to recombinant cynomolgus monkey CD33 with a Kd of less than 10 nM, less than 7 nM, less than 5 nM, or less than 3 nM, less than 2 nM, or less than 1 nM.

**[0007]** In ~~some embodiments~~one aspect, an isolated antibody that binds to CD33 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:112; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:113; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:114; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:111; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:115; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:116; (c) HVR-H3

g) a heavy chain variable region comprising the sequence of SEQ ID NO: 86 and a light chain variable region comprising the sequence of SEQ ID NO: 85;

h) a heavy chain variable region comprising the sequence of SEQ ID NO: 88 and a light chain variable region comprising the sequence of SEQ ID NO: 87;

i) a heavy chain variable region comprising the sequence of SEQ ID NO: 90 and a light chain variable region comprising the sequence of SEQ ID NO: 89;

j) a heavy chain variable region comprising the sequence of SEQ ID NO: 92 and a light chain variable region comprising the sequence of SEQ ID NO: 91;

k) a heavy chain variable region comprising the sequence of SEQ ID NO: 94 and a light chain variable region comprising the sequence of SEQ ID NO: 93;

l) a heavy chain variable region comprising the sequence of SEQ ID NO: 96 and a light chain variable region comprising the sequence of SEQ ID NO: 95;

m) a heavy chain variable region comprising the sequence of SEQ ID NO: 98 and a light chain variable region comprising the sequence of SEQ ID NO: 97; or

n) a heavy chain variable region comprising the sequence of SEQ ID NO: 100 and a light chain variable region comprising the sequence of SEQ ID NO: 99.

**[00011]** In some embodiments, an isolated antibody that binds to CD33 is provided. In some embodiments, the antibody binds to CD33 and has one or more of the following characteristics:

a) binds to recombinant human CD33;

b) binds to recombinant cynomolgus monkey CD33;

c) binds to endogenous CD33 on the surface of human peripheral blood mononucleocytes (PBMCs);

d) binds to recombinant human CD33 with a Kd of less than 10 nM, less than 7 nM, less than 5 nM, less than 3 nM, less than 2 nM, or less than 1 nM; and/or

e) binds to recombinant cynomolgus monkey CD33 with a Kd of less than 10 nM, less than 7 nM, less than 5 nM, less than 3 nM, less than 2 nM, or less than 1 nM.

**[00012]** In ~~some embodiments~~one aspect, an isolated antibody that binds to CD33 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16.

**[00013]** In some embodiments, an antibody that binds CD33 comprises:

a) a heavy chain variable region comprising the sequence of SEQ ID NO: 70 and a light chain variable region comprising the sequence of SEQ ID NO: 69;

b) a heavy chain variable region comprising the sequence of SEQ ID NO: 72 and a light chain variable region comprising the sequence of SEQ ID NO: 71;

c) a heavy chain variable region comprising the sequence of SEQ ID NO: 74 and a light chain variable region comprising the sequence of SEQ ID NO: 73; or

d) a heavy chain variable region comprising the sequence of SEQ ID NO: 76 and a light chain variable region comprising the sequence of SEQ ID NO: 75.

**[00014]** In ~~some embodiments~~one aspect, an isolated antibody that binds to CD33 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:24; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

**[00015]** In ~~some embodiments~~one aspect, an isolated antibody that binds to CD33 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:24; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:26; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

**[00016]** In ~~some embodiments~~one aspect, an isolated antibody that binds to CD33 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16.

**[00017]** In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody. In some embodiments, the antibody is an antibody fragment that binds CD33. In some embodiments, CD33 is human CD33 has the sequence of SEQ ID NO: 1, with or without a signal sequence (*e.g.*, with or without amino acids 1-17).

**[00018]** In ~~some embodiments~~one aspect, an isolated nucleic acid encoding an antibody described herein is provided. In ~~some embodiments~~one aspect, a host cell comprising the nucleic acid is provided. In ~~some embodiments~~one aspect, a method of producing an antibody comprising culturing the host cell so that the antibody is produced is provided.

