MATERIALS AND METHODS FOR BINDING SIGLEC-3/CD33

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority of U.S. provisional patent application serial number 62/989093 filed on March 13, 2020, U.S. provisional patent application serial number 62/989071 filed on March 13, 2020, U.S. provisional patent application serial number 62/989120 filed on March 13, 2020, U.S. provisional patent application serial number 62/989187 filed on March 13, 2020 and U.S. provisional patent application serial number 62/989230 filed on March 13, 2020.

TECHNICAL FIELD

The invention provides antigen binding domains that bind myeloid cell surface antigen CD33 protein comprising the antigen binding domains that bind CD33, polynucleotides encoding them, vectors, host cells, methods of making and using them.

BACKGROUND

Myeloid cell surface antigen CD33, also known as Sialic acid-binding immunoglobulin-like lectin 3 (Siglec-3), is a member of the sialic acid binding family of proteins. CD33 is a type-I single pass transmembrane protein with an N-terminal sialic acid binding domain in its extracellular domain (ECD) and intracellular immunoreceptor tyrosine-based inhibitor motif (ITIM) motifs (Uniprot.org) [4].

In normal cells, CD33 in involved in immune cell maintenance and cell-cell interactions [5]. CD33 protein is expressed in at least the bone marrow, lung, skin, appendix, spleen, lymph nodes, and tonsils. Expression of CD33 mRNA is found in a wide variety of tissues and organs, including but not limited to, bone marrow, the brain, eye, lung, endocrine tissues, salivary glands, esophagus, stomach, colon, rectum, liver, gallbladder, pancreas, kidney, bladder, male and female reproductive tissues, prostate, breast, heart, smooth muscle, skeletal muscle, adipose tissue, skin, thymus, appendix, spleen, lymph nodes, tonsil, granulocytes, and blood (e.g., PBMC, dendritic cells, and lymphocytes). See, e.g., www.proteinatlas.org/ENSG00000105383-CD33/tissue.

CD33 is expressed in 90% of AML patients [6] and is one of the most widely explored surface antigens for AML immunotherapy. Several approaches have been explored to target CD33, including antibody-drug/antibody-toxin conjugates, chimeric antigen receptors (CARs) and T-cell redirector bispecific antibodies [7]. There is a need for additional CD33 binding domains for therapeutic and diagnostic purposes.

Acute Myeloid Leukemia (AML) is a type of leukemia of the myeloid cell lineage characterized by rapid development and progression [1]. AML is a heterogeneous disease with widely varying molecular

changes along with different clinical outcomes. Moreover, AML is characterized by the ability of AML blasts, or leukemia cells, to self-renew, continuously proliferate and escape the immune system resulting in disease relapse from minor clones [2]. Accordingly, AML has historically been considered to have one of the lowest long-term survival rates, with an overall 5-year survival rate of adult AML at about 27%. Survival rates have a strong inverse relationship with age [3]. Historically, the primary AML treatment strategies involve chemotherapy, along with stem cell transplantation. In recent years however, there has been aggressive effort to develop immunotherapy for AML exploring key surface antigens. One of the surface antigens highly expressed in AML cells is CD33.

SUMMARY

In one aspect, the disclosure provides an isolated protein that binds CD33, wherein the isolated protein comprises:

a) a heavy chain complementarity determining region (HCDR) 1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27,

or a CD33 binding fragment thereof.

In some embodiments, the isolated protein comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;

b) SEQ ID NOs: 2, 7, and 13, respectively;

c) SEQ ID NOs: 1, 8, and 14, respectively;

d) SEQ ID NOs: 3, 9, and 13, respectively;

e) SEQ ID NOs: 4, 10, and 15, respectively;

f) SEQ ID NOs: 5, 11, and 16, respectively; or

g) SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH. In some embodiments, the isolated protein is the VHH. In some embodiments, the isolated

protein is the Fab. In some embodiments, the isolated protein is the scFv. In some embodiments, the isolated protein comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In another aspect, the disclosure provides an isolated protein that binds CD33, where the isolated protein comprises a VHH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In another aspect, the disclosure provides an isolated protein that binds CD33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In some embodiments, the isolated protein comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively;

j) SEQ ID NOs: 35, 42, and 51, respectively

k) SEQ ID NOs: 253, 256, and 259, respectively:

l) SEQ ID NOs: 254, 257 and 260, respectively: or

m) SEQ ID NOs: 255, 258 and 261, respectively.

In another aspect, the disclosure provides an isolated protein that binds CD33, wherein the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 29, 37, and 46, respectively;
b) SEQ ID NOs: 32, 40, and 49, respectively;
c) SEQ ID NOs: 34, 43, and 51, respectively;
d) SEQ ID NOs: 253, 256, and 259, respectively;
e) SEQ ID NOs: 254, 257, and 260, respectively; or
f) SEQ ID NOs: 255, 258, and 261, respectively.

In some embodiments, the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the isolated protein comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;

g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;

h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;

j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively:

k) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively:

l) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively: or

m) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In some embodiments, the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH. In some embodiments, the isolated protein is the Fab. In some embodiments, the isolated protein is the VHH. In some embodiments, the isolated protein is the scFv. In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

In some embodiments, the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140. In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the isolated protein comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304,

305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272,273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In some embodiments, the isolated protein comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;

k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;

m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;

n) the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;

o) the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;

p) the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;

q) the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;

r) the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;

s) the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;

t) the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;

u) the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;

v) the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;

w) the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;

x) the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;

y) the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;

z) the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;

aa) the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;

ab) the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;
ac) the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;
ad) the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;
ae) the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;
af) the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271;
ag) the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271;
ah) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;
ai) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.
aj) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;
ak) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;
al) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
am) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
an) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;
ao) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
ap) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
aq) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
ar) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
as) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
at) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
au) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
av) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;
aw) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
ax) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
ay) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
az) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
ba) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
bb) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;
bc) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
bd) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
be) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
bf) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
bg) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
bh) the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
bi) the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;

bj) the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
bk) the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
bl) the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
bm) the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
bn) the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
bo) the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
bp) the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
bq) the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;
br) the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
bs) the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;
bt) the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
bu) the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
bv) the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272;
bw) the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.
bx) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
by) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
bz) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
ca) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
cb) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
cc) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
cd) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;
ce) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
cf) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
cg) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
ch) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
ci) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
cj) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;
ck) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
cl) the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
cm) the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
cn) the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
co) the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
cp) the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
cq) the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;

cr) the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
cs) the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
ct) the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
cu) the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
cv) the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
cw) the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
cx) the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
cy) the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;
cz) the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85;
da) the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85;
db) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
dc) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
dd) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;
di) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
dj) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;
dk) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;
dl) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or
dm) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

In another aspect, the disclosure provides an isolated protein that binds CD33, where the isolated protein comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;
n) the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;
o) the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;
p) the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;
q) the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;
r) the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;
s) the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;
t) the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;
u) the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;
v) the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;
w) the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;
x) the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;
y) the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;
z) the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;
aa) the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;
ab) the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;
ac) the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;
ad) the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;
ae) the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;
af) the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271;
ag) the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271;
ah) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;
ai) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.
aj) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;
ak) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;
al) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
am) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
an) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;

ao) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
ap) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
aq) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
ar) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
as) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
at) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
au) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
av) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;
aw) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
ax) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
ay) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
az) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
ba) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
bb) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;
bc) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
bd) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
be) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
bf) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
bg) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
bh) the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
bi) the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;
bj) the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
bk) the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
bl) the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
bm) the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
bn) the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
bo) the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
bp) the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
bq) the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;
br) the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
bs) the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;
bt) the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
bu) the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
bv) the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272;

bw) the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.
bx) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
by) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
bz) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
ca) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
cb) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
cc) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
cd) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;
ce) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
cf) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
cg) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
ch) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
ci) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
cj) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;
ck) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
cl) the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
cm) the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
cn) the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
co) the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
cp) the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
cq) the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;
cr) the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
cs) the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
ct) the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
cu) the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
cv) the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
cw) the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
cx) the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
cy) the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;
cz) the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85;
da) the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85;
db) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
dc) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
dd) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;

de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;
di) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
dj) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;
dk) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;
dl) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or
dm) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

In some embodiments, the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH. In some embodiments, the isolated protein is the Fab. In some embodiments, the isolated protein is the VHH. In some embodiments, the isolated protein is the scFv. In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises

a) about 5-50 amino acids;
b) about 5-40 amino acids;
c) about 10-30 amino acids; or
d) about 10-20 amino acids.

In some embodiments, the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140. In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the isolated protein comprises the amino acid sequence of any one of SEQ ID NO: 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221 274, 275 or 276. In particular, the amino acid sequence of SEQ ID NO: 213, 216, 219, 274, 275 or 276.

In another aspect the disclosure provides an isolated protein that binds myeloid CD33, where the isolated protein comprises:

a) a heavy chain complementarity determining region (HCDR) 1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 196.

In some embodiments, the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 89, 90, and 92, respectively;

b) SEQ ID NOs: 89, 90, and 93, respectively;

c) SEQ ID NOs: 33, 91, and 94, respectively

d) SEQ ID NOs: 167, 168, and 169, respectively;

e) SEQ ID NOs: 172, 173, and 174, respectively;

f) SEQ ID NOs: 176, 177, and 178, respectively;

g) SEQ ID NOs: 89, 90, and 180, respectively; or

h) SEQ ID NOs: 89, 90, and 93, respectively.

In another aspect the disclosure provides an isolated protein of claim 32 or 33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

In some embodiments, the isolated protein comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

In some embodiments, the isolated protein is the Fab.

In some embodiments, the isolated protein is the VHH.

In some embodiments, the isolated protein is the scFv.

In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

In some embodiments, the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the isolated protein comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the isolated protein comprises:

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In another aspect, the disclosure provides an isolated protein that that binds CD33, where the isolated protein comprises:

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH. In some embodiments, the isolated protein is the Fab. In some embodiments, the isolated protein is the VHH. In some embodiments, the isolated protein is the scFv. In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH). In some embodiments, the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

In some embodiments, the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128,

129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140. In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

The disclosure provides any isolated protein described above, where the protein is conjugated to a half-life extending moiety. In some embodiments, the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol. In some embodiments, the isolated protein is a monospecific protein. In some embodiments, the isolated protein is a multispecific protein. In some embodiments, the multispecific protein is a bispecific protein. In some embodiments, the multispecific protein is a trispecific protein. In some embodiments, the multispecific protein further comprises an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof. In some embodiments, the fragment of the Ig constant region comprises a Fc region. In some embodiments, the fragment of the Ig constant region comprises a CH2 domain. In some embodiments, the fragment of the Ig constant region comprises a CH3 domain. In some embodiments, the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain. In some embodiments, the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain. In some embodiments, the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain. In some embodiments, the isolated protein is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region. In some embodiments, the isolated protein is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region. In some embodiments, the isolated protein is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2). In some embodiments, the L2 comprises the amino acid sequence of any one of SEQ ID NOs:108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

In some embodiments, the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte. In some embodiments, the lymphocyte is a T cell. In some embodiments, the T cell is a $CD8^+$ T cell. In some embodiments, the lymphocyte is a natural killer (NK) cell. In some embodiments, the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C. In some embodiments, the multispecific protein comprises an antigen binding domain that binds CD3ε. In some embodiments, the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

In some embodiments, the multispecific protein comprises an antigen binding domain that binds TRGV9. In some embodiments, the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype. In some embodiments, the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR). In some embodiments, the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-

deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index. In some embodiments, the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR. In some embodiments, the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index. In some embodiments, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof. In some embodiments, the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein. In some embodiments, the at least one mutation that modulates the half-life of the protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index. In some embodiments, the protein comprises at least one mutation in a CH3 domain of the Ig constant region. In some embodiments, the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

The disclosure provides any isolated protein described above, where the protein is a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises

a) an extracellular domain comprising any of the isolated proteins described above;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

In some embodiments, the CAR further comprises a CD8a-hinge region. In some embodiments,

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

In some embodiments,

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163 and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

In some embodiments, the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, or any combinations thereof.

In another aspect, the disclosure provides an isolated multispecific protein comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen. In some embodiments, the lymphocyte antigen is a T cell antigen. In some embodiments, the T cell antigen is a $CD8^+$ T cell antigen. In some embodiments, the lymphocyte antigen is a NK cell antigen. In some embodiments, the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C. In certain embodiments, the lymphocyte antigen is CD3ε. In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH. In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab. In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH. In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv. In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH). In some embodiments, the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

In some embodiments, the L1 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128,

129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140. In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108. In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17. In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;
b) SEQ ID NOs: 2, 7, and 13, respectively;
c) SEQ ID NOs: 1, 8, and 14, respectively;
d) SEQ ID NOs: 3, 9, and 13, respectively;
e) SEQ ID NOs: 4, 10, and 15, respectively;
f) SEQ ID NOs: 5, 11, and 16, respectively; or
g) SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27. In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, or 35, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, or 44, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, or 51, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, or 68, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, or 74, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, or 80. In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively:
k) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively:
l) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively: or
m) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273. In some embodiments, the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199. In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;
g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;
h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or
i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises

a) the HCDR1 of SEQ ID NO: 141, the HCDR2 of SEQ ID NO: 142, the HCDR3 of SEQ ID NO: 143, the LCDR1 of SEQ ID NO: 144, the LCDR2 of SEQ ID NO: 145 and the LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

In some embodiments, the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region. In certain embodiments, the multispecific protein further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region. In some embodiments, the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140. In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype. In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR. In some embodiments, the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and

S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR). In some embodiments, the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index. In some embodiments, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof. In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein. In some embodiments, the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index. In some embodiments, the isolated multispecific protein comprises at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region. In some embodiments, the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A,Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index. In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

a) L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

b) L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

The disclosure provides an immunoconjugate comprising any of the above isolated proteins conjugated to a therapeutic agent or an imaging agent. The disclosure provides a pharmaceutical composition comprising any of the above isolated proteins and a pharmaceutically acceptable carrier. The disclosure provides a polynucleotide encoding any of the above isolated proteins. The disclosure also provides a vector comprising a polynucleotide encoding any of the above isolated proteins. The disclosure also provides a host cell comprising a vector comprising a polynucleotide encoding any of the above isolated proteins.

In another aspect the disclosure provides a method of producing any of the above isolated proteins, the method comprising culturing the host cell in conditions that the protein is expressed, and recovering the protein produced by the host cell. In another aspect the disclosure provides an immunoconjugate comprising any of the above isolated multispecific proteins conjugated to a therapeutic agent or an imaging agent.

In another aspect the disclosure provides a method of producing any of the above isolated multispecific proteins, comprising culturing any host cell described above in conditions that the multispecific protein is expressed, and recovering the multispecific protein produced by the host cell.

In another aspect the disclosure provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of any of the above isolated proteins, any of the above isolated multispecific proteins, any of the above immunoconjugates, or any of the above pharmaceutical compositions to the subject for a time sufficient to treat the CD33 expressing cancer.

In another aspect the disclosure provides a method of reducing the amount of CD33 expressing cells in a subject, comprising administering any of the above isolated proteins, any of the above isolated multispecific proteins, any of the above immunoconjugates, or any of the above pharmaceutical compositions to the subject for a time sufficient to reduce the amount of CD33 expressing cells. In some embodiments, the CD33 expressing cells are cancerous cells. In some embodiments, the CD33 expressing cells are not cancerous cells.

In another aspect the disclosure provides a method of preventing establishment of a CD33 expressing cancer in a subject, comprising administering any of the above isolated proteins, any of the above isolated multispecific proteins, any of the above immunoconjugates, or any of the above pharmaceutical compositions to the subject to prevent establishment of the CD33 expressing cancer in the subject.

In another aspect the disclosure provides a method of treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer, comprising administering any of the above isolated proteins, any of the above isolated multispecific proteins, any of the above immunoconjugates, or any of the above pharmaceutical compositions to the subject to the subject to treat the noncancerous condition.

In some embodiments of the above three aspects, the CD33 expressing cancer is a hematologic cancer. In some embodiments, the hematologic cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodiments, the hematologic 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 (DPDCN). In some embodiments, the isolated protein or the isolated multispecific protein is administered in combination with a second therapeutic agent. In some embodiments, the second therapeutic agent is surgery, chemotherapy, or radiation, or any combination thereof. The disclosure also provides a method of detecting the presence of a hematologic cancer in a subject, comprising administering any of the above immunoconjugates to a subject suspected to have the hematologic cancer and visualizing the biological structures to which the immunoconjugate is bound, thereby detecting the presence of the hematologic cancer.

The disclosure also provides a kit comprising any of the above isolated proteins, any of the above isolated multispecific proteins, any of the above immunoconjugates, or any of the above pharmaceutical compositions.

The disclosure also provides an anti-idiotypic antibody binding to any of the above isolated proteins.

The disclosure also provides a chimeric antigen receptor (CAR) comprising:

a) an extracellular domain comprising an antigen binding domain that binds CD33;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

In some embodiments, the CAR further comprises a CD8a-hinge region.

a) In some embodiments, the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

In some embodiments,

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163, and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17.

In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;

b) SEQ ID NOs: 2, 7, and 13, respectively;

c) SEQ ID NOs: 1, 8, and 14, respectively;

d) SEQ ID NOs: 3, 9, and 13, respectively;

e) SEQ ID NOs: 4, 10, and 15, respectively;

f) SEQ ID NOs: 5, 11, and 16, respectively; or

g) SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27. In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, 35, 253, 254 or 255, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, 44, 256, 257 or 258, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 259, 260 or 261, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, 68, 265, or 266, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, 74, 267 or 268, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, 80, 269 or 270. In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;
k) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively:
l) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively: or
m) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, 273. In some embodiments, the antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199. In some embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the antigen binding domain that binds CD33 is a VHH.

In some embodiments, the antigen binding domain that binds CD33 is a scFv.

In some embodiments, the scFv comprises a first linker (L1) between the VL and the VH.

In certain embodiments, the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the extracellular domain of the CAR comprising the antigen binding domain that binds CD33 further comprises a signal polypeptide. In some embodiments, the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, and a combination thereof. In some embodiments, the CD137 component comprises the amino acid sequence of SEQ ID NO: 163. In some embodiments, the CD3z component comprises an

amino acid sequence of SEQ ID NO: 164. In some embodiments, the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 165. In some embodiments, the CD8a-TM polypeptide comprises an amino acid sequence of SEQ ID NO: 162. In some embodiments, the CD8a-hinge region comprises an amino acid sequence of SEQ ID NO: 157.

In some embodiments, the CAR is a multispecific protein. In some embodiments, the multispecific protein is a bispecific protein. In some embodiments, the multispecific protein is a trispecific protein. In some embodiments, the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte. In some embodiments, the lymphocyte is a T cell. In some embodiments, the T cell is a $CD8^+$ T cell. In some embodiments, the lymphocyte is a natural killer (NK) cell. In some embodiments, the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C. In some embodiments, the multispecific protein comprises an antigen binding domain that binds CD3ε. In some embodiments, the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;
b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;
c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or
d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

In some embodiments, the multispecific protein comprises an antigen binding domain that binds TRGV9. In some embodiments, the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;
b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

In another aspect, the disclosure provides an isolated lymphocyte expressing any of the CARs above. In some embodiments, the lymphocyte is a T lymphocyte. In some embodiments, the lymphocyte is a natural killer (NK) cell. In another aspect, the disclosure provides an isolated polynucleotide encoding any of the above CARs. The disclosure also provides a vector comprising an isolated polynucleotide encoding any of the above CARs. The disclosure provides a host cell comprising the isolated polynucleotide or the vector. The disclosure also provides a pharmaceutical composition comprising any of the above lymphocytes and a pharmaceutically acceptable excipient.

In another aspect, the disclosure provides a method of treating a subject having a CD33 expressing cancer, the method comprising: administering a therapeutically effective amount of any of the above lymphocytes to a subject in need thereof, whereby the lymphocyte mediates killing of the CD33 expressing cancer in the subject. In some embodiments, the CD33 expressing cancer is a hematologic cancer. In some embodiments, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodiments, the CD33 expressing 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 (DPDCN).

In another aspect is provided a method of targeted killing of a cancer cell, the method comprising: contacting the cancer cell with any of the above lymphocytes, whereby the lymphocyte induces targeted killing of the cancer cell. In some embodiments, the cancer cell is a hematologic cancer. In some embodiments, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodiments, the CD33 expressing 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 (DPDCN).

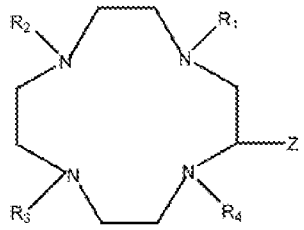
In another aspect is provided a method of detecting the presence of a cancer in a subject, comprising:

a) contacting a cell sample obtained from the subject with any of the above CARs, thereby forming a CAR-cell complex, and

b) detecting the CAR-cell complex, wherein detection of the CAR-cell complex is indicative of the presence of the cancer in the subject.

In some embodiments, the cancer cell is a hematologic cancer. In some embodiments, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodiments, the CD33 expressing 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 (DPDCN).

In another aspect is provided an immunoconjugate comprising any of the above isolated proteins conjugated to a radioactive agent. In some embodiments, the radioactive agent comprises a radiometal ion. In some embodiments, the radiometal ion is ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{225}Ac, ^{255}Fm, ^{227}Th, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, or ^{111}In. In certain embodiments, the radiometal ion is ^{225}Ac, ^{111}In or ^{89}Zr. In certain embodiments, the radiometal ion is ^{225}Ac. In some embodiments, the radiometal ion of the immunoconjugate is conjugated to a chelating moiety. In some embodiments, the chelating moiety comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1-C_4 alkyl; and

Z is $(CH2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner; alternatively, Z is hydrogen; and

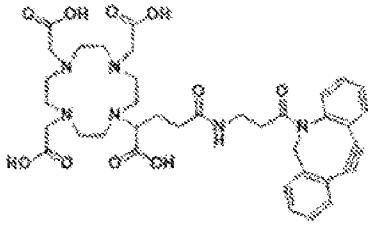
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

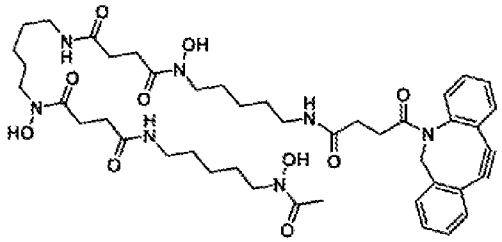
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand.

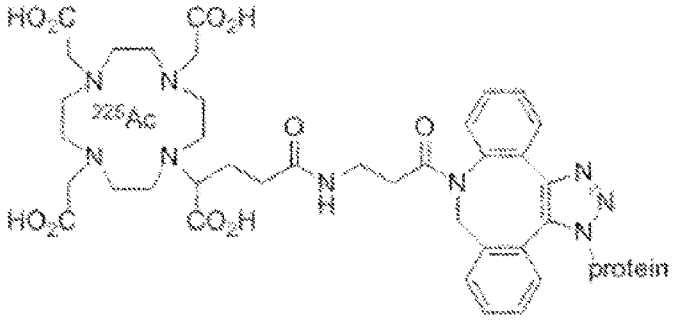
In some embodiments, the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



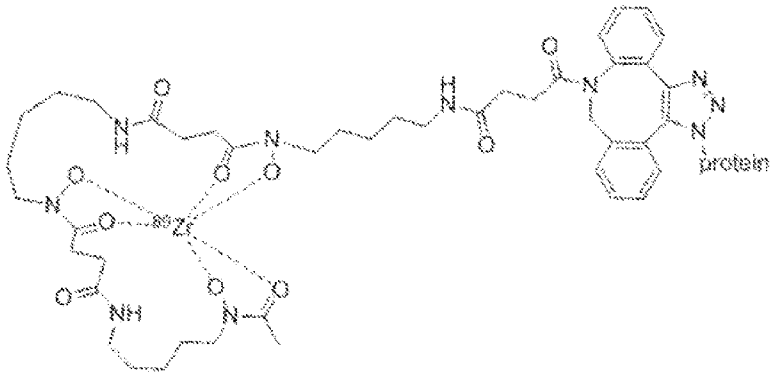
The disclosure also provides an immunoconjugate of formula (IV),



formula (IV)

where the protein is any of the above isolated proteins.

The disclosure also provides an immunoconjugate of formula (V),



formula (V)

where the protein is any of the above isolated proteins.

The disclosure provides a pharmaceutical composition comprising a means for treating a CD33 expressing cancer in a subject.

The disclosure provides a pharmaceutical composition comprising a means for reducing the amount of CD33 expressing tumor cells in a subject.

The disclosure provides a pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject.

The disclosure provides a pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer.

The disclosure provides a method of treating a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cancer cell in the subject.

The disclosure provides a method of reducing the amount of CD33 expressing tumor cells in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to one or more of the CD33 expressing tumor cells in the subject.

The disclosure provides a pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

The disclosure provides a pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

This patent application file contains at least one drawing executed in color. Copies of this patent application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 shows representative BIAcore 8K sensorgrams of anti-CD33 vHHs interactions against hu CD33 antigens (FL ECD and IgC2 domain).

FIGS. 2A and **2B** show binding of CD33 antibodies to target cell lines. Graphs show the non-linear regression profiles of anti-human CD33 antibodies at 4°C. Median fluorescence intensity (MFI) versus log concentration of antibody are plotted. Binding was performed by incubating the cells with the serial dilutions of the antibody at 4°C followed by detection with PE conjugated Goat F(ab')2 Anti-Human IgG - Fc antibody. The top hit for each cell line is indicated in red, and Lintuzumab binding is shown in blue curves.

FIGS. 3 show time and temperature dependent binding of different CD33 antibodies to target cell lines. Graphs show the non-linear regression profiles of binding of anti-human CD33 antibodies at 37°C and 4°C. Median fluorescence intensity (MFI) versus log concentrations of antibody are plotted. Binding was performed by incubating the cells with the serial dilutions of the directly conjugated antibodies at 37°C and 4°C.

FIGS. 4A-4D show internalization of CD33 antibodies on high, medium and low expression target cell lines. Cells were cultured with serial dilutions of the CD33 antibodies along with Protein A-MMAF for 96hrs. After 96 hours, CellTitre-Glo reagent was added to cells to measure cell viability. Wells with Protein A-MMAF alone were considered as 100% viable. HDLM-2 cells were used as a no expression cell line. A decrease in cell viability indicates antibody internalization. Graphs indicate non-linear regression analysis for cell viability against log concentration of antibody.

FIG. 5 shows kinetics of CD33 antibody internalization. CD33 antibodies were labeled with pHAb dyes and incubated with the cells at a concentration of 100nM at 37°C for the indicated time points following which the cells were washed and acquired on the flow cytometer. Graphs show MFI values at indicated time points.

FIG. 6 shows CD33 expression on target cell lines. High, medium and low CD33 expression cell lines were identified using anti-human CD33 antibody from Biolegend. MOLM-13, Kasumi-1, OCI-AML-3 and HDLM2 cells were stained with the antibody (orange histograms) or the mouse IgG isotype control (blue histograms) and fold change over isotype for each cell line was calculated.

FIG. 7 shows antibody integrity and DAR values post conjugation with pHAb dyes. Antibodies were labeled with pHAb dyes and analyzed on SDS-PAGE. Drug to antibody ratios (DAR) were calculated by measuring the OD at 280 and 530 nm and correcting for the dye absorbance at 280nm.

FIGS. 8A and 8B show specificity of pHAb mediated antibody internalization kinetics determination. In FIG. 8A, CD33 antibodies were labeled with pHAb dyes and incubated with the Kasumi-1 or OCI-AML-3 cells at a concentration of 100nM at 4°C for the indicated time points following which the cells were washed and acquired on the flow cytometer. Graphs show MFI values at indicated

time points. No internalization was observed at 4C. In FIG. 8B, antibodies were also incubated with HDLM-2 cells at 37°C. No increase in MFI was observed.

FIG. 9 shows a summary of the binding EC_{50} values of CD33 antibodies to high (MOLM-13), medium (Kasumi-1) and low (OCI-AML-3) cell lines. Binding was carried out at 4°C with serial dilutions of the antibody.

FIG. 10 shows a summary of CD33 antibody internalization on various target cell lines. For MOLM-13 and OCI-AML-3, EC_{50} values are indicated. For Kasumi-1, % viability at 100nM concentration.

FIG. 11 shows a schematic demonstrating the binding of an anti-TRGV9/anti-CD33 bispecific antibody to recruit γδ T-cells to a cancer cell that is CD33+ and to induce cancer cell death.

FIG. 12 shows the results of an assay of the integrity of the VG4 bispecific antibody by an SDS-PAGE (non-reducing) gel.

FIGS. 13-15 show the results of assays of the binding of anti-CD33 antibody (clone C33B904) to a panel of tumor cell lines were measured by FACS. The EC50 for: MOLM-13 (high receptor density) was 134.3 nM, Kasumi-1 (moderate density) was 82.2 nM and OCI-AML-3 (low surface density) was 16.4 nM.

FIGS. 16-17 show the results of an experiment demonstrating that the anti-TRGV9/anti-CD33 bispecific antibody mediates γδ T cell cytotoxicity against CD33 expressing Kasumi-3 cells *in vitro*. In the experiment, enriched γδ T cells (Effectors), isolated from PBMCs cultured with Zoledronic acid + IL-2 + IL-15 for 12 days, were co-cultured with CFSE labelled Kasumi-3 cells (Targets) at 1:1 and 5:1 E:T ratios in the presence of various concentrations of the bispecific antibody for 24 hours. Dose response curves show anti-TRGV9/anti-CD33 and anti-TRGV9/anti-NULL bispecific antibodies mediated γδ T cell cytotoxicity against CD33 expressing kasumi-3 cells in a dose dependent manner at 1:1 and 5:1 E:T ratios. Cytotoxicity values represented here were subtracted of basal cytotoxicity value observed in the absence of bispecific antibody. EC_{50} values were calculated as described in methods. Representative data are shown here are from a single experiment.

FIG. 18 shows the results of experiments demonstrating that the anti-TRGV9/anti-CD33 bispecific antibody mediates γδ T cell cytotoxicity against CD33 expressing Kasumi-3 cells *in vitro*. Healthy donor derived PBMCs (Effectors), cultured with Zoledronic acid + IL-2 + IL-15 for 12 days, were co-cultured with CFSE labelled Kasumi-3 cells (Targets) at 1:1 E:T ratios in the presence of various concentrations of the bispecific antibody for 24 hours.

FIG. 19 shows in silico analysis of potential Post-Translational Modifications (PTMs) risk. Some potential PTM risk motifs (DG, NG, DP) are highlighted.

FIG. 20 shows the results of assays to evaluate the thermostability of scFvs generated from NNK library based on C33B1475 (SEQ ID NO: 274). ELISAs were performed on each variant in order to assess thermostability. E. coli supernatants were heat treated at 55C, 60C, 65C and luminescence signals were normalized to untreated room temperature of the same clone.

FIG. 21 shows the results of assay to evaluate the thermostability of scFv generated from NNK library based on C33B1516 (SEQ ID NO: 275). ELISAs were performed on each variant in order to assess thermostability. E. coli supernatants were heat treated at 55C, 60C, 65C and luminescence signals were normalized to untreated room temperature of the same clone.

FIG. 22 shows the results of assay to evaluate the thermostability of scFv generated from NNK library based on C33B1517 (SEQ ID NO: 216). ELISAs were performed on each variant in order to assess thermostability. E. coli supernatants were heat treated at 55C, 60C, 65C and luminescence signals were normalized to untreated room temperature of the same clone.

FIG. 23 shows the results of assay to evaluate the thermostability of scFv generated from NNK library based on C33B1522 (SEQ ID NO: 213). ELISAs were performed on each variant in order to assess thermostability. E. coli supernatants were heat treated at 55C, 60C, 65C and luminescence signals were normalized to untreated room temperature of the same clone.

FIG. 24 is a table listing variant VH and VL sequences identified during library screen of NNK library.

DETAILED DESCRIPTION

The disclosed methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods are not limited to the specific methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods.

All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

When a list is presented, unless stated otherwise, it is to be understood that each individual element of that list, and every combination of that list, is a separate embodiment. For example, a list of embodiments presented as "**A, B, or C**" is to be interpreted as including the embodiments, "A," "B," "C," "A or B," "A or C," "B or C," or "A, B, or C."

As used in this specification and the appended claims, the singular forms "**a**," "**an**," and "**the**" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

The transitional terms "**comprising**," "**consisting essentially of**," and "**consisting of**" are intended to connote their generally accepted meanings in the patent vernacular; that is, (i) "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps; (ii) "consisting of" excludes any element, step, or ingredient not specified in the claim; and (iii) "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. Embodiments described in terms of the phrase "comprising" (or its equivalents) also provide as embodiments those independently described in terms of "consisting of" and "consisting essentially of."

"**About**" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, "about" means within one standard deviation per the practice in the art, or a range of up to 5%, whichever is larger.

"**Activation**" or "**stimulation**" or "**activated**" or "**stimulated**" refers to induction of a change in the biologic state of a cell resulting in expression of activation markers, cytokine production, proliferation or mediating cytotoxicity of target cells. Cells may be activated by primary stimulatory signals. Co-stimulatory signals can amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity. A "**co-stimulatory signal**" refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell and/or NK cell proliferation and/or upregulation or downregulation of key molecules.

"**Alternative scaffold**" refers to a single chain protein framework that contains a structured core associated with variable domains of high conformational tolerance. The variable domains tolerate variation to be introduced without compromising scaffold integrity, and hence the variable domains can be engineered and selected for binding to a specific antigen.

"**Antibody-dependent cellular cytotoxicity**", "**antibody-dependent cell-mediated cytotoxicity**" or "**ADCC**" refers to the mechanism of inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells (NK), monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells.

"**Antibody-dependent cellular phagocytosis**" or "**ADCP**" refers to the mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells.

"**Antigen**" refers to any molecule (e.g., protein, peptide, polysaccharide, glycoprotein, glycolipid, nucleic acid, portions thereof, or combinations thereof) capable of being bound by an antigen binding domain or a T-cell receptor that is capable of mediating an immune response. Exemplary immune responses include antibody production and activation of immune cells, such as T cells, B cells or NK cells. Antigens may be expressed by genes, synthetized, or purified from biological samples such as a tissue sample, a tumor sample, a cell or a fluid with other biological components, organisms, subunits of proteins/antigens, killed or inactivated whole cells or lysates.

"**Antigen binding fragment**" or "**antigen binding domain**" 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', $F(ab')_2$, 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) 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.

"**Antibodies**" is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antigen binding fragments, multispecific antibodies, such as bispecific, trispecific, tetraspecific etc., dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity. "Full length antibodies" are comprised of two heavy chains (HC) and two light chains (LC) inter-connected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (comprised of domains CH1, hinge, CH2 and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The VH and the VL regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged

from amino-to-carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. Immunoglobulins may be assigned to five major classes, IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Antibody light chains of any vertebrate species may be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"**Bispecific**" refers to a molecule (such as an antibody) that specifically binds two distinct antigens or two distinct epitopes within the same antigen. The bispecific molecule may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca cynomolgus* (cynomolgus, cyno) or *Pan troglodytes*, or may bind an epitope that is shared between two or more distinct antigens.

"**Cancer**" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

"**Chimeric antigen receptor**" (CAR) as used herein is defined as a cell-surface receptor comprising an extracellular target-binding domain, a transmembrane domain and an intracellular signaling domain, all in a combination that is not naturally found together on a single protein. This includes receptors wherein the extracellular domain and the intracellular signaling domain are not naturally found together on a single receptor protein. CARs are intended primarily for use with lymphocyte such as T cells and natural killer (NK) cells.

"**Complement-dependent cytotoxicity**" or "**CDC**", refers to the mechanism of inducing cell death in which the Fc effector domain of a target-bound protein binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate CDC by binding complement receptors (e.g., CR3) on leukocytes

"**Complementarity determining regions**" (CDR) are antibody regions that bind an antigen. There are three CDRs in the VH (HCDR1, HCDR2, HCDR3) and three CDRs in the VL (LCDR1, LCDR2, LCDR3). CDRs may be defined using various delineations such as Kabat (Wu et al. (1970) J Exp Med 132: 211-50; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991), Chothia (Chothia et al. (1987) J Mol Biol 196: 901-17), IMGT (Lefranc et al. (2003) Dev Comp Immunol 27: 55-77), AbM (Martin and Thornton J Bmol Biol 263: 800-15, 1996), and Contact, which is based on an analysis of the available complex crystal structures (MacCallum, R. M., Martin, A. C. R. and Thornton, J. T. "Antibody-antigen

interactions: Contact analysis and binding site topography" J. Mol. Biol. 262:732-745). The correspondence between the various delineations and variable region numbering is described (see e.g. Lefranc et al. (2003) Dev Comp Immunol 27: 55-77; Honegger and Pluckthun, J Mol Biol (2001) 309:657-70; International ImMunoGeneTics (IMGT) database; Web resources, www.imgt.org). Available programs such as abYsis by UCL Business PLC may be used to delineate CDRs. The term "CDR", "HCDR1", "HCDR2", "HCDR3", "LCDR1", "LCDR2" and "LCDR3" as used herein includes CDRs defined by any of the methods described supra, Kabat, Chothia, IMGT, AbM, or Contact, unless otherwise explicitly stated in the specification. The CDRs of the sequences with SEQ ID NO. 1 to 17, 28 to 51, 62 to 80, 89 to 94, 98 to 104, 141 to 146, 149 to 154, 167 to 169, 172 to 174, 176 to 178, 180, 182 to 184, 186, 187, 189 to 191, 193 to 195, 197 to 199, 201, 236 to 244, 253 to 261 and 265 to 270 are defined according to Kabat. The CDRs of the sequences with SEQ ID NO. 399 to 434 are defined according to AbM. The CDRs of the sequences with SEQ ID NO. 435 to 470, are defined according to Chothia. The CDRs of the sequences with SEQ ID NO. 471 to 506are defined according to IMGT. The CDRs of the sequences with SEQ ID NO. 507 to 542are defined according to Contact.