**[00019]** In ~~some embodiments~~one aspect, an immunoconjugate comprising an antibody described herein and a cytotoxic agent is provided. In some embodiments, the immunoconjugate has the formula Ab-(L-D)p, wherein:

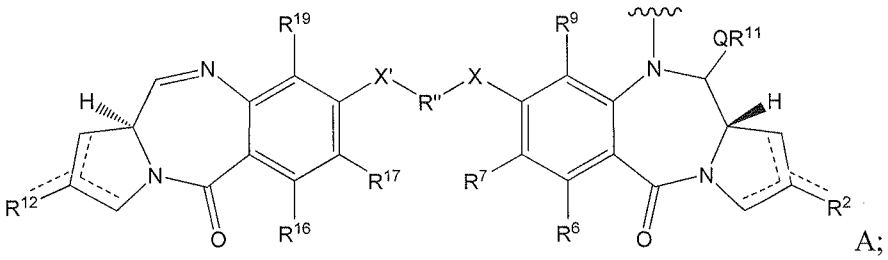
(a) Ab is the antibody of any one of claim 1 to 15;

(b) L is a linker;

(c) D is a cytotoxic agent; and

(d) p ranges from 1-8.

**[00020]** In some embodiments, the cytotoxic agent is selected from a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative. In some embodiments, D is a pyrrolobenzodiazepine of Formula A:




A;

wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

$R^2$ is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, $O\text{-}SO_2\text{-}R$, $CO_2R$ and COR, and optionally further selected from halo or dihalo, wherein $R^D$ is independently selected from R, $CO_2R$, COR, CHO, $CO_2H$, and halo;

$R^6$ and $R^9$ are independently selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', $NO_2$, $Me_3Sn$ and halo;

$R^7$ is independently selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', $NO_2$, $Me_3Sn$ and halo;

Q is independently selected from O, S and NH;

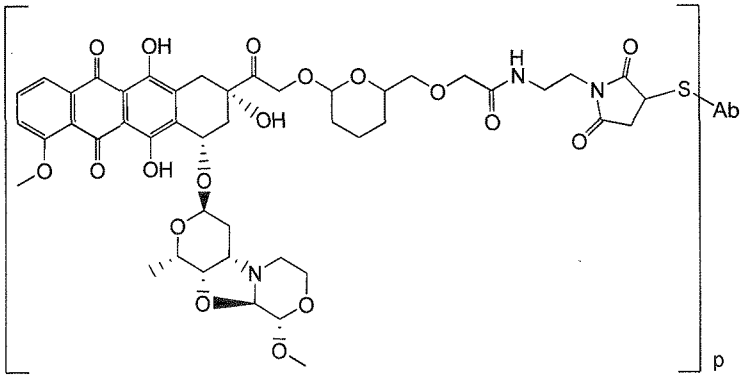
$R^{11}$ is either H, or R or, where Q is O, $SO_3M$, where M is a metal cation;

R and R' are each independently selected from optionally substituted $C_{1-8}$ alkyl, $C_{3-8}$ heterocyclyl and $C_{5-20}$ aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

$R^{12}$, $R^{16}$, $R^{19}$ and $R^{17}$ are as defined for $R^2$, $R^6$, $R^9$ and $R^7$ respectively;

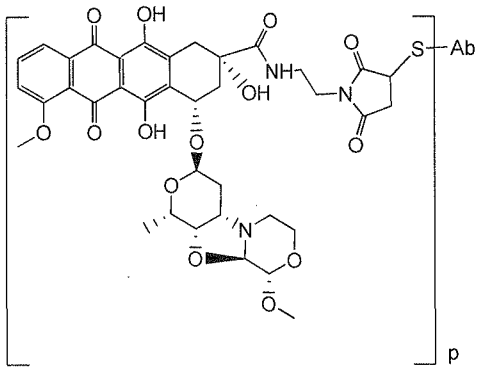
R'' is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

X and X' are independently selected from O, S and N(H).




;

and




.

**[00025]** In any of the immunoconjugate embodiments described herein, p ranges from 2-5.

**[00026]** In some embodiments, pharmaceutical formulations are provided. In ~~some embodiments~~one aspect, a pharmaceutical formulation ~~comprises~~ comprising an immunoconjugate described herein and a pharmaceutically acceptable carrier is provided. In some embodiments, the pharmaceutical formulation comprises an additional therapeutic agent.

**[00027]** In one aspect, use of the immunoconjugate described herein or the pharmaceutical formulation described herein in the manufacture of a medicament for treating an individual having a CD33-positive cancer is provided. In some embodiments, methods of treatment are provided. In some embodiments, methods of treating CD33-positive cancers are provided. In some embodiments, a method of treatment comprises administering to an individual an effective amount of an immunoconjugate described herein or a pharmaceutical formulation described herein. In some embodiments, the CD33-positive cancer is AML. In some embodiments, the method comprises administering an additional therapeutic agent to the individual.