"**Decrease,**" "**lower,**" "**lessen**," "**reduce**," or "**abate**" refers generally to the ability of a test molecule to mediate a reduced response (*i.e.,* downstream effect) when compared to the response mediated by a control or a vehicle. Exemplary responses are T cell expansion, T cell activation or T-cell mediated tumor cell killing or binding of a protein to its antigen or receptor, enhanced binding to a Fcγ or enhanced Fc effector functions such as enhanced ADCC, CDC and/or ADCP. Decrease may be a statistically significant difference in the measured response between the test molecule and the control (or the vehicle), or a decrease in the measured response, such as a decrease of about 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 30 fold or more, such as 500, 600, 700, 800, 900 or 1000 fold or more (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.).

"**Differentiation**" refers to a method of decreasing the potency or proliferation of a cell or moving the cell to a more developmentally restricted state.

"**Encode**" or "**encoding**" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

"**Enhance,**" "**promote,**" "**increase,**" "**expand**" or "**improve**" refers generally to the ability of a test molecule to mediate a greater response (*i.e.*, downstream effect) when compared to the response mediated by a control or a vehicle. Exemplary responses are T cell expansion, T cell activation or T-cell mediated tumor cell killing or binding of a protein to its antigen or receptor, enhanced binding to a Fcγ or enhanced Fc effector functions such as enhanced ADCC, CDC and/or ADCP. Enhance may be a statistically significant difference in the measured response between the test molecule and control (or vehicle), or an increase in the measured response, such as an increase of about 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 30 fold or more, such as 500, 600, 700, 800, 900 or 1000 fold or more (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.).

"**Expansion**" refers to the outcome of cell division and cell death.

"**Express**" and "**expression**" refers the to the well-known transcription and translation occurring in cells or *in vitro*. The expression product, *e.g.*, the protein, is thus expressed by the cell or *in vitro* and may be an intracellular, extracellular or a transmembrane protein.

"**Expression vector**" refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

"**dAb**" or "**dAb fragment**" refers to an antibody fragment composed of a VH domain (Ward et al., Nature 341:544 546 (1989)).

"**Fab**" or "**Fab fragment**" refers to an antibody fragment composed of VH, CH1, VL and CL domains.

"**F(ab')$_2$**" or "**F(ab')$_2$ fragment**" refers to an antibody fragment containing two Fab fragments connected by a disulfide bridge in the hinge region.

"**Fd**" or "**Fd fragment**" refers to an antibody fragment composed of VH and CH1 domains.

"**Fv**" or "**Fv fragment**" refers to an antibody fragment composed of the VH and the VL domains from a single arm of the antibody.

"**Full length antibody**" is comprised of two heavy chains (HC) and two light chains (LC) inter-connected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable domain (VH) and a heavy chain constant domain, the heavy chain constant domain comprised of subdomains CH1, hinge, CH2 and CH3. Each light chain is comprised of a light chain variable domain (VL) and a light chain constant domain (CL). The VH and the VL may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-to-carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

"**Genetic modification**" refers to the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences operably linked to polynucleotide encoding the chimeric antigen receptor, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "genetically engineered." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or from a different genus or species.

"**Heterologous**" refers to two or more polynucleotides or two or more polypeptides that are not found in the same relationship to each other in nature.

"**Heterologous polynucleotide**" refers to a non-naturally occurring polynucleotide that encodes two or more neoantigens as described herein.

"**Heterologous polypeptide**" refers to a non-naturally occurring polypeptide comprising two or more neoantigen polypeptides as described herein.

"**Host cell**" refers to any cell that contains a heterologous nucleic acid. An exemplary heterologous nucleic acid is a vector (*e.g.*, an expression vector).

"**Human antibody**" refers to an antibody that is optimized to have minimal immune response when administered to a human subject. Variable regions of human antibody are derived from human immunoglobulin sequences. If human antibody contains a constant region or a portion of the constant region, the constant region is also derived from human immunoglobulin sequences. Human antibody comprises heavy and light chain variable regions that are "derived from" sequences of human origin if the variable regions of the human antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice or rats carrying human immunoglobulin loci. "Human antibody" typically contains amino acid differences when compared to the immunoglobulins expressed in humans due to differences between the systems used to obtain the human antibody and human immunoglobulin loci, introduction of somatic mutations or intentional introduction of substitutions into the frameworks or CDRs, or both. Typically, "human antibody" is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical in amino acid sequence to an amino acid sequence encoded by human germline immunoglobulin or rearranged immunoglobulin genes. In some cases, "human antibody" may contain consensus framework sequences derived from human framework

sequence analyses, for example as described in Knappik et al., (2000) J Mol Biol 296:57-86, or a synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi et al., (2010) J Mol Biol 397:385-96, and in Int. Patent Publ. No. WO2009/085462. Antibodies in which at least one CDR is derived from a non-human species are not included in the definition of "human antibody".

"**Humanized antibody**" refers to an antibody in which at least one CDR is derived from non-human species and at least one framework is derived from human immunoglobulin sequences. Humanized antibody may include substitutions in the frameworks so that the frameworks may not be exact copies of expressed human immunoglobulin or human immunoglobulin germline gene sequences.

"**In combination with**" means that two or more therapeutic agents are be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

"**Intracellular signaling domain**" or "**cytoplasmic signaling domain**" refers to an intracellular portion of a molecule. It is the functional portion of the protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell.

"**Isolated**" refers to a homogenous population of molecules (such as synthetic polynucleotides or polypeptides) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. "Isolated" refers to a molecule that is substantially free of other cellular material and/or chemicals and encompasses molecules that are isolated to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

"**Modulate**" refers to either enhanced or decreased ability of a test molecule to mediate an enhanced or a reduced response (*i.e.,* downstream effect) when compared to the response mediated by a control or a vehicle.

"**Monoclonal antibody**" refers to an antibody obtained from a substantially homogenous population of antibody molecules, i.e., the individual antibodies comprising the population are identical except for possible well-known alterations such as removal of C-terminal lysine from the antibody heavy chain or post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation. Monoclonal antibodies typically bind one antigenic epitope. A bispecific monoclonal antibody binds two distinct antigenic epitopes. Monoclonal antibodies

may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific such as bispecific, monovalent, bivalent or multivalent.

"**Multispecific**" refers to a molecule, such as an antibody that specifically binds two or more distinct antigens or two or more distinct epitopes within the same antigen. Multispecific molecule may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno) or *Pan troglodytes*, or may bind an epitope that is shared between two or more distinct antigens.

"**Natural killer cell**" and "**NK cell**" are used interchangeably and synonymously herein. NK cell refers to a differentiated lymphocyte with a $CD16^+CD56^+$ and/or $CD57^+$ TCR^- phenotype. NK cells are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

"**Operatively linked**" and similar phrases, when used in reference to nucleic acids or amino acids, refers to the operational linkage of nucleic acid sequences or amino acid sequence, respectively, placed in functional relationships with each other. For example, an operatively linked promoter, enhancer elements, open reading frame, 5' and 3' UTR, and terminator sequences result in the accurate production of a nucleic acid molecule (e.g., RNA) and in some instances to the production of a polypeptide (i.e., expression of the open reading frame). Operatively linked peptide refers to a peptide in which the functional domains of the peptide are placed with appropriate distance from each other to impart the intended function of each domain.

"**Pharmaceutical combination**" refers to a combination of two or more active ingredients administered either together or separately.

"**Pharmaceutical composition**" refers to a composition that results from combining an active ingredient and a pharmaceutically acceptable carrier.

"**Pharmaceutically acceptable carrier**" or "excipient" refers to an ingredient in a pharmaceutical composition, other than the active ingredient, which is nontoxic to a subject. Exemplary pharmaceutically acceptable carriers are a buffer, stabilizer or preservative.

"**Polynucleotide**" or "**nucleic acid**" refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide. Polynucleotide may be a DNA or a RNA molecule.

"**Prevent,**" "**preventing,**" "**prevention,**" or "**prophylaxis**" of a disease or disorder means preventing that a disorder occurs in a subject.

"**Proliferation**" refers to an increase in cell division, either symmetric or asymmetric division of cells.

"**Promoter**" refers to the minimal sequences required to initiate transcription. Promoter may also include enhancers or repressor elements which enhance or suppress transcription, respectively.

"**Protein**" or "**polypeptide**" are used interchangeably herein are refers to a molecule that comprises one or more polypeptides each comprised of at least two amino acid residues linked by a peptide bond. Protein may be a monomer, or may be protein complex of two or more subunits, the subunits being identical or distinct. Small polypeptides of less than 50 amino acids may be referred to as "peptides". Protein may be a heterologous fusion protein, a glycoprotein, or a protein modified by post-translational modifications such as phosphorylation, acetylation, myristoylation, palmitoylation, glycosylation, oxidation, formylation, amidation, citrullination, polyglutamylation, ADP-ribosylation, pegylation or biotinylation. Protein may be recombinantly expressed.

"**Recombinant**" refers to polynucleotides, polypeptides, vectors, viruses and other macromolecules that are prepared, expressed, created or isolated by recombinant means.

"**Regulatory element**" refers to any cis-or trans acting genetic element that controls some aspect of the expression of nucleic acid sequences.

"**Relapsed**" refers to the return of a disease or the signs and symptoms of a disease after a period of improvement after prior treatment with a therapeutic.

"**Refractory**" refers to a disease that does not respond to a treatment. A refractory disease can be resistant to a treatment before or at the beginning of the treatment, or a refractory disease can become resistant during a treatment.

"**Single chain Fv**" or "**scFv**" refers to a fusion protein comprising at least one antibody fragment comprising a light chain variable region (VL) and at least one antibody fragment comprising a heavy chain variable region (VH), wherein the VL and the VH are contiguously linked via a polypeptide linker, and capable of being expressed as a single chain polypeptide. Unless specified, as used herein, a scFv may have the VL and VH variable regions in either order, e.g., with respect to the N- terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

"**Specifically binds,**" "**specific binding,**" "**specifically binding**" or "**binds**" refer to a proteinaceous molecule binding to an antigen or an epitope within the antigen with greater affinity than for other antigens. Typically, the proteinaceous molecule binds to the antigen or the epitope within the antigen with an equilibrium dissociation constant (K_D) of about $1x10^{-7}$ M or less, for example about $5x10^{-8}$ M or less, about $1x10^{-8}$ M or less, about $1x10^{-9}$ M or less, about $1x10^{-10}$ M or less, about $1x10^{-11}$ M or less, or about $1x10^{-12}$ M or less, typically with the K_D that is at least one hundred fold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein).

"**Subject**" includes any human or nonhuman animal. "Nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. The terms "subject" and "patient" can be used interchangeably herein.

"**T cell**" and "**T lymphocyte**" are interchangeable and used synonymously herein. T cell includes thymocytes, naïve T lymphocytes, memory T cells, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; $CD4^+$ T cell) $CD4^+$ T cell, a cytotoxic T cell (CTL; $CD8^+$ T cell), a tumor infiltrating cytotoxic T cell (TIL; $CD8^+$ T cell), $CD4^+CD8^+$ T cell, or any other subset of T cells. Also included are "NKT cells", which refer to a specialized population of T cells that express a semi-invariant αβ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include $NK1.1^+$ and $NK1.1^-$, as well as $CD4^+$, $CD4^-$, $CD8^+$ and $CD8^-$ cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the MHC I-like molecule CD Id. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance. Also included are "gamma-delta T cells (γδ T cells)," which refer to a specialized population that to a small subset of T cells possessing a distinct TCR on their surface, and unlike the majority of T cells in which the TCR is composed of two glycoprotein chains designated α- and β-TCR chains, the TCR in γδ T cells is made up of a γ-chain and a δ-chain . γδ T cells can play a role in immunosurveillance and immunoregulation, and were found to be an important source of IL-17 and to induce robust $CD8^+$ cytotoxic T cell response. Also included are "regulatory T cells" or "Tregs" which refer to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. Tregs are typically transcription factor Foxp3-positive $CD4^+$T cells and can also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing $CD4^+$T cells.

"**Therapeutically effective amount**" or "**effective amount**" used interchangeably herein, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Example indicators of an effective therapeutic or combination of therapeutics that include, for example, improved wellbeing of the patient, reduction of a tumor burden, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

"**Transduction"** refers to the introduction of a foreign nucleic acid into a cell using a viral vector.

"**Treat,**" "**treating**" or "**treatment**" of a disease or disorder such as cancer refers to accomplishing one or more of the following: reducing the severity and/or duration of the disorder, inhibiting worsening of symptoms characteristic of the disorder being treated, limiting or preventing recurrence of the disorder in subjects that have previously had the disorder, or limiting or preventing recurrence of symptoms in subjects that were previously symptomatic for the disorder.

"**Tumor cell**" or a "**cancer cell**" refers to a cancerous, pre-cancerous or transformed cell, either *in vivo*, *ex vivo*, or in tissue culture, that has spontaneous or induced phenotypic changes. These changes do not necessarily involve the uptake of new genetic material. Although transformation may arise from infection with a transforming virus and incorporation of new genomic nucleic acid, uptake of exogenous nucleic acid or it can also arise spontaneously or following exposure to a carcinogen, thereby mutating an endogenous gene. Transformation/cancer is exemplified by morphological changes, immortalization of cells, aberrant growth control, foci formation, proliferation, malignancy, modulation of tumor specific marker levels, invasiveness, tumor growth in suitable animal hosts such as nude mice, and the like, *in vitro*, *in vivo*, and *ex vivo*.

"**Variant,**" "**mutant**" or "**altered**" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications, for example one or more substitutions, insertions or deletions.

The numbering of amino acid residues in the antibody constant region throughout the specification is according to the EU index as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), unless otherwise explicitly stated.

Mutations in the Ig constant regions are referred to as follows: L351Y_F405A_Y407V refers to L351Y, F405A and Y407V mutations in one immunoglobulin constant region. L351Y_F405A_Y407V/T394W refers to L351Y, F405A and Y407V mutations in the first Ig constant region and T394W mutation in the second Ig constant region, which are present in one multimeric protein.

COMPOSITIONS OF MATTER

Antigen binding domains that bind CD33

The disclosure provides antigen binding domains that bind CD33, monospecific and multispecific proteins comprising the antigen binding domains that bind CD33, chimeric antigen receptors (CAR) comprising the antigen binding domains that bind CD33, polynucleotides encoding the foregoing, vectors, host cells and methods of making and using the foregoing. The antigen binding domains that bind CD33 identified herein demonstrated improved properties in terms of improved thermostability.

The disclosure provides an isolated protein comprising an antigen binding domain that binds CD33, wherein the antigen binding domain that binds CD33comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27.

Methods and techniques for identifying CDRs (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3) are known in the art and can be used to identify CDRs within any of the heavy chain variable regions and/or any of the light chain variable regions disclosed herein. Conventions that can be used to identify the boundaries of CDRs include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition, the IMGT definition and the Contact definition. Without wishing to be bound by theory, the Kabat definition is based on sequence variability, the Chothia definition is based on location of structural loop regions, and the AbM definition is a compromise between the Chothia and Kabat approaches.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein isolated protein comprises the HCDR1, the HCDR2, the HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is a Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some

embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, wherein the antigen binding domain that binds CD33 comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In certain embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56.

In other embodiments, the the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262.

In other embodiments, the the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

Exemplary HCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264.

In some embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 272

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;
b) SEQ ID NOs: 29, 37, and 46, respectively;
c) SEQ ID NOs: 30, 38, and 47, respectively;
d) SEQ ID NOs: 31, 39, and 48, respectively;
e) SEQ ID NOs: 32, 40, and 49, respectively;
f) SEQ ID NOs: 33, 41, and 50, respectively;
g) SEQ ID NOs: 34, 42, and 51, respectively;
h) SEQ ID NOs: 34, 43, and 51, respectively;
i) SEQ ID NOs: 34, 44, and 51, respectively; or
j) SEQ ID NOs: 35, 42, and 51, respectively;
k) SEQ ID NOs: 253, 256 and 259, respectively;
l) SEQ ID NOs: 254, 257 and 260, respectively; or
m) SEQ ID NOs: 255, 258 and 261 respectively.

In certain embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 29, 37, and 46, respectively.

In other embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 32, 40, and 49, respectively.

In other embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 34, 43, and 51, respectively.

In other embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 253, 256, and 259, respectively.

In other embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 254, 257, and 260, respectively.

In other embodiments, the isolated protein comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 255, 258, and 361, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264.

In some embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 272

In other embodiments, the antigen binding domain that binds CD33 comprises the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an antigen binding domain that binds myeloid cell surface antigen CD33, wherein the antigen binding domain that binds CD33 comprises a heavy chain complementarity determining region (HCDR) 1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85; an HCDR1, an

HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

Exemplary HCDR and LCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In certain embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272.

In other embodiments, the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$.

In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an antigen binding domain that binds myeloid cell surface antigen CD33, wherein the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;
k) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively;
l) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively;
m) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In certain embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively.

In other embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively.

In other embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively.

In other embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively.

In other embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively.

In other embodiments, the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In some embodiments, the antigen binding domain that binds CD33 comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;

k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or

m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the

CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the isolated protein comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the isolated protein comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the isolated protein comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the isolated protein comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the isolated protein comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the isolated protein comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the

CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises a) an

HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105; b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106; c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107; d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185; e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188; f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192; g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196; h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;
g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;
h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;
i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the

antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises a VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and a VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105; the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106; the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107; the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185; the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188; the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192; the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196; the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds myeloid cell surface antigen CD33, where the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105; the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106; the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107; the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185; the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188; the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192; the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196; the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202. In some embodiments, the antigen binding domain that binds CD33 is an scFv. In some embodiments, the antigen binding domain that binds CD33 is an $(scFv)_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fv. In some embodiments, the antigen binding domain that binds CD33 is an Fab. In some embodiments, the antigen binding domain that binds CD33 is an $F(ab')_2$. In some embodiments, the antigen binding domain that binds CD33 is an Fd. In some

embodiments, the CD33 antigen binding domain is a dAb. In some embodiments, the CD33 antigen binding domain is a VHH.

CD33 binding scFvs

Any of the VH and the VL domains identified herein that bind CD33 may be engineered into scFv format in either VH-linker-VL or VL-linker-VH orientation. Any of the VH and the VL domains identified herein may also be used to generate sc$(Fv)_2$ structures, such as VH-linker-VL-linker-VL-linker-VH, VH-linker-VL-linker-VH-linker-VL. VH-linker-VH-linker-VL-linker-VL. VL-linker-VH-linker-VH-linker-VL. VL-linker-VH-linker-VL-linker-VH or VL-linker-VL-linker-VH-linker-VH.

The VH and the VL domains identified herein may be incorporated into a scFv format and the binding and thermostability of the resulting scFv to CD33 may be assessed using known methods. Binding may be assessed using ProteOn XPR36, Biacore 3000 or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. Binding may be evaluated using purified scFvs or E. coli supernatants or lysed cells containing the expressed scFv. The measured affinity of a test scFv to CD33 may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other binding parameters (e.g., K_D, K_{on}, K_{off}) are typically made with standardized conditions and standardized buffers. Thermostability may be evaluated by heating the test scFv at elevated temperatures, such as at 50°C, 55°C or 60°C for a period of time, such as 5 minutes (min), 10 min, 15 min, 20 min, 25 min or 30 min and measuring binding of the test scFv to CD33. The scFvs retaining comparable binding to CD33 when compared to a non-heated scFv sample are referred to as being thermostable.

In recombinant expression systems, the linker is a peptide linker and may include any naturally occurring amino acid. Exemplary amino acids that may be included into the linker are Gly, Ser Pro, Thr, Glu, Lys, Arg, Ile, Leu, His and The. The linker should have a length that is adequate to link the VH and the VL in such a way that they form the correct conformation relative to one another so that they retain the desired activity, such as binding to CD33.

The linker may be about 5-50 amino acids long. In some embodiments, the linker is about 10-40 amino acids long. In some embodiments, the linker is about 10-35 amino acids long. In some embodiments, the linker is about 10-30 amino acids long. In some embodiments, the linker is about 10-25 amino acids long. In some embodiments, the linker is about 10-20 amino acids long. In some embodiments, the linker is about 15-20 amino acids long. In some embodiments, the linker is 6 amino acids long. In some embodiments, the linker is 7 amino acids long. In some embodiments, the linker is 8 amino acids long. In some embodiments, the linker is 9 amino acids long. In some embodiments, the linker is 10 amino acids long. In some embodiments, the linker is 11 amino acids long. In some embodiments, the linker is 12 amino acids long. In some embodiments, the linker is 13 amino acids long.

In some embodiments, the linker is 14 amino acids long. In some embodiments, the linker is 15 amino acids long. In some embodiments, the linker is 16 amino acids long. In some embodiments, the linker is 17 amino acids long. In some embodiments, the linker is 18 amino acids long. In some embodiments, the linker is 19 amino acids long. In some embodiments, the linker is 20 amino acids long. In some embodiments, the linker is 21 amino acids long. In some embodiments, the linker is 22 amino acids long. In some embodiments, the linker is 23 amino acids long. In some embodiments, the linker is 24 amino acids long. In some embodiments, the linker is 25 amino acids long. In some embodiments, the linker is 26 amino acids long. In some embodiments, the linker is 27 amino acids long. In some embodiments, the linker is 28 amino acids long. In some embodiments, the linker is 29 amino acids long. In some embodiments, the linker is 30 amino acids long. In some embodiments, the linker is 31 amino acids long. In some embodiments, the linker is 32 amino acids long. In some embodiments, the linker is 33 amino acids long. In some embodiments, the linker is 34 amino acids long. In some embodiments, the linker is 35 amino acids long. In some embodiments, the linker is 36 amino acids long. In some embodiments, the linker is 37 amino acids long. In some embodiments, the linker is 38 amino acids long. In some embodiments, the linker is 39 amino acids long. In some embodiments, the linker is 40 amino acids long. Exemplary linkers that may be used are Gly rich linkers, Gly and Ser containing linkers, Gly and Ala containing linkers, Ala and Ser containing linkers, and other flexible linkers.

Other linker sequences may include portions of immunoglobulin hinge area, CL or CH1 derived from any immunoglobulin heavy or light chain isotype. Alternatively, a variety of non-proteinaceous polymers, including polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers. Exemplary linkers that may be used are shown in Table 1. Additional linkers are described for example in International Patent Publication No. WO2019/060695, which is incorporated by reference herein in its entirety.

In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL).

In some embodiments, the scFv comprises, from the N-to C-terminus, the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 109.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 110.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 111.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 112.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 113.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 114.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 115.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 116.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 117.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 118.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 119.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 120.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 121.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 122.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 123.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 124.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 125.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 126.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 127.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 128.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 129.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 130.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 131.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 132.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 133.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 134.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 135.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 136.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 137.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 138.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 139.
In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 140.

Table 1.

Linker name	Amino acid sequence	SEQ ID NO:
Linker 1	GGSEGKSSGSGSESKSTGGS	108
Linker 2	GGGSGGGS	109
Linker 3	GGGSGGGSGGGS	110
Linker 4	GGGSGGGSGGGSGGGS	111
Linker 5	GGGSGGGSGGGSGGGSGGGS	112

Linker name	Amino acid sequence	SEQ ID NO:
Linker 6	GGGGSGGGGSGGGGS	113
Linker 7	GGGGSGGGGSGGGGSGGGGS	114
Linker 8	GGGGSGGGGSGGGGSGGGGSGGGGS	115
Linker 9	GSTSGSGKPGSGEGSTKG	116
Linker 10	IRPRAIGGSKPRVA	117
Linker 11	GKGGSGKGGSGKGGS	118
Linker 12	GGKGSGGKGSGGKGS	119
Linker 13	GGGKSGGGKSGGGKS	120
Linker 14	GKGKSGKGKSGKGKS	121
Linker 15	GGGKSGGKGSGKGGS	122
Linker 16	GKPGSGKPGSGKPGS	123
Linker 17	GKPGSGKPGSGKPGSGKPGS	124
Linker 18	GKGKSGKGKSGKGKSGKGKS	125
Linker 19	STAGDTHLGGEDFD	126
Linker 20	GEGGSGEGGSGEGGS	127
Linker 21	GGEGSGGEGSGGEGS	128
Linker 22	GEGESGEGESGEGES	129
Linker 23	GGGESGGEGSGEGGS	130
Linker 24	GEGESGEGESGEGESGEGES	131
Linker 25	GSTSGSGKPGSGEGSTKG	132
Linker 26	PRGASKSGSASQTGSAPGS	133
Linker 27	GTAAAGAGAAGGAAAGAAG	134
Linker 28	GTSGSSGSGSGGSGSGGGG	135
Linker 29	GKPGSGKPGSGKPGSGKPGS	136
Linker 30	GSGS	137
Linker 31	APAPAPAPAP	138
Linker 32	APAPAPAPAPAPAPAPAPAP	139
Linker 33	AEAAAKEAAAKEAAAAKEAAAAKEAAAAKAAA	140

In certain embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108 (e.g. consists of the amino acid sequence of SEQ ID NO: 108).

In some embodiments, the scFv comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the scFv comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In certain embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

Exemplary HCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In some embodiments, the scFv comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264.

In certain embodiments, the scFv comprises the VH of SEQ ID NO: 53.

In other embodiments, the scFv comprises the VH of SEQ ID NO: 56.

In other embodiments, the scFv comprises the VH of SEQ ID NO: 59.

In other embodiments, the scFv comprises the VH of SEQ ID NO: 262.

In other embodiments, the scFv comprises the VH of SEQ ID NO: 263.

In other embodiments, the scFv comprises the VH of SEQ ID NO: 264.

In some embodiments, the scFv comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In certain embodiments, the scFv comprises the VL of SEQ ID NO: 82.

In other embodiments, the scFv comprises the VL of SEQ ID NO: 85.

In other embodiments, the scFv comprises the VL of SEQ ID NO: 87.

In other embodiments, the scFv comprises the VL of SEQ ID NO: 271.

In other embodiments, the scFv comprises the VL of SEQ ID NO: 272.

In other embodiments, the scFv comprises the VL of SEQ ID NO: 273.

In some embodiments, the scFv comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273. In some embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively;

j) SEQ ID NOs: 35, 42, and 51, respectively;

k) SEQ ID NOs: 253, 256 and 259, respectively;

l) SEQ ID NOs: 254, 257 and 260, respectively; or

m) SEQ ID NOs: 255, 258 and 261, respectively.

In certain embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 29, 37, and 46, respectively.

In other embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 32, 40, and 49, respectively.

In other embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 34, 43, and 51, respectively.

In other embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 253, 256, and 259, respectively.

In other embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 254, 257, and 260, respectively.

In other embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 255, 258, and 261, respectively.In some embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO:

262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In certain embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272.

In other embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

Exemplary HCDR and LCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In some embodiments, the scFv comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;

g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;

h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or

j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;

k) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively;

l) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively;

m) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In certain embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively.

In other embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively.

In other embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively.

In other embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively.

In other embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively.

In other embodiments, the scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

In some embodiments, the scFv comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;

k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or

m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the scFv comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 53, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 82 (VH-L1-VL). In other embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 82, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 53 (VL-L1-VH).

In other embodiments, the scFv comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 56, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 85 (VH-L1-VL). In other

embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 56, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 85 (VL-L1-VH).

In other embodiments, the scFv comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 59, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 87 (VH-L1-VL). In other embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 59, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 87 (VL-L1-VH).

In other embodiments, the scFv comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 262, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 271 (VH-L1-VL). In other embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 271, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 262 (VL-L1-VH).

In other embodiments, the scFv comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 263, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 272 (VH-L1-VL). In other embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 272, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 263 (VL-L1-VH).

In other embodiments, the scFv comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273. In certain such embodiments, the scFv comprises a linker sequence of the amino acid sequence of SEQ ID NO: 108. Thus, in some embodiments, the scFv comprises, from the N- to C-terminus, a VH of SEQ ID NO: 264, a first linker (L1) of SEQ ID NO: 108 and a VL of SEQ ID: 273 (VH-L1-VL). In other embodiments, the scFv comprises from the N- to C- terminus, a VL of SEQ ID NO: 273, a first linker of SEQ ID NO: 108 and a VL of SEQ ID NO: 264 (VL-L1-VH).

In some embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

In some embodiments, the scFv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively.

In some embodiments, the scFv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and an LCDR1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

In some embodiments, the scFv comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the scFv comprises a VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and a VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the scFv comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105; the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106; the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107; the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185; the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188; the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192; the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196; the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 203.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 204.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 205.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 206.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 207.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 208.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 209.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 210.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 211.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 212.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 213.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 214.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 215.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 216.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 217.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 218.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 219.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 220.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 221.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 274.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 275.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 276.

In certain embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 213.

In other embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 216.

In other embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 219.

In other embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 274.

In other embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 275.

In other embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 276.

Other antigen binding domains that bind CD33

Any of the VH and the VL domains identified herein that bind CD33 may also be engineered into Fab, $F(ab')_2$, Fd or Fv format and their binding to CD33 and thermostability may be assessed using the assays described herein. In certain embodiments, the VH and VL domains identified herein are engineered into a Fab.

In some embodiments, the Fab comprises an HCDR1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the Fab comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the Fab comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In certain embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

Exemplary HCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In some embodiments, the Fab comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264. In some embodiments, the Fab comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273. In some embodiments, the Fab comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88, 271, 272 or 273.

In some embodiments, the Fab comprises an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively; or

j) SEQ ID NOs: 35, 42, and 51, respectively; or

l) SEQ ID NOs: 253, 256, and 259, respectively;

m) SEQ ID NOs: 254, 257, and 260, respectively;

n) SEQ ID NOs: 255, 258, and 261, respectively.

In some embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

Exemplary HCDR and LCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In certain embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272.

In other embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the Fab comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;
k) SEQ ID NOs: 253, 256, 259, 265, 74, and 269, respectively;
l) SEQ ID NOs: 254, 257, 260, 66, 267, and 76, respectively; or
m) SEQ ID NOs: 255, 258, 261, 266, 268, and 270, respectively.

In some embodiments, the Fab comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2

and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

In some embodiments, the Fab comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively.

In some embodiments, the Fab comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and an LCDR1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

In some embodiments, the Fab comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the Fab comprises a VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and a VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the Fab comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105; the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106; the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107; the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185; the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188; the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192; the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196; the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In other embodiments, the VH and VL domains identified herein are engineered into a $F(ab')_2$.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the $F(ab')_2$ comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264. In some embodiments, the $F(ab')_2$ comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273. In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively;

j) SEQ ID NOs: 35, 42, and 51, respectively;

k) SEQ ID NOs: 253, 256 and 259, respectively

l) SEQ ID NOs: 254, 257 and 260, respectively

m) SEQ ID NOs: 255, 258 and 261 respectively.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO:

87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88 an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;
k) SEQ ID NOs: 253, 256, 259, 265, 74, and 269, respectively;
l) SEQ ID NOs: 254, 257, 260, 66, 267, and 76, respectively; or
m) SEQ ID NOs: 255, 258, 261, 266, 268, and 270, respectively.

In some embodiments, the $F(ab')_2$ comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;

k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or

m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively.

In some embodiments, the $F(ab')_2$ comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and an LCDR1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

In some embodiments, the F(ab')$_2$ comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

In some embodiments, the F(ab')$_2$ comprises a VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 196, and a VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202. In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105; the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106; the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107; the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185; the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188; the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192; the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196; the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In certain embodiments, the VH and VL domains identified herein are engineered into a Fv.

In some embodiments, the Fv comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the Fv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the Fv comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In certain embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In some embodiments, the Fv comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264. In some embodiments, the Fv comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, or 273. In some embodiments, the Fv comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60,r 61, 262, 263 or 264, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, 273.

In some embodiments, the Fv comprises an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;
c) SEQ ID NOs: 30, 38, and 47, respectively;
d) SEQ ID NOs: 31, 39, and 48, respectively;
e) SEQ ID NOs: 32, 40, and 49, respectively;
f) SEQ ID NOs: 33, 41, and 50, respectively;
g) SEQ ID NOs: 34, 42, and 51, respectively;
h) SEQ ID NOs: 34, 43, and 51, respectively;
i) SEQ ID NOs: 34, 44, and 51, respectively;
j) SEQ ID NOs: 35, 42, and 51, respectively;
k) SEQ ID NOs: 253, 256 and 259, respectively
l) SEQ ID NOs: 254, 257 and 260, respectively
m) SEQ ID NOs: 255, 258 and 261 respectively.

In some embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In certain embodiment, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271.

In other embodiments the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272.

In other embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the Fv comprises an HCDR1, an HCDR2, an HCDR3, an LCDR1, an LCDR2 and an LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;

g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;

h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or

j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

In some embodiments, the Fv comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;

k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or

m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

In some embodiments, the Fv comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively.

In some embodiments, the Fv comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and an LCDR1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

In certain embodiments, the VH and VL domains identified herein are engineered into a Fd.

In some embodiments, the Fd comprises an HCDR1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO:

19; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27. In some embodiments, the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the Fd comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 1, 6, and 12, respectively; SEQ ID NOs: 2, 7, and 13, respectively; SEQ ID NOs: 1, 8, and 14, respectively; SEQ ID NOs: 3, 9, and 13, respectively; SEQ ID NOs: 4, 10, and 15, respectively; SEQ ID NOs: 5, 11, and 16, respectively; or SEQ ID NOs: 5, 11, and 17, respectively.

In some embodiments, the Fd comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60;

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61;

k) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

l) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

m) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In certain embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

In some embodiments, the Fd comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264.

In some embodiments, the Fd comprises an HCDR1, an HCDR2, and an HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively;

j) SEQ ID NOs: 35, 42, and 51, respectively;

k) SEQ ID NOs: 253, 256 and 259, respectively

l) SEQ ID NOs: 254, 257 and 260, respectively

m) SEQ ID NOs: 255, 258 and 261 respectively.

In some embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In certain embodiment, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271.

In other embodiments the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272.

In other embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

In some embodiments, the Fd comprises:

a) the VH of SEQ ID NO: 52;

b) the VH of SEQ ID NO: 53;

c) the VH of SEQ ID NO: 54;

d) the VH of SEQ ID NO: 55;

e) the VH of SEQ ID NO: 56;

f) the VH of SEQ ID NO: 57;

g) the VH of SEQ ID NO: 58;

h) the VH of SEQ ID NO: 59;

i) the VH of SEQ ID NO: 60;

j) the VH of SEQ ID NO: 61;

k) the VH of SEQ ID NO: 262;

l) the VH of SEQ ID NO: 263; or

m) the VH of SEQ ID NO: 264.

In some embodiments, the Fd comprises an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179; an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181; or an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

In some embodiments, the Fd comprises an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 92, respectively; an HCDR1, an HCDR2, and the HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 33, 91, and 94, respectively;

an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 167, 168, and 169, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 172, 173, and 174, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 176, 177, and 178, respectively; an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 180, respectively; or an HCDR1, an HCDR2, and an HCDR3 of SEQ ID NOs: 89, 90, and 93, respectively.

Homologous antigen binding domains and antigen binding domains with conservative substitutions

Variants of the antigen binding domains that bind CD33 are within the scope of the disclosure. For example, variants may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acid substitutions in the antigen binding domain that bind CD33 as long as they retain or have improved functional properties when compared to the parent antigen binding domains. For instance, in some embodiments, the variant antigen binding domain that binds CD33 has improved thermostability when compared to the parent antigen binding domain in the same assay. In some embodiments, the variant antigen binding domain that binds CD33 has reduced likelihood of post-translational modification when compared to the parent binding domain. A reduced likelihood of post-translation modification may arise due to the substitution of a motif such as NG, DG or DP. In some embodiments, the sequence identity may be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to the antigen binding domains that bind CD33 of the disclosure. In some embodiments, the variation is in the framework regions. In some embodiments, variants are generated by conservative substitutions.

For example, the antigen binding domains that bind CD33 may comprise substitutions at residue positions P41, I49, M70, and A88 in the VH (residue numbering according to the hu11B6_VH of SEQ ID NO: 5) and S80, L82, A88 and Y91 in the VL (residue numbering according to the hu11B6_VL of SEQ ID NO: 2). Conservative substitutions may be made at any indicated positions and the resulting variant antigen binding domains that bind CD33 are tested for their desired characteristics in the assays described herein.

Also provided are antigen binding domains that bind CD33 comprising the VH which are at least 80% identical to:

the VH of SEQ ID NO: 18;

the VH of SEQ ID NO: 19;

the VH of SEQ ID NO: 20;

the VH of SEQ ID NO: 21;

the VH of SEQ ID NO: 22;

the VH of SEQ ID NO: 23;

the VH of SEQ ID NO: 24;

the VH of SEQ ID NO: 25;
the VH of SEQ ID NO: 26;
the VH of SEQ ID NO: 27;
the VH of SEQ ID NO: 52;
the VH of SEQ ID NO: 53;
the VH of SEQ ID NO: 54;
the VH of SEQ ID NO: 55;
the VH of SEQ ID NO: 56;
the VH of SEQ ID NO: 57;
the VH of SEQ ID NO: 58;
the VH of SEQ ID NO: 59;
the VH of SEQ ID NO: 60;
the VH of SEQ ID NO: 61;
the VH of SEQ ID NO: 95;
the VH of SEQ ID NO: 96;
the VH of SEQ ID NO: 97;
the VH of SEQ ID NO: 170;
the VH of SEQ ID NO: 171;
the VH of SEQ ID NO: 175;
the VH of SEQ ID NO: 179;
the VH of SEQ ID NO: 181;
the VH of SEQ ID NO: 96
the VH of SEQ ID NO: 262;
the VH of SEQ ID NO: 263; or
the VH of SEQ ID NO: 264.

In certain embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 53.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 56.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 59.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 262.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 263.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 264.

In some embodiments, the identity is 85%. In some embodiments, the identity is 90%. In some embodiments, the identity is 91%. In some embodiments, the identity is 91%. In some embodiments, the identity is 92%. In some embodiments, the identity is 93%. In some embodiments, the identity is 94%. In some embodiments, the identity is 94%. In some embodiments, the identity is 95%. In some embodiments, the identity is 96%. In some embodiments, the identity is 97%. In some embodiments, the identity is 98%. In some embodiments, the identity is 99%.

Also provided are antigen binding domains that bind CD33 comprising the VH and the VL which are at least 80% identical to

the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200;
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202;
the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the antigen binding domain that binds to CD33 comprises a VH and VL that are at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 53 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 82. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 82 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 53.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 56 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 85. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 85 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 56.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 59 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 87. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 87 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 59.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 262 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 271. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 271 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 262.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 263 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 272. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 272 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 263.

In other embodiments, the antigen binding domain that binds to CD33 comprises a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273. For instance, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 264 and a VL that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VL of SEQ ID NO: 273. For instance, the antigen binding domain that binds to CD33 comprises a VL of SEQ ID NO: 273 and a VH that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH of SEQ ID NO: 264.

The inventors have found that positions in the VH and/or VL domains may be mutated to remove motifs that are liable to post-translational modification. Post-translational modification may increase the

likelihood of, for example, deamidation, isomerization and/or fragmentation of the antigen binding domain that binds to CD33. These mutations may also enhance thermostability of an antigen binding domain that binds to CD33.

Examples of sequence motifs that are liable to post-translation modification, for example, NG, DG, and DP.

Accordingly, in some embodiments, the antigen binding domain that binds to CD33 comprises a VH and/or VL domain comprising one or two amino acid substitutions in one or more NG motifs. In some embodiments, the N residue of the NG motif is substituted. In other embodiments, the G residue of the NG motif is substituted. In some embodiments the N and G residues of the NG motif are substituted. In certain such embodiments, the one or two amino acid substitutions in the one or more NG motifs are the only substitutions within the VH and/or VL domain.

In some embodiments, the antigen binding domain that binds to CD33 comprises a VH and/or VL domain comprising one or two amino acid substitutions in one or more DG motifs. In some embodiments, the D residue of the DG motif is substituted. In other embodiments, the G residue of the DG motif is substituted. In some embodiments the D and G residues of the DG motif are substituted. In certain such embodiments, the one or two amino acid substitutions in the one or more DG motifs are the only substitutions within the VH and/or VL domain.

In some embodiments, the antigen binding domain that binds to CD33 comprises a VH and/or VL domain comprising one or two amino acid substitutions in one or more DP motifs. In some embodiments, the D residue of the DP motif is substituted. In other embodiments, the P residue of the DP motif is substituted. In some embodiments the D and P residues of the DP motif are substituted. In certain such embodiments, the one or two amino acid substitutions in the one or more DP motifs are the only substitutions within the VH and/or VL domain.

An exemplary antigen binding domain that binds to CD33 of the invention is a protein (for example, an scFv) comprising the VH of SEQ ID NO: 262 and VL of SEQ ID NO: 271. The inventors have found that mutating the DG motif within the VH of SEQ ID NO: 262 can reduce the likelihood of post-translation modification whilst maintaining CD33 binding properties. These mutations may also enhance thermostability.

An antigen binding domain that binds to CD33 having the VH of SEQ ID NO: 262 and VL of SEQ ID NO: 271 may be mutated to remove a DG motif. The VH of SEQ ID NO: 262 has a DG motif at positions 54-55 (using residue numbering according to EU index).

Thus, in some embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 262 in which the D residue at position 54 is substituted (for example, D54G, D54A, D54Y, D54P, D54N, D54S, D54R, D54L, D54V, D54T, D54F or D54W). In

other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 262 in which the G residue at position 55 is substituted (for example, G55V, G55P, G55R, G55A, G55L, G55Q, G55T or G55H). In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 262 in which the D residue at position 54 (for example, D54G, D54A, D54Y, D54P, D54N, D54S, D54R, D54L, D54V, D54T, D54F or D54W) is substituted and the G residue at position 55 is substituted (for example, G55V, G55P, G55R, G55A, G55L, G55Q, G55T or G55H). In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309 or 310.

In certain embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises:the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271; or

the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271.

Another exemplary antigen binding domain that binds to CD33 of the invention is a protein (for example, an scFv) comprising the VH of SEQ ID NO: 263 and VL of SEQ ID NO: 272. The inventors have found that mutating one or more NG motifs within the VH of SEQ ID NO: 263 and VL of SEQ ID

NO: 272 can reduce the likelihood of post-translation modification whilst maintaining CD33 binding properties. These mutations may also enhance thermostability.

An antigen binding domain that binds to CD33 having the VH of SEQ ID NO: 263 and VL of SEQ ID NO: 272 may be mutated to remove an NG motif. The VH of SEQ ID NO: 263 has a NG motif at positions 99-100 (using residue numbering according to EU index) and the VL of SEQ ID NO: 272 has a NG motif at positions 97-98 (using residue numbering according to EU index).

Thus, in some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 263 in which the N residue at position 99 is substituted (for example, N99V, N99S, N99E, N99H, N99A, N99G, N99K, N99T, N99F, N99V, N99R, N99L, N99H or N99I). In other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 263 in which the G residue at position 100 is substituted (for example, G100S, G100A). In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 263 in which the N residue at position 99 is substituted (for example, N99V, N99S, N99E, N99H, N99A, N99G, N99K, N99T, N99F, N99V, N99R, N99L, N99H or N99I) and the G residue at position 100 is substituted (for example, G100S, G100A). In some embodiments, the antigen binding domain that binds to CD33 comprises a VH of SEQ ID NO: 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, or 352.

In some embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 272 in which the N residue at position 97 is substituted (for example, N97V, N97R, N97P, N97T, N97G, N97Q, N97S, N97A, N97L, N97Y, N97E, N97F, N97D, N97I or N97H). In other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 272 in which the G residue at position 98 is substituted (for example, G98V, G98P, G98R, G98D, G98E, G98W, G98Y, G98A, G98N, G98K, G98L). In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 272 in which the N residue at position 97 is substituted and the G residue at position 98 is substituted. In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 311, 312 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335 or 336.

In certain embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises:

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;

the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272; or
the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.

Another exemplary antigen binding domain that binds to CD33 of the invention is a protein (for example, an scFv) comprising the VH of SEQ ID NO: 56 and VL of SEQ ID NO: 85. The inventors have found that mutating one or more NG motifs within the VH of SEQ ID NO: 56 and VL of SEQ ID NO: 85 can reduce the likelihood of post-translation modification whilst maintaining CD33 binding properties. These mutations may also enhance thermostability.

An antigen binding domain that binds to CD33 having the VH of SEQ ID NO: 56 and VL of SEQ ID NO: 85 may be mutated to remove an NG motif. The VH of SEQ ID NO: 56 has a NG motif at positions 99-100 (using residue numbering according to EU index) and the VL of SEQ ID NO: 85 has a NG motif at positions 97-98 (using residue numbering according to EU index).

Thus, in some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 56 in which the N residue at position 99 is substituted (for example, N99R, N99P, N99G, N99V, N99L, N99D, N99E, N99A, N99Q or N99T). In other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 56 in which the G residue at position 100 is substituted (for example, G100S, G100A, G100L, G100P, G100T or G100Q). In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 56 in which the N residue at position 99 is substituted (for example, N99R, N99P, N99G, N99V, N99L, N99D, N99E, N99A, N99Q or N99T) and the G residue at position 100 is substituted (for example, G100S, G100A, G100L, G100P, G100T or G100Q). In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH of SEQ ID NO: 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381 or 382.

In some embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 85 in which the N residue at position 97 is substituted (for example, N97G, N97D, N97P, N97K, N97E, N97A, N97R, N97Q, N97I, N97V, N97H, N97K, N97W or N97S). In other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 85 in which the G residue at position 98 is substituted. In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 85 in which the N residue at position 97 is substituted (for example, N97G, N97D, N97P, N97K, N97E, N97A, N97R, N97Q, N97I, N97V, N97H, N97K, N97W or N97S) and the G residue at position 98 is

substituted. In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, or 366.

In certain embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises:

the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85; or
the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85.

Another exemplary antigen binding domain that binds to CD33 of the invention is a protein (for example, an scFv) comprising the VH of SEQ ID NO: 53 and VL of SEQ ID NO: 82. The inventors have found that mutating the NG motif within the VH of SEQ ID NO: 53 and VL of SEQ ID NO: 82 can reduce the likelihood of post-translation modification whilst maintaining CD33 binding properties. These mutations may also enhance thermostability.

An antigen binding domain that binds to CD33 having the VH of SEQ ID NO: 53 and VL of SEQ ID NO: 82 may be mutated to an NG motif. The VL of SEQ ID NO: 82 has a NG motif at positions 97-98 (using residue numbering according to EU index).

In some embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 82 in which the N residue at position 97 is substituted (for example, N97S, N97Q, N97V, N97G, N97W, N97T, N97I, N97E, N97A, N97R or N97L). In other embodiments, an antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 272 in which the G residue at position 98 is substituted (for example, G98V, G98A, G98P, G98L or G98T). In other embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 82 in which the N residue at position 97 is substituted (for example, N97S, N97Q, N97V, N97G, N97W, N97T, N97I, N97E, N97A, N97R or N97L) and the G residue at position 98 is substituted (for example, G98V, G98A, G98P, G98L or G98T). In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL of SEQ ID NO: 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In certain embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises:

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH domain of SEQ ID NO: 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382.

In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VL domain of SEQ ID NO: 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In some embodiments, the antigen binding domain that binds to CD33 (for example, an scFv) comprises a VH domain of SEQ ID NO: 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382 and a VL domain of SEQ ID NO: 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In some embodiments, the identity is 85%. In some embodiments, the identity is 90%. In some embodiments, the identity is 91%. In some embodiments, the identity is 91%. In some embodiments, the identity is 92%. In some embodiments, the identity is 93%. In some embodiments, the identity is 94%. In some embodiments, the identity is 94%. In some embodiments, the identity is 95%. In some embodiments, the identity is 96%. In some embodiments, the identity is 97%. In some embodiments, the identity is 98%. In some embodiments, the identity is 99%.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number 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 amino acid sequences may 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 which has been

incorporated into the GAP program in the GCG software package (available at www_gcg_com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In some embodiments, variant antigen binding domains that bind CD33 comprise one or two conservative substitutions in any of the CDR regions, while retaining desired functional properties of the parent antigen binding fragments that bind CD33.

"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 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, 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 invention 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.

Methods of generating antigen binding fragment that bind CD33

Antigen binding domains that bind CD33 provided in the disclosure may be generated using various technologies. For example, the hybridoma method of Kohler and Milstein may be used to identify VH/VL pairs that bind CD33. In the hybridoma method, a mouse or other host animal, such as a hamster, rat or chicken is immunized with human and/or cyno CD33, followed by fusion of spleen cells from immunized animals with myeloma cells using standard methods to form hybridoma cells. Colonies arising from single immortalized hybridoma cells may be screened for production of the antibodies containing the antigen binding domains that bind CD33 with desired properties, such as specificity of binding, cross-reactivity or lack thereof, affinity for the antigen, and any desired functionality.

Antigen binding domains that bind CD33 generated by immunizing non-human animals may be humanized. Exemplary humanization techniques including selection of human acceptor frameworks include CDR grafting (U.S. Patent No. 5,225,539), SDR grafting (U.S. Patent No. 6,818,749), Resurfacing (Padlan, (1991) *Mol Immunol* 28:489-499), Specificity Determining Residues Resurfacing (U.S. Patent Publ. No. 2010/0261620), human framework adaptation (U.S. Patent No. 8,748,356) or superhumanization (U.S. Patent No. 7,709,226). In these methods, CDRs or a subset of CDR residues of parental antibodies are transferred onto human frameworks that may be selected based on their overall homology to the parental frameworks, based on similarity in CDR length, or canonical structure identity, or a combination thereof.

Humanized antigen binding domains may be further optimized to improve their selectivity or affinity to a desired antigen by incorporating altered framework support residues to preserve binding affinity (backmutations) by techniques such as those described in Int. Patent Publ. Nos. WO1990/007861 and WO1992/22653, or by introducing variation at any of the CDRs for example to improve affinity of the antigen binding domain.

Transgenic animals, such as mice, rat or chicken carrying human immunoglobulin (Ig) loci in their genome may be used to generate antigen binding fragments that bind CD33, and are described in for example U.S. Patent No. 6,150,584, Int. Patent Publ. No. WO1999/45962, Int. Patent Publ. Nos. WO2002/066630, WO2002/43478, WO2002/043478 and WO1990/04036. The endogenous immunoglobulin loci in such animal may be disrupted or deleted, and at least one complete or partial human immunoglobulin locus may be inserted into the genome of the animal using homologous or non-homologous recombination, using transchromosomes, or using minigenes. Companies such as Regeneron (www_regeneron_com), Harbour Antibodies (www_harbourantibodies_com), Open Monoclonal Technology, Inc. (OMT) (www_omtinc_net), KyMab (www_kymab_com), Trianni (www.trianni_com) and Ablexis (www_ablexis_com) may be engaged to provide human antibodies directed against a selected antigen using technologies as described above.

Antigen binding domains that bind CD33 may be selected from a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single chain antibodies (scFv), or unpaired or paired antibody variable regions. The antigen binding domains that bind CD33 may be isolated for example from phage display library expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein as described in Shi *et al.*, (2010) *J Mol Biol* 397:385-96, and Int. Patent Publ. No. WO09/085462). The libraries may be screened for phage binding to human and/or cyno CD33 and the obtained positive clones may be further characterized, the Fabs isolated from the clone lysates, and converted to scFvs or other configurations of antigen binding fragments.

Preparation of immunogenic antigens and expression and production of antigen binding domains of the disclosure may be performed using any suitable technique, such as recombinant protein production. The immunogenic antigens may be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen may be formed *de novo* in the animal's body from nucleic acids encoding said antigen or a portion thereof.

Conjugation to half-life extending moieties

The antigen binding domains that bind CD33 of the disclosure may be conjugated to a half-life extending moiety. Exemplary half-life extending moieties are albumin, albumin variants, albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof, immunoglobulins (Ig) or fragments thereof, such as Fc regions. Amino acid sequences of the aforementioned half-life extending moieties are known. Ig or fragments thereof include all isotypes, *i.e.,* IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE.

Additional half-life extending moieties that may be conjugated to the antigen binding domains that bind CD33 of the disclosure include polyethylene glycol (PEG) molecules, such as PEG5000 or PEG20,000, fatty acids and fatty acid esters of different chain lengths, for example laurate, myristate, stearate, arachidate, behenate, oleate, arachidonate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like, polylysine, octane, carbohydrates (dextran, cellulose, oligo- or polysaccharides) for desired properties. These moieties may be direct fusions with the antigen binding domains that bind CD33 of the disclosure and may be generated by standard cloning and expression techniques. Alternatively, well known chemical coupling methods may be used to attach the moieties to recombinantly produced antigen binding domains that bind CD33 of the disclosure.

A pegyl moiety may for example be conjugated to the antigen binding domain that bind CD33 of the disclosure by incorporating a cysteine residue to the C-terminus of the antigen binding domain that bind CD33 of the disclosure, or engineering cysteines into residue positions that face away from the CD33 binding site and attaching a pegyl group to the cysteine using well known methods.

In some embodiments, the antigen binding fragment that binds CD33 is conjugated to a half-life extending moiety.

In some embodiments, the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol. In some embodiments, the half-life extending moiety is an Ig constant region.

In some embodiments, the half-life extending moiety is the Ig.

In some embodiments, the half-life extending moiety is the fragment of the Ig.

In some embodiments, the half-life extending moiety is the Ig constant region.

In some embodiments, the half-life extending moiety is the fragment of the Ig constant region.

In some embodiments, the half-life extending moiety is the Fc region.

In some embodiments, the half-life extending moiety is albumin.

In some embodiments, the half-life extending moiety is the albumin binding domain.

In some embodiments, the half-life extending moiety is transferrin.

In some embodiments, the half-life extending moiety is polyethylene glycol.

The antigen binding domains that bind CD33 conjugated to a half-life extending moiety may be evaluated for their pharmacokinetic properties utilizing known *in vivo* models.

Conjugation to immunoglobulin (Ig) constant regions or fragments of the Ig constant regions

The antigen binding domains that bind CD33 of the disclosure may be conjugated to an Ig constant region or a fragment of the Ig constant region to impart antibody-like properties, including Fc effector functions C1q binding, complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis or down regulation of cell surface receptors (e.g., B cell receptor; BCR). The Ig constant region or the fragment of the Ig constant region functions also as a half-life extending moiety as discussed herein. The antigen binding domains that bind CD33 of the disclosure may be engineered into conventional full length antibodies using standard methods. The full length antibodies comprising the antigen binding domain that binds CD33 may further be engineered as described herein.

Immunoglobulin heavy chain constant region comprised of subdomains CH1, hinge, CH2 and CH3. The CH1 domain spans residues A118-V215, the CH2 domain residues A231-K340 and the CH3 domain residues G341-K447 on the heavy chain, residue numbering according to the EU Index. In some instances G341 is referred as a CH2 domain residue. "Hinge" is generally defined as including E216 and terminating at P230 of human IgG1. Ig Fc region comprises at least the CH2 and the CH3 domains of the Ig constant region, and therefore comprises at least a region from about A231 to K447 of Ig heavy chain constant region.

The invention also provides an antigen binding domain that binds CD33 conjugated to an immunoglobulin (Ig) constant region or a fragment of the Ig constant region. In certain such embodiments, the Ig constant region or a fragment of the Ig constant region is derived from IgG1.

In some embodiments, the Ig constant region is a heavy chain constant region. In certain such embodiments, the Ig constant region is an IgG1 heavy chain constant region.

In some embodiments, the Ig constant region is a light chain constant region.

In some embodiments, the fragment of the Ig constant region comprises a Fc region. In certain such embodiments, the Ig constant region comprises an IgG1 Fc region. In some embodiments, the

fragment of the Ig constant region comprises a CH2 domain. In certain such embodiments, the Ig constant region comprises an IgG1 CH2 domain.

In some embodiments, the fragment of the Ig constant region comprises a CH3 domain. In certain such embodiments, the Ig constant region comprises an IgG1 CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain. In certain such embodiments, the Ig constant region comprises an IgG1 CH2 and CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain. In certain such embodiments, at least portion of a hinge, the CH2 domain and the CH3 domain from IgG1. Portion of the hinge refers to one or more amino acid residues of the Ig hinge.

In some embodiments, the fragment of the Ig constant region comprises the hinge, the CH2 domain and the CH3 domain. In certain such embodiment, the hinge, the CH2 domain and the CH3 domain from IgG1.

In certain embodiments, the fragment of the Ig constant region comprises an amino acid sequence of SEQ ID NO: 278.

The cysteine residue at position 220 of the heavy chain may be mutated to prevent the formation of a cysteine bridge between the constant region and a light chain. Thus, in certain embodiments, the Ig constant region comprises a residue that is not cysteine at position 220. In one embodiment, the Ig constant region comprises a serine at position 220.

In certain embodiments, the fragment of the Ig constant region comprises an amino acid sequence of SEQ ID NO: 279.

In some embodiments, the antigen binding domain that binds CD33 is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

In some embodiments, the antigen binding domain that binds CD33 is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

In some embodiments, the antigen binding domain that binds CD33 is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

The antigen binding domains that binds CD33 of the disclosure conjugated to Ig constant region or the fragment of the Ig constant region may be assessed for their functionality using several known assays. Binding to CD33 may be assessed using methods described herein. Altered properties imparted

by the Ig constant domain or the fragment of the Ig constant region such as Fc region may be assayed in Fc receptor binding assays using soluble forms of the receptors, such as the FcγRI, FcγRII, FcγRIII or FcRn receptors, or using cell-based assays measuring for example ADCC, CDC or ADCP.

ADCC may be assessed using an *in vitro* assay using CD33 expressing cells as target cells and NK cells as effector cells. Cytolysis may be detected by the release of label (e.g. radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. In an exemplary assay, target cells are used with a ratio of 1 target cell to 4 effector cells. Target cells are pre-labeled with BATDA and combined with effector cells and the test antibody. The samples are incubated for 2 hours and cell lysis measured by measuring released BATDA into the supernatant. Data is normalized to maximal cytotoxicity with 0.67% Triton X-100 (Sigma Aldrich) and minimal control determined by spontaneous release of BATDA from target cells in the absence of any antibody.

ADCP may be evaluated by using monocyte-derived macrophages as effector cells and any CD33 expressing cells as target cells which are engineered to express GFP or other labeled molecule. In an exemplary assay, effector:target cell ratio may be for example 4:1. Effector cells may be incubated with target cells for 4 hours with or without the antibody of the invention. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescence in the $CD11^{+}CD14^{+}$ macrophages using standard methods.

CDC of cells may be measured for example by plating Daudi cells at 1×10^{5} cells/well (50 µL/well) in RPMI-B (RPMI supplemented with 1% BSA), adding 50 µL of test protein to the wells at final concentration between 0-100 µg/mL, incubating the reaction for 15 min at room temperature, adding 11 µL of pooled human serum to the wells, and incubation the reaction for 45 min at 37° C. Percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods.

Proteins comprising the antigen binding domains that bind CD33 of the disclosure

The antigen binding domains that bind CD33 of the disclosure may be engineered into monospecific or multispecific proteins of various designs using standard methods.

The disclosure also provides a monospecific protein comprising the antigen binding domain that binds CD33 of the disclosure.

In some embodiments, the monospecific protein is an antibody.

The disclosure also provides a multispecific protein comprising the antigen binding domain that binds CD33 of the disclosure.

In some embodiments, the multispecific protein is bispecific.

In some embodiments, the multispecific protein is trispecific.

In some embodiments, the multispecific protein is tetraspecific.

In some embodiments, the multispecific protein is monovalent for binding to CD33.

In some embodiments, the multispecific protein is bivalent for binding to CD33.

The disclosure also provides an isolated multispecific protein comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen.

In some embodiments, the lymphocyte antigen is a T cell antigen.

In some embodiments, the T cell antigen is a $CD8^+$ T cell antigen.

In some embodiments, the lymphocyte antigen is a NK cell antigen.

In some embodiments, the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

In a certain embodiments, the multispecific protein is a bispecific protein comprising a first antigen binding domain that binds CD33 and second antigen binding domain that binds to TRGV9.TRGV9 is also known as Vγ9, and is expressed on γδT cells. As used herein, the term "TRGV9" refers to a polypeptide capable of forming a T cell receptor when expressed on the surface of γδ T cells. TRGV9-expressing γδ T cells are among the first T cells to develop in the human fetus and are the predominant γδ T cell subset in healthy adult peripheral blood cells. The term "TRGV9" includes any TRGV9 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. Unless noted, preferably the TRGV9 is a human TRGV9. A human TRGV9 amino acid sequence is provided by GenBank Accession Number NG_001336.2. Vγ9Vδ2 T lymphocytes, a major γ/δ T cell subset in humans, can recognize phosphoantigens, certain tumor cells, and cells treated with aminobisphosphonates. Vγ9Vδ2 T lymphocytes can display cytolytic activity against various tumor cells. Those having ordinary skill appreciate that γ/δ TCR is an heterodimeric TCR complex composed of covalently bound γ and δ chains involved in antigen recognition and the non-covalently associated monomorphic proteins CD3δ, γ, ε, and ζ chains. The Vγ9 TCR is a variant of the TCR γ chain expressed on a subset of γ/δ T cells.

Without wishing to be bound by theory, a bispecific antibody expressing both TRGV9 antigen and a CD33 antigen could recruit γδT cells to the cancerous cells expressing CD33. A multispecific antibody (e.g., a bispecific antibody) can bridge the effector cell (e.g., γδ T cell) and target cells together so as to resulting in cancer cell killing. γδ T-cells may have innate immunity. Without wishing to be bound by theory, γδ T-cells represent only a minor proportion of the peripheral $CD3^+$ T cells (about 1%–5%), but constitute a major subset (about 20%–50%) of T-cells in epithelial tissues. Circulating γδ T-cells mainly express heterodimers of Vγ9 (TRGV9) and Vδ2 (TRVD2) chains whereas tissue γδ T-cells preferentially express Vδ1 chains associated with different Vγ chains.

In humans, γδ T-cells are endowed with potent anti-cancer functions (high cytotoxicity and interferon γ secretion). Moreover, γδ T-cells are capable of phagocytosis, a function previously exclusive to innate myeloid lineage cells, and behave as efficient antigen-presenting cells for αβ T-cells and induce adaptive immune response. γδ T-cells may infiltrate cancerous tissues, tumors and cancerous cells. A bispecific antibody expressing both TRGV9 antigen and a CD33 antigen may exhibit less or no significant T cell redirection, less pan activation of T cells, and have increased induction of potent cancer lysis via selective recruitment of γδ T cells. Accordingly, a bispecific antibody expressing both TRGV9 antigen and a CD33 antigen may not lead to severe side effects that might arise by cytokine storm induction.

In some embodiments, the lymphocyte antigen is CD3ε.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the Fab.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the $F(ab')_2$.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the VHH.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the Fv.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the Fd.

In some embodiments, the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen (for example, TRGV9) comprise the scFv.

In some embodiments, the first antigen binding domain that binds CD33 comprises an scFv and the second binding domain that binds the lymphocyte antigen (for example, TRGV9) comprises a VHH.

In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises about 5-50 amino acids.

In some embodiments, the L1 comprises about 5-40 amino acids.

In some embodiments, the L1 comprises about 10-30 amino acids.

In some embodiments, the L1 comprises about 10-20 amino acids.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 109.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 110.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 111.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 112.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 113.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 114.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 115.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 116.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 117.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 118.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 119.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 120.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 121.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 122.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 123.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 124.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 125.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 126.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 127.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 128.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 129.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 130.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 131.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 132.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 133.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 134.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 135.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 136.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 137.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 138.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 139.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 140.

In certain embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 1, 2, 3, 4, 5, 28, 29, 30, 31, 32, 33, 34, 35, 89, 167, 172, 176, 253, 254 or 255, the HCDR2 of SEQ ID NOs: 6, 7, 8, 9, 10, 11, 36, 37, 38, 39, 40, 41, 42, 43, 44, 90, 91, 168, 173, 177, 256, 257 or 258 the HCDR3 of SEQ ID NOs: 12, 13, 14, 15, 16, 17, 45, 46, 47, 48, 49, 50, 51, 92, 93, 94, 169, 174, 178, 180, 259, 260 or 261 the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, 68, 98, 99, 100, 182, 186, 189, 193, 197, 201, 265 or 266 the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, 74, 101, 102, 103, 183, 187, 190, 194, 198, 267 or 268 and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, 80, 104, 184, 191, 195, 199, 269 or 270.

In some embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;

SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;

SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively;

SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively;

SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively;

SEQ ID NOs: 253, 256, 259, 265, 74 and 269 respectively;

SEQ ID NOs: 254, 257, 260, 66, 267 and 76 respectively

SEQ ID NOs: 255, 258, 261, 266, 268 and 270 respectively.

In certain embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively. In certain such embodiments, the first antigen binding domain is an scFv.

In certain embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 253,

256, 259, 265, 74 and 269, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273. In certain such embodiments, the first antigen binding domain is an scFv. In certain embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82 and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85 and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87 and the second antigen binding binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271 and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272 and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273 and the second antigen binding domain binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In some embodiments, the first antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

In some embodiments, the first antigen binding domain that binds CD33 comprises:

the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 19.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 24.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60 61, 262, 263 or 264.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 52.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 53.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 54.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 55.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 57.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 58.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 59.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 60.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 61.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 262.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 263.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 264.In certain embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 53. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 56. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 59. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 262. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 263. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 264. In certain such embodiments, the first antigen binding domain is an scFv.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 95.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 96.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 97.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 170.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 171.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 175.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 179.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 181.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 96.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 81.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 82.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 83.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 84.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 85.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 86.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 87.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 88.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 271.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 272.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 273.

In certain embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 82. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 85. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 87. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 271. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 272. In certain such embodiments, the first antigen binding domain is an scFv.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 273. In certain such embodiments, the first antigen binding domain is an scFv.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 105.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 106.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 107.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 185.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 188.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 192.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 196.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 200.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 202.In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 221, 274, 275 or 276.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 203.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 204.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 205.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 206.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 207.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 208.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 209.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 210.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 211.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 212.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 213.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 214.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 215.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 216.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 217.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 218.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 219.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 220.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 221.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 274.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 275.

In some embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 276.

In certain embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 213.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 216.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 219.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 274.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 275.

In other embodiments, the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 276.

In certain embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 213 and the second antigen binding domain that binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 216 and the second antigen binding domain that binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 219 and the second antigen binding domain that binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 274 and the second antigen binding domain that binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 275 and the second antigen binding domain that binds to TRGV9 (for example, wherein the second binding domain is a VHH).

In other embodiments, the first antigen binding domain that binds CD33 is an scFv comprising the amino acid sequence of SEQ ID NO: 276 and the second antigen binding domain that n binds to TRGV9 (for example, wherein the second binding domain is a VHH). In some embodiments, the second antigen binding domain that binds a lymphocyte antigen comprises

the HCDR1 of SEQ ID NO: 141, the HCDR2 of SEQ ID NO: 142, the HCDR3 of SEQ ID NO: 143, the LCDR1 of SEQ ID NO: 144, the LCDR2 of SEQ ID NO: 145 and the LCDR3 of SEQ ID NO: 146;

the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises:

a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

In some embodiments, the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a Fc region.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a CH2 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a CH3 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises the CH2 domain and the CH3 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises the hinge, the CH2 domain and the CH3 domain.

In some embodiments, the multispecific protein further comprises a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG2 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG3 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG4 isotype.

The first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region can further be engineered as described herein.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR. In certain such embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.

In some embodiments, the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S,

S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

In certain embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise L234A/L235A/D265S mutations, wherein residue numbering is according to the EU index. In certain such embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

In some embodiments, the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L, M252Y/S254T/T256E and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

In certain embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the M252Y/S254T/T256E mutations, wherein residue numbering is according to the EU index. In certain such embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the L234A/L235A/D265S and the M252Y/S254T/T256E mutations, wherein residue numbering is according to the EU index. In certain embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.In some embodiments, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

In some embodiments, the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one

mutation that modulates binding to protein A. Such modifications may be advantageous for purification purposes during antibody production.

In certain embodiments, the at least one mutation that modulates binding to protein A is H435/Y436F, wherein residue numbering is according to the EU index. In certain such embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the L234A/L235A/D265S, the M252Y/S254T/T256E and the H435/Y436F mutations, wherein residue numbering is according to the EU index. In certain embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotypeIn some embodiments, the multispecific protein comprises at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

In some embodiments, the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations

L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

Generation of multispecific proteins that comprise antigen binding fragments that bind CD33

The antigen binding fragments that bind CD33 of the disclosure may be engineered into multispecific antibodies which are also encompassed within the scope of the invention.

The antigen binding fragments that bind CD33 may be engineered into full length multispecific antibodies which are generated using Fab arm exchange, in which substitutions are introduced into two monospecific bivalent antibodies within the Ig constant region CH3 domain which promote Fab arm

exchange *in vitro*. In the methods, two monospecific bivalent antibodies are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. 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- 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 of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

CH3 mutations that may be used include technologies such as Knob-in-Hole mutations (Genentech), electrostatically-matched mutations (Chugai, Amgen, NovoNordisk, Oncomed), the Strand Exchange Engineered Domain body (SEEDbody) (EMD Serono), Duobody® mutations (Genmab), and other asymmetric mutations (e.g. Zymeworks).

Knob-in-hole mutations are disclosed for example in WO1996/027011 and include mutations on the interface of CH3 region in which an amino acid with a small side chain (hole) is introduced into the first CH3 region and an amino acid with a large side chain (knob) is introduced into the second CH3 region, resulting in preferential interaction between the first CH3 region and the second CH3 region. Exemplary CH3 region mutations forming a knob and a hole are T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

Heavy chain heterodimer formation may be promoted by using electrostatic interactions by substituting positively charged residues on the first CH3 region and negatively charged residues on the second CH3 region as described in US2010/0015133, US2009/0182127, US2010/028637 or US2011/0123532.

Other asymmetric mutations that can be used to promote heavy chain heterodimerization are L351Y_F405A_Y407V/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 US2012/0149876 or US2013/0195849 (Zymeworks).

SEEDbody mutations involve substituting select IgG residues with IgA residues to promote heavy chai heterodimerization as described in US20070287170.

Other exemplary mutations that may be used are R409D_K370E/D399K_E357K, S354C_T366W/Y349C_ T366S_L368A_Y407V, Y349C_T366W/S354C_T366S_L368A_Y407V, T366K/L351D, L351K/Y349E, L351K/Y349D, L351K/L368E, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, K392D/D399K, K392D/ E356K, K253E_D282K_K322D/D239K_E240K_K292D, K392D_K409D/D356K_D399K as described in WO2007/147901, WO 2011/143545, WO2013157954, WO2013096291 and US2018/0118849.

Duobody® mutations (Genmab) are disclosed for example in US9150663 and US2014/0303356 and include mutations F405L/K409R, wild-type/F405L_R409K, T350I_K370T_F405L/K409R, K370W/K409R, D399AFGHILMNRSTVWY/K409R, T366ADEFGHILMQVY/K409R, L368ADEGHNRSTVQ/K409AGRH, D399FHKRQ/K409AGRH, F405IKLSTVW/K409AGRH and Y407LWQ/K409AGRH.

Additional bispecific or multispecific structures into which the antigen binding domains that bind CD33 can be incorporated include Dual Variable Domain Immunoglobulins (DVD) (Int. Pat. Publ. No. WO2009/134776; DVDs are full length antibodies comprising the heavy chain having a structure VH1-linker-VH2-CH and the light chain having the structure VL1-linker-VL2-CL; linker being optional), structures that include various dimerization domains to connect the two antibody arms with different specificity, such as leucine zipper or collagen dimerization domains (Int. Pat. Publ. No. WO2012/022811, U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441), two or more domain antibodies (dAbs) conjugated together, diabodies, heavy chain only antibodies such as camelid antibodies and engineered camelid antibodies, Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star) and CovX-body (CovX/Pfizer), IgG-like Bispecific (InnClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idec) and TvAb (Roche), ScFv/Fc Fusions (Academic Institution), SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual$(ScFv)_2$-Fab (National Research Center for Antibody Medicine--China), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (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 Biotech), dual targeting nanobodies (Ablynx), dual targeting heavy chain only domain antibodies.

The antigen binding domains that bind CD33 of the disclosure may also be engineered into multispecific proteins which comprise three polypeptide chains. In such designs, at least one antigen

binding domain is in the form of a scFv. Exemplary designs include (in which "1" indicates the first antigen binding domain, "2" indicates the second antigen binding domain and "3" indicates the third antigen binding domain:

Design 1: Chain A) scFv1- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 2: Chain A) scFv1- hinge- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 3: Chain A) scFv1- CH1-hinge- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 4: Chain A) CH2-CH3-scFv1; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

CH3 engineering may be incorporated to the Designs 1-4, such as mutations L351Y_F405A_Y407V/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 US2012/0149876 or US2013/0195849 (Zymeworks).

Isotypes, allotypes and Fc engineering

The Ig constant region or the fragment of the Ig constant region, such as the Fc region present in the proteins of the disclosure may be of any allotype or isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG1 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG2 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG3 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG4 isotype.

The Ig constant region or the fragment of the Ig constant region may be of any allotype. It is expected that allotype has no influence on properties of the Ig constant region, such as binding or Fc-mediated effector functions. Immunogenicity of therapeutic proteins comprising Ig constant regions of fragments thereof is associated with increased risk of infusion reactions and decreased duration of therapeutic response (Baert *et al.*, (2003) *N Engl J Med* 348:602-08). The extent to which therapeutic proteins comprising Ig constant regions of fragments thereof induce an immune response in the host may be determined in part by the allotype of the Ig constant region (Stickler *et al.*, (2011) *Genes and Immunity*

12:213-21). Ig constant region allotype is related to amino acid sequence variations at specific locations in the constant region sequences of the antibody. Table 2 shows select IgG1, IgG2 and IgG4 allotypes.

Table 2.

Allotype	Amino acid residue at position of diversity (residue numbering: EU Index)							
	IgG2		IgG4		IgG1			
	189	282	309	422	214	356	358	431
G2m(n)	T	M						
G2m(n-)	P	V						
G2m(n)/(n-)	T	V						
nG4m(a)			L	R				
G1m(17)					K	E	M	A
G1m(17,1)					K	D	L	A

C-terminal lysine (CTL) may be removed from the Ig constant region by endogenous circulating carboxypeptidases in the blood stream (Cai *et al.*, (2011) *Biotechnol Bioeng* 108:404-412). During manufacturing, CTL removal may be controlled to less than the maximum level by control of concentration of extracellular Zn^{2+}, EDTA or EDTA – Fe^{3+} as described in U.S. Patent Publ. No. US20140273092. CTL content of proteins may be measured using known methods.

In some embodiments, the antigen binding fragment that binds CD33 conjugated to the Ig constant region has a C-terminal lysine content from about 10% to about 90%. In some embodiments, the C-terminal lysine content is from about 20% to about 80%. In some embodiments, the C-terminal lysine content is from about 40% to about 70%. In some embodiments, the C-terminal lysine content is from about 55% to about 70%. In some embodiments, the C-terminal lysine content is about 60%.