**[00028]** In some embodiments, methods of inhibiting proliferation of a CD33-positive cell are provided. In some embodiments, the method comprises exposing the cell to an immunoconjugate

described herein under conditions permissive for binding of the immunoconjugate to CD33 on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, the cell is an AML cancer cell. In one aspect, an *in vitro* method of inhibiting proliferation of a CD33-positive cell is provided, the method comprising exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to CD33 on the surface of the cell, thereby inhibiting proliferation of the cell. In one aspect, use of the immunoconjugate described herein in the manufacture of a medicament for inhibiting proliferation of a CD33-positive cell is provided, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD33 on the surface of the cell, thereby inhibiting proliferation of the cell.

**[0010]** In ~~some embodiments~~one aspect, a method of detecting human CD33 in a biological sample is provided~~. In some embodiments, a method comprises~~ comprising contacting the biological sample with an anti-CD33 antibody described herein under conditions permissive for binding of the anti-CD33 antibody to a naturally occurring human CD33, and detecting whether a complex is formed between the anti-CD33 antibody and a naturally occurring human CD33 in the biological sample. In some embodiments, an anti-CD33 antibody is an antibody described herein. In some embodiments, the biological sample is an AML cancer sample.

**[0011]** In ~~some embodiments~~another aspect, a method for detecting a CD33-positive cancer is provided~~. In some such embodiments, a method~~ comprise~~s~~ing (i) administering a labeled anti-CD33 antibody to a subject having or suspected of having a CD33-positive cancer, wherein the labeled anti-CD33 antibody comprises the anti-CD33 antibody described herein, and (ii) detecting the labeled anti-CD33 antibody in the subject, wherein detection of the labeled anti- CD33 antibody indicates a CD33-positive cancer in the subject. In some embodiments, an anti-CD33 antibody is an antibody described herein. In some such embodiments, the labeled anti-CD33 antibody comprises an anti-CD33 antibody conjugated to a positron emitter. In some embodiments, the positron emitter is $^{89}Zr$.

## BRIEF DESCRIPTION OF THE FIGURES

**[0012]** **Figure 1A-B** shows alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of 7A1, 9C2, 10D3, and 15G15.

**[0013]** **Figure 2A-B** shows alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of 15G15, 15G15.33, 15G15.37, 15G15.83, 15G15.88, 15G15.7, 15G15.17, 15G15.30, 15G15.31, 15G15.39, and 15G15.84.

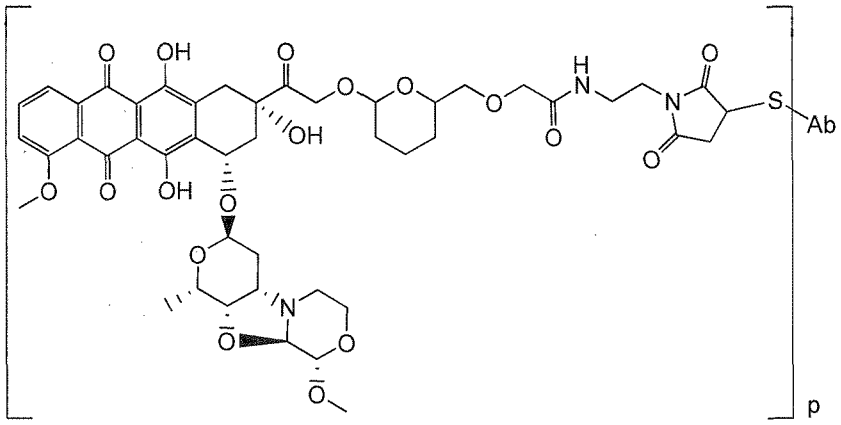
**[0014]** **Figure 3A-B** shows alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of 23E4, 27C6, 33F3, 33F9, and 33H4.

**[0015]** **Figure 4A-B** shows alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of 9C3, 9C3.2, 9C3.3, and 9C3.4.

**[0016]** **Figure 5A-D** show species cross-reactivity of anti-CD33 antibodies to recombinant CD33. Anti-CD33 antibody binding to 293 cells expressing recombinant human (hu) CD33 (A, C) and cynomolgus (cyno) CD33 (B, D) is shown.