Fc region mutations may be made to the antigen binding domains that bind CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region to modulate their effector functions such as ADCC, ADCP and/or ADCP and/or pharmacokinetic properties. This may be achieved by introducing mutation(s) into the Fc that modulate binding of the mutated Fc to activating FcγRs (FcγRI, FcγRIIa, FcγRIII), inhibitory FcγRIIb and/or to FcRn.

In some embodiments, the antigen binding domain that binds CD33s conjugated to the Ig constant region or the fragment of the Ig constant region comprises at least one mutation in the Ig constant region or in the fragment of the Ig constant region.

In some embodiments, the at least one mutation is in the Fc region.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen mutations in the Fc region.

In some embodiments, the antigen binding domain that binds CD33s conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that modulates binding of the antibody to FcRn.

Fc positions that may be mutated to modulate half-life (e.g. binding to FcRn) include positions 250, 252, 253, 254, 256, 257, 307, 376, 380, 428, 434 and 435. Exemplary mutations that may be made singularly or in combination are mutations T250Q, M252Y, I253A, S254T, T256E, P257I, T307A, D376V, E380A, M428L, H433K, N434S, N434A, N434H, N434F, H435A and H435R. Exemplary singular or combination mutations that may be made to increase the half-life are mutations M428L/N434S, M252Y/S254T/T256E, T250Q/M428L, N434A and T307A/E380A/N434A. Exemplary singular or combination mutations that may be made to reduce the half-life are mutations H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises M252Y/S254T/T256E mutation.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that reduces binding of the protein to an activating Fcγ receptor (FcγR) and/or reduces Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP).

Fc positions that may be mutated to reduce binding of the protein to the activating FcγR and subsequently to reduce effector function include positions 214, 233, 234, 235, 236, 237, 238, 265, 267, 268, 270, 295, 297, 309, 327, 328, 329, 330, 331 and 365. Exemplary mutations that may be made singularly or in combination are mutations K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, A330S and P331S in IgG1, IgG2, IgG3 or IgG4. Exemplary combination mutations that result in proteins with reduced ADCC are mutations L234A/L235A on IgG1, L234A/L235A/D265S on IgG1, V234A/G237A/ P238S/H268A/V309L/A330S/P331S on IgG2, F234A/L235A on IgG4, S228P/F234A/ L235A on IgG4, N297A on all Ig isotypes, V234A/G237A on IgG2, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M on IgG1, H268Q/V309L/A330S/P331S on IgG2, S267E/L328F on IgG1, L234F/L235E/D265A on IgG1, L234A/L235A/G237A/P238S/H268A/A330S/P331S on IgG1, S228P/F234A/L235A/G237A/P238S on IgG4, and S228P/F234A/L235A/G236-deleted/G237A/P238S on IgG4. Hybrid IgG2/4 Fc domains may also be used, such as Fc with residues 117-260 from IgG2 and residues 261-447 from IgG4.

Exemplary mutation that result in proteins with reduced CDC is a K322A mutation.

Well-known S228P mutation may be made in IgG4 to enhance IgG4 stability.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation selected from the group consisting of K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, K322, A330S and P331S.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises L234A/L235A/D265S mutation.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises L234A/L235A mutation.

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that enhances binding of the protein to an Fcγ receptor (FcγR) and/or enhances Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and/or phagocytosis (ADCP).

Fc positions that may be mutated to increase binding of the protein to the activating FcγR and/or enhance Fc effector functions include positions 236, 239, 243, 256,290,292, 298, 300, 305, 312, 326, 330, 332, 333, 334, 345, 360, 339, 378, 396 or 430 (residue numbering according to the EU index). Exemplary mutations that may be made singularly or in combination are G236A, S239D, F243L, T256A, K290A, R292P, S298A, Y300L, V305L, K326A, A330K, I332E, E333A, K334A, A339T and P396L. Exemplary combination mutations that result in proteins with increased ADCC or ADCP are a S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E.

Fc positions that may be mutated to enhance CDC include positions 267, 268, 324, 326, 333, 345 and 430. Exemplary mutations that may be made singularly or in combination are S267E, F1268F, S324T, K326A, K326W, E333A, E345K, E345Q, E345R, E345Y, E430S, E430F and E430T. Exemplary combination mutations that result in proteins with increased CDC are K326A/E333A, K326W/E333A, H268F/S324T, S267E/H268F, S267E/S324T and S267E/H268F/S324T.

The specific mutations described herein are mutations when compared to the IgG1, IgG2 and IgG4 wild-type amino acid sequences of SEQ ID NOs: 222, 223 and 224, respectively.

SEQ ID NO: 222, wild-type IgG1

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK

SEQ ID NO: 223; wild-type IgG2

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN
GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVE
WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

SEQ ID NO: 224; wild-type IgG4

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL
SLGK

Binding of the antibody to FcγR or FcRn may be assessed on cells engineered to express each receptor using flow cytometry. In an exemplary binding assay, $2x10^5$ cells per well are seeded in 96-well plate and blocked in BSA Stain Buffer (BD Biosciences, San Jose, USA) for 30 min at 4°C. Cells are incubated with a test antibody on ice for 1.5 hour at 4°C. After being washed twice with BSA stain buffer, the cells are incubated with R-PE labeled anti-human IgG secondary antibody (Jackson Immunoresearch Laboratories) for 45 min at 4°C. The cells are washed twice in stain buffer and then resuspended in 150 µL of Stain Buffer containing 1:200 diluted DRAQ7 live/dead stain (Cell Signaling Technology, Danvers, USA). PE and DRAQ7 signals of the stained cells are detected by Miltenyi MACSQuant flow cytometer (Miltenyi Biotec, Auburn, USA) using B2 and B4 channel respectively. Live cells are gated on DRAQ7 exclusion and the geometric mean fluorescence signals are determined for at least 10,000 live events collected. FlowJo software (Tree Star) is used for analysis. Data is plotted as the logarithm of antibody concentration versus mean fluorescence signals. Nonlinear regression analysis is performed.

Glycoengineering

The ability of the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region to mediate ADCC can be enhanced by engineering the Ig constant region or the fragment of the Ig constant region oligosaccharide component. Human IgG1 or

IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known biantennary G0, G0F, G1, G1F, G2 or G2F forms. Ig constant region containing proteins may be produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region enhances the ADCC of the protein via improved FcγRIIIa binding without altering antigen binding or CDC activity. Such proteins can be achieved using different methods reported to lead to the successful expression of relatively high defucosylated immunoglobulins bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.,* Cytotechnology 64(:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al., J Biol Chem* 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al., MAbs*;2(4): 405-415, 2010; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al., J Biol Chem* 278:3466-3473, 2003), introduction of small interfering RNA specifically against the a 1,6-fucosyltrasferase (*FUT8)* gene (Mori *et al., Biotechnol Bioeng* 88:901-908, 2004), or coexpression of β-1,4-*N*-acetylglucosaminyltransferase III and Golgi α-mannosidase II or a potent alpha-mannosidase I inhibitor, kifunensine (Ferrara *et al., J Biol Chem* 281:5032-5036, 2006, Ferrara *et al., Biotechnol Bioeng* 93:851-861, 2006; Xhou *et al., Biotechnol Bioeng* 99:652-65, 2008).

In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region of the disclosure has a biantennary glycan structure with fucose content of about between 1% to about 15%, for example about 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%. In some embodiments, the antigen binding domain that binds CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region has a glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, or 20%.

"Fucose content" means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Int Pat. Publ. No. WO2008/077546 2); 2) by enzymatic release of the Asn297 glycans with subsequent derivatization and detection/ quantitation by HPLC (UPLC) with fluorescence detection and/or HPLC-MS (UPLC-MS); 3) intact protein analysis of the native or reduced mAb, with or without treatment of the Asn297 glycans with Endo S or other enzyme that cleaves between the first and the second GlcNAc monosaccharides, leaving the fucose attached to the first GlcNAc; 4) digestion of the mAb to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS); 5) Separation of the mAb

oligosaccharides from the mAb protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides thus released can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosaccharide forms according to hydrophilicity criteria by normal-phase HPLC (GlycoSep N), and separation and quantification of the oligosaccharides by high performance capillary electrophoresis-laser induced fluorescence (HPCE-LIF).

"Low fucose" or "low fucose content" as used herein refers to the antigen binding domain that bind CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region with fucose content of about between 1%-15%.

"Normal fucose" or 'normal fucose content" as used herein refers to the antigen binding domain that bind CD33 conjugated to the Ig constant region or to the fragment of the Ig constant region with fucose content of about over 50%, typically about over 80% or over 85%.

Anti-idiotypic antibodies

Anti-idiotypic antibodies are antibodies that specifically bind to the antigen binding domain that binds CD33 of the disclosure.

The invention also provides an anti-idiotypic antibody that specifically binds to the antigen binding domain that binds CD33 of the disclosure.

The invention also provides an anti-idiotypic antibody that specifically binds to the antigen binding domain that binds CD33 comprising

the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200;
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202;
the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the anti-idiotypic antibody specifically binds to an antigen binding domain that binds CD33 comprising the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

An anti-idiotypic (Id) antibody is an antibody which recognizes the antigenic determinants (*e.g.* the paratope or CDRs) of the antibody. The Id antibody may be antigen-blocking or non-blocking. The antigen-blocking Id may be used to detect the free antigen binding domain in a sample (*e.g.* the antigen binding domain that binds CD33 of the disclosure). The non-blocking Id may be used to detect the total antibody (free, partially bond to antigen, or fully bound to antigen) in a sample. An Id antibody may be prepared by immunizing an animal with the antibody to which an anti-Id is being prepared.

An anti-Id antibody may also be used as an immunogen to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id may be epitopically identical to the original antigen binding domain which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of the antigen binding domain, it is possible to identify other clones expressing antigen binding domains of identical specificity. Anti-Id antibodies may be varied (thereby producing anti-Id antibody variants) and/or derivatized by any suitable technique, such as those described elsewhere herein.

Immunoconjugates

The antigen binding domains that bind CD33 of the disclosure, the proteins comprising the antigen binding domains that bind CD33 or the multispecific proteins that comprise the antigen binding domains that bind CD33 (collectively referred herein as to CD33 binding proteins) may be conjugated to a heterologous molecule. CD33 binding protein also includes chimeric antigen receptors (CAR) which comprises the antigen binding domains that bind CD33 of the disclosure.

In some embodiments, the heterologous molecule is a detectable label or a cytotoxic agent.

The invention also provides an antigen binding domain that binds CD33 conjugated to a detectable label.

The invention also provides a protein comprising an antigen binding domain that binds CD33 conjugated to a detectable label.

The invention also provides a multispecific protein comprising an antigen binding domain that binds CD33 conjugated to a detectable label.

The invention also provides a CAR comprising an antigen binding domain that binds CD33 conjugated to a detectable label.

The invention also provides an antigen binding domain that binds CD33 conjugated to a cytotoxic agent.

The invention also provides a protein comprising an antigen binding domain that binds CD33 conjugated to a cytotoxic agent.

The invention also provides a multispecific protein comprising an antigen binding domain that binds CD33 conjugated to a cytotoxic agent.

The invention also provides a CAR comprising an antigen binding domain that binds CD33 conjugated to a cytotoxic agent.

CD33 binding proteins of the disclosure may be used to direct therapeutics to CD33 expressing cells, such as those of a myeloid lineage. Alternatively, CD33 expressing cells may be targeted with a CD33 binding protein of the disclosure coupled to a therapeutic intended to modify cell function once internalized.

In some embodiments, the detectable label is also a cytotoxic agent.

The CD33 binding proteins of the disclosure conjugated to a detectable label may be used to evaluate expression of CD33 on a variety of samples.

Detectable label includes compositions that when conjugated to the CD33 binding proteins of the disclosure renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, scintillates, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni^{2+}, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

Any of the labels disclosed in International Patent Publication No. WO2019/125982, incorporated by reference herein, may be used.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases, the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ-emitting, Auger-emitting, β-emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ^{3}H, ^{11}C, ^{13}C, ^{15}N, ^{18}F, ^{19}F, ^{55}Co, ^{57}Co, ^{60}Co, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{72}As, ^{75}Br, ^{86}Y, ^{89}Zr, ^{90}Sr, ^{94m}Tc, ^{99m}Tc, ^{115}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{211}At, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{226}Ra, ^{225}Ac and ^{227}Ac.

In some embodiments, the radioactive isotope is used for therapeutic purposes, such as by enhancing cell killing. Radioactive isotopes used for enhancing cell killing include, but are not limited to, ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{255}Fm and ^{227}Th. Without wishing to be bound by theory, CD33 binding proteins comprising a radioisotope used for therapeutic purposes may be internalized by the target cell and killed by the target cell.

Radioimmunotherapy using a CD33 binding protein conjugated with a radioactive isotope can be beneficial for treating local or diffuse tumors. In some embodiments, the subject is treated by administration of radiolabeled monoclonal antibodies that are directed specifically against tumor-associated antigens or against the tumor microenvironment. Radiolabeled (e.g Ac225) therapy may be highly effective requiring low doses for cell killing, such as in the context of treating hematologic malignancies with cells expressing CD33.

In some embodiments, the radioactive isotope is used for enhancing imaging, such as for testing to indicate whether the CD33 binding protein is being targeted to tissues of interest. Exemplary isotopes used for enhancing imaging include, but are not limited to, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, and ^{111}In.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic

atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms, thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1, +2, or +3 oxidation states, such as Ba^{2+}, Bi^{3+}, Cs^{+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Co^{3+}, Cu^{+}, Cu^{2+}, Cu^{3+}, Ga^{3+}, Gd^{3+}, Au^{+}, Au^{3+}, Fe^{2+}, Fe^{3+}, F^{3+}, Pb^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}, Hg^{2+}, Ni^{2+}, Ni^{3+}, Ag^{+}, Sr^{2+}, Sn^{2+}, Sn^{4+}, and Zn^{2+}. The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyanate (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The antigen binding domain that binds CD33 conjugated to a detectable label may be used as an imaging agent.

The protein comprising an antigen binding domain that binds CD33 conjugated to a detectable label may be used as an imaging agent.

The multispecific protein comprising an antigen binding domain that binds CD33 conjugated to a detectable label may be used as an imaging agent.

The CAR comprising an antigen binding domain that binds CD33 conjugated to a detectable label may be used as an imaging agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elicit their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxin such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancer and antifungal activity. The dolastatin or auristatin drug moiety may be attached to the antibody of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO02/088172), or via any cysteine engineered into the antibody.

The CD33 binding proteins of the disclosure may be conjugated to a detectable label using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

In some embodiments, the detectable label is conjugated to the CD33 binding proteins of the disclosure via a linker.

The detectable label or the cytotoxic moiety may be linked directly, or indirectly, to the CD33 binding proteins of the disclosure using known methods. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10,tetraacetic acid (DOTA), derivatives of diethylenetriaminepentaacetic avid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclodocedan-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

In some embodiments, the CD33 binding proteins of the disclosure is removed from the blood via renal clearance.

Kits

The invention also provides a kit comprising any of the antigen binding domains that bind CD33 described herein.

The invention also provides a kit comprising the protein comprising any of the antigen binding domains that bind CD33 described herein.

The invention also provides a kit comprising any of the multispecific proteins described herein that comprise an antigen binding domain that binds CD33.

The invention also provides a kit comprising the CAR comprising any of the antigen binding domains described herein that bind CD33.

The kit may be used for therapeutic uses and as diagnostic kits.

The kit may be used to detect the presence of CD33in a sample.

In some embodiments, the kit comprises the CD33binding protein of the disclosure and reagents for detecting the CD33binding protein. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices

or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the antigen binding domain that binds CD33 in a container and instructions for use of the kit.

In some embodiments, the kit comprises the protein comprising an antigen binding domain that binds CD33in a container and instructions for use of the kit.

In some embodiments, the kit comprises the multispecific protein comprising an antigen binding domain that binds CD33in a container and instructions for use of the kit.

In some embodiments, the antigen binding domain that binds CD33in the kit is labeled.

In some embodiments, the protein comprising an antigen binding domain that binds CD33in the kit is labeled.

In some embodiments, the multispecific protein comprising an antigen binding domain that binds CD33in the kit is labeled.

In some embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the kit comprises the antigen binding domain (for example, an scFv) that binds CD33 comprising the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising

a) the VH of SEQ ID NO: 18;

b) the VH of SEQ ID NO: 19;

c) the VH of SEQ ID NO: 20;

d) the VH of SEQ ID NO: 21;

e) the VH of SEQ ID NO: 22;

f) the VH of SEQ ID NO: 23;

g) the VH of SEQ ID NO: 24;

h) the VH of SEQ ID NO: 25; or

i) the VH of SEQ ID NO: 26; or

j) the VH of SEQ ID NO: 27.

In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27. In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263 or 264. In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272 or 273. In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 95, 96, 97, 170, 171,

175, 179, 181, or 96. In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

In some embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NOs: 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 221, 274, 275 or 276.

In certain embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 213.

In other embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 216.

In other embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 219.

In other embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 274.

In other embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 275.

In other embodiments, the kit comprises the antigen binding domain that binds CD33 comprising SEQ ID NO: 276.

Methods of detecting CD33

The invention also provides a method of detecting CD33 in a sample, comprising obtaining the sample, contacting the sample with the antigen binding domain that binds CD33 of the disclosure and detecting the bound CD33 in the sample.

In some embodiments, the sample may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, synovial fluid, circulating cells, cells that are not tissue associated (*i.e.*, free cells), tissues (*e.g.*, surgically resected tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

The antigen binding domain that binds CD33 of the disclosure may be detected using known methods. Exemplary methods include direct labeling of the antibodies using fluorescent or chemiluminescent labels, or radiolabels, or attaching to the antibodies of the invention a moiety which is readily detectable, such as biotin, enzymes or epitope tags. Exemplary labels and moieties are ruthenium, ^{111}In-DOTA, ^{111}In- diethylenetriaminepentaacetic acid (DTPA), horseradish peroxidase, alkaline phosphatase and beta-galactosidase, poly-histidine (HIS tag), acridine dyes, cyanine dyes, fluorone dyes, oxazin dyes, phenanthridine dyes, rhodamine dyes and Alexafluor® dyes.

The antibodies can be directly labeled with any of the radiolabels and radioactive agents disclosed in International Patent Publication No. WO2019/125982, incorporated by reference herein, may be used.

The antigen binding domain that binds CD33 of the disclosure may be used in a variety of assays to detect CD33 in the sample. Exemplary assays are western blot analysis, radioimmunoassay, surface plasmon resonance, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

Chimeric antigen receptors (CAR) comprising the antigen binding domains that bind CD33 of the disclosure

Any of the antigen binding domains that bind CD33 identified herein may be engineered into a chimeric antigen receptor (CAR) to provide CARs that bind CD33. The disclosure provides for CARs that target CD33, cells comprising such CARs, and methods of treating CD33 producing cancer, such as hematologic cancer, using the CARs described herein.

The CARs of the invention have antigen specificity for CD33. The phrases "have antigen specificity" and "elicit antigen-specific response" as used herein mean that the CAR can specifically bind to and immunologically recognize an antigen, such that binding of the CAR to the CD33 antigen elicits an immune response. Methods of testing the CARs for antigen specificity and for the ability to recognize target cells are known in the art.

The disclosure relates to the use of T cells which have been genetically modified to stably express a desired chimeric antigen receptor. A chimeric antigen receptor (CAR) is an artificially constructed heterologous protein or polypeptide containing an antigen binding domain of an antibody (such as scFv) linked to a T-cell signaling domain. Characteristics of CARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigens independent of antigen processing, thus bypassing a major mechanism of tumor evasion. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

The CARs described herein provide recombinant polypeptide constructs comprising at least an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain (also referred to herein as a cytoplasmic signaling domain) comprising a functional signaling domain derived from a stimulatory molecule as described herein. T cells expressing a CAR are referred to herein as CAR T cells, CAR-T cells or CAR modified T cells, and these terms are used interchangeably herein. The cell can be genetically modified to stably express an antibody binding domain on its surface, conferring novel antigen specificity that is MHC independent.

In some instances, the T cell is genetically modified to stably express a CAR that combines an antigen recognition domain of an antibody with an intracellular domain of the CD3-zeta chain or FcγRI

protein into a single chimeric protein. In one embodiment, the stimulatory molecule is the zeta chain associated with the T cell receptor complex.

An "**intracellular signaling domain**," refers to an intracellular portion of a molecule. It is the functional portion of the protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell. Examples of immune effector function, e.g., in a CAR-T cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In one embodiment, the intracellular signaling domain comprises a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In one embodiment, the intracellular signaling domain comprises a co-stimulatory intracellular domain. Exemplary co-stimulatory intracellular signaling domains include those derived from molecules responsible for co-stimulatory signals, or antigen independent stimulation. F or example, in the case of a CAR-T, a primary intracellular signaling domain comprises a cytoplasmic sequence of a T cell receptor, and a co-stimulatory intracellular signaling domain comprises a cytoplasmic sequence from co-receptor or co-stimulatory molecule.

A primary intracellular signaling domain comprises a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Exemplary ITAM containing primary cytoplasmic signaling sequences include those derived from CD3-zeta, FcRγ, FcR beta, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, CD66d, DAP10 and DAP12.

The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" is defined as the protein provided as GenBank Acc. No. BAG36664.1, or the equivalent residues from a nonhuman species, e.g., murine, rabbit, primate, mouse, rodent, monkey, ape and the like, and a "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" is defined as the amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect, the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO: 28, or a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99%, sequence identity with SEQ ID NO: 164.

SEQ ID NO: 164 CD3-zeta stimulatory domain

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

The term "co-stimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-stimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Co-stimulatory molecules include, but are not limited to an MHC class 1 molecule, BTLA and a Toll ligand receptor, as well as OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137).

A co-stimulatory intracellular signaling domain can be the intracellular portion of a co-stimulatory molecule. A co-stimulatory molecule can be represented in the following protein families: TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), and activating NK cell receptors. Exemplary such molecules include CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, MyD88, CD40, ICOS, BAFFR, HVEM, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, and a ligand that specifically binds with CD83, and the like.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

The term "4-1BB" or alternatively "CD137" refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a "4-1BB co-stimulatory domain" is defined as amino acid residues 214-255 of GenBank accession no. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the "4-1BB co-stimulatory domain" or "CD137 co-stimulatory domain" is the sequence provided as **SEQ ID NO: 163** (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL; **SEQ ID NO: 163**) or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, or a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with SEQ ID NO: 163.

In one embodiment, a transmembrane domain that is naturally associated with one of the other domains in the CAR is used. In another embodiment, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In one embodiment, the transmembrane domain comprises the CD8α hinge domain.

In some embodiments, the cytoplasmic signaling domain further comprises one or more

functional signaling domains derived from at least one co-stimulatory molecule as defined herein. In one embodiment, the co-stimulatory molecule is chosen from 4-1BB (*i.e.,* CD137), CD27, CD3-zeta and/or CD28. CD28 is a T cell marker important in T cell co-stimulation. CD27 is a member of the tumor necrosis factor receptor superfamily and acts as a co-stimulatory immune checkpoint molecule. 4-1BB transmits a potent co-stimulatory signal to T cells, promoting differentiation and enhancing long-term survival of T lymphocytes. CD3-zeta associates with TCRs to produce a signal and contains immunoreceptor tyrosine-based activation motifs (ITAMs). In another embodiment, the co-stimulatory molecule is MyD88 or CD40.

In one embodiment, the CAR comprises an intracellular hinge domain comprising CD8 and an intracellular T cell receptor signaling domain comprising CD28, 4-1BB, and CD3-zeta. In another embodiment, the CAR comprises an intracellular hinge domain and an intracellular T cell receptor signaling domain comprising CD28, 4-1BB, and CD3-zeta, wherein the hinge domain comprises all or part of the extracellular region of CD8, CD4 or CD28; all or part of an antibody constant region; all or part of the FcγRIIIA receptor, an IgG hinge, an IgM hinge, an IgA hinge, an IgD hinge, an IgE hinge, or an Ig hinge. The IgG hinge may be from IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof.

CARs described herein provide recombinant polypeptide constructs comprising at least an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain (also referred to herein as "a cytoplasmic signaling domain") comprising, *e.g.,* a functional signaling domain derived from a stimulatory molecule as defined below

In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule.

The CARs of the disclosure can be designed to comprise the CD28 and/or 4-1BB signaling domain by itself or be combined with any other desired cytoplasmic domain(s) useful in the context of the CARs of the invention. In one embodiment, the cytoplasmic domain of the CAR can further comprise the signaling domain of CD3-zeta. For example, the cytoplasmic domain of the CAR can include but is not

limited to CD3-zeta, 4-1BB and CD28 signaling modules and combinations thereof. Accordingly, the invention provides CAR T cells and methods of their use for adoptive therapy.

The disclosure further provides variants, e.g., functional variants, of the CARs. The term "functional variant" as used herein refers to a CAR having substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR for which it is a variant. Functional variants encompass, e.g., those variants of the CAR that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for example, be at least about 30%, about 40%, about 50%, about 60%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent CAR.

A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. In one embodiment, the functional variant comprises the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, the non-conservative amino acid substitution may not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant such that the biological activity of the functional variant is increased as compared to the parent CAR.

Conservative amino acid substitutions are known in the art, and described herein.

The CAR of the disclosure can consist essentially of the specified amino acid sequence or sequences described herein, such that other components e.g., other amino acids, do not materially change the biological activity of the functional variant.

The CARs of the disclosure (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to an antigen, detect diseased cells (e.g., cancer cells) in a host, or treat or prevent disease in a host, etc. For example, the CARs can be about 50 to about 5000 amino acids long, such as about 50, about 70, about 75, about 100, about 125, about 150, about 175, about 200, about 225, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, about 1000 or more amino acids in length.

The CARs of the disclosure (including functional portions and functional variants of the invention) may comprise synthetic amino acids in place of one or more naturally-occurring amino acids.

Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, N′-benzyl-N′-methyl-lysine, N′,N′-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, and α-tert-butylglycine.

The CARs of the disclosure (including functional portions and functional variants) can be subject to post-translational modifications. They can be glycosylated, esterified, N-acylated, amidated, carboxylated, phosphorylated, esterified, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt. In some embodiments, they are dimerized or polymerized, or conjugated.

The CARs of the disclosure (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., *Fmoc Solid Phase Peptide Synthesis*, Oxford University Press, Oxford, United Kingdom, 2000; *Peptide and Protein Drug Analysis*, ed. Reid, R., Marcel Dekker, Inc.,2000; and *Epitope Mapping*, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001. Also, the CARs can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. Further, some of the CARs (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, etc. Methods of isolation and purification are known in the art. Alternatively, the CARs, of the disclosure (including functional portions and functional variants thereof) can be commercially synthesized. In this respect, the CARs, polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

The disclosure also provides an antibody or an antigen binding fragment thereof that binds to an epitope of the CAR of the disclosure. The antibody or the antigen binding fragment thereof may have any level of affinity or avidity for the functional portion of the CAR. In some embodiments, the antibody or the antigen binding fragment may bind CD33 with a range of affinities (K_D). In one embodiment the antibody binds to CD33 with the K_D equal to or less than about 10^{-7} M, such as but not limited to, 1-9.9 (or any range or value therein, such as 1, 2, 3, 4, 5, 6, 7, 8, or 9)$\times 10^{-8}$ M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M, 10^{-15} M or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. One exemplary affinity is equal to or less than 1×10^{-8} M. Another exemplary affinity is equal to or less than 1×10^{-9} M.

Methods of testing antibodies for their ability to bind to any functional portion of the CARs of the disclosure are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), Western blot, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, and competitive inhibition assays.

The portion of the CAR comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a scFv and a human chimeric or humanized antibody (Harlow et al., 1999, *In: Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, N.Y.; Harlow et al., 1989, *In: Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y.; Houston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; Bird et al., 1988, *Science* 242:423-426). In one aspect, the antigen binding domain of a CAR of the disclosure comprises an antibody fragment. In one embodiment, the CAR of the disclosure comprises an antibody fragment that comprises a scFv.

The disclosure also provides a CAR comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33.

The disclosure also provides a CAR comprising

an extracellular domain comprising an antigen binding domain that binds CD33;

a transmembrane domain; and

an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

In some embodiments, the CAR further comprise a CD8a-hinge region.

In some embodiments, the CAR comprises a CD8a transmembrane region (CD8a-TM) polypeptide and the intracellular signaling domain comprising a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

In some embodiments, the CAR comprises

the CD8a-hinge region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 157;

the transmembrane domain comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 162; and/or

the intracellular signaling domain comprising a co-stimulatory domain having an amino acid sequence that is at least 90% identical to SEQ ID NO: 163, and a primary signaling domain having an amino acid sequence that is at least 90% identical to SEQ ID NO: 164.

The disclosure also provides a CAR comprising an extracellular antigen binding domain, a

transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises:

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 81 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 52;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 82 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 53;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 83 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 54;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 84 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 55;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 85 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 56;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 86 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 57;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 87 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 58;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 87 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 59;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 87 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 60;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 88 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 61;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 105 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 95;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 106 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 96;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 107 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 97;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 185 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 170;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 188 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 171;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 192 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 175;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 196 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 179;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 200 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 181;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 202 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 96;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 271 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 262;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 272 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 263; or

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 273 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 264.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 82 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 53.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises the the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 85 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 56.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 87 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 59.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 271 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 262.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds CD33 and comprises the the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 272 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 263.

In certain embodiments, the CAR comprises an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding

domain binds CD33 and comprises the LCDR1, the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 273 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 264.

Exemplary HCDR and LCDR sequences are described in Tables 4a, 4b, 4c, 4d, 4e and 4f.

In some embodiments, the CDRs are defined according to Kabat.

In some embodiments, the CDRs are defined according to Chothia.

In some embodiments, the CDRs are defined according to IMGT.

In some embodiments, the CDRs are defined according to AbM.

In some embodiment, the CDRs are defined according to Contact.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, 35, 253, 254 or 255, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, 44, 256, 257 or 258, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 259, 260 or 261 the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, 68, 265 or 266, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, 74, 267 or 268. and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, 80, 269 or 270.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NOs: 104, 184, 191, 195, or 199.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, 35, 253, 254 or 255, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, 44, 256, 257 or 258, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 259, 260 or 261, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, 68, 265 or 266 or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, 74, 267 or 268, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR3 of SEQ ID NOs: 71 or 146, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular

antigen binding domain that binds CD33 comprises the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, 80, 269 or 270, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the LCDR3 of SEQ ID NOs: 104, 184, 191, 195, or 199, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular

antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 253, 256, 259, 265, 74, and 269, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 254, 257, 260, 66, 267, and 76, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 258, 261, 266, 268, and 270, respectively.

In some embodiments, the extracellular antigen binding domain that binds CD33 comprises the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

In some embodiments, the extracellular antigen binding domain that binds CD33 comprises the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

In some embodiments, the extracellular antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;

In certain embodiments, the extracellular antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In other embodiments, the extracellular antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85.

In other embodiments, the extracellular antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87.

In other embodiments, the extracellular antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271.

In other embodiments, the extracellular antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272.

In other embodiments, the extracellular antigen binding domain that binds CD33 comprises the the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the extracellular antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

The extracellular antigen-binding domain may also be a variant the CD33 binding domains as described elsewhere (for example, a variant domain as described in Examples 19-20).

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 52 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 81.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 53 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 82.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 54 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 83.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 55 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 84.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 56 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 85.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 57 and the VL comprising the amino acid sequence that is at least 50%, at

least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 86.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 58 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 87.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 59 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 87.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 60 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 87.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 61 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 88.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 95 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 105.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 96 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 106.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 97 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 107.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 170 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 185.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 171 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 188.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 175 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 192.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 179 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 196.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 181 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 200.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 96 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 202.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 262 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 271.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 263 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 272.

In some embodiments, the extracellular antigen-binding domain comprises the VH comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 264 and the VL comprising the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 273.

In some embodiments, the extracellular antigen binding domain that binds CD33 comprises a scFv. In some embodiments, the scFv comprises a linker polypeptide between the VL and the VH.

In some embodiments, the linker polypeptide comprises the amino acid sequence of SEQ ID NO: 7. In some embodiments, the linker polypeptide comprises an amino acid sequence having at least

50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with SEQ ID NO: 108.

In some embodiments, the linker polypeptide comprises the amino acid sequence of SEQ ID NO: 108 (e.g. wherein the linker polypeptide consists of the amino acid sequence of SEQ ID NO: 108).

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 and 276.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 203.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 204.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 205.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 206.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 207.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 208.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 209.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 210.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 211.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 212.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 213.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 214.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 215.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 216.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 217.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 218.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 219.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 220.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 221.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 274.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 275.

In some embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 276.

In certain embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 213.

In other embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 216.

In other embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 219.

In other embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 274.

In other embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 275.

In other embodiments, the extracellular antigen binding domain that binds CD33 is a scFv which comprises an amino acid sequence SEQ ID NO: 276.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 203.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 204.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 205.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 206.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 207.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 208.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 209.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 210.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 211.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 212.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 213.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 214.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 215.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 216.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 217.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 218.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 219.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 220.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 221.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 274.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 275.

In some embodiments, the scFv comprises the amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 276.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence selected from the group

consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 or 276.

In certain embodiments, the CAR comprises an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 213, 216, 219, 274, 275 or 276.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 18.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 18.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 19.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 19.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 20.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 20.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 21.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 21.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 22.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 22.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 23.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 23.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 24.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 24.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 25.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 25.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 26.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 26.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 27.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 27.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 203.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 203.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 204.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 204.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 205.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 205.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 206.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 206.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 207.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 207.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 208.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 208.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 209.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 209.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 210.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 210.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 211.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 211.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 212.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 212.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 213.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 213.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 214.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 214.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 215.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 215.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 216.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 216.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 217.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 217.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 218.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 218.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 219.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 219.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 220.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 220.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 221.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 221.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 274.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 274.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 275.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 275.

The disclosure provides a CAR comprising an extracellular antigen binding domain that comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 276.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds CD33, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NO: 276.

In some embodiments, the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member (such as, e.g., CD28 or inducible T-cell co-stimulator (ICOS) component, and a combination thereof.

In some embodiments, the CD137 component comprises an amino acid sequence of SEQ ID NO: 163. In some embodiments, the CD137 component comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 163.

In some embodiments, the CD3z component comprises an amino acid sequence of SEQ ID NO: 164. In some embodiments, the CD3z component comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 164.

In some embodiments, the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 165. In some embodiments, the intracellular signaling domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 165.

SEQ ID NO: 165

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

In some embodiments, the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide. In some embodiments, the CD8a-TM polypeptide comprises an amino acid sequence of **SEQ ID NO: 162**. (IYIWAPLAGTCGVLLLSLVITLYC; **SEQ ID NO: 162**).

In some embodiments, the CD8a-TM polypeptide comprises an amino acid sequence that is least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the transmembrane domain comprises at least the transmembrane region(s) of) the α, β or ζ chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD33, CD37, CD40, CD64, CD80, CD86, CD134, CD137, CD154. In some

embodiments, the transmembrane domain comprises at least the transmembrane domain of ζ, η or FcεR1γ and -β, MB1 (Igα.), B29 or CD3- γ, ζ, or η. In some embodiments, the transmembrane domain is synthetic, e.g., comprising predominantly hydrophobic residues such as leucine and valine, a triplet of phenylalanine, or tryptophan.

In some embodiments, the CAR further comprises a hinge region linking the transmembrane domain to the extracellular antigen binding domain that binds CD33. In some embodiments, the hinge region is a CD8a-hinge region. In some embodiments, CD8a-hinge region comprises an amino acid sequence of **SEQ ID NO: 157** (TSTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD; SEQ ID NO: 157).

In some embodiments, the CD8a-hinge region comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of **SEQ ID NO: 157**. In some embodiments, the hinge region comprises the amino acid sequence EPKSCDKTHTCPPCP (SEQ ID NO: 158), or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% amino acid sequence identity with EPKSCDKTHTCPPCP (SEQ ID NO: 158). In some embodiments, the hinge region comprises the sequence ERKCCVECPPCP (SEQ ID NO: 159) or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ERKCCVECPPCP (SEQ ID NO: 159). In some embodiments, the hinge region comprises the sequence ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$, (SEQ ID NO: 160) or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$ (SEQ ID NO: 160). In some embodiments, the hinge region comprises the sequence ESKYGPPCPSCP (SEQ ID NO: 161), or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ESKYGPPCPSCP (SEQ ID NO: 161).

CAR constructs and immunoresponsive cells expressing CARs

The disclosure also provides isolated polynucleotides encoding the CARs described herein.

Unless otherwise specified, a "polynucleotide encoding an amino acid sequence" includes all polynucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase polynucleotide that encodes a protein or a RNA may also include introns to the

extent that the polynucleotide encoding the protein may in some variants contain an intron(s) that is spliced into the expressed protein.

The disclosure also provides an expression vector comprising the nucleic acid molecule encoding the CAR of the disclosure.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an *in vitro* expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The disclosure also provides isolated immunoresponsive cells comprising the CAR of the disclosure. In some embodiments, the isolated immunoresponsive cell is transduced with the CAR, for example, the CAR is constitutively expressed on the surface of the immunoresponsive cell. In certain embodiments, the isolated immunoresponsive cell is further transduced with at least one co-stimulatory ligand such that the immunoresponsive cell expresses the at least one co-stimulatory ligand. In certain embodiments, the at least one co-stimulatory ligand is selected from the group consisting of 4-1BBL, CD48, CD70, CD80, CD86, OX40L, TNFRSF14, and combinations thereof. In certain embodiments, the isolated immunoresponsive cell is further transduced with at least one cytokine such that the immunoresponsive cell secretes the at least one cytokine. In certain embodiments, the at least cytokine is selected from the group consisting of IL-2, IL-3, IL-6, IL-7, IL-11, IL-12, IL-15, IL-17, IL-21, and combinations thereof. In some embodiments, the isolated immunoresponsive cell is selected from the group consisting of a T lymphocyte (T cell), a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell (Treg), a human embryonic stem cell, a lymphoid progenitor cell, a T cell-precursor cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.

In some embodiments, the isolated immunoresponsive cell is the T cell.

For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to bone marrow, blood, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD8^+$ T cells (e.g., cytotoxic T cells), $CD4^+$ helper T cells, e.g., Th1 and Th2 cells, peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor

infiltrating cells, memory T cells, naïve T cells, and the like. The T cell may be a $CD8^+$ T cell or a $CD4^+$ T cell.

In some embodiments, the isolated immunoresponsive cell is the NK cell.

In some embodiments, the isolated immunoresponsive cell is the CTL.

In some embodiments, the isolated immunoresponsive cell is the Treg.

In some embodiments, the isolated immunoresponsive cell is the human embryonic stem cell.

In some embodiments, the isolated immunoresponsive cell is the lymphoid progenitor cell.

In some embodiments, the isolated immunoresponsive cell is the pluripotent stem cell.

In one embodiment, the CAR T cells of the disclosure can be generated by introducing a lentiviral vector comprising a desired CAR, for example, a CAR comprising an extracellular domain comprising an antigen binding domain that binds CD33, CD8α hinge and transmembrane domain, and human 4-1BB and CD3-zeta signaling domains, into the cells. The CAR T cells of the disclosure are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

Embodiments of the invention further provide host cells comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, or algae, fungi, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5α *E.coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, e.g., a DH5α cell. For purposes of producing a recombinant CAR, polypeptide, or protein, the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL). The host cell may be a T cell.

Also provided are a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, an erythrocyte, a neutrophil, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal

population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

Polynucleotides, host cells and vectors

The disclosure also provides an isolated polynucleotide encoding any of the CD33 binding proteins of the disclosure. The CD33 binding protein includes the antigen binding domains that bind CD33, the proteins comprising the antigen binding domains that bind CD33, the multispecific proteins that comprise the antigen binding domains that bind CD33 and the chimeric antigen receptors (CAR) comprising the antigen binding domains that bind CD33 of the disclosure.

The invention also provides an isolated polynucleotide encoding any of CD33 biding proteins or fragments thereof.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382.

In certain embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 53.

In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 56.

In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 59.

In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 262.

In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 263.

In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 264.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87,88, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In certain embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 82.

In other embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 85.

In other embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 87.

In other embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 271.

In other embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 272.

In other embodiments, the isolated polynucleotide encodes the VL of SEQ ID NO: 273. The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 95, 96, 97, 170, 171, 175, 179, 181, or 96.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the isolated polynucleotide encodes a VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382 and a VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

The invention also provides for an isolated polynucleotide encoding

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272 or
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. In other embodiments, the isolated

polynucleotide encodes the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In other embodiments, the isolated polynucleotide encodes the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

The invention also provides for an isolated polynucleotide encoding

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 221, 274, 275 or 276.The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 18.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 19.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 20.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 21.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 22.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 23.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 24.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 25.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 26.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 27.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 203.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 204.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 205.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 206.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 207.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 208.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 209.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 210.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 211.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 212.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 213.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 214.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 215.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 216.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 217.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 218.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 219.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 220.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 221.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 274.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 275.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 276.

In certain embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 213. In other embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 216. In other embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 219. In other embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 274. In other embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 275. In other embodiments, the isolated polynucleotide encodes the polypeptide of SEQ ID NO: 276.

The disclosure also provides an isolated or purified polynucleotide that encodes any of the CAR of the disclosure, or functional portions or functional variants thereof.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 or 276.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 18.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 19.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 20.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 21.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 22.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 23.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 24.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 25.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 26.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 27.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 203.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 204.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 205.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 206.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 207.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 208.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 209.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 210.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 211.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 212.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 213.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 214.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 215.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 216.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 217.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 218.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 219.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 220.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 221.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 274.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 275.

The invention also provides an isolated polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 276.

Some embodiments of the disclosure also provide an isolated or purified nucleic acid comprising a polynucleotide which is complementary to the polynucleotides encoding the CD33 binding proteins of the disclosure or polynucleotides which hybridize under stringent conditions to the polynucleotides encoding the CD33 binding proteins of the disclosure.

The polynucleotides which hybridize under stringent conditions may hybridize under high stringency conditions. By “high stringency conditions” is meant that the polynucleotide specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-12 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the

equivalent, at temperatures of about 50-70° C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the CARs described herein. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

The polynucleotide sequences of the disclosure may be operably linked to one or more regulatory elements, such as a promoter or enhancer, that allow expression of the nucleotide sequence in the intended host cell. The polynucleotide may be a cDNA. The promoter bay be a strong, weak, tissue-specific, inducible or developmental-specific promoter. Exemplary promoters that may be used are hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin, human myosin, human hemoglobin, human muscle creatine, and others. In addition, many viral promoters function constitutively in eukaryotic cells and are suitable for use with the described embodiments. Such viral promoters include Cytomegalovirus (CMV) immediate early promoter, the early and late promoters of SV40, the Mouse Mammary Tumor Virus (MMTV) promoter, the long terminal repeats (LTRs) of Maloney leukemia virus, Human Immunodeficiency Virus (HIV), Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV), and other retroviruses, and the thymidine kinase promoter of Herpes Simplex Virus. Inducible promoters such as the metallothionein promoter, tetracycline-inducible promoter, doxycycline-inducible promoter, promoters that contain one or more interferon-stimulated response elements (ISRE) such as protein kinase R 2',5'-oligoadenylate synthetases, Mx genes, ADAR1, and the like may also be sued.

The invention also provides a vector comprising the polynucleotide of the invention. The disclosure also provide an expression vector comprising the polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the synthetic polynucleotide of the invention into a given organism or genetic background by any means. Polynucleotides encoding the CD33 binding proteins of the disclosure may be operably linked to control sequences in the expression vector(s) that ensure the expression of the CD33 binding proteins. Such regulatory elements may include a transcriptional promoter, sequences encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. Expression vectors may also include one or more nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, other 5' or 3' flanking nontranscribed sequences, 5' or 3' nontranslated sequences (such as necessary ribosome binding sites), a polyadenylation site, splice donor and acceptor sites, or transcriptional termination sequences. An origin of replication that confers the ability to replicate in a host may also be incorporated.

The expression vectors can comprise naturally-occurring or non-naturally-occurring

internucleotide linkages, or both types of linkages. The non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the CD33 binding proteins of the disclosure encoded by the incorporated polynucleotides. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. Exemplary vectors may be constructed as described by Okayama and Berg, 3 *Mol. Cell. Biol.* 280 (1983).

Vectors of the disclosure may also contain one or more Internal Ribosome Entry Site(s) (IRES). Inclusion of an IRES sequence into fusion vectors may be beneficial for enhancing expression of some proteins. In some embodiments, the vector system will include one or more polyadenylation sites (e.g., SV40), which may be upstream or downstream of any of the aforementioned nucleic acid sequences. Vector components may be contiguously linked or arranged in a manner that provides optimal spacing for expressing the gene products (i.e., by the introduction of "spacer" nucleotides between the ORFs) or positioned in another way. Regulatory elements, such as the IRES motif, may also be arranged to provide optimal spacing for expression.

Vectors of the disclosure may be circular or linear. They may be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColE1, SV40, 2μ plasmid, λ, bovine papilloma virus, and the like.

The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

The vectors may also comprise selection markers, which are well known in the art. Selection markers include positive and negative selection marker. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Exemplary marker genes include antibiotic resistance genes (e.g., neomycin resistance gene, a hygromycin resistance gene, a kanamycin resistance gene, a tetracycline resistance gene, a penicillin resistance gene, histidinol resistance gene, histidinol x resistance gene), glutamine synthase genes, HSV-TK, HSV-TK derivatives for ganciclovir selection, or bacterial purine nucleoside

phosphorylase gene for 6-methylpurine selection (Gadi et al., 7 *Gene Ther.* 1738-1743 (2000)). A nucleic acid sequence encoding a selection marker or the cloning site may be upstream or downstream of a nucleic acid sequence encoding a polypeptide of interest or cloning site.

Exemplary vectors that may be used are Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia), pEE6.4 (Lonza) and pEE12.4 (Lonza). Additional vectors include the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λGT10, λGT11, λEMBL4, and λNM1149, λZapII (Stratagene) can be used. Exemplary plant expression vectors include pBI01, pBI01.2, pBI121, pBI101.3, and pBIN19 (Clontech). Exemplary animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). The expression vector may be a viral vector, e.g., a retroviral vector, e.g., a gamma retroviral vector.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382.

In certain embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 53. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 56. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 59. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 262. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 263. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 264.

In some embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In certain embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 82. In other embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 85. In other embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 87. In other embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 271. In

other embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 272. In other embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 273.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 95, 96, 97, 170, 171, 175, 179, 181, or 96.

In some embodiments, the vector comprises the polynucleotide encoding the VL of SEQ ID NO: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

In some embodiments, the vector comprises the polynucleotide encoding for an isolated polynucleotide encoding

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
k) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;
m) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In other embodiments, the vector comprises the

polynucleotide encoding the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In other embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In some embodiments, the vector comprises the polynucleotide encoding for an isolated polynucleotide encoding

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NOs: SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 or 276.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 18.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 20.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 21.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 22.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 23.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 24.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 25.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 26.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 27.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 203.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 204.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 205.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 206.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 207.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 208.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 209.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 210.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 211.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 212.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 213.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 214.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 215.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 216.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 217.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 218.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 219.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 220.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 221.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 274.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 275.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NO: 276.

In some embodiments, the vector comprises the polynucleotide encoding any of the CAR of the disclosure, or functional portions or functional variants thereof.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 or 276.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 24.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 203.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 204.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 205.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 206.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 207.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 208.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 209.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 210.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 211.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 212.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 213.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 214.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 215.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 216.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 217.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 218.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 219.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 220.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 221.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 274.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 275.

In some embodiments, the vector comprises the polynucleotide encoding the CAR comprising the amino acid sequence of SEQ ID NO: 276.

The invention also provides for a host cell comprising one or more vectors of the invention. "Host cell" refers to a cell into which a vector has been introduced. It is understood that the term host cell is intended to refer not only to the particular subject cell but to the progeny of such a cell, and also to a stable cell line generated from the particular subject cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Such host cells may be eukaryotic cells, prokaryotic cells, plant cells or archeal cells. *Escherichia coli*, bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species are examples of prokaryotic host cells. Other microbes, such as yeast, are also useful for expression. Saccharomyces (e.g., S. cerevisiae) and Pichia are examples of suitable yeast host cells. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATTC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary

(CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

The disclosure also provides a method of producing the CD33 binding protein of the disclosure comprising culturing the host cell of the disclosure in conditions that the K2 binding protein is expressed, and recovering the CD33 binding protein produced by the host cell. Methods of making proteins and purifying them are known. Once synthesized (either chemically or recombinantly), the CD33 binding proteins may be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, Protein Purification (Springer- Verlag, N.Y., (1982)). A subject protein may be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or at least about 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules, etc. other than the subject protein.

The polynucleotides encoding the CD33 binding proteins of the disclosure may be incorporated into vectors using standard molecular biology methods. Host cell transformation, culture, antibody expression and purification are done using well known methods.

Accordingly, the invention provides a method of producing a polypeptide comprising expressing a nucleotide of the invention that encodes for a polypeptide of the invention. In certain embodiments, the polynucleotide encodes the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In other embodiments, the polynucleotide encodes the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In other embodiments, the polynucleotide encodes the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In other embodiments, the polynucleotide encodes the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 82. In other embodiments, the polynucleotide encodes the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In other embodiments, the polynucleotide encodes the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82.

In certain embodiments of this method, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 53 and a polypeptide comprising the VL of SEQ ID NO: 82. In other embodiments, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 56 and a polypeptide comprising the VL of SEQ ID NO: 85. In other embodiments, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 59 and a polypeptide comprising the VL of SEQ ID NO: 87. In other embodiments, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 262 and a polypeptide comprising the VL of SEQ ID NO: 271. In other embodiments, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 263 and a polypeptide comprising the VL of SEQ ID NO: 272. In other embodiments, the polynucleotide encodes a polypeptide comprising the VH of SEQ ID NO: 264 and a polypeptide comprising the VL of SEQ ID NO: 273.

Where the polypeptide is formed of separate chains which are encoded by different nucleic acids, the invention provides a method of producing a polypeptide comprising expressing nucleotides of the invention that encode for a polypeptide of the invention. In certain embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 53 and a second polynucleotide of the invention encodes a polypeptide comprising the VL of SEQ ID NO: 82. In other embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 56 and a second polynucleotide of the invention encodes a polypeptide comprising the VL of SEQ ID NO: 85. In other embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 59 and a second polynucleotide of the invention encodes a polypeptide comprising the VL of of SEQ ID NO: 87. In other embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 262 and a second polynucleotide of the invention encodes a polypeptide comprising the VL of SEQ ID NO: 271. In other embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 263and a second polynucleotide of the invention encodes a polypeptide comprising the VL of SEQ ID NO: 272. In other embodiments, a first polynucleotide of the invention encodes a polypeptide comprising the VH of SEQ ID NO: 264 and a second polynucleotide of the invention encodes a polypeptide comprising the VL of SEQ ID NO: 273.

Modified nucleotides may be used to generate the polynucleotides of the disclosure. Exemplary modified nucleotides are 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5″-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queuosine, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

Pharmaceutical Compositions/Administration

The disclosure also provides a pharmaceutical composition comprising the CD33 binding protein of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the antigen binding domain that binds CD33 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the protein comprising the

antigen binding domain that binds CD33 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the multispecific protein comprising the antigen binding domain that binds CD33 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the CAR comprising the antigen binding domain that binds CD33 of the disclosure and a pharmaceutically acceptable carrier. For therapeutic use, the CD33 binding protein of the disclosure may be prepared as pharmaceutical compositions containing an effective amount of the antibody as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody of the invention is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine may be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the antibodies of the invention in such pharmaceutical formulation may vary, from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and may be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration of the CD33 binding protein of the disclosure may be any suitable route such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, transmucosal (oral, intranasal, intravaginal, rectal) or other means appreciated by the skilled artisan, as well known in the art.

The CD33 binding protein of the disclosure of the invention may also be administered prophylactically in order to reduce the risk of developing a disease such as cancer.

Thus, a pharmaceutical composition of the invention for intramuscular injection may be prepared to contain 1 ml sterile buffered water, and between about 1 ng to about 100 mg/kg, *e.g.* about 50 ng to about 30 mg/kg or more preferably, about 5 mg to about 25 mg/kg, of the CD33 binding protein of the disclosure of the invention.

In embodiments of the present disclosure, the CAR-expressing cells may be provided in compositions, e.g., suitable pharmaceutical composition(s) comprising the CAR-expressing cells and a pharmaceutically acceptable carrier. In one aspect, the present disclosure provides pharmaceutical compositions comprising an effective amount of a lymphocyte expressing one or more of the CARs described and a pharmaceutically acceptable excipient. Pharmaceutical compositions of the present disclosure may comprise a CAR-expressing cell, e.g., a plurality of CAR-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, excipients or diluents. A pharmaceutically acceptable carrier can be an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to the subject.

A pharmaceutically acceptable carrier can include a buffer, excipient, stabilizer, or preservative. Examples of pharmaceutically acceptable carriers are solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible, such as salts, buffers, antioxidants, saccharides, aqueous or non-aqueous carriers, preservatives, wetting agents, surfactants or emulsifying agents, or combinations thereof. The amounts of pharmaceutically acceptable carrier(s) in the pharmaceutical compositions may be determined experimentally based on the activities of the carrier(s) and the desired characteristics of the formulation, such as stability and/or minimal oxidation.

Pharmaceutical compositions may comprise buffers such as acetic acid, citric acid, formic acid, succinic acid, phosphoric acid, carbonic acid, malic acid, aspartic acid, histidine, boric acid, Tris buffers, HEPPSO, HEPES, neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); antibacterial and antifungal agents; and preservatives.

Pharmaceutical compositions of the present disclosure can be formulated for a variety of means of parenteral or non-parenteral administration. In one embodiment, the compositions can be formulated for infusion or intravenous administration. Pharmaceutical compositions disclosed herein can be provided, for example, as sterile liquid preparations, e.g., isotonic aqueous solutions, emulsions, suspensions, dispersions, or viscous compositions, which may be buffered to a desirable pH. Formulations suitable for oral administration can include liquid solutions, capsules, sachets, tablets, lozenges, and troches, powders liquid suspensions in an appropriate liquid and emulsions.

The term “pharmaceutically acceptable,” as used herein with regard to pharmaceutical compositions, means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals and/or in humans.

METHODS OF TREATMENT AND USES

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the antigen binding domain that binds CD33 of the disclosure to the subject in need thereof for a time sufficient to treat the CD33 expressing cancer.

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the protein comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to treat the CD33 expressing cancer

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to treat the CD33 expressing cancer.

In certain embodiments, the method comprises administering a therapeutically effective amount of a multispecific protein (for example, a bispecific protein) comprising a first antigen binding domain that binds CD33 of the disclosure and a second antigen binding domain that binds TRGV9 to the subject for a time sufficient to treat the CD33 expressing cancer. In certain such embodiments, the first antigen binding domain that binds CD33 is an scFv and, optionally, the second binding domain that binds TRGV9 is a VHH.

In some embodiments, the method comprises administering a therapeutically effective amount of a multispecific protein (for example, a bispecific protein) comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds TRGV9 to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the first antigen binding domain that binds CD33 comprises:

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or

the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain such embodiments, the first antigen binding domain that binds CD33 is an scFv and, optionally, the second binding domain that binds TRGV9 is a VHH.

In some embodiments, the method comprises administering a therapeutically effective amount of a multispecific protein (for example, a bispecific protein) comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds TRGV9 to the subject for a time sufficient to

treat the CD33 expressing cancer, wherein the first antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 213, 216, 219, 274, 275 or 276. In certain such embodiments the second binding domain that binds TRGV9 is a VHH.

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the CAR comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to treat the CD33 expressing cancer.

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the immunoconjugate comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to treat the CD33 expressing cancer.

The disclosure also provides a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the pharmaceutical composition comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to treat the CD33 expressing cancer. In certain such embodiments, the pharmaceutical composition comprises a multispecific protein (for example, a bispecific protein) comprising a first antigen binding domain that binds CD33 of the disclosure and a second antigen binding domain that binds TRGV9. In some embodiments the first antigen binding domain that binds CD33 is an scFv and, optionally, the second binding domain that binds TRGV9 is a VHH.

In some embodiments, the method comprises administering a therapeutically effective amount of a pharmaceutical composition comprising a multispecific protein (for example, a bispecific protein), wherein the multispecific protein comprises a first antigen binding domain that binds CD33 and a second antigen binding domain that binds TRGV9 to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the first antigen binding domain that binds CD33 comprises:

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain such embodiments, the first antigen binding domain that binds CD33 is an scFv and, optionally, the second binding domain that binds TRGV9 is a VHH.

In some embodiments, the method comprises administering a therapeutically effective amount of a pharmaceutical composition comprising a multispecific protein (for example, a bispecific protein),

wherein the multispecific protein comprises a first antigen binding domain that binds CD33 and a second antigen binding domain that binds TRGV9 to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the first antigen binding domain that binds CD33 comprise the amino acid sequence of SEQ ID NOs: 213, 216, 219, 274, 275 or 276. In certain such embodiments the second binding domain that binds TRGV9 is a VHH.

The disclosure also provides a method of administering a genetically modified T cell expressing a CAR for the treatment of a subject having cancer or at risk of having cancer using lymphocyte infusion.

In at least one embodiment, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a subject in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the subject.

In one aspect, the disclosure relates generally to the treatment of a subject at risk of developing cancer. The invention also includes treating a malignancy or an autoimmune disease in which chemotherapy and/or immunotherapy results in significant immunosuppression in a subject, thereby increasing the risk of the subject developing cancer.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the antigen binding domain that bind CD33 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the protein comprising the antigen binding domain that bind CD33 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the multispecific protein comprising the antigen binding domain that bind CD33 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the immunoconjugate of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the pharmaceutical composition of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the CAR of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of preventing CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of any antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer.

The disclosure also provides a method of preventing a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the protein comprising any antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer

The disclosure also provides a method of preventing a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer.

The disclosure also provides a method of preventing a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the CAR comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer.

The disclosure also provides a method of preventing a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the immunoconjugate comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer.

The disclosure also provides a method of preventing a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the pharmaceutical composition comprising any antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to prevent the CD33 expressing cancer.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering any antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering the protein comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering the multispecific protein comprising the antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering the CAR that comprises any antigen binding domain that binds CD33 of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering the immunoconjugate of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

The disclosure also provides a method of reducing the amount of CD33 expressing tumor cells in a subject, comprising administering the pharmaceutical composition of the disclosure to the subject for a time sufficient to reduce the amount of CD33 expressing tumor cells.

In some embodiments, the CD33 expressing cancer is hematologic cancer.

In some embodiments, the CD33 expressing cancer is leukemia.

In some embodiments, the CD33 expressing cancer is lymphoma.

In some embodiments, the CD33 expressing cancer is multiple myeloma.

In some embodiments, the CD33 expressing cancer is acute myeloid leukemia (AML).

In some embodiments, the CD33 expressing cancer is myelodysplastic syndrome (MDS).

In some embodiments, the CD33 expressing cancer is acute lymphocytic leukemia (ALL).

In some embodiments, the CD33 expressing cancer is diffuse large B-cell lymphoma (DLBCL).

In some embodiments, the CD33 expressing cancer is chronic myeloid leukemia (CML).

In some embodiments, the CD33 expressing cancer is blastic plasmacytoid dendritic cell neoplasm (DPDCN).

In certain embodiments, the CD33 expressing cancer is acute myeloid leukemia (AML).

In some embodiments, the cancer is relapsed, refractory, malignant, or any combination thereof.

The disclosure also provides a method of treating hematologic cancer (e.g., leukemia (in particular, AML), lymphoma or multiple myeloma) in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 to the subject for a time sufficient to treat the hematologic cancer, wherein the antigen binding domain that bind CD33 comprises:

the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87;
the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88;
the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272; or
the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

In certain embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82. In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85. In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87. In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271. In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272. In other embodiments, the antigen binding domain that binds CD33 comprises the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273.

The disclosure also provides a method of treating hematologic cancer (e.g., leukemia (in particular, AML), lymphoma or multiple myeloma) in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 to the subject for a time sufficient to treat the hematologic cancer, wherein the antigen binding domain that bind CD33 comprises:

the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

The disclosure also provides a method of treating hematologic cancer (e.g., leukemia (in particular, AML), lymphoma or multiple myeloma) in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 to the subject for a time sufficient to treat the hematologic cancer, wherein the antigen binding domain that bind CD33 comprises:

the VH of SEQ ID NO: 18;
the VH of SEQ ID NO: 19;
the VH of SEQ ID NO: 20;
the VH of SEQ ID NO: 21;
the VH of SEQ ID NO: 22;
the VH of SEQ ID NO: 23;
the VH of SEQ ID NO: 24;
the VH of SEQ ID NO: 25;
the VH of SEQ ID NO: 26; or
the VH of SEQ ID NO: 27.

The disclosure also provides a method of treating hematologic cancer (e.g., leukemia (in particular, AML), lymphoma or multiple myeloma) in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds CD33 to the subject for a time sufficient to treat the hematologic cancer, wherein the antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 274, 275 or 276.

In certain embodiments, the antigen binding domain that binds CD33 comprises the amino acid sequence of SEQ ID NOs: 213, 216, 219, 274, 275 or 276.

In one aspect, the present disclosure provides methods of preventing cancer, the methods comprising administering an amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof.

In one aspect, the present disclosure provides methods of treating a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof, whereby the lymphocyte induces or modulates killing of cancer cells in the subject.

In another aspect, the present disclosure provides methods of reducing tumor burden in a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described herein to a subject in need thereof, whereby the lymphocyte induces killing of hematologic cancer cells in the subject. In another aspect, the present disclosure provides methods of increasing survival of a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof, whereby the survival of the subject is lengthened. Generally, the lymphocytes expressing the CAR(s) induce killing of hematologic cancer cells in the subject and result in

reduction or eradication of the hematologic tumors/cancer cells in the subject. A non-limiting list of hematologic cancers, inclusive of metastatic lesions, that can be targeted, includes leukemia, lymphoma, multiple myeloma. In some embodiments, the hematologic 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 (DPDCN).

In one aspect, methods of treating a subject having cancer are provided that comprise administering a therapeutically effective amount of a lymphocyte expressing a CAR, the CAR having an extracellular antigen-binding domain that binds the CD33 antigen, to a subject in need thereof, whereby the lymphocyte induces killing of cancer cells in the subject.

In one aspect, a method of targeted killing of a cancer cell is disclosed, the method comprising contacting the cancer cell with a lymphocyte expressing one or more of the CARs described, whereby the lymphocyte induces killing of the cancer cell. A non-limiting list of cancer cells, inclusive of hematologic cancer cells, that can be targeted includes leukemia, lymphoma, multiple myeloma. In some embodiments, the hematologic 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 (DPDCN).

When a therapeutically effective amount is indicated, the precise amount of the CARs or CAR-Ts of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the subject. It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of about 10^4 to about 10^{10} cells/kg body weight, in some instances about 10^5 to about 10^6 cells/kg body weight, including all integer values within those ranges. In some embodiments, a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of about 10^6 cells/kg body weight. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., *New Eng. J. of Med.* 319:1676, 1988).

Delivery systems useful in the context of the CAR-T of the invention may include time-released, delayed release, and sustained release delivery systems such that the delivery of the T cell compositions occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain composition embodiments of the invention.

Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polyesteramides,

polyorthoesters, polycaprolactones, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di- and tri-glycerides; sylastic systems; peptide based systems; hydrogel release systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775; 4,667,014; 4,748,034; and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480 and 3,832,253. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

In certain aspects, it may be desirable to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate the T cells according to the present disclosure, and reinfuse the subject with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 10 cc to 400 cc. In certain aspects, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc.

The administration of the CAR-T cells and compositions may be carried out in any manner, e.g., by parenteral or nonparenteral administration, including by aerosol inhalation, injection, infusions, ingestion, transfusion, implantation or transplantation. For example, the CAR-T cells and compositions described herein may be administered to a patient trans-arterially, intradermally, subcutaneously, intratumorally, intramedullary, intranodally, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the compositions of the present disclosure are administered by i.v. injection. In one aspect, the compositions of the present disclosure are administered to a subject by intradermal or subcutaneous injection. The compositions of T cells may be injected, for instance, directly into a tumor, lymph node, tissue, organ, or site of infection.

Administration can be autologous or non-autologous. For example, immunoresponsive cells expressing a CD33-specific CAR can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived T cells of the present disclosure, or expanded T cells (e.g., *in vivo, ex vivo* or *in vitro* derived) can be administered via, e.g., intravenous injection, localized injection, systemic injection, catheter administration, or parenteral administration.

In particular embodiments, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted *ex vivo* to select and/or isolate the cells of interest, e.g., T cells. These T cell isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the

present disclosure may be introduced, thereby creating a CAR-T cell. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR-T cells. In one aspect, expanded cells are administered before or following surgery.

The dosage administered to a patient having a malignancy is sufficient to alleviate or at least partially arrest the disease being treated ("therapeutically effective amount"). The dosage of the above treatments to be administered to a subject will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to practices generally accepted in the art.

The CAR T cells of the invention can undergo *in vivo* T cell expansion and can establish CD33-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some instances, the CAR T cells of the invention infused into a subject can eliminate cancer cells, e.g., hematologic cancer cells, *in vivo* in subjects with advanced chemotherapy-resistant cancer.

In one embodiment, a CAR of the present disclosure is introduced into T cells, e.g., using *in vitro* transcription, and the subject (e.g., human) receives an initial administration of CAR-T cells of the disclosure, and one or more subsequent administrations of the CAR-T cells, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR-T cells are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CAR-T cells are administered per week. In one embodiment, the subject receives more than one administration of the CAR-T cells per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR-T cell administrations, and then one or more additional administration of the CAR-T cells (e.g., more than one administration of the CAR-T cells per week) is administered to the subject. In another embodiment, the subject receives more than one cycle of CAR-T cells, and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR-T cells are administered every other day for 3 administrations per week. In one embodiment, the CAR-T cells are administered for at least two, three, four, five, six, seven, eight or more weeks.

In one embodiment, administration may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose.

The CAR-T cells may be administered in the methods of the invention by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

In one embodiment, CAR-T cells are generated using lentiviral viral vectors, such as lentivirus. CAR-T cells generated with such viral vectors will generally have stable CAR expression.

In one embodiment, CAR-T cells transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be affected by RNA CAR vector delivery. In one embodiment, the CAR RNA is transduced into the T cell by electroporation.

If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CAR-T infusion breaks should not last more than ten to fourteen days.

Combination therapies

The CD33 binding proteins of the disclosure may be administered in combination with at least one additional therapeutics.

In some embodiments the at least one additional therapeutic is surgery, chemotherapy, or radiation, or any combination thereof.

In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

In one embodiment, other therapeutic agents such as factors may be administered before, after, or at the same time (simultaneous with) as the CD33 binding proteins such as CAR-T cells, including, but not limited to, interleukins, as well as colony stimulating factors, such as G-, M- and GM-CSF, and interferons.

The CD33 binding proteins such as CAR-expressing cells described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions,

or sequentially. For sequential administration, the CD33 binding proteins such as CAR-expressing cells described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

In further embodiments, the CD33 binding proteins such as CAR-expressing cells described herein may be used in a treatment regimen in combination with surgery, radiation, chemotherapy, immunosuppressive agents, antibodies, or other immunoablative agents. In one embodiment, the subject can be administered an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule.

The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

EXAMPLES

The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing from the invention in spirit or in scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

Example 1: Antigen Generation

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 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-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 3.

Human and cyno CD33 full-length ECD or sub-domain expression constructs were transiently transfected into HEK293 derived cells, Expi293 (Gibco/Thermo Fisher Scientific) using Expifectamine according to manufacturer protocol. Cells were incubated 5 days at 37°C with 8% CO_2 on an orbital shaker before harvesting. The expressed cells were removed by centrifugation and the soluble CD33 proteins with his-tags were purified from the media using immobilized metal affinity chromatography

using Ni Sepharose 6 Fast Flow resin (GE Healthcare) and subsequently buffer-exchanged into 1X Dubelcco's Phosphate Saline buffer pH 7.2 without calcium or magnesium using Zeba™ Spin Desalting Columns, 7K MWCO, 10 mL; ThermoScientific Catalog number: 89893 per the manufacturer's specifications.

Table 3. Amino acid sequences of the CD33 antigens.

Protein AA ID	Description	Amino Acid Sequence
C33W1	HSA N-terminal Fusion; CynoCD33ECD-HSA(C34S)-6xHis	DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFR EGAIVSLDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRPQILIPGALD PDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIITP RPQDHGTNLTCQVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDG SGKQGVVQGSGSGSENLYFQGVRSSSDAHKSEVAHRFKDLGEENF KALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDK SLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDD NPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRL KCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVH TECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSH CIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEF KPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTL VEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKT PVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADI CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEK CCKADDKETCFAEEGKKLVAASQAALGLGGGSHHHHHH (SEQ ID NO: 225)
C33W2	HSA N-terminal Fusion; Human CD33ECD-Tev-HSA(C34S)-6xHis	DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFR EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE PGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITP RPQDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGD GSGKQETRAGVVHGSGSGSENLYFQGVRSSSDAHKSEVAHRFKD LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTD LTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAK VFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQ VSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETF TFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFA AFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGSHHHHHH (SEQ ID NO: 226)
C33W3	HSA N-terminal Fusion; hCD33 Ig-like V-type domain	DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFR EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRDAHKSEVA HRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTC

Protein AA ID	Description	Amino Acid Sequence
	(Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus	VADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPE RNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEI ARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRD EGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEV SKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLK ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAE AKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAAD PHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVP KEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLK AVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLHHH HHH (SEQ ID NO: 227)
C33W4	HSA N-terminal Fusion; hCD33 Ig-like C2-type domain (Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus	VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSA APTSLGPRTTHSSVLIITPRPQDHGTNLTCQVKFAGAGVTTERTIQL NVTYVPQNPTTGIFPGDGSGKQETRAGVVHDAHKSEVAHRFKDL GEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQ HKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSA KQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLL EKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAK VFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQ VSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETF TFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFA AFVEKCCKADDKETCFAEEGKKLVAASQAALGLHHHHHH (SEQ ID NO: 228)
C33W8	hCD33_Ig-like V-Type domain	DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFR EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRGSHHHHHH (SEQ ID NO: 229)
C33W9	hCD33 Ig-like C2-type domain	VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSA APTSLGPRTTHSSVLIITPRPQDHGTNLTCQVKFAGAGVTTERTIQL NVTYVPQNPTTGIFPGDGSGKQETRAGVVHGSHHHHHH (SEQ ID NO: 230)
C33W10	MuIgV_Hu IgC2 chimera CD33-6xHis_v1	QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLRK GVSLHEDSPVATSDPRQLVQKATQGRFQLLGDPQKHDCSLFIRDA QKNDTGMYFFRVVREPFVRYSYKKSQLSLHVTSLSRTPKILIPGTL EPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITP RPQDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGD GSGKQETRAGVVHGSHHHHHH (SEQ ID NO: 231)

Protein AA ID	Description	Amino Acid Sequence
C33W12	MuIgV_cyno IgC2 chimera CD33-6xHis_v1	QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLRK GVSLHEDSPVATSDPRQLVQKATQGRFQLLGDPQKHDCSLFIRDA QKNDTGMYFFRVVREPFVRYSYKKSQLSLHVTSLSRTPQILIPGAL DPDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIIT PRPQDHGTNLTCQVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGD GSGRKARKQGVVQGSHHHHHH (SEQ ID NO: 232)
C33W49	Human CD33 ECD (D18-H259) from Uniprot P20138 with C-terminal 6xHis tag	DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFR EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE PGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITP RPQDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGD GSGKQETRAGVVHGSHHHHHH (SEQ ID NO: 233)
C33W50	Cyno CD33 ECD (D37-Q274) from XP_005590138.1 with C-terminal 6xHis tag	DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFR EGAIVSLDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRPQILIPGALD PDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIITP RPQDHGTNLTCQVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDG SGKQGVVQGSHHHHHH (SEQ ID NO: 234)
C33W51	Human CD33 ECD (D18-G241) from Uniprot P20138 with 6xHis tag	DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFR EGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDA RRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLE PGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITP RPQDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGHHHH HH (SEQ ID NO: 235)

Example 2. Generation of anti-CD33 antibodies

Antibodies were generated using either the Open Monoclonal Technologies (OMT) or the Ablexis Transgenic mice technologies as follows. Both the AlivaMab and OMT transgenic mice were engineered to produce human/mouse immunoglobulins. AlivaMab or OMT transgenic mice were immunized with recombinant human CD33 protein (a selection of antigens from Table-1). Lymphocytes were extracted from secondary lymphoid organs and either fused with FO mouse myeloma cell line for hybridoma generation or subjected to single cell sorting via FACS. Hybridoma supernatants were screened by MSD electrochemiluminescence for binding to over-expressing human CD33 ECD HEK cells. The samples identified from the screening were further assayed with FACS for binding to over-expressing human CD33 ECD HEK cells (positive signal) compared to parental HEK cells (negative signal). Confirmed cell binders were light chain isotyped using ELISA. Single cell sorting supernatants

were screened by MSD electrochemiluminescence for binding to recombinant human CD33 protein. Several hits with the desired binding profile were selected and sequenced.

V region cloning was performed as follows. Both RNA purified by the Qiagen® RNeasy Plus Mini Kit and B cell lysate were used to perform cDNA synthesis using the Smarter cDNA synthesis kit (Clontech, Mount View, CA). To facilitate cDNA synthesis, oligodT was used to prime reverse transcription of all messenger RNAs, followed by "5' capping" with a Smarter IIA oligonucleotide. Subsequent amplification of the VH and VL fragments was performed using a 2-step PCR amplification using 5' primers targeting the Smarter IIA cap and 3' primers targeting consensus regions in CH1. Briefly, each 50 µl PCR reaction consists of 20 µM of forward and reverse primer mixes, 25 µl of PrimeStar Max DNA polymerase premix (Clontech), 2 µl of unpurified cDNA, and 21 µl of double-distilled H2O. The cycling program starts at 94 °C for 3 min, followed by 35 cycles (94 °C for 30 Sec, 55°C for 1 min, 68 °C for 1 min), and ends at 72 °C for 7 min. A second round of PCR was performed using VL and VH second round primers that contained 15bp complementary extensions that "overlap" respective regions in their respective Lonza mother vector (VH and VL). Second round PCR was performed with the following program: 94 °C for 3 min; 35 cycles (94 °C for 30 Sec, 55°C for 1 min, 68 °C for 1 min), and ends at 72 °C for 7 min. In-Fusion® HD Cloning Kit (Clonetech, U.S.A.) was used for directional cloning of VL gene into Lonza huIgK or Lambda vector and VH gene into Lonza huIgG1 vector. To facilitate In-Fusion® HD Cloning, PCR products were treated with Cloning Enhancer before performing In-Fusion® HD Cloning. The cloning and transformation were performed according to manufacturer's protocol (Clonetech, U.S.A.). Mini-prep DNAs were subjected to Sanger sequencing to confirm that complete V-gene fragments were obtained.

Table 4 shows the amino acid sequences of the heavy and light chains of each of the antibodies generated by the above methods.