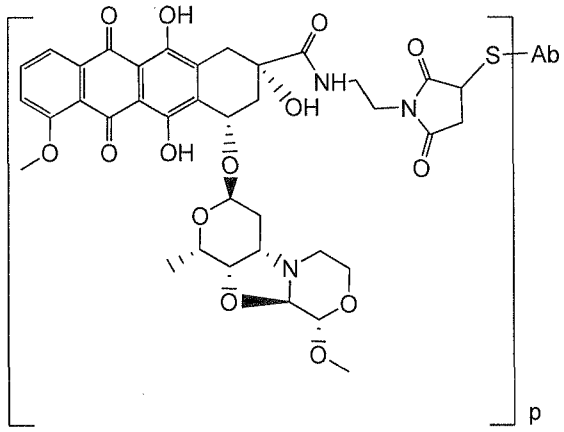
**[0017]** **Figure 6A-D** shows anti-CD33 antibody binding to endogenous huCD33 expressed in Molm-13 (A, C) and AML (B, D) cells.

**[0018]** **Figure 7A-D** shows species cross-reactivity of anti-CD33 antibodies to endogenous CD33. Anti-CD33 antibody binding to huCD33+ (A, C) and cynoCD33+ (B, D) myeloid cells.



;

and



33. The immunoconjugate of any one of claims 23 to 32, wherein p ranges from 2-5.

34. The immunoconjugate of any one of claims 23 to 33, comprising an antibody of any one of claims 15 to 17.

35. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 23 to 34 and a pharmaceutically acceptable carrier.

36. The pharmaceutical formulation of claim 39, further comprising an additional therapeutic agent.

37. ~~A method of treating an individual having a CD33-positive cancer, the method comprising administering to the individual an effective amount~~Use of the immunoconjugate of any one of claims 23 to 34 or the pharmaceutical formulation of claim 35 in the manufacture of a medicament for treating an individual having a CD33-positive cancer.

38. The ~~method~~ use of claim 37, wherein the CD33-positive cancer is AML.

39. The ~~method~~ use of claim 37 or claim 38, ~~further comprising administering~~wherein the medicament is to be administered with an additional therapeutic agent ~~to the individual~~.

40. An *in vitro* method of inhibiting proliferation of a CD33-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 23 to 34 under conditions permissive for binding of the immunoconjugate to CD33 on the surface of the cell, thereby inhibiting proliferation of the cell.

41. The method of claim 40, wherein the cell is an AML cancer cell.

42. The antibody of any one of claims 1 to 18 conjugated to a label.

43. The antibody of claim 42, wherein the label is a positron emitter.

44. The antibody of claim 43, wherein the positron emitter is $^{89}Zr$.

45. A method of detecting human CD33 in a biological sample comprising contacting the biological sample with the anti-CD33 antibody of any one of claims 1 to 18 under conditions permissive for binding of the anti-CD33 antibody to a naturally occurring human CD33, and detecting whether a complex is formed between the anti-CD33 antibody and a naturally occurring human CD33 in the biological sample.

46. The method of claim 45, wherein the anti-CD33 antibody is an antibody as in any one of claims 15 to 17.

47. The method of claim 46, wherein the biological sample is a AML endometrial cancer sample.

48. A method for detecting a CD33-positive cancer comprising (i) administering a labeled anti-CD33 antibody to a subject having or suspected of having a CD33-positive cancer, wherein the labeled anti-CD33 antibody comprises the anti-CD33 antibody of any one of claims 1 to 18, and (ii) detecting the labeled anti-CD33 antibody in the subject, wherein detection of the labeled anti-CD33 antibody indicates a CD33-positive cancer in the subject.

49. The method of claim 48, wherein the labeled anti-CD33 antibody is an antibody as in claim 8 or claim 14 that is labeled.

50. The method of claim 48 or claim 49, wherein the labeled anti-CD33 antibody comprises an anti-CD33 antibody conjugated to a positron emitter.

51. The method of claim 50, wherein the positron emitter is $^{89}Zr$.

52. Use of the immunoconjugate of any one of claims 23 to 34 in the manufacture of a medicament for inhibiting proliferation of a CD33-positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD33 on the surface of the cell, thereby inhibiting proliferation of the cell.

53. The use of claim 52, wherein the cell is an AML cancer cell.