Table 4.

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
C33B1474	VH: QVQLVESGGGLVQPGGSLRLSCAASGFTFS**SFGMS**WVRQAPGKGLEWVA **NIKRDGSEKYYVDSVKG**RFTISRDNAKNSLFLQMSSLRAEDTAVYYCVR**D YGYFDF**WGQGTLVTVSS (SEQ ID NO: 52) VL: DIQMTQSPSSLSASVGDRVTITC**QASQAISNYLN**WYQQKPGKAPKLLIY**DA SNLET**GVPSRFSGSGSGTDFTFTISSLQPEDFSTYFC**QHYDNLPYT**FGQGTK LEIK (SEQ ID NO: 81)

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
C33B1522	VH: QVQLQESGPGLVKPSETLSLTCTVSGGSIS**SYYWG**WIRQPPGKGLEWIG**YIY YSGSTNYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**MWEIL GFDP**WGQGTLVTVS (SEQ ID NO: 53) VL: QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSNPVN**WYQQLPGTAPKLLIY**SN NQRPS**GVPDRFSGSKSGTSASLAISGLQSEDEADYFC**AAWDDSLNGPV**FGG GTKLTVL (SEQ ID NO: 82)
C33B1607	VH: EVQLQQSGPELVKPGSSVKISCKASGYTFT**DYNIN**WVKQSHGKNLEWIG**TI NPNNGVTFYNQRFKG**QATLTVDKSSSTAYMELRSLTSEDSAVYYCSR**DG YDTYYAMDY**WGQGTTLTVSS (SEQ ID NO: 54) VL: DIVMTQSHKFMSTSVGDRVSITC**KASQDVRTAEA**WYQQKPGQSPKLLIY**S ASNRYT**GVPDRFTGSGSGTDFTFTISSVQAEDLAVYYC**QQYYTTPRT**FGG GTKLEIK (SEQ ID NO: 83)
C33B1601	VH: EVQLVESGGGLVRPGGSLKLSCAASGFTFS**NYAMS**WVRQTPEKRLEWVA**G ISNSGYSLYYPDTLKG**RFTISRDNARNTLYLQMISLRSEDTAMYYCAR**DG GSYPYAMDY**WGHGTSVTVSS (SEQ ID NO: 55) VL: DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTYVA**WYQQKPGHSPKALIY **SASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLADYFC**QQYKSYPLT**FGA GTKLELK (SEQ ID NO: 84)
C33B1517	VH: QVQLQESGPGLVKPSETLSLTCSVSGASIR**NYYWS**WIRQTAGKGLEWLG**HI YSTGNIHYNPSLKS**RVTMSVDTSNNQFSLKLRSVTAADTAVYYCAR**DNGA ALFDY**WGQGTLVTVSS (SEQ ID NO: 56) VL: QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSNIVN**WYQQFPGTAPKLLIY**SNN QRPS**GVPDRVSGSKSGTSASLAISGLQSEDEADYYC**AAWDDSLNGPV**FGP GTKVTVL (SEQ ID NO: 85)
C33B1495	VH: EVQLVESGGGLVQPGGSLRLSCTASGFTFS**SYWMS**WVRQAPGKGLDWVA **NIKRDGSEKHYVDSVKG**RFTISRDNAKNSLYLQMNSLRAEDSAVYYCTR**D YGYFDY**WGQGTLVTVSS (SEQ ID NO: 57) VL:

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
	DIQLTQSPSFLSASVGDRVTITC**RASQGISSYLA**WYQQKPGKAPKVLIY**AAS TLQS**GVPSRFSGSGSGTEFTLTISSLQPEDFATYFC**QQLNSYPVT**FGGGTKV EIK (SEQ ID NO: 86)
C33B1478	VH: EVQLVESGGGLVQPGGSLKVSCEASGFTFS**VSAIH**WVRQASGKGLEWIG**RI RSKGNSYATAYDVSVKG**RFTISRDDSKNTAYLQMDSLKTEDTAVYYCTR **HNDKWNYYGLDV**WGQGTTVTVSS (SEQ ID NO: 58) VL: DIVMTQSPLSLPVTPGEPASISC**KSSQSLLFSNGYKFLD**WYLQRPGQSPQLL IY**LGSYRAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGLYYC**MQALQTPPT**F GGGTKVEIK (SEQ ID NO: 87)
C33B1477	VH: EVQLVESGGGLVQPGGSLKVSCEASGFTFS**VSAIH**WVRQASGKGLEWIG**RI RSKGNSYATAYAASVKG**RFTISRDDSKNTAYLQMDSLKTEDTAVYYCTR **HNDKWNYYGLDV**WGQGTTVTVSS (SEQ ID NO: 59) VL: DIVMTQSPLSLPVTPGEPASISC**KSSQSLLFSNGYKFLD**WYLQRPGQSPQLL IY**LGSYRAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGLYYC**MQALQTPPT**F GGGTKVEIK (SEQ ID NO: 87)
C33B1476	VH: EVQLVESGGGLVQPGGSLKVSCEASGFTFS**VSAIH**WVRQASGKGLEWIG**RI RSKGNSYATAYNVSVKG**RFTISRDDSKNTAYLQMDSLKTEDTAVYYCTR **HNDKWNYYGLDV**WGQGTTVTVSS (SEQ ID NO: 60) VL: DIVMTQSPLSLPVTPGEPASISC**KSSQSLLFSNGYKFLD**WYLQRPGQSPQLL IY**LGSYRAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGLYYC**MQALQTPPT**F GGGTKVEIK (SEQ ID NO: 87)
C33B1484	VH: EVQLVESGGGLVQPGGSLKVSCEASGFTFS**VFAIH**WVRQASGKGLEWIG**RI RSKGNSYATAYDVSVKG**RFTISRDDSKNTAYLQMDSLKTEDTAVYYCTR **HNDKWNYYGLDV**WGQGTTVTVSS (SEQ ID NO: 61) VL: DIVMTQSPLSLPVTPGEPASISC**KSSQSLLFSNGYKFLD**WYLQRPGQSPQLL IY**LGSYRAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGLYYC**MQALQTPPT**F GQGTKVEIK (SEQ ID NO: 88)

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
C33B933	VH: QVQLVESGGGVVQPGRSLRLSCAASGFTFS**SYGMH**WVRQSPGKGLEWVA **VISYDGSNKYYADSVKG**RFTISRDNSKSTLYLQMNSLRAEDTAVYYCAK**D** **FRSLDWLPPDSTSYDGMDV**WGQGTTVTVSS (SEQ ID NO: 95) VL: SYELTQPPSVSVSPGQTASITCSGHKLGDKYASWYQQKPGQSPVVVIYKDS KRPSGIPERFSGSNFGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTK LTVL (SEQ ID NO: 105)
C33B919	VH: QVQLVESGGGVVQPGRSLRLSCAASGFTFS**SYGMH**WVRQAPGKGLEWVA **VISYDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK**D** **FRSFDWLPPDSTSYYGMDV**WGQGTTVTVSS (SEQ ID NO: 96) VL: SYELTQPPSVSVSPGQTASITCSGDKLGDKYVSWYQQKPGQSPVVVIHQDR KRPSGIPERFSGSNFGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTK LTVL (SEQ ID NO: 106)
C33B931	VH: EVQLVESGGGFVQPGGSLRLSCAASGFTFS**SYWMS**WVRQAPGKGLEWVA **NIKQHGSEKYYVDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR **DRDLGYFDY**WGQGTLVTVSS (SEQ ID NO: 97) VL: SYELTQPPSVSVSPGQTASITC**SGDKLGSKFAS**WYQQKPGQSPVLVIY**QDS** **KRPS**GIPERFSGSNSGNTATLTISGTQAMDEADYYC**QAWDSSTVV**FGGGT KLTVL (SEQ ID NO: 107)
C33B836	VH: QVQLVQSGSELRKPGASVKVSCKASGYTFT**NYAMN**WVRQAPGQGLEWM G**WINTNTGNPTYAQGFTG**RFVFSLDTSVSSAYLQISSLKAEDTAMYYCAT **DRDRGTDY**WGQGTLVTVSS (SEQ ID NO: 170) VL: QSALTQPASVSGSPGQSITISC**TGTSSDVGDYNYVS**WYQQHPGKVPKLMIY **DVSNRPS**GVSNRFSGSMSGNTASLTISGLQAEDEADYYC**SSYSSSSALEV**FG GGTKLTVL (SEQ ID NO: 185)
C33B782	VH: EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYWMS**WVRQAPGKGLEWVA **NIKQHGSEKYYVDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR **DRDLGYFDY**WGQGTLVTVSS (SEQ ID NO: 171)

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
	VL: SYELTQPPSVSVSPGQTASITC**SGNKLGAKFAS**WYQQKPGQSPVLVIY**QDN KRPS**GIPERFSGSNSGNTATLTISGTQAVDEADYYC**QAWDSSTVV**FGGGTK LTVL (SEQ ID NO: 188)
C33B806	VH: QLQLQESGPGLVKPSETLSLTCTVSGGSIS**SSSYYWG**WIRQPPGKGLDWIG**S INYSGSTYYNPSLKS**RVTISVDTSKIQFSLKLRSVTAADTAVYYCAR**LDGY ESPFDY**WGQGTLVTVSS (SEQ ID NO: 175) VL: DIQMTQSPSSVSASVGDRVTITC**RASQGISSWLA**WYQQKPGKAPKLLIY**AA SSLQS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQANSFPFT**FGPGTK VDIK (SEQ ID NO: 192)
C33B904	VH: EVQLVESGGGLVQPGRSLRLSCAASGFTFD**DYAMH**WVRQAPGKGLEWVS **GIGWSGGSIVYADSVKG**RFTISRDNAKNSLYLQMNSLRAEDTALYYCAK**D SPYGDFFDY**WGQGTLVTVSS (SEQ ID NO: 179) VL: DIVMTQSPDSLAVSLGERATINC**KSSQTVFYSSNNKNYLA**WYQQKPGQPP KLLIS**WASTRKS**GVPDRFSGSGSGTDFTLTVSSLQAEDVAVYYC**QHYYSTP YT**FGQGTKLEIK (SEQ ID NO: 196)
C33B937	VH: QVQLVESGGGVVQPGRSLRLSCVASGFTFS**SYGMH**WVRQAPGKGLEWVA **VISYDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEGTAVYYCAK**D FRSFDWLPPDSASYHGMDV**WGQGTTVTVSS (SEQ ID NO: 181) VL: SYELTQPPSVSVSPGQTASITC**SGDKLGDKYAS**WYQQKPGQSPVLVIY**QDG KRPS**GIPERFSGSNFGNKATLTISGTQAMDEADYYC**QAWDRNTVV**FGGGT KLTVL (SEQ ID NO: 200)
C33B125	VH: QVQLVESGGGVVQPGRSLRLSCAASGFTFS**SYGMH**WVRQAPGKGLEWVA **VISYDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK**D FRSFDWLPPDSTSYYGMDV**WGQGTTVTVSS (SEQ ID NO: 96) VL: SYELTQPPSVSVSPGQTASITC**SGDKLGDKYVC**WYQQKPGQSPVVVIH**QD RKRPS**GIPERFSGSNFGNTATLTISGTQAMDEADYYC**QAWDSSTVV**FGGG TKLTVL (SEQ ID NO: 202)

Antibody Name	Amino acid sequence of Heavy and Light Chains (CDRs according to the Kabat system in bold and underlined)
C33B1475	VH: EVQLVESGGGLVQPGGSLRLSCVVSGFTFS**SYWMT**WVRQAPGKGLEWVA **NIKQDGSERYYVDSVKG**RFTISRDSAKNSLYLQMNSLRAEDTAVYYCAR**E** **VGYNWNQGGYFDY**WGQGTLVTVSS (SEQ ID NO: 262) VL: DIVMTQSPLSLPVTPGEPASISC**RSSQSLLHSDGYNYLD**WYLQKSGQSPQLL IY**LGSYRAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGIYYC**MQVLQTPWT**F GQGTKVEIK (SEQ ID NO: 271)
C33B1516	VH: QVQLQESGPGLVKPSETLSLTCSVSGGSIR**NYYWS**WIRQSAGKELEWFG**HI** **FSTGHINYDSSLKS**RVTMSVDTSNNQFSLKLRSVTAADTAVYYCAR**DNGA** **ALFDF**WGQGTLVTVSS (SEQ ID NO: 263) VL: QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSNIVN**WYQQFPGTAPKLLLY**SD** **NQRPS**GVPDRFSGSKSGTSASLAISGLQSEDEADYYC**AAWDDSLNGPV**FG TGTKVTVL (SEQ ID NO: 272)
C33B1481	VH: QVQLVESGGGLVQPGGSLRLSCAASGITFS**NYWMS**WVRQAPGKGLEWVA **SIKRDGSDKYYVDSVKG**RFTISRDNAKNSLSLQMHSLRAEDTAVYYCAK**G** **EFDY**WGQGTLVTVSS (SEQ ID NO: 264) VL: DVVMTQSPLSLPVTLGQPASISC**RSSQSLVYSDGNTYLN**WFQQRPGQSPRR LIY**KVSTRDS**GVPDRFSGSGSGTDFTLKISRVEAEDVAVYYC**LQGTHWPW** **T**FGQGTKVEIK (SEQ ID NO: 273)

Table 4a shows the amino acid sequences of the CDR sequences of the C33B1522 antibody (an antibody having the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4a.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GGSISSYY WG** (SEQ ID NO: 399)	**YIYYSGSTN** (SEQ ID NO: 405)	**MWEILGF DP** (SEQ ID NO: 411)	**SGSSSNIGSN PVN** (SEQ ID NO: 417)	**SNNQRPS** (SEQ ID NO: 423)	**AAWDDSL NGPV** (SEQ ID NO: 429)
Kabat	**SYYWG** (SEQ ID NO: 29)	**YIYYSGSTNY NPSLKS** (SEQ ID NO: 37)	**MWEILGF DP** (SEQ ID NO: 46)	**SGSSSNIGSN PVN** (SEQ ID NO: 63)	**SNNQRPS** (SEQ ID NO: 70)	**AAWDDSL NGPV**

	HC1	HC2	HC3	LC1	LC2	LC3
						(SEQ ID NO: 76)
Chothia	**GGSISSY** (SEQ ID NO: 435)	**YYSGS** (SEQ ID NO: 441)	**MWEILGFD** (SEQ ID NO: 447)	**SSSNIGSNP** (SEQ ID NO: 453)	**SNN** (SEQ ID NO: 459)	**WDDSLNGP** (SEQ ID NO: 465)
IMGT	**GGSISSYY** (SEQ ID NO: 471)	**IYYSGST** (SEQ ID NO: 477)	**ARMWEILGFDP** (SEQ ID NO: 483)	**SSNIGSNP** (SEQ ID NO: 489)	**SNN** (SEQ ID NO: 495)	**AAWDDSLNGPV** (SEQ ID NO: 501)
Contact	**SSYYWG** (SEQ ID NO: 507)	**WIGYIYYSGSTN** (SEQ ID NO: 513)	**ARMWEILGFD** (SEQ ID NO: 519)	**IGSNPVNWY** (SEQ ID NO: 525)	**LLIYSNNQRP** (SEQ ID NO: 531)	**AAWDDSLNGP** (SEQ ID NO: 537)

Table 4b shows the amino acid sequences of the CDR sequences of the C33B1477 antibody (an antibody having the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4b.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GFTFSVSAIH** (SEQ ID NO: 400)	**RIRSKGNSYATA** (SEQ ID NO: 406)	**HNDKWNYYGLDV** (SEQ ID NO: 412)	**KSSQSLLFSNGYKFLD** (SEQ ID NO: 418)	**LGSYRAS** (SEQ ID NO: 424)	**MQALQTPPT** (SEQ ID NO: 430)
Kabat	**VSAIH** (SEQ ID NO: 34)	**RIRSKGNSYATAYAASVKG** (SEQ ID NO: 43)	**HNDKWNYYGLDV** (SEQ ID NO: 51)	**KSSQSLLFSNGYKFLD** (SEQ ID NO: 68)	**LGSYRAS** (SEQ ID NO: 74)	**MQALQTPPT** (SEQ ID NO: 80)
Chothia	**GFTFSVS** (SEQ ID NO: 436)	**RSKGNSYA** (SEQ ID NO: 442)	**HNDKWNYYGLD** (SEQ ID NO: 448)	**SQSLLFSNGYKF** (SEQ ID NO: 454)	**LGS** (SEQ ID NO: 460)	**ALQTPP** (SEQ ID NO: 466)
IMGT	**GFTFSVSA** (SEQ ID NO: 472)	**IRSKGNSYAT** (SEQ ID NO: 478)	**TRHNDKWNYYGLDV** (SEQ ID NO: 484)	**QSLLFSNGYKF** (SEQ ID NO: 490)	**LGS** (SEQ ID NO: 496)	**MQALQTPPT** (SEQ ID NO: 502)
Contact	**SVSAIH** (SEQ ID NO: 508)	**WIGRIRSKGNSYATA** (SEQ ID NO: 514)	**TRHNDKWNYYGLD** (SEQ ID NO: 520)	**LFSNGYKFLDWY** (SEQ ID NO: 526)	**LLIYLGSYRA** (SEQ ID NO: 532)	**MQALQTPP** (SEQ ID NO: 538)

Table 4c shows the amino acid sequences of the CDR sequences of the C33B1475 antibody (an antibody having the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4c.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GFTFSSY WMT** (SEQ ID NO: 401)	**NIKQDGSER Y** (SEQ ID NO: 407)	**EVGYNWN QGGYFDY** (SEQ ID NO: 413)	**RSSQSLLHSD GYNYLD** (SEQ ID NO: 419)	**LGSYRA S** (SEQ ID NO: 425)	**MQVLQTP WT** (SEQ ID NO: 431)
Kabat	**SYWMT** (SEQ ID NO: 253)	**NIKQDGSER YYVDSVKG** (SEQ ID NO: 256)	**EVGYNWN QGGYFDY** (SEQ ID NO: 259)	**RSSQSLLHSD GYNYLD** (SEQ ID NO: 265)	**LGSYRA S** (SEQ ID NO: 74)	**MQVLQTP WT** (SEQ ID NO: 269)
Chothia	**GFTFSSY** (SEQ ID NO: 437)	**KQDGSE** (SEQ ID NO: 443)	**EVGYNWN QGGYFD** (SEQ ID NO: 449)	**SQSLLHSDGY NY** (SEQ ID NO: 455)	**LGS** (SEQ ID NO: 461)	**VLQTPW** (SEQ ID NO: 467)
IMGT	**GFTFSSY W** (SEQ ID NO: 473)	**IKQDGSER** (SEQ ID NO: 479)	**AREVGYN WNQGGYF DY** (SEQ ID NO: 485)	**QSLLHSDGYN Y** (SEQ ID NO: 491)	**LGS** (SEQ ID NO: 497)	**MQVLQTP WT** (SEQ ID NO: 503)
Contact	**SSYWMT** (SEQ ID NO: 509)	**WVANIKQDG SERY** (SEQ ID NO: 515)	**AREVGYN WNQGGYF D** (SEQ ID NO: 521)	**LHSDGYNYLD WY** (SEQ ID NO: 527)	**LLIYLGS YRA** (SEQ ID NO: 533)	**MQVLQTP W** (SEQ ID NO: 539)

Table 4d shows the amino acid sequences of the CDR sequences of the C33B1517 antibody (an antibody having the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4d.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GASIR NYYWS** (SEQ ID NO: 402)	**HIYSTGNIH** (SEQ ID NO: 408)	**DNGAALFD Y** (SEQ ID NO: 414)	**SGSSSNIGSNI VN** (SEQ ID NO: 420)	**SNNQRPS** (SEQ ID NO: 426)	**AAWDDSLN GPV** (SEQ ID NO: 432)
Kabat	**NYYWS** (SEQ ID NO: 32)	**HIYSTGNIHYN PSLKS** (SEQ ID NO: 40)	**DNGAALFD Y** (SEQ ID NO: 49)	**SGSSSNIGSNI VN** (SEQ ID NO: 66)	**SNNQRPS** (SEQ ID NO: 70)	**AAWDDSLN GPV** (SEQ ID NO: 76)
Chothia	**GASIR NY** (SEQ ID NO: 438)	**YSTGN** (SEQ ID NO: 444)	**DNGAALFD** (SEQ ID NO: 450)	**SSSNIGSNI** (SEQ ID NO: 456)	**SNN** (SEQ ID NO: 462)	**WDDSLNGP** (SEQ ID NO: 468)
IMGT	**GASIR NYY** (SEQ ID NO: 474)	**IYSTGNI** (SEQ ID NO: 480)	**ARDNGAAL FDY** (SEQ ID NO: 486)	**SSNIGSNI** (SEQ ID NO: 492)	**SNN** (SEQ ID NO: 498)	**AAWDDSLN GPV** (SEQ ID NO: 504)
Contact	**RNYY WS** (SEQ ID	**WLGHIYSTGN IH** (SEQ ID NO: 516)	**ARDNGAAL FD** (SEQ ID NO: 522)	**IGSNIVNWY** (SEQ ID NO: 528)	**LLIYSNNQ RP** (SEQ ID NO: 534)	**AAWDDSLN GP** (SEQ ID NO: 540)

	HC1	HC2	HC3	LC1	LC2	LC3
	NO: 510)					

Table 4e shows the amino acid sequences of the CDR sequences of the C33B1516 antibody (an antibody having the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4e.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GGSIR NYYWS** (SEQ ID NO: 403)	**HIFSTGHIN** (SEQ ID NO: 409)	**DNGAALFD F** (SEQ ID NO: 415)	**SGSSSNIGSN IVN** (SEQ ID NO: 421)	**SDNQRPS** (SEQ ID NO: 427)	**AAWDDSLN GPV**(SEQ ID NO: 433)
Kabat	**NYYWS** (SEQ ID NO: 254)	**HIFSTGHINYD SSLKS** (SEQ ID NO: 257)	**DNGAALFD F** (SEQ ID NO: 260)	**SGSSSNIGSN IVN** (SEQ ID NO: 66)	**SDNQRPS** (SEQ ID NO: 267)	**AAWDDSLN GPV** (SEQ ID NO: 76)
Chothia	**GGSIR NY** (SEQ ID NO: 439)	**FSTGH** (SEQ ID NO:445)	**DNGAALFD** (SEQ ID NO: 451)	**SSSNIGSNI** (SEQ ID NO: 457)	**SDN** (SEQ ID NO: 463)	**WDDSLNGP** (SEQ ID NO: 469)
IMGT	**GGSIR NYY** (SEQ ID NO: 475)	**IFSTGHI** (SEQ ID NO: 481)	**ARDNGAAL FDF** (SEQ ID NO: 487)	**SSNIGSNI** (SEQ ID NO: 493)	**SDN** (SEQ ID NO: 499)	**AAWDDSLN GPV** (SEQ ID NO: 505)
Contact	**RNYY WS** (SEQ ID NO: 511)	**WFGHIFSTGH IN** (SEQ ID NO: 517)	**ARDNGAAL FD** (SEQ ID NO: 523)	**IGSNIVNWY** (SEQ ID NO: 529)	**LLLYSDN QRP** (SEQ ID NO: 535)	**AAWDDSLN GP** (SEQ ID NO: 541)

Table 4f shows the amino acid sequences of the CDR sequences of the C33B1481 antibody (an antibody having the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273) as defined according to the AbM, Kabat, Chothia, IMGT and Contact systems.

Table 4f.

	HC1	HC2	HC3	LC1	LC2	LC3
AbM	**GITFSN YWMS** (SEQ ID NO: 404)	**SIKRDGSDKY** (SEQ ID NO: 410)	**GEFDY** (SEQ ID NO: 416)	**RSSQSLVYSDG NTYLN**(SEQ ID NO: 422)	**KVSTRDS** (SEQ ID NO: 428)	**LQGTHWP WT** (SEQ ID NO: 434)
Kabat	**NYWMS** (SEQ ID NO: 255)	**SIKRDGSDKYY VDSVKG** (SEQ ID NO: 258)	**GEFDY** (SEQ ID NO: 261)	**RSSQSLVYSDG NTYLN** (SEQ ID NO: 266)	**KVSTRDS** (SEQ ID NO: 268)	**LQGTHWP WT** (SEQ ID NO: 270)

	HC1	HC2	HC3	LC1	LC2	LC3
Chothia	**GITFSN Y** (SEQ ID NO: 440)	**KRDGSD** (SEQ ID NO: 446)	**GEFD** (SEQ ID NO: 452)	**SQSLVYSDGNT Y** (SEQ ID NO: 458)	**KVS** (SEQ ID NO: 464)	**GTHWPW** (SEQ ID NO: 470)
IMGT	**GITFSN YW** (SEQ ID NO: 476)	**IKRDGSDK** (SEQ ID NO: 482)	**AKGEFD Y** (SEQ ID NO: 488)	**QSLVYSDGNT Y** (SEQ ID NO: 494)	**KVS** (SEQ ID NO: 500)	**LQGTHWP WT**(SEQ ID NO: 506)
Contact	**SNYWM S** (SEQ ID NO: 512)	**WVASIKRDGSD KY** (SEQ ID NO: 518)	**AKGEFD** (SEQ ID NO: 524)	**VYSDGNTYLN WF** (SEQ ID NO: 530)	**RLIYKVS TRD** (SEQ ID NO: 536)	**LQGTHWP W** (SEQ ID NO: 542)

Antibodies were generated in llamas as follows. Three llamas were injected subcutaneously with hCD33-IgC2 antigen from Table 3 for three injections (Days 0, 14 and 28), with the first injection in Complete Freund's Adjuvant and subsequent injections in Incomplete Freund's Adjuvant). The first injection was followed by three injections of hCD33-FL ECD antigen from Table 3 (days 61, 76 and 91). PBMCs for single B cell sorting were acquired on days 14, 35 and 86 and frozen. Frozen PBMCs were thawed and stained with recombinant antigen baits, either biotinylated or conjugated with Alexa Fluor 647. The antigen baits were recombinant human CD33-ECD and recombinant human CD33-IgC domain. Biotinylated bait was detected using PE-labelled Streptavidin (SA). Antigen-specific cells (positive for both antigen baits) were bulk sorted directly into RLT lysis buffer (Qiagen) using BD FACSAria Fusion sorter. The cell lysate was frozen at -80 °C and shipped to Genewiz for further processing.

Llama vHH V- region cloning and mono-Fc fusion construct design were performed as follows. B-cells were lysed in RealTime Ready Cell Lysis Buffer (Roche). RNA were then purified with the RNeasy Plus® Mini Kit (Qiagen 74134). Fresh RNA was used directly for cDNA synthesis using the SuperScript® cDNA kit (Invitrogen 18091-050). To facilitate cDNA synthesis, oligodT was used to prime reverse transcription of all messenger RNAs. Subsequent amplification of the VHH fragments was performed using a 2-step PCR amplification using Llama SPs and 3' primers targeting consensus regions in CH2. Briefly, each 50 µl PCR reaction consisted of 20 µM of forward and reverse primer mixes, 25 µl of PrimeStar Max DNA polymerase premix (Clontech), 2 µl of unpurified cDNA, and 21 µl of double-distilled H_2O. The cycling program started at 94 °C for 3 min, followed by 35 cycles (94 °C for 30 Sec, 55°C for 1 min, 68 °C for 1 min), and ended at 72 °C for 7 min.

VHH DNA from first round PCR were gel-purified. Second round VHH PCR was performed with second round primers containing 15bp complementary extensions that "overlap" respective regions in their respective Lonza mother vector. Second round PCR was performed with the following program: 94 °C for 3 min; 35 cycles (94 °C for 30 Sec, 55°C for 1 min, 68 °C for 1 min), and ending at 72 °C for 7 min. An In-Fusion® HD Cloning Kit (Clonetech, U.S.A.) was used for directional cloning of VHH gene

into Lonza vector with mono-Fc. To facilitate In-Fusion® HD Cloning, PCR products were treated with Cloning Enhancer before In-Fusion HD Cloning. Cloning and transformation were performed according to manufacturer's protocol (Clonetech, U.S.A.). Mini-prep DNAs were subjected to Sanger sequencing to confirm that complete V-gene fragments were obtained.

Table 5 shows the amino acid sequences of the heavy chain of the llama antibodies generated by the above method.

Table 5.

Antibody Name	Amino acid sequence of Heavy Chain (CDR sequences underlined in bold)
C33B1785	VH: QVQLVESGGGSVQPGGSLRLSCAVFGFSLS**DYAIG**WFRQAPGKEREKVS**CISESDGRTYLSDSVKS**RFTISRDNGKNTVYLQMNNLKPDDTGVYYCAT**VDQAISESSYHCEKGFGS**WGQGTQVTVSS (SEQ ID NO: 18)
C33B1784	VH: EVQLVESGGGLVQAGGSLRLSCAASGRTFS**TYVMG**WFRQAPGQERELVA**AIVSGRNPTYADSVKS**RFTISRDNAKNTIYLHMNSLQPEDTAVYYCHM**YHYSSQY**WGQGTQVTVSS (SEQ ID NO: 19)
C33B1787	VH: EVQLVESGGGLVQAGGSLRLSCAASGFTFD**DYAIG**WFRQAPGKEREGVS**CISSSDGSTYYVDSVKG**RFTISSDNAKNTVYLQMNSLKPEDTAVYYCTA**LIIMYGSGSERAAAACRYKYEY**WGQGTQVTVSS (SEQ ID NO: 20)
C33B1786	VH: EVQVVESGGGVVQSGGSLTLSCEASESILS**FSTMD**WFRQAPGKEREGVS**CIRPRYSTTTHADSIKG**RFKMYSDNAKNTIYLHMNSLQPEDTAVYYCHM**YHYSSQY**WGQGTQVTVSS (SEQ ID NO: 21)
C33B1781	VH: EVQVVESGGGLVQGGGSLRLSCAASGSIFS**INAMG**WYHQAPGKQRELVA**RITGGGATDYVDSVKG**RFTISLDSAKSTVYLQMNSLKPEDTAVYHCYA**DLVTRVGSDLLYDDY**WGQGTQVTVSS (SEQ ID NO: 22)

Antibody Name	Amino acid sequence of Heavy Chain (CDR sequences underlined in bold)
C33B1780	VH: EVQLVESGGGLVQPGGSLRLSCAVFGFSLS**DYAIG**WFRQAPGKEREKVS**CI SESDGRTYLSDSVKS**RFTISRDNGKNTVYLQMNNLKPDDTGVYYCAT**VDQ AISESSYHCEKGFGS**WGQGTQVTVSS (SEQ ID NO: 23)
C33B1783	VH: EVQLVESGGGLVQAGGSLRLSCAASGFTFD**DYAVG**WFRQVPGKEREGVS**C ISSSDGATYYADSVKG**RFTISSDNAKNTVYLQMNSLKPEDTAVYYCTA**IIV RGSGWERAAAACRYEYSY**WGQGTQVTVSS (SEQ ID NO: 24)
C33B1782	VH: EVQLVESGGGLVQAGGSLRLSCAASGFTFD**DYAVG**WFRQAPGKEREGIS**CI SSSDGATYYADSVKG**RFTISTDNAKNTAFLQMNSLKPEDTAVYYCTA**VIV RGTGWERAAAACRYEYAY**WGQGTQVTVSS (SEQ ID NO: 25)
C33B1789	VH: QVQLVESGGGLVQAGGSLRLSCAVFGFSLS**DYAIG**WFRQAPGKEREKVS**CI SESDGRTYLSDSVKS**RFTISRDNGKNTVYLQMNNLKPDDTGVYYCAT**VDQ AISESSYHCEKGFGS**WGQGTQVTVSS (SEQ ID NO: 26)
C33B1788	VH: QVQLVESGGGLVQPGGSLRLSCAVFGFSLS**DYAIG**WFRQAPGKEREKVS**CI SESDGRTYLSDSVKS**RFTISRDNGKNTVYLQMNNLKPDDTGVYYCAT**VDQ AISESSYHCEKGFGS**WGQGTQVTVSS (SEQ ID NO: 27)

The anti-CD33 antibodies and vHH-monoFc fusion proteins were expressed in ExpiCHO-S™ cells (ThermoFisher Scientific; Waltham, MA, Cat # A29127) by transient transfection with purified plasmid DNA encoding the proteins following the manufacturer's recommendations. Briefly, ExpiCHO-S™ cells were maintained in suspension in ExpiCHO™ expression medium (ThermoFisher Scientific, Cat # A29100) in an orbital shaking incubator set at 37°C, 8% CO_2 and 125 RPM. The cells were passaged and diluted prior to transfection to 6.0 x 10^6 cells per ml, maintaining cell viability at 99.0% or better. Transient transfections were done using the ExpiFectamine™ CHO transfection kit (ThermoFisher Scientific, Cat # A29131). For each ml of diluted cells to be transfected, 0.5 microgram of scFv Fc fusion encoding DNA and 0.5 microgram of pAdVAntage DNA (Promega, Cat# E1711) was used and diluted into OptiPRO™

SFM complexation medium. ExpiFectamine™ CHO reagent was used at a 1:4 ratio (v/v, DNA:reagent) and diluted into OptiPRO™. The diluted DNA and transfection reagent were combined for one minute, allowing DNA/lipid complex formation, and then added to the cells. After overnight incubation, ExpiCHO™ feed and ExpiFectamine™ CHO enhancers were added to the cells as per the manufacturer's Standard protocol. Cells were incubated with orbital shaking (125 rpm) at 37°C for seven days prior to harvesting the culture broth. The culture supernatant from the transiently transfected ExpiCHO-S™ cells was clarified by centrifugation (30 min, 3000rcf) followed by filtration (0.2µm PES membrane, Corning; Corning, NY).

Protein Purification was performed as follows. The filtered cell culture supernatant was loaded onto a pre-equilibrated (1xDPBS, pH 7.2) MabSelect Sure Protein A column (GE Healthcare) using an AKTAXpress chromatography system. After loading, the column was washed with 10 column volumes of 1xDPBS, pH7.2. The protein was eluted with 10 column volumes of 0.1 M sodium (Na)-Acetate, pH 3.5. Protein fractions were neutralized immediately by the addition of 2.5 M Tris HCl, pH 7.5 to 20% (v/v) of the elution fraction volume. Peak fractions were pooled and filtered (0.2 µm). The quality of the purified protein was assessed by analytical size exclusion HPLC (Agilent HPLC system).

Example 3. Characterization of anti-CD33 antibodies and Llama vHHs by Surface Plasmon Resonance (SPR; Biacore)

Affinities (K_D) for the interaction of anti-CD33 antibodies human CD33 (full length ECD and IgC2 domain) were obtained by SPR. The anti-CD33 antibodies were captured using an anti-human Fc antibody and the antigens were injected in solution. The data is shown in Table 6 below.

Table 6.

Protein ID #	binding to hu CD33 (FL ECD) K_D (nM)	binding to hu CD33 (IgC2) K_D (nM)
C33B1474	2.47	3.99
C33B1517	4.44	no binding
C33B919	0.5	no binding
C33B1522	>10	no binding
C33B1607	1.49	no binding
C33B933	0.5	Not measured
C33B125	0.1	>10
C33B931	0.4	Not measured
C33B937	0.3	56
C33B1601	1.1	6.8

The binding affinity of anti-CD33 antibodies and Llama vHHs to the recombinant human CD33 (FL ECD or IgC2 domain) was determined by surface plasmon resonance (SPR) using Biacore 8K instrument. The Fc fused scFvs or mono-Fc fused vHHs were captured using a goat anti-Fc antibody-modified GLC chip and titrated with 5-fold serial dilutions of CD33 antigen spanning concentrations of 40nM-1.6nM. The association and dissociation were monitored for 2 and 30 minutes, respectively, using a flow rate of 50 μL/min. Raw binding data was referenced by subtracting the analyte binding signals from blanks and analyzed using a 1:1 Langmuir binding model using the Biacore software to obtain the kinetics which were used to calculate the binding affinity.

Affinities (K_D) for the interaction of anti-CD33 Llama vHHs with human CD33 (FL ECD or IgC2 domain). The anti-CD33 Llama vHHs on a mono-Fc backbone were captured using an anti-human Fc antibody and the antigens were injected in solution. The data are shown in Table 7 below. Representative Biacore 8K sensorgrams of anti-CD33 vHHs interactions against hu CD33 antigens (FL ECD and IgC2 domain) are also shown in **FIG. 1**.

Table 7.

vHH Protein ID	binding to hu CD33 (FL ECD) **KD (M)**	binding to hu CD33 (IgC2) **KD (M)**
C33B1783	8.59E-11	no/low binding
C33B1782	2.06E-10	no/low binding
C33B1786	3.57E-10	no/low binding
C33B1785	6.61E-10	3.31E-09
C33B1789	6.70E-10	3.03E-09
C33B1788	7.07E-10	4.34E-09
C33B1787	7.92E-10	no/low binding
C33B1780	8.47E-10	3.38E-09
C33B1781	2.21E-09	5.59E-11
C33B1784	low nM binder; low response at this conc	no/low binding

The thermal stability of anti-CD33 scFv-Fc fusion antibodies and Llama VHHs was determined by nanoDSF on the Prometheus® NT.Plex instrument (NanoTemper Technologies). Measurements were made by loading samples into Standard Capillaries (NanoTemper Technologies) from a 384 well sample plate. Duplicate or triplicate runs were performed. Thermal unfolding was monitored in a 1.0°C/minute thermal ramp from 20^0C to 95^0C. Thermal inflection temperatures (midpoint Tms) and onset of aggregation temperature (Tagg) were determined automatically by PR. ThermControl Software (NanoTemper Technologies) and further analyzed using the PR.Stability Analysis Software (NanoTemper Technologies). The data are shown in Table 8 below.

Table 8. Thermal stability data for anti-CD33 scFvs and vHH antibodies (Llama vHHs on mono-Fc backbone) as obtained using a nanoDSF instrument.

vHH Protein ID	Tm1 (^{0}C)	Tm2 (^{0}C)	Tm3 (^{0}C)
C33B1780	50.2	72.1	
C33B1781	52.6	53	
C33B1782	50.3	66.5	
C33B1783	51.4	64.7	
C33B1784	48.4	58.5	
C33B1785	49.9	63	
C33B1786	48.0	63.6	
C33B1787	50.4	59.3	
C33B1788	50.2	63.5	75.7
C33B1789	50.2	63.8	81.5

Table 8a: Thermal stability data for anti-CD33 scFvs and vHHs antibodies as obtained using a nanoDSF instrument. An IgG1 Fc backbone of SEQ ID NO: 277 was used. scFvs were attached directly to this IgG1 backbone without the use of a linker sequence.

scFvs on human IgG1 Fc backbone

scFv-Fc fusion protein ID	scFv clone ID	Protein Description	Parental mAb	Average Tm1—ScFv (^{0}C)	Average Tm2 (^{0}C)	Average Tm3 (^{0}C)	Average Tagg (^{0}C)	Average Onset (^{0}C)
C33B1689	C33B1617	C33B1617_LH_Fc	C33B937	58	67.7	80.6	71.8	53.6
C33B1699	C33B1617	C33B1617_HL_Fc	C33B937	56.4	67.8	80.7	77.9	51.7
C33B1690	C33B1618	C33B1618_LH_Fc	C33B933	63.1		80.7	77.7	52.5
C33B1700	C33B1618	C33B1618_HL_Fc	C33B933	63.7		80.5	79.6	52.2
C33B1691	C33B1619	C33B1619_LH_Fc	C33B931	61.2	68.3	80.1	78.5	56.6
C33B1701	C33B1619	C33B1619_HL_Fc	C33B931	61.6	68.4	80.6	78.7	56.5
C33B1698	C33B1620	C33B1620_LH_Fc	C33B919	63.5	68.8	81.3	77.6	59.2
C33B1702	C33B1620	C33B1620_HL_Fc	C33B919	64.9		80.4	79.2	55.5
C33B1693	C33B1646	C33B1646_LH_Fc	C33B1607	66.5		81.2	66.2	60.5
C33B1703	C33B1646	C33B1646_HL_Fc	C33B1607	64.7	81		64.1	58.9
C33B1694	C33B1651	C33B1651_LH_Fc	C33B1601	65.2		81.2	63.4	56.9
C33B1704	C33B1651	C33B1651_HL_Fc	C33B1601	64.6	81.6		70.7	56.2
C33B1696	C33B1659	C33B1659_LH_Fc	C33B1517	68.1		81.1	76.3	56.1
C33B1706	C33B1659	C33B1659_HL_Fc	C33B1517	68.1		80.9	79.8	60.7
C33B1697	C33B1680	C33B1680_LH_Fc	C33B1474	56.7	67.1	80.6	69	48.2
C33B1707	C33B1680	C33B1680_HL_Fc	C33B1474	54.6	67.2	80.2	78.7	46.2
C33B1692	C33B1682	C33B1682_LH_Fc	C33B125	65		81	77.7	55.4
C33B1708	C33B1682	C33B1682_HL_Fc	C33B125	63.9		80.2	80.5	58.8

Example 4: Construction and expression of CD33 scFv CARs

CD33 CAR constructs comprising the following scFv sequences are constructed:

QSALTQPASVSGSPGQSITISCTGTSSDVGDYNYVSWYQQHPGKVPKLMIYDVSNRPSGVSNRF SGSMSGNTASLTISGLQAEDEADYYCSSYSSSSALEVFGGGTKLTVLGGSEGKSSGSGSESKST GGSQVQLVQSGSELRKPGASVKVSCKASGYTFTNYAMNWVRQAPGQGLEWMGWINTNTGN PTYAQGFTGRFVFSLDTSVSSAYLQISSLKAEDTAMYYCATDRDRGTDYWGQGTLVTVSS (SEQ ID NO: 203)
SYELTQPPSVSVSPGQTASITCSGNKLGAKFASWYQQKPGQSPVLVIYQDNKRPSGIPERFSGSN SGNTATLTISGTQAVDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSEV QLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDS VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS (SEQ ID NO: 204)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQANSFPFTFGPGTKVDIKGGSEGKSSGSGSESKSTGGSQL QLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLDWIGSINYSGSTYYNPSLKS RVTISVDTSKIQFSLKLRSVTAADTAVYYCARLDGYESPFDYWGQGTLVTVSS (SEQ ID NO: 205)
DIVMTQSPDSLAVSLGERATINCKSSQTVFYSSNNKNYLAWYQQKPGQPPKLLISWASTRKSG VPDRFSGSGSGTDFTLTVSSLQAEDVAVYYCQHYYSTPYTFGQGTKLEIKGGSEGKSSGSGSES KSTGGSEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGIGWSG GSIVYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSPYGDFFDYWGQGTLVTV SS (SEQ ID NO: 206)
SYELTQPPSVSVSPGQTASITCSGHKLGDKYASWYQQKPGQSPVVVIYKDSKRPSGIPERFSGSN FGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQV QLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQSPGKGLEWVAVISYDGSNKYYADSV

KGRFTISRDNSKSTLYLQMNSLRAEDTAVYYCAKDFRSLDWLPPDSTSYDGMDVWGQGTTVT VSS (SEQ ID NO: 207)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYASWYQQKPGQSPVLVIYQDGKRPSGIPERFSGSN FGNKATLTISGTQAMDEADYYCQAWDRNTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQ VQLVESGGGVVQPGRSLRLSCVASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEGTAVYYCAKDFRSFDWLPPDSASYHGMDVWGQGTT VTVSS (SEQ ID NO: 208)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYVSWYQQKPGQSPVVVIHQDRKRPSGIPERFSGS NFGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQ VQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDFRSFDWLPPDSTSYYGMDVWGQGTT VTVSS (SEQ ID NO: 209)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYVCWYQQKPGQSPVVVIHQDRKRPSGIPERFSGS NFGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQ VQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDFRSFDWLPPDSTSYYGMDVWGQGTT VTVSS (SEQ ID NO: 210)
SYELTQPPSVSVSPGQTASITCSGDKLGSKFASWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSN SGNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSEV QLVESGGGFVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDS VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS (SEQ ID NO: 211)
DIQMTQSPSSLSASVGDRVTITCQASQAISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSG SGSGTDFTFTISSLQPEDFSTYFCQHYDNLPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSQV

QLVESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVANIKRDGSEKYYVDSV KGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCVRDYGYFDFWGQGTLVTVSS (SEQ ID NO: 212)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSG SKSGTSASLAISGLQSEDEADYFCAAWDDSLNGPVFGGGTKLTVLGGSEGKSSGSGSESKSTGG SQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWGWIRQPPGKGLEWIGYIYYSGSTNYNPSL KSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARMWEILGFDPWGQGTLVTVS (SEQ ID NO: 213)
DIVMTQSHKFMSTSVGDRVSITCKASQDVRTAEAWYQQKPGQSPKLLIYSASNRYTGVPDRFT GSGSGTDFTFTISSVQAEDLAVYYCQQYYTTPRTFGGGTKLEIKGGSEGKSSGSGSESKSTGGSE VQLQQSGPELVKPGSSVKISCKASGYTFTDYNINWVKQSHGKNLEWIGTINPNNGVTFYNQRF KGQATLTVDKSSSTAYMELRSLTSEDSAVYYCSRDGYDTYYAMDYWGQGTTLTVSS (SEQ ID NO: 214)
DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTYVAWYQQKPGHSPKALIYSASYRYSGVPDRF TGSGSGTDFTLTISNVQSEDLADYFCQQYKSYPLTFGAGTKLELKGGSEGKSSGSGSESKSTGG SEVQLVESGGGLVRPGGSLKLSCAASGFTFSNYAMSWVRQTPEKRLEWVAGISNSGYSLYYPD TLKGRFTISRDNARNTLYLQMISLRSEDTAMYYCARDGGSYPYAMDYWGHGTSVTVSS (SEQ ID NO: 215)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSG SKSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGPGTKVTVLGGSEGKSSGSGSESKSTG GSQVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPS LKSRVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDYWGQGTLVTVSS (SEQ ID NO: 216)
DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKVLIYAASTLQSGVPSRFSGS GSGTEFTLTISSLQPEDFATYFCQQLNSYPVTFGGGTKVEIKGGSEGKSSGSGSESKSTGGSEVQ LVESGGGLVQPGGSLRLSCTASGFTFSSYWMSWVRQAPGKGLDWVANIKRDGSEKHYVDSV KGRFTISRDNAKNSLYLQMNSLRAEDSAVYYCTRDYGYFDYWGQGTLVTVSS (SEQ ID NO: 217)

DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPD RFSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKST GGSEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYAT AYDVSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVT VSS (SEQ ID NO: 218)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPD RFSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKST GGSEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYAT AYAASVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVT VSS (SEQ ID NO: 219)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPD RFSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKST GGSEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYAT AYNVSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVT VSS (SEQ ID NO: 220)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPD RFSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGQGTKVEIKGGSEGKSSGSGSESKST GGSEVQLVESGGGLVQPGGSLKVSCEASGFTFSVFAIHWVRQASGKGLEWIGRIRSKGNSYAT AYDVSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVT VSS (SEQ ID NO: 221)
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKSGQSPQLLIYLGSYRASGVPD RFSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTPWTFGQGTKVEIKGGSEGKSSGSGSESKST GGSEVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSERY

YVDSVKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVT VSS (SEQ ID NO: 274)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSG SKSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVLGGSEGKSSGSGSESKSTG GSQVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSS LKSRVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDFWGQGTLVTVSS (SEQ ID NO: 275)
DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYKVSTRDSGVP DRFSGSGSGTDFTLKISRVEAEDVAVYYCLQGTHWPWTFGQGTKVEIKGGSEGKSSGSGSESK STGGSQVQLVESGGGLVQPGGSLRLSCAASGITFSNYWMSWVRQAPGKGLEWVASIKRDGSD KYYVDSVKGRFTISRDNAKNSLSLQMHSLRAEDTAVYYCAKGEFDYWGQGTLVTVSS (SEQ ID NO: 276)

Dynabeads Human T-Expander CD3/CD28 stimulated T cells are subjected to electroporation, then washed three times with OPTI-MEM (Invitrogen), and resuspended in OPTI-MEM at the final concentration of 50E6/ml. Subsequently, 0.1 ml of the cells (5E6) are mixed with 10ug of IVT CAR encoding RNA and electroporated in a 2-mm Gap cuvette (Harvard Apparatus BTX, Holliston, MA, USA) using BTX ECM830 (Harvard Apparatus BTX, Holliston, MA, USA) by pressing the "pulse" button one time. (Settings: 500 Volts, 750 μsec Pulse Length and single(1) pulse, 100mSec interval.) Post electroporation, the T cells are transferred to a 6-well plate and immediately put back into a 37°C incubator. Primary human T cells are electroporated with no mRNA (MOCK) or 10μg of mRNA expressing either the CD33 scFv CAR or irrelevant control CAR. 24 hours post-electroporation CAR surface expression is measured by flow cytometry following staining with 2 μg/ml biotinylated L-protein and streptavidin-conjugated PE, or biotinylated CD33 (1 μg/ml) and streptavidin-conjugated PE.

Twenty-four hours post electroporation, the T cells are counted. 1E5 T cells are collected for each. The cells are washed with FACS buffer twice using 200 μL/well of FACS buffer for microtiter plates, with the supernatant discarded. All wells are stained with 100 μl staining buffer containing Protein L (Genscript, Cat. No. M000971:500;2ug/ml), and incubated for at least 30 minutes at 4°C while being protected from light. The cells are washed by adding FACS Buffer twice, using 150 μL/well for microtiter plates with FACS buffer. Centrifugation at 400x g is performed for 4 minutes at room temperature. The supernatant is then discarded. All wells are stained with 100 μl Streptavidin-R-

Phycoerythrin (SA-PE;1:250) and Live/dead Fixable Near-IR Dead Cell Stain dye (1:1000), incubated for at least 30 minutes at 4°C while being protected from light. The cells are then ready for flow cytometry analysis.

Observation of protein L staining is observed for the CD33 CARs, whereas only the background staining (~5.5%) is seen in the control T cells that were T cells without mRNA electroporation. CAR expression on primary human T cells also can be detected via J&J internal biotin-labeled recombinant KLK2 protein followed by SA-PE. As shown, T cells efficiently express CD33 CARs as measured by flow cytometry, whereas only the background staining is seen in the control T cells that are T cells without mRNA electroporation or undisclosed control CAR (non-CD33 specific).

Example 5: Tumor cell killing by CD33 CAR-T cells

Co-culture for CellTrace Violet (CTV, Thermo Fisher Scientific Catalog number: C34557) based cytotoxicity assay using flow cytometer is performed as follows.

The T cells are prepared as follows. Twenty-four hours post EP, T cells are counted and resuspended at the concentration needed for the most concentrated/desired E:T. The T cells are added at 100 µl/well of assay ($2x10^6$ cells/ml; plated 100 µl in a 10:1 E:T ratio, i.e., 2E5 T cells per 2E4 target cells). A stock of the 10:1 E:T concentration is made, with two-fold serial dilutions made with complete T cell media (Optimizer w/CTS, 5% Human Serum, 1% GlutaMax) to 0.3125:1. The T cells are plated (100ul/well) in triplicate using a 96 well round bottom tissue culture treated plate.

CTV labeled target cells are prepared as follows. 20 µL DMSO is added to a vial of CTV staining solution. This stock solution is diluted into 20 mL of PBS (warmed to 37°C) for a 5 µM staining solution. 10E6 tumor cells are collected, washed with PBS twice and resuspended in 4E6/ml (2.5ml). An equal volume (2.5ml) of CTV staining solution is added. The cells are incubated for 20 minutes in a 37°C incubator. 40 ml PRMI+20% FBS are added to the cells to absorb any unbound dye. The cells are incubated for 5 minutes. The cells re centrifuged for 5 minutes at 400 x g. The cell pellet is resuspended in pre-warmed RPMI+10%FBS medium. In the meantime, T cells are seeded at the desired E/T ratio described above. A CD33+ tumor cell lines and a CD33- tumor cell line are recounted, and then the cells are resuspended in 2E5/ml and 100ul in duplicate. The cells are co-incubated with labelled tumor cell lines in a flat-bottom 96-well plate.

A cytotoxicity assay is performed as follows using a flow cytometer. After 20 hours of co-culture, all of the cells are transferred to a U-bottom 96-well plate and washed. After 20 hours of co-culture all of the cells are collected from a flat-bottom 96-well plate and transferred to a U-bottom 96-well plate, and then washed. 30 µl of 0.25% trypsin is added to all the wells and incubated for 5 minutes in a 37°C incubator. After 5 minutes, all of the tumor cells are collected to a U-bottom 96-well plate. The

cells are centrifuged and washed for 5 minutes at 400 x g twice. The cell pellet is then resuspended in diluted (1:1000) LIVE/DEAD™ Fixable Near-IR staining dye (100 µl). The cells are incubated for 30 mins at 4 °C, and washed with FACS buffer twice by centrifuging the cells for 5 minutes at 400 x g. After washing, all of the cells are fixed for 10 minutes using 100 µl of BD Cytofix™ Fixation Buffer (50ul FACS buffer +50ulFixation Buffer). The cells are centrifuged and washed for 5 minutes at 400 x g once. The cell pellet is resuspended in FACS buffer. Stained samples are analyzed by multicolor flow cytometry after the end of the incubation period. The percentage of cytotoxic activity is calculated using the following equation:

% specific death = % Near IR+CTV+(dead) cells - % spontaneous Near IR +CTV+ / (100% - % spontaneous Near IR +CTV+ (dead) cells) x 100%.

Twenty-four hours after transient transfection, target cells (CD33 positive Vcap and CD33 negative DU145 cells) are labeled with Cell Trace Violet (CTV) fluorescent dye and then co-cultured with CD33 CAR-T cells. Mock T cells serve as negative effector controls. Cells are co-cultured for 20 hours at the effector-to-target cell (E/T) ratios ranging from 0.3125:1 to 10:1. The percent killing is measured as the ratio of the absolute number of live (viability dye negative) target (CTV positive) cells remaining in the co-culture relative to the number of live target cells cultured without CAR-T cells. As shown, CD33 CAR T cells specifically and efficiently lyse the CD33(+) human cancer cell lines, but not CD33(-) cells at E/T ratios of 10:1 to 0.3125:1, whereas only the background cytotoxicity is seen in the T cells that were Mock or CD33 CAR.

CD33 CAR-T cells are also tested for real-time cytotoxicity using xCELLigence as a real-time cell analysis system as a potency assay for immune cell-mediated cytolysis of target cells.

50 µL of target cancer cell culturing media is added to each well of the 96-well E-Plates (ACEA Biosciences), and the background impedance is measured and displayed as a Cell Index. Then, adherent target cells CD33(+) and CD33(-) are dissociated and seeded at a density of 5E4 (VCap), 5E3 cells/well of the E-Plate in a volume of 100 µL, and allowed to passively adhere on the electrode surface. Post seeding, the E-Plate is kept at ambient temperature inside a laminar flow hood for 30 minutes and then transferred to the RTCA MP instrument inside a cell culture incubator. Data recording is initiated immediately at 15-minute intervals for the entire duration (96 hours) of the experiment.

At the time treatment is applied (24 hours post cancer cells seeding), data acquisition is paused, 50 µL of media is removed from each well, and effector CAR-T cells are added at different effector to target (E:T) ratios in a volume of 50 µL. CD33(+) CAR-T and undisclosed control CAR (non-CD33 specific) T cells are resuspended. Two-fold dilutions are then performed in duplicate in a 96-well plate (from 5:1 to 0.156:1 E/T ratio). Target plus Mock effector controls (no RNA electroporation T cells) are also added to the target cells.

Target cells CD33(+) and CD33(-) are incubated with Mock, 10μg mRNA electroporated (24 hours post transfection) into CD33(+) and CD33(-) CAR-T cells at different E/T ratios for approximately 72 hours. Normalized cell index (CI) plots for CD33(+) and CD33(-) are generated. When seeded alone, target cells adhered to the plate and proliferated, increasing the CI readout.

Example 6: Cytokine production by CD33 CAR-T cells

IFN-γ produced by cytotoxic T cells allows for exertion of immune surveillance of tumors, which can directly inhibit proliferation and induce apoptosis of some malignancies *in vivo* and *in vitro.* To determine whether CD33 CAR-T cells are able to recognize and be activated by CD33 (+) tumor cells, the supernatant was collected from xCELLigence-based killing assay, as described in Example 5. After about 70 hours co-culture, the supernatant was collected and assayed by ELISA according to the directions provided with the ELISA kit (Human IFN-γ ELISA MAX™ Deluxe, BioLegend, Cat#:430106).

IFN-γ production of antigen-stimulated CAR-T cells occurs. CD33 CAR and control CAR-T cells secreted IFN-γ during co-culture with CD33-expressing cells in an E:T ratio-dependent manner, but not during co-culture with CD33-negative cells. Undisclosed control CAR secreted much higher amount of IFN- γ due to the much higher antigen expression level than CD33.

Example 7: Tumor cell killing by CD33 CAR-T cells

CD33 CAR-T cells are evaluated in the real-time IncuCyte tumor killing assay for antigen-dependent cytotoxicity. CD33 CAR-T cells are co-incubated with target cells for 88 hours at effector:target ratio of 1:1 or 0.5:1 which was calculated based on CAR expression data. Target cells are identified that are stably expressing a red nuclear dye as measured by IncuCyte imaging system in a real-time fashion. The following calculation is performed: Tumor cell growth inhibition (%) = (Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%).

Example 8: Cytokine production by CD33 CAR-T cells

Supernatant is collected from overnight (approximately 20 hours) co-culture of CD33 CAR-T cells with cells at 1:1 of E/T ratio and was analyzed using 13-plex Milliplex Human High Sensitivity T cell kit (HSTCMAG28SPMX13). CD33 CAR modified T cells secreted cytokines during co-culture with CD33-expressing cells, but minimal for un-transduced T cells (UTD).

Supernatant was collected from overnight (approximately 20 hours) co-culture of CD33 CAR-T cells with cells at 1:1 of E/T ratio. CD33 CAR-T cells secreted IFN-γ during co-culture with CD33-expressing cells, but not during co-culture with CD33-negative cells. CD3/28 beads stimulated T

cells and T cells only were used as positive and negative controls, respectively. IFN-γ release by CD33 CAR-T cells. Mean IFN-γ concentration ± SD (pg/ml) from duplicate cultures is shown. Different thermally stabilized CAR-T cells produced different amount of IFN- γ when co-culture with CD33 (+) cells.

Example 9: Proliferation of CD33 CAR-T cells

CD33 CAR-T cells are evaluated in a proliferation assay. T-cell proliferation is an important *in vitro* parameter of *in vivo* immune function. To further evaluate the function of CD33 CAR-T cells, the CD33 CAR -T cells are labeled with CTV to assess T cells proliferation.

CD33 CAR-T and un-transduced (UTD) T cells are labelled with CellTrace Violet (CTV; 5μM) and co-cultured with CD33 (+) and CD33 (-) cells. Five days post co-culture, cells are harvested and stained with CD3, CD25, NearIR live/dead Dye and CD33 CAR. Flow cytometric analysis is performed on a Fortessa flow cytometer with Flowjo software. Lymphocytes are identified by live CD3, and the frequencies of CAR-T cells with CTV dye dilution and activation marker CD25 are determined. By gating on CD33 CAR+ T cells, as shown, the CD33 (+) cells but not CD33 (-) cells promote the all CAR constructs. CD3/28 beads stimulated T cells and T cells only are used as positive and negative controls, respectively. T cells only without any stimulation do not proliferate and CD3/28 beads stimulated T cells displayed equivalent proliferation pattern. CD33 CAR-T cells proliferated more robustly than CD3/28 beads positive control after 5 days of co-culture with cells. Different CAR constructs engineered T cells have different proliferation activity and displayed different CAR-T cells counts. The CAR-T cells counts are based on mean absolute cell count +/- SEM from three technical replicates.

The protocol is performed as follows. The tumor cells are collected, washed twice with PBS, and resuspended in 10E6/ml in PBS containing 100ug/ml Mitomycin C (MMC) for 1.5 hours in a 37°C incubator so as to block tumor cells proliferation. 20 μL of DMSO is added to a vial of CTV staining solution. 5 μl of the solution is diluted into 5mL (1:1000) PBS (warmed to 37°C) to provide a 5 μM staining solution. The 2E6 T cells are counted, collected, washed with PBS twice, and resuspended in 4E6/ml (0.5ml). An equal volume (0.5 ml) of CTV staining solution is added. The cells are incubated for 20 minutes at 37°C. Then, 4 ml PRMI+20% FBS is added to the cells to absorb any unbound dye. The cells are incubated for 5 minutes, and centrifuged for 5 minutes at 400 x g. The cell pellet is resuspended in pre-warmed RPMI+10%FBS medium. The T cells are counted, and 1E5 cells (100ul) are seeded in 96-wells flat bottom-plate.

In the meantime, MMC-treated tumor cells are collected and counted after 1.5 hours, and then resuspended at 1E6/ml. 1E5 of the cells (100 μl) are cocultured with T cells in a 96-well plate. T cells

alone, and T cells added 3:1 CD3/28 beads to cells ratio, are used as negative and positive controls, respectively.

After 5 days of co-culture, all of the cells are collected from each well. The cells are centrifuged and washed for 5 minutes at 400 x g twice, then stained CD33 CAR, CD3, CD8 and CD25, live/dead (Near-IR) in 96-well U bottom plate. After washing, all cells are fixed for 10 minutes using 100 µl BD Cytofix™ Fixation Buffer (50ul FACS buffer +50ulFixation Buffer). The stained samples are analyzed by multicolor flow cytometry after the end of the incubation period.

Data analysis is performed as follows. A CTV histogram is prepared. The CTV undiluted gate is set to encompass the far-right peak (CTV bright) of T cells cultured alone, and the CTV diluted gate to capture the rest of the population. This is applied to all samples.

Example 10: Characterization of CAR-T cells transduced with CAR17, CAR18, CAR19 or CAR20.

The generated CAR-T cells are evaluated in the JNL reporter assay for antigen-dependent activity. Briefly, Jurkat cells containing the luciferase gene driven by the signaling-responsive NFAT promoter (termed JNL cells) were transduced with CAR17 (KL2B413_HL), CAR18 (KL2B413_LH), CAR19 (KL2B359_HL) or CAR20 (KL2B359_LH) constructs. Expression of each CAR is determined by biotinylated CD33 followed by streptavidin-conjugated PE.

Binding between the CD33 CAR construct and its cognate cellular antigen (CD33 on target cells) leads to luciferase expression in the JNL cells. To that end, JNL cells transduced with the test CAR constructs or untransduced JNL cells (UTD) were co-cultured with target tumor cell lines and luciferase activity was measured as luminescence intensity. Constructs were considered active when the luminescence intensity exceeded 1.5-fold the level of UTD cells in the presence of antigen-expressing cells. No antigen-dependent activation was found for the tested CAR constructs.

CAR-T cells mediate tumor cell killing in antigen-dependent manner

CAR-T cells are transduced with CAR17, CAR18, CAR19 and CAR20 are co-incubated with CD33 positive cells and CD33 negative cells for 96 hours at effector-to-target (ET) ratio of 1:1 or 0.5:1 which was calculated based on CAR expression on T cells. Target cells were stably expressing a red nuclear dye, which was measured by IncuCyte imaging system in a real-time fashion. Tumor cell growth inhibition (TGI) (%) = (Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%). Tested CAR-T cells achieved approximately 100% TGI whereas the untransduced control did not demonstrate any TGI. No TGI was observed with the tested CAR-T cells in CD33 negative cells.

CAR-T cells produce cytokines upon antigen stimulation

IFN-γ produced by cytotoxic T cells is critical for exerting immune surveillance of tumors, which can directly inhibit proliferation and induce apoptosis of some malignancies *in vivo* and *in vitro.* To determine whether CD33 CAR-modified human T cells were able to recognize and become activated by CD33 positive tumor cells, primary T cells transduced with indicated CAR clones and control untransduced T cells (UTD) were co-cultured with target cells lines and supernatant were collected for IFN-γ concentration measurement. CAR-T cells transduced with CD33 CARs cells secreted IFN-γ during co-culture with LNCaP cells recombinantly express CD33 cells and also during co-culture with very low CD33-expressing cells but not CD33-negative cells.

CAR-T cells are activated and upregulate markers of degranulation in antigen-dependent manner

Tumor cells can be recognized and killed by cytotoxic lymphocytes, such as CD8+ T lymphocytes and natural killer (NK) cells mainly through the immune secretion of lytic granules that kill the target tumor cells. This process involves the fusion of the granule membrane with the cytoplasmic membrane of the immune effector cell, resulting in surface exposure of lysosomal-associated proteins that are typically present on the lipid bilayer surrounding lytic granules, such as CD107a. Therefore, membrane expression of CD107a constitutes a marker of immune cell activation and cytotoxic degranulation.

The degranulation assay is performed as described below. Target cells (5×10^4) were co-cultured with an equal number of effector cells in 0.1ml per well in a 96-well plate. Control wells contained T cells alone. Anti-CD107a (5μl per well) are added in addition to 1μl/sample of monensin (BD Biosciences) and incubated for 4 hours at 37°C. Cells are washed two times with PBS, stained for expression of the CD33 CAR, CD3, and CD8 and analyzed on a flow cytometer BD Fortessa.

CD33 CAR-T cells proliferate in antigen-dependent manner

CAR-T cells are evaluated for their proliferation using T-cell proliferation assay protocol described in Example 9. CD33 CAR-T and untransduced (UTD) T cells were labelled with CellTrace Violet(CTV; 5μM) and co-cultured with CD33 positive and CD33 negative cells. Five days post co-culture, cells were harvested and stained with CD3, CD25, NearIR live/dead Dye and CD33 CAR. Flow cytometric analysis was performed on a Fortessa flow cytometer with Flowjo software. Lymphocytes were identified by live CD3, and the frequencies of CAR-T cells with CTV dye dilution and activation marker CD25 were determined. By gating on CD3+ T cells, the CD33 positive cells but not CD33 negative cells promoted proliferation of each tested CAR-T cell line. T cells only without any stimulation did not proliferate and CD3/28 beads stimulated T cells displayed equivalent proliferation pattern. CD33 CAR+ T cells proliferated more robustly than CD3/28 beads positive control after 5 days of coculture with cells. Different tested CAR-T cells had different proliferation activity and displayed different CAR-

T cells counts. The percentage of proliferating T cells and CD25 expressing T cells was based on mean absolute cell count +/- SEM from duplicate.

Example 11: Generation of bispecific CD33xCD3 antibodies

The VH/VL regions of the anti-CD33 antibodies generated in the Examples above and the VH/VL regions of the anti-CD3 antibodies CD3B219 and CD3B376 are engineered into bispecific format and expressed as IgG1. CD3B219 and CD3B376 are engineered as Fabs and the CD33 VH/VL regions are engineered as scFvs in both orientation into the bispecific antibodies, yielding CD33 biding arm in a format scFv-hinge-CH2-CH3 and a CD3 binding arm in a format of heavy chain: VH-CH1-linker-CH2-CH3 and light chain: VL-CL. Alternatively, the VH/VL regions of the anti-CD3 antibodies are engineered as scFvs and the VH/VL regions of the anti-CD33 antibodies are engineered as Fabs. The linker that is used in the scFv is the linker of **SEQ ID NO: 7**. CD3B219 has been described in U.S. Patent No. 9,850,310.

T350V_L351Y_F405A_Y407V CH3 mutations are engineered into one heavy chain and T350V_T366L_K392L_T394W CH3 mutations are engineered into the other heavy chain. In addition, both CD33 and CD3 binding arms are engineered to contain Fc effector silencing mutations L235A_L235A_D265S.

The engineered chains are expressed and the resulting bispecific antibodies purified using standard methods. The bispecific antibodies are characterized for their binding to CD33 and CD3, and their *in vitro* and *in vivo* cytotoxicity as described herein. **Table 10** shows the amino acid sequences of the anti-CD3 antibodies CD3B219 and CD3B376.

Table 10.

Antibody	Region	Amino acid sequence	SEQ ID NO:
CD3B219	HCDR1	TYAMN	108
	HCDR2	RIRSKYNNYATYYAASVKG	109
	HCDR3	HGNFGNSYVSWFAY	110
	LICDR1	RSSTGAVTTSNYAN	111
	LCDR2	GTNKRAP	112
	LCDR3	ALWYSNLWV	113
	VH (CD3B219VH)	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTY AMNWVRQAPGKGLEWVARIRSKYNNYATY YAASVKGRFTISRDDSKNSLYLQMNSLKTED TAVYYCARHGNFGNSYVSWFAYWGQGTLVT VSS	114
	VL (CD3B219VL)	QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTS NYANWVQQKPGQAPRGLIGGTNKRAPGTPA RFSGSLLGGKAALTLSGVQPEDEAEYYCALW YSNLWVFGGGTKLTVL	115
CD3B376	HCDR1	NNNAAWS	116
	HCDR2	RTYYRSKWLYDYAVSVKS	117
	HCDR3	GYSSSFDY	118
	LICDR1	TGTSSNIGTYKFVS	119
	LCDR2	EVSKRPS	120
	LCDR3	VSYAGSGTLL	121
	VH (CD3H219)	QVQLQQSGPRLVRPSQTLSLTCAISGDSVFNN NAAWSWIRQSPSRGLEWLGRTYYRSKWLYD YAVSVKSRITVNPDTSRNQFTLQLNSVTPEDT ALYYCARGYSSSFDYWGQGTLVTVSS	122
	VL (CD3L150)	QSALTQPASVSGSPGQSITISCTGTSSNIGTYKF VSWYQQHPDKAPKVLLYEVSKRPSGVSSRFS GSKSGNTASLTISGLQAEDQADYHCVSYAGS GTLLFGGGTKLTVL	123

Example 12: Generated bispecific CD33xCD3 antibodies are cytotoxic *in vitro*

T-cell-mediated cytotoxicity assay is used to evaluate the cytotoxicity potential of the generated bispecific antibodies *in vitro,* using live-time lapse imaging on the Incucyte platform. The bispecific antibodies are tested in CD33 positive cell line cells, in the presence of isolated pan human CD3+ T cells from healthy donors at a (Effector:Target) effector:target ratio (E:T ratio) of 3:1. Cell death by apoptosis is monitored by measuring the fluorescence signal from a dye which is stably expressed by target cells. The bispecific antibodies promote a dose-dependent reduction of viable cells with increasing time and hence induce T cell mediated death of the tumor cells. Percent of tumor cell inhibition %= (Number of initial plated tumor cells – Current number of viable tumor cells) / (Number of initial plated tumor cells) *100%

Example 13: Generation and characterization of ADC

Antibodies or antigen binding domains that bind CD33 are to be labeled with various radiometals such as In-111, Zr-89, Lu-177, or Ac-225 and in vivo tumor biodistribution or tumor efficacy is evaluated in established subcutaneous (SC) human prostate models including LNCaP, VCaP or C4-2B in male NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG, The Jackson Laboratory, Bar Harbor, ME) or in Balb-c or SHO Nude mice. Various doses of antibodies or antigen binding domains that bind CD33 or isotype control antibodies are labeled with different amounts of radioactivity (10-1000 nCi) and tumor uptake or efficacy is measured.

Example 14: Testing Anti-CD33 antibodies conjugated with drugs for internalization

The following tests were performed to characterize the degree of internalization of anti-CD33 antibodies and to determine a therapeutic effect of anti-CD33 antibodies conjugated to drugs. Without wishing to be bound by theory, internalization of an anti-CD33 antibody is suggestive of increased therapeutic benefit of the anti-CD33 antibody when conjugated to a radioactive isotope, e.g. In-111, Zr-89, Lu-177, or Ac-225.

Materials and Methods

Cell culture was performed as follows. MOLM-13, Kasumi-1, OCI-AML-3 and HDLM2 cells were cultured in RPMI 1640 (Gibco) media with 10 % FBS (HiMedia). Cells were stained with anti-CD33 antibody (clone WM53, Biolegend) at saturating concentration to determine receptor density on each cell line. Receptor density was determined using BD Quantibrite PE beads (BD Biosciences).

MOLM-13, Kasumi-1, OCI-AML-3 and HDLM-2 cells were stained for viability by fixable violet dead cell stain (L34955, ThermoFisher) according to the manufacturer's protocol. Cells were

harvested, washed once with PBS and then stained with the fixable viability dye at room temperature for 30min. Following incubation, cells were washed twice with FACS buffer (PBS+2% FBS). Cells were then used for binding assay with test antibodies. 100,000 cells were stained with the test antibodies in a 100ul staining volume. Antibodies for staining were diluted in FACS buffer and incubated with the cells for 40 mins on ice, followed by washing 2X with FACS buffer. Test antibodies were then detected using the PE conjugated Goat F(ab')2 Anti-Human IgG - Fc (ab98596; Abcam) for 40 minutes on ice. Following washing, data was acquired on the Novocyte flow cytometer (ACEA). Cells were gated on FSC/SSC, followed by live cell gating and doublet discrimination. Data was analyzed using Flow Jo (BD).

Median fluorescence intensity values were used to plot a 4-parameter non-linear regression analysis curve after subtracting the MFI of the secondary only sample.

For kinetics binding studies at 37°C, cells were processed as above for staining with the test antibodies. Test antibodies were conjugated to Alexa Fluor 647 (AF647) dye using the AF647 labeling kit (A20186, ThermoFisher) using the manufacturer's protocol. Post antibody addition, cells were incubated at 37°C in a thermomixer (Eppendorf) for the indicated time points. Samples were then fixed using Cytofix (BD Biosciences) and acquired on the Novocyte (ACEA Biosciences). Data was analyzed as above.

Antibody Internalization assays were performed as follows:

A Protein A-MMAF assay was performed as follows. Protein A-MMAF was purchased from Levena Biopharma. MOLM-13, Kasumi-1, OCI-AML3 and HDLM2 cells were seeded as 4000 cells per well of a 96 well flat bottom dark well plate with transparent bottom (Corning) in a 50ul volume. Antibody stocks were prepared as 4X conc. (400nM) and serially diluted. 25 µl of each antibody dilution was added to the cells. Protein A-MMAF stock solution was also prepared at 4X concentration (400nM), and 25 µl was added to each well. Wells with cells and Protein A-MMAF, but no antibody, were considered as 100% viable cells. Cells were incubated with antibodies and Protein A-MMAF for 96 hours. 100ul of CellTitre-Glo (Promega) was added to the cells at the end of the incubation period and luminescence was read in a Tecan plate reader. The % viability was calculated as below and used to plot a 4-parameter non-linear regression curve using Graph Pad Prism.

$$\% \text{ Viability} = \frac{\text{Cells + MMAF +Ab}}{\text{Cells + MMAF}} \times 100$$

A pHAb dye-based internalization assay was performed as follows. pHAb amine reactive dyes were purchased from Promega. 400ug of each test antibody was conjugated to pHAb dyes. Briefly reconstituted the amine reactive pHAb dye (Promega, #G9845) in 12.5uL DMSO + 12.5uL water (total

volume 25uL) for a final concentration of 10mg/mL (11.3mM). Conjugation reactions were run in a 10 fold molar excess of the dye.

Reaction mix contained antibody (500ug in a 250ul volume+3ul of pHAb dye+30ul of 0.5M sodium borate buffer+ water to make the final reaction volume to 300ul. Reactions were incubated at 37C for 2hrs with agitation followed by quenching by addition of 1M Tris pH 7.5 to a final concentration of 10mM. Dye labeled samples were exchanged into PBS with Zeba columns - 2mL, 40kDa cutoff (Thermo 87768). Antibody concentration and DAR values were calculated as follows:

Measured concentrations by NanoDrop

- pHAb concentration (uM) = (Absorbance / (EC_pHAb * path length)) x 10^6, where:
  - Absorbance = A532
  - EC_pHAb= 75,000
- Ab concentration (uM) = (Absorbance / (EC-Ab * path length)) x 10^6, where:
  - Absorbance = A280-(0.256*A532)
    - 0.256 is correction factor for residual pHAb absorbance at A280
  - EC_Ab= 210,000
- Dye:Ab = conc. pHAb (uM) / conc. Ab (uM)

To analyze the kinetics of pHAb dye labeled CD33 antibodies, MOLM-13, Kasumi-1, OCI-AML-3 and HDLM2 cells were incubated with 100nM of pHAb conjugated test antibody for 45 minutes on ice. Antibody dilutions were prepared in FACS buffer. Post labeling, cells were washed with chilled FACS buffer and incubated at 37˚C for the indicated time points. At the end of the incubation period, cells were acquired on the Novocyte flow cytometer. An unstained sample was included for every time to account for background fluorescence.

Results

Binding was performed by incubating the cells with the serial dilutions of the CD33 antibody at 4˚C followed by detection with PE conjugated Goat F(ab')2 Anti-Human IgG - Fc antibody. The data showing binding of CD33 antibodies to target cell lines is shown in **FIGS. 2A** and **2B** as graphs which show the non-linear regression profiles of anti-human CD33 antibodies at 4˚C. Median fluorescence intensity (MFI) versus log concentration of antibody are plotted. The top hit for each cell line is indicated in red, and Lintuzumab binding is shown in blue curves.

Time and temperature dependent binding of CD33 antibodies to target cell lines was assayed. Binding was performed by incubating the cells with the serial dilutions of the directly conjugated antibodies at 37˚C and 4˚C. The data is shown in **FIGS. 3A** and **3B**. The graphs in these figures show

the non-linear regression profiles of binding of anti-human CD33 antibodies at 37°C and 4°C. Median fluorescence intensity (MFI) versus log concentrations of antibody are plotted.

Internalization of CD33 antibodies on high, medium and low expression target cell lines was assayed. Cells were cultured with serial dilutions of the CD33 antibodies along with Protein A-MMAF for 96hrs. After 96 hours, CellTitre-Glo reagent was added to cells to measure cell viability. Wells with Protein A-MMAF alone were considered as 100% viable. HDLM-2 cells were used as a no expression cell line. The results are shown in **FIGS. 4A-4D**. A decrease in cell viability indicates antibody internalization. Graphs indicate non-linear regression analysis for cell viability against log concentration of antibody.

The kinetics of CD33 antibody internalization were assessed. CD33 antibodies were labeled with pHAb dyes and incubated with the cells at a concentration of 100nM at 37°C for the indicated time points following which the cells were washed and acquired on the flow cytometer. The results are shown in **FIG. 5**, in which the graphs show MFI values at indicated time points.

CD33 expression on target cell lines was assayed. High, medium and low CD33 expression cell lines were identified using anti-human CD33 antibody from Biolegend. MOLM-13, Kasumi-1, OCI-AML-3 and HDLM2 cells were stained with the antibody (orange histograms) or the mouse IgG isotype control (blue histograms) and fold change over isotype for each cell line was calculated. The data are shown in **FIG. 6**.

Antibody integrity and DAR values post conjugation with pHAb dyes were assessed. Antibodies were labeled with pHAb dyes and analyzed on SDS-PAGE. Drug to antibody ratios (DAR) were calculated by measuring the OD at 280 and 530 nm and correcting for the dye absorbance at 280nm. The data are shown in **FIG. 7**.

The specificity of pHAb mediated antibody internalization kinetics was determined. CD33 antibodies were labeled with pHAb dyes and incubated with the Kasumi-1 or OCI-AML-3 cells at a concentration of 100nM at 4°C for the indicated time points following which the cells were washed and acquired on the flow cytometer. The data are shown in **FIG. 8A**. Graphs show MFI values at indicated time points. No internalization was observed at 4 °C. Antibodies were also incubated with HDLM-2 cells at 37°C. The data are shown in **FIG. 8B**. No increase in MFI was observed.

The binding EC_{50} values of CD33 antibodies to high (MOLM-13), medium (Kasumi-1) and low (OCI-AML-3) cell lines were assayed. Binding was carried out at 4°C with serial dilutions of the antibody. The data are shown in **FIG. 9**.

CD33 antibody internalization on various target cell lines was assayed, with the data shown in **FIG. 10**. For MOLM-13 and OCI-AML-3, EC_{50} values are indicated. For Kasumi-1, % viability at 100nM concentration is indicated.

Example 15: Effect of CD33 Antibodies on CD33 Cell Surface Levels

An assay is performed to test the effect of anti-CD33 and CD33 bispecific antibodies on the cell surface level of CD33 on monocytes, macrophages, dendritic cells, neutrophils, T cells, and/or microglia. In the assay, anti-CD33 antibodies are evaluated for their ability to reduce cell surface levels of CD33 on the histiocytic lymphoma cell line U937, as well as CD33-expressing CHO cells, human primary monocytes, human primary macrophages derived from peripheral blood monocytes, human primary dendritic cells derived from peripheral blood monocytes, human microglial cells derived from peripheral blood monocytes, and human primary T cells.

Human microglial cells are prepared from peripheral blood monocytes by cell culture. Monocytes from peripheral human blood samples are isolated using monocyte isolation antibodies, differentiated into dendritic cells, and cultured for five days. Cells are cultured in media containing 10% fetal calf scrum at 37° C in 5% CO_2. Non-adherent cells are collected and used for phagocytosis experiments. To generate human macrophages, monocytes from peripheral human blood samples are isolated and differentiated into macrophages or into dendritic cells.

Cell samples are plated at 200,000 cells per ml in 2 ml of RPMI supplemented with 10% Hyclone FBS, 2 mM glutamine, penicillin, streptomycin, and non-essential amino acids. CD33 antibodies or control isotypes are added at 1.0 μg/ml, and incubated for 24 hours at 37° C. with 5% CO_2.

To assess receptor dynamics, antibodies are allowed to bind cells for one hour, and then are washed out. Surface levels of CD33 are determined at least 24 hours later. Cell surface receptor expression is detected by FACS analysis. Cells are incubated with an anti-CD33 antibody conjugated with FITC and with a control surface marker. The cells are washed 2× in FACS buffer (PBS+2% FBS, 2 mM EDTA) and flow cytometry is performed. For analysis, the data is calculated as a percent of receptor expression in the absence of antibody using WI values for the respective fluorophores.

Example 16: Assay of anti-CD33 antibodies and CD33 bispecific antibodies for induction or inhibition of signaling molecule phosphorylation

Various cells (e.g., J774, RAW 264.7, BMM cells, human primary monocytes, macrophages, dendritic cells, T cells Microglia or osteoclasts) are cultured individually. The cells are removed from culture dishes and counted. The cells are then incubated with (i) an anti-CD33 antibody, (ii) an anti-CD33 bispecific antibody, or (iii) an isotype-matched control antibody. The cells are then lysed and centrifuged to remove any insoluble materials. Immunoprecipitation reactions are performed on each supernatant with one or more different antibodies against each of DAP12, ERK, AKT, protein A-

agarose, and protein G-agarose. The beads are extensively washed with RIPA buffer. The proteins are separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes by Western blotting. The beads are then incubated with antibodies that specifically recognize phosphorylated tyrosine or phosphorylated form of DAP12, ERK, Syk, LCK, FYN, C-Cbl, VAV, or AKT. The beads are visualized with an enhanced chemiluminescence (ECL) system so as to quantify the degree of phosphorylation of DAP12, ERK, Syk, LCK, FYN, C-Cbl, VAV, and AKT.

Example 17: *In vivo* Analysis of the Anti-Cancer Effect of CD33 Antibodies and/or Bispecific Antibodies

Groups of 10 mice at 8 weeks (+/−2 weeks) of age, either regular mice or mice that overexpress the human CD33 gene, are challenged subcutaneously with tumor cells (e.g. 1×10^5 to 1×10^6 MC38, Lewis Lung, or B16 cells) suspended in 1.00 µl PBS. The mice are anesthetized with isoflurane prior to implant. Starting at day 2, groups of mice are injected i.p. every 3 days for 4 doses with 200 µg of each of antagonistic anti-CD33 antibodies. Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm^3 or 60 days. Tumor growth and % survival are the outcome measures. If the anti-CD33 has an anti-cancer effect, one or more of the following is observed: reduced tumor take and growth rate, reduced number of tumor infiltrating immune suppressor macrophages, and increased effector T cell influx into the tumor.

Example 18: Anti-TRGV9 and Anti-CD33 Bispecific Antibodies

γ/δ T cell stimulation and expansion was performed. Expansion of Vγ9-Vδ2 T cells was carried out by treating PBMCs in complete RPMI media containing rhIL-2 (1000 IU/mL), rhIL-15 (10 ng/mL) and Zoledronic acid (5 µM) for 14 days.

De novo sequencing of the anti-Vγ9 monoclonal antibodies was performed as follows. The mouse IgG1 anti-human T cell receptor anti-TRGV9 clone 7A5 was obtained from Abcam (cat # ab171109). Sample preparation and LC-MS/MS analysis were performed by Lake Pharma (San Carlos, CA). The sample was reduced and alkylated, divided into seven aliquots, and proteolytically digested with Trypsin/LysC, Chymotrypsin, LysC, Pepsin, and AspN, Elastase, and Proteinase K enzymes. Resulting peptides were desalted using a ZipTip C18 Pipette Tips and separated on-line using reverse phase chromatography. Mass spectrometry was performed on Thermo Q-Exactive spectrometer using HCD fragmentation. MS data sets were analyzed using PEAKS software by matching *de novo* sequence tags to an IMGT-based antibody sequences database. Gaps in the sequence were assigned using Contig

sequence assembly of *de novo* identified peptides. All CDRs and hyper-mutations were confirmed by inspecting the MS/MS spectra.

The sequences of four monoclonal antibodies generated according to the above, as well as their CDR sequences, are shown in Tables 11-15 below.

Table 11: CDR Sequences of anti-TRGV9 mAb (LP7A5_1)

Antibody	**HCDR1**	**SEQ ID NO:**	**HCDR2**	**SEQ ID NO:**	**HCDR3**	**SEQ ID NO:**
LP7A5_1	DHYIN	236	QIYPGDGNTYYNQKFKG	237	NYGDYTIDF	238
Antibody	**LCDR1**	**SEQ ID NO:**	**LCDR2**	**SEQ ID NO:**	**LCDR3**	**SEQ ID NO:**
LP7A5_1	KSSQSLLYSSNQKNYLA	239	WASTRES	240	QQYYRYHT	241

Table 12: CDR Sequences of anti-TRGV9 mAb.

Antibody	**HCDR1**	**SEQ ID NO:**	**HCDR2**	**SEQ ID NO:**	**HCDR3**	**SEQ ID NO:**
LP7A5_2	DHYIN	236	QIYPGDGNTYYNQKFKG	237	NMGMYTIDF	242
Antibody	**LCDR1**	**SEQ ID NO:**	**LCDR2**	**SEQ ID NO:**	**LCDR3**	**SEQ ID NO:**
LP7A5_2	KSSQSLLYSSNQKNYLA	239	WASTRES	240	QQYYRYHT	241

Table 13: CDR Sequences of anti-TRGV9 mAb.

Antibody	**HCDR1**	**SEQ ID NO:**	**HCDR2**	**SEQ ID NO:**	**HCDR3**	**SEQ ID NO:**
LP7A5_3	DHYIN	236	QIYPGDGNTYYNQKFKG	237	NMGMYTLDF	243
Antibody	**LCDR1**	**SEQ ID NO:**	**LCDR2**	**SEQ ID NO:**	**LCDR3**	**SEQ ID NO:**
LP7A5_3	KSSQSLLYSSNQKNYLA	239	WASTRES	240	QQYYRYHT	241

Table 14: CDR Sequences of anti-TRGV9 mAb.

Antibody	**HCDR1**	**SEQ ID NO:**	**HCDR2**	**SEQ ID NO:**	**HCDR3**	**SEQ ID NO:**
LP7A5_4	DHYIN	236	QIYPGDGNTYYNQKFKG	237	NYGDYTLDF	244
Antibody	**LCDR1**	**SEQ ID NO:**	**LCDR2**	**SEQ ID NO:**	**LCDR3**	**SEQ ID NO:**

Antibody	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:
LP7A5_4	KSSQSLLYSSNQKNYLA	239	WASTRES	240	QQYYRYHT	241

Table 15: Heavy chain and light chain sequences of anti-TRGV9 mAb

mAb	Heavy Chain Amino Acid Sequence (CDR sequences bold and underlined)	SEQ ID NO:
LP7A5_1	EVQLQQSGAELARPGASVKLSCKASGFTFT**DHYIN**WVKQRTGQGLEWIG**QI YPGDGNTYYNQKFKG**KATLTADKSSSTAYMQLSSLTSEDSAVYFCAP**NYG DYTIDF**WGQGTSVTVSS	245
LP7A5_2	EVQLQQSGAELARPGASVKLSCKASGFTFT**DHYIN**WVKQRTGQGLEWIG**QI YPGDGNTYYNQKFKG**KATLTADKSSSTAYMQLSSLTSEDSAVYFCAP**NMG MYTIDF**WGQGTSVTVSS	246
LP7A5_3	EVQLQQSGAELARPGASVKLSCKASGFTFT**DHYIN**WVKQRTGQGLEWIG**QI YPGDGNTYYNQKFKG**KATLTADKSSSTAYMQLSSLTSEDSAVYFCAP**NMG MYTLDF**WGQGTSVTVSS	247
LP7A5_4	EVQLQQSGAELARPGASVKLSCKASGFTFT**DHYIN**WVKQRTGQGLEWIG**QI YPGDGNTYYNQKFKG**KATLTADKSSSTAYMQLSSLTSEDSAVYFCAP**NYG DYTLDF**WGQGTSVTVSS	248
	Light Chain Amino Acid Sequence	**SEQ ID NO:**
LP7A5_1	DIVMSQSPSSLAVSVGEKVTMSC**KSSQSLLYSSNQKNYLA**WYQQKPGQSPK LLIY**WASTRES**GVPDRFTGSGSGTDFTLTISSVKAEDLAVYYC**QQYYRYHT**F GTGTKLEIK	249

Preparation of the Vγ9 x CD33 bispecific antibody was performed as follows. The variable region sequence of 7A5 (anti-TRGV9) and C33B904 (anti-CD33 antibody) was used to generate a bispecific antibody to be tested for T cell re-directed killing of acute myeloid leukemia (AML) cells. The bispecific antibodies VG4 (anti-TRGV9 x CD33) and VG3 (anti-TRGV9 x Null) were produced as full-length antibodies in the knob-into-hole format as human IgG4. Nucleic acid sequences encoding variable regions were sub-cloned into a custom mammalian expression vectors containing constant region of human IgG4 expression cassettes using standard PCR restriction enzyme based standard cloning techniques, and sequenced verified. The bispecific antibodies were expressed by transient transfection in a Chinese hamster ovary (CHO) cell line. The sequences of the bispecific antibodies expressed in the CHO cells are shown in Table 16 below.

Table 16: Sequences of antibodies expressed in CHO cells

mAb ID	'Knob' arm and 'hole' arm amino acid sequence	SEQ ID NO:
ANTI-TRGV9 ('hole' arm)	MAWVWTLLFLMAAAQSIQADIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYS SNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSV KAEDLAVYYCQQYYRYHTFGTGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEG KSSGSGSESKSTGGSEVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWV KQRTGQGLEWIGQIYPGDGNTYYNQKFKGKATLTADKSSSTAYMQLSSLTS EDSAVYFCAPNYGDYTIDFWGQGTSVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQKSLSLSLGK	250
anti-CD33 ('knob' arm)	MAWVWTLLFLMAAAQSIQADIVMTQSPDSLAVSLGERATINCKSSQTVFYS SNNKNYLAWYQQKPGQPPKLLISWASTRKSGVPDRFSGSGSGTDFTLTVSSL QAEDVAVYYCQHYYSTPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTE GKSSGSGSESKSTGGSEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMH WVRQAPGKGLEWVSGIGWSGGSIVYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTALYYCAKDSPYGDFFDYWGQGTLVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL GK	251
Anti-RSV ('knob' arm)	MAWVWTLLFLMAAAQSIQAEIVLTQSPGTLSLSPGERATLSCRASQSVSSSY LAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQDYGFPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEGKSSGSGS ESKSTGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWISWVRQMPGK GLEWMGIIDPSDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYY CARGDGSTDLDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	252

The antibodies were initially purified by Mab Select SuRe Protein A column (GE Healthcare). The column was equilibrated with PBS pH 7.2 and loaded with fermentation supernatant at a flow rate of 2 mL/min. After loading, the column was washed with 4 column volumes of PBS followed by elution in

30 mM sodium acetate, pH 3.5. Fractions containing protein peaks as monitored by absorbance at 280 nm were pooled and neutralized to pH 5.0 by adding 1% 3 M sodium acetate pH 9.0. The bispecific mAbs were further purified on a preparative Superdex 200 10/300 GL (GE healthcare) size exclusion chromatography (SEC) column equilibrated with PBS buffer. The integrity of the sample was assessed by endotoxin measurement and SDS-PAGE under reducing and non-reducing conditions. A representative gel for VG68 is shown in FIG. 12. The final protein concentrations were 1.0 mg/ml for anti-TRGV9/anti-CD33 and 1.0 mg/mL for ANTI-TRGV9/Null. The final EU levels of ANTI-TRGV9/anti-CD33 and ANTI-TRGV9/Null based on these were <3.0 EU/mg.

The binding activity of anti-CD33 antibody on target cell lines was assessed. The binding of anti-CD33 clone C33B904 to a panel CD33+ cell lines were measured by FACS. The EC50 and EC90 were calculated for MOLM-13 (FIG. 13), Kasumi-1 (FIG. 14) and OCI-AML-3 (FIG. 16).

Characterization of Vγ9+ (γδ) T cells and Pan T-cells was performed as follows. Zoledronic acid was used to selectively expand $V\gamma9^+$ γδ T cells from whole PBMCs. The PBMCs were isolated from whole fresh PBMCs using the EasySep™ Human γδ T cell isolation kit (Stem cell Technologies; Vancouver, CA) according to the manufacturer's instructions. Isolated PBMCs were cultured in RPMI-10 (RPMI supplemented with 10%FBS, 1x Pen/Strep) medium with recombinant human IL-2 (rhIL-2) to a final concentration of 1000IU/mL, recombinant human IL-15 (rhIL-15) to a final concentration of 10ng/mL, and Zoledronic acid to a final concentration of 5μM for 14 days.

Evaluation of binding and cytotoxic properties of the anti-TRGV9/anti-CD33 bispecific antibody using Kasumi-3 cells and human γδ T cells was performed as follows. Enriched γδ T cells (Effectors), isolated from PBMCs cultured with Zoledronic acid + IL-2 + IL-15 for 12 days, were co-cultured with CFSE labelled Kasumi-3 cells (Targets) at 1:1 and 5:1 E:T ratios in the presence of various concentrations of the bispecific antibody for 24 hours. Dose response curves show anti-TRGV9/anti-CD33 and anti-TRGV9/anti-NULL bispecific mediated γδ T cell cytotoxicity against CD33 expressing kasumi-3 cells in a dose dependent manner at 1:1 (Fig.6) and 5:1 (Fig.7) E:T ratios. Cytotoxicity values represented here were subtracted of basal cytotoxicity value observed in the absence of bispecific antibody. EC_{50} values were calculated as described in methods. Representative data shown from a single experiment are provided in **FIGS. 16-17**, which show that the anti-TRGV9/anti-CD33 bispecific antibody mediates γδ T cell cytotoxicity against CD33 expressing Kasumi-3 cells *in vitro*.

Healthy donor derived PBMCs (Effectors), cultured with Zoledronic acid + IL-2 + IL-15 for 12 days, were co-cultured with CFSE labelled MOLM-13 cells (Targets) at 1:1 E:T ratios in the presence of various concentrations of the bispecific antibody for 24 hours. **FIG. 18** shows that the anti-TRGV9/anti-CD33 bispecific antibody mediates γδ T cell cytotoxicity (from whole PBMCs) against CD33 expressing

MOLM-13 cells *in vitro*. Dose response curves in **FIG. 18** show anti-TRGV9/anti-CD33 and anti-TRGV9/anti-NULL bispecific mediated γδ T cell cytotoxicity against CD33 expressing kasumi-3 cells in a dose dependent manner. Cytotoxicity values represented there were subtracted of basal cytotoxicity value observed in the absence of bispecific antibody. EC_{50} values were calculated as described in methods. Representative data shown here are from a single experiment.

Example 19: *In silico* analysis and NKK library design

In silico analysis of the VH and VL domains identified herein was performed to evaluate the potential for these VH and VL domains to undergo post-translational modifications (PTMs). An NNK library was generated at certain PTM sites and screened to determine the effects of mutation of amino acids in a potential PTM.

Potential PTM risks were identified using internal sequence analysis tool focused on identifying motifs including NG, DG, and DP. NNK saturation mutagenesis libraries were designed for the first and second positions of each motif. The NNK library utilized codons (N=A, G, C, T; K=G, T) such that all 20 amino acids are represented in the library. Libraries were cloned into an *E.coli* expression plasmid by infusion cloning. Libraries were plated and colonies were isolated to represent mutated PTMs. **FIG. 19** shows *in silico* analysis of PTMs, with PTMs selected for NNK library in boxes.

Example 20: Testing thermostability of scFv variants generated from NNK library

The thermostability of parental and mutated scFv clones generated in Example 19 was evaluated. Cultures of parental clone and mutagenesis libraries were inoculated and grown overnight. Expression was induced and supernatants were analyzed by ELISA. CD33 was plated on 96 well plates and a binding ELISA was performed. Supernatants were split into 4 separate plates for heating. The assay was performed on untreated RT, 55° C, 60° C, 65° C to assess thermal stability. Luminescence signals were normalized to untreated room temperature of the same clone.

FIG. 20 shows the thermostability of scFvs generated from NNK library based on C33B1475 (SEQ ID NO: 277). **FIG. 21** shows the thermostability of scFv generated from NNK library based on C33B1516 (SEQ ID NO: 278). **FIG. 22** shows the thermostability of scFv generated from NNK library based on C33B1517SEQ (SEQ ID NO: 216). **FIG. 23** shows the thermostability of scFv generated from NNK library based on C33B1522 (SEQ ID NO: 213). **FIG. 24** is a table with preferred residues identified during library screen of NNK library.

These figures illustrate that removal of potential PTM sites by mutation did not impair binding to CD33 or reduce thermostability. Unexpected improvements in stability were seen in several scFv clones.

Numbered embodiments

The invention also provides the following numbered embodiments:

List of numbered embodiments (A)

1) An isolated protein that binds CD33, the isolated protein comprising
   a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18;
   b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19;
   c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20;
   d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21;
   e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22;
   f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23;
   g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24;
   h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25;
   i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or
   j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27,
   or a CD33 binding fragment thereof.
2) The isolated protein of embodiment 1, comprising the HCDR1, the HCDR2, the HCDR3 of
   a) SEQ ID NOs: 1, 6, and 12, respectively;
   b) SEQ ID NOs: 2, 7, and 13, respectively;
   c) SEQ ID NOs: 1, 8, and 14, respectively;
   d) SEQ ID NOs: 3, 9, and 13, respectively;
   e) SEQ ID NOs: 4, 10, and 15, respectively;
   f) SEQ ID NOs: 5, 11, and 16, respectively; or
   g) SEQ ID NOs: 5, 11, and 17, respectively.
3) The isolated protein of embodiments 1 or 2, wherein isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.
4) The isolated protein of embodiment 3, wherein the isolated protein is the VHH.
5) The isolated protein of embodiment 3, wherein the isolated protein is the Fab.
6) The isolated protein of embodiment 3, wherein the isolated protein is the scFv.

7) The isolated protein of any of embodiments 1-6, wherein the isolated protein comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

8) An isolated protein that binds CD33, wherein the isolated protein comprises a VHH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27, or a CD33 binding fragment thereof.

9) An isolated protein that binds CD33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61.

10) The isolated protein of embodiment 9, comprising the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively; or

j) SEQ ID NOs: 35, 42, and 51, respectively.

11) The isolated protein of embodiment 9 or 10, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88.

12) The isolated protein of embodiment 11, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

13) The isolated protein of any one of embodiments 9-12, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

14) The isolated protein of embodiment 13, wherein the isolated protein is the Fab.

15) The isolated protein of embodiment 13, wherein the isolated protein is the VHH.

16) The isolated protein of embodiment 13, wherein the isolated protein is the scFv.

17) The isolated protein of embodiment 16, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

18) The isolated protein of embodiment 17, wherein the L1 comprises

a) about 5-50 amino acids;
b) about 5-40 amino acids;
c) about 10-30 amino acids; or
d) about 10-20 amino acids.

19) The isolated protein of embodiment 18, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

20) The isolated protein of embodiment 19, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

21) The isolated protein of any one of embodiments 9-20, wherein the isolated protein comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

22) The isolated protein of embodiment 21, wherein the isolated protein comprises:
a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

23) An isolated protein that binds CD33, wherein the isolated protein comprises
a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

24) The isolated protein of embodiment 23, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

25) The isolated protein of embodiment 24, wherein the isolated protein is the Fab.

26) The isolated protein of embodiment 24, wherein the isolated protein is the VHH.

27) The isolated protein of embodiment 24, wherein the isolated protein is the scFv.

28) The isolated protein of embodiment 27, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

29) The isolated protein of embodiment 28, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

30) The isolated protein of embodiment 29, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

31) The isolated protein of embodiment 30, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

32) An isolated protein that binds CD33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

33) The isolated protein of embodiment 32, comprising the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 89, 90, and 92, respectively;

b) SEQ ID NOs: 89, 90, and 93, respectively;

c) SEQ ID NOs: 33, 91, and 94, respectively

d) SEQ ID NOs: 167, 168, and 169, respectively;

e) SEQ ID NOs: 172, 173, and 174, respectively;

f) SEQ ID NOs: 176, 177, and 178, respectively;

g) SEQ ID NOs: 89, 90, and 180, respectively; or

h) SEQ ID NOs: 89, 90, and 93, respectively.

34) The isolated protein of embodiment 32 or 33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

35) The isolated protein of embodiment 34, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

36) The isolated protein of any one of embodiments 32-35, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

37) The isolated protein of embodiment 36, wherein the isolated protein is the Fab.

38) The isolated protein of embodiment 36, wherein the isolated protein is the VHH.

39) The isolated protein of embodiment 36, wherein the isolated protein is the scFv.

40) The isolated protein of embodiment 39, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

41) The isolated protein of embodiment 40, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

42) The isolated protein of embodiment 41, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

43) The isolated protein of embodiment 42, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

44) The isolated protein of any one of embodiments 32-43, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

45) The isolated protein of embodiment 44, wherein the antigen binding domain that binds CD33 comprises:

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

46) An isolated protein that binds CD33, wherein the isolated protein comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

47) The isolated protein of embodiment 46, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

48) The isolated protein of embodiment 47, wherein the isolated protein is the Fab.

49) The isolated protein of embodiment 47, wherein the isolated protein is the VHH.

50) The isolated protein of embodiment 47, wherein the isolated protein is the scFv.

51) The isolated protein of embodiment 50, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

52) The isolated protein of embodiment 51, wherein the L1 comprises
a) about 5-50 amino acids;
b) about 5-40 amino acids;
c) about 10-30 amino acids; or
d) about 10-20 amino acids.

53) The isolated protein of embodiment 52, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

54) The isolated protein of embodiment 53, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

55) The isolated protein of any one of embodiments 1-54, wherein the protein is conjugated to a half-life extending moiety.

56) The isolated protein of embodiment 55, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol.

57) The isolated protein of any one of embodiments 1-56, wherein the isolated protein is a monospecific protein.

58) The isolated protein of any one of embodiments 1-57, wherein the isolated protein is a multispecific protein.

59) The isolated protein of embodiment 58, wherein the multispecific protein is a bispecific protein.

60) The isolated protein of embodiment 59, wherein the multispecific protein is a trispecific protein.

61) The isolated protein of any one of embodiments 1-60, further comprising an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof.

62) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises a Fc region.

63) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises a CH2 domain.

64) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises a CH3 domain.

65) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.

66) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

67) The isolated protein of embodiment 61, wherein the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain.

68) The isolated protein of any one of embodiments 61-67, comprising an antigen binding domain that binds CD33 that is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

69) The isolated protein of any one of embodiments 61-67, comprising an antigen binding domain that binds CD33 that is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

70) The isolated protein of any one of embodiments 61-69, comprising an antigen binding domain that binds CD33 that is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

71) The isolated protein of embodiment 70, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs:108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

72) The isolated protein of any one of embodiments 58-71, wherein the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte.

73) The isolated protein of embodiment 72, wherein the lymphocyte is a T cell.

74) The isolated protein of embodiment 72, wherein the T cell is a $CD8^+$ T cell

75) The isolated protein of embodiment 72, wherein the lymphocyte is a natural killer (NK) cell.

76) The isolated protein of any one of embodiments 58-75, wherein the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

77) The isolated protein of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds CD3ε.

78) The isolated protein of embodiment 77, wherein the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

79) The isolated protein of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds TRGV9.

80) The isolated protein of embodiment 79, wherein the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining

region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

81) The isolated protein of any one of embodiments 61-80, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype.

82) The isolated protein of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR).

83) The isolated protein of embodiment 82, wherein the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

84) The isolated protein of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR.

85) The isolated protein of embodiment 84, wherein the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

86) The isolated protein of any one of embodiments 82-85, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

87) The isolated protein of any one of embodiments 61-86, wherein the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein.

88) The isolated protein of embodiment 87, wherein the at least one mutation that modulates the half-life of the protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H,

M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

89) The isolated protein of any one of the embodiments 61-88, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region.

90) The isolated protein of embodiment 89, wherein the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

91) The isolated protein of any one of embodiments 1-54, wherein the protein is a chimeric antigen receptor (CAR).

92) The isolated protein of embodiment 91, wherein the CAR comprises

a) an extracellular domain comprising the antigen binding domain that binds CD33 of any one of embodiments 1-54;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

93) The isolated protein of embodiment 91 or 92, wherein the CAR further comprises a CD8a-hinge region.

94) The isolated protein of any one of embodiments 91-93, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

95) The isolated protein of any one of embodiments 91-93, wherein

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163 and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

96) The isolated protein of embodiment 95, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, or any combinations thereof.

97) A pharmaceutical composition comprising the isolated protein of any one of embodiments 1-96 and a pharmaceutically acceptable carrier.

98) A polynucleotide encoding the isolated protein of any one of embodiments 1-96.

99) A vector comprising the polynucleotide of embodiment 98.

100) A host cell comprising the vector of embodiment 99.

101) A method of producing the isolated protein of any one of embodiments 1-96, comprising culturing the host cell of embodiment 100 in conditions that the protein is expressed, and recovering the protein produced by the host cell.

102) An anti-idiotypic antibody binding to the isolated protein of any one of embodiments 1-96.

103) A kit comprising the isolated protein of any one of embodiments 1-96.

104) A pharmaceutical composition comprising a means for treating a CD33 expressing cancer in a subject.

105) A pharmaceutical composition comprising a means for reducing the amount of CD33 expressing tumor cells in a subject.

106) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject.

107) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer.

List of numbered embodiments (B)

1) An isolated multispecific protein comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen.

2) The isolated multispecific protein of embodiment 1, wherein the lymphocyte antigen is a T cell antigen.

3) The isolated multispecific protein of embodiment 1, wherein the T cell antigen is a $CD8^+$ T cell antigen.

4) The isolated multispecific protein of embodiment 1, wherein the lymphocyte antigen is a NK cell antigen.

5) The isolated multispecific protein of any one of embodiments 1-4, wherein the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

6) The isolated multispecific protein of embodiment 5, wherein the lymphocyte antigen is CD3ε.

7) The isolated multispecific protein of any one of embodiments 1-6, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

8) The isolated multispecific protein of embodiment 7, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab.

9) The isolated multispecific protein of embodiment 7, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH.

10) The isolated multispecific protein of embodiment 7, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv.

11) The isolated multispecific protein of embodiment 10, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

12) The isolated multispecific protein of embodiment 11, wherein the L1 comprises
    a) about 5-50 amino acids;
    b) about 5-40 amino acids;
    c) about 10-30 amino acids; or
    d) about 10-20 amino acids.

13) The isolated multispecific protein of embodiment 12, wherein the L1 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

14) The isolated multispecific protein of embodiment 13, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

15) The isolated multispecific of any one of embodiments 1-14, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17.

16) The isolated multispecific protein of any one of 1-15, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;
b) SEQ ID NOs: 2, 7, and 13, respectively;
c) SEQ ID NOs: 1, 8, and 14, respectively;
d) SEQ ID NOs: 3, 9, and 13, respectively;
e) SEQ ID NOs: 4, 10, and 15, respectively;
f) SEQ ID NOs: 5, 11, and 16, respectively; or
g) SEQ ID NOs: 5, 11, and 17, respectively,

or a CD33 binding fragment thereof.

17) The isolated multispecific protein of any one of embodiments 1-16, wherein the first antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

18) The isolated multispecific protein of any one of embodiments 1-14, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, or 35, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, or 44, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, or 51, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, or 68, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, or 74, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, or 80.

19) The isolated multispecific protein of any one of embodiments 1-14 and 18, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

20) The isolated multispecific protein of any one of embodiments 1-14, 18, and 19, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

21) The isolated multispecific protein of any one of embodiments 1-14 and 18-20, wherein the first antigen binding domain that binds CD33 comprises
   a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
   b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
   c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
   d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
   e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
   f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
   g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
   h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
   i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
   j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

22) The isolated multispecific protein of any one of embodiments 1-14, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199.

23) The isolated multispecific protein of any one of embodiments 1-14 and 22, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
   a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
   b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
   c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
   d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
   e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
   f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;
   g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;
   h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or
   i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

24) The isolated multispecific protein of any one of embodiments 1-14, 22 and 23, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202.

25) The isolated multispecific protein of any one of embodiments 1-14 and 22-24, wherein the first antigen binding domain that binds CD33 comprises
   a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
   b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
   c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
   d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
   e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
   f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
   g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
   h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
   i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

26) The isolated multispecific protein of any one of embodiments 1-25, wherein the second antigen binding domain that binds the lymphocyte antigen comprises
   a) the HCDR1 of SEQ ID NO: 141, the HCDR2 of SEQ ID NO: 142, the HCDR3 of SEQ ID NO: 143, the LCDR1 of SEQ ID NO: 144, the LCDR2 of SEQ ID NO: 145 and the LCDR3 of SEQ ID NO: 146;
   b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;
   c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or
   d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

27) The isolated multispecific protein of any one of embodiments 1-25, wherein the second antigen binding domain that binds the lymphocyte antigen comprises
   a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;
   b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;
   c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining

region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

28) The isolated multispecific protein of any one of embodiments 1-27, wherein the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

29) The isolated multispecific protein of embodiment 28, further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

30) The isolated multispecific protein of embodiment 29 wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

31) The isolated multispecific protein of any one of embodiments 27-29, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

32) The isolated multispecific protein of embodiment 28-31, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR.

33) The isolated multispecific protein of embodiment 32, wherein the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/

P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

34) The isolated multispecific protein of any one of embodiments 28-31, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

35) The isolated multispecific protein of embodiment 34, wherein the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

36) The isolated multispecific protein of any one of embodiments 32-35, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

37) The isolated multispecific protein of any one of embodiments 28-36, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

38) The isolated multispecific protein of embodiment 37, wherein the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

39) The isolated multispecific protein of any one of the embodiments 28-38, comprising at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

40) The isolated multispecific protein of embodiment 39, wherein the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A,Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A,

T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

41) The isolated multispecific protein of any one of embodiments 28-40, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

a) L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

b) L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

41) A kit comprising the isolated multispecific protein of any one of embodiments 1-41.

42) A pharmaceutical composition comprising a means for treating a CD33 expressing cancer in a subject.

43) A pharmaceutical composition comprising a means for reducing the amount of CD33 expressing tumor cells in a subject.

44) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject.

45) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer.

List of numbered embodiments (C):

1) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27,

or a CD33 binding fragment thereof.

2) The immunoconjugate of embodiment 1, wherein the isolated protein comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;

b) SEQ ID NOs: 2, 7, and 13, respectively;

c) SEQ ID NOs: 1, 8, and 14, respectively;

d) SEQ ID NOs: 3, 9, and 13, respectively;

e) SEQ ID NOs: 4, 10, and 15, respectively;

f) SEQ ID NOs: 5, 11, and 16, respectively; or

g) SEQ ID NOs: 5, 11, and 17, respectively.

3) The immunoconjugate of embodiments 1 or 2, wherein the antigen binding domain that binds CD33 is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

4) The immunoconjugate of embodiment 3, wherein the antigen binding domain that binds CD33 is the VHH.

5) The immunoconjugate of embodiment 3, wherein the antigen binding domain that binds CD33 is the Fab.

6) The immunoconjugate of embodiment 3, wherein the antigen binding domain that binds CD33 is the scFv.

7) The immunoconjugate of any of embodiments 1-6, wherein the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

8) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, wherein the isolated protein comprises a VHH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

9) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, and wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61.

10) The immunoconjugate of embodiment 9, wherein the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 28, 36, and 45, respectively;

b) SEQ ID NOs: 29, 37, and 46, respectively;

c) SEQ ID NOs: 30, 38, and 47, respectively;

d) SEQ ID NOs: 31, 39, and 48, respectively;

e) SEQ ID NOs: 32, 40, and 49, respectively;

f) SEQ ID NOs: 33, 41, and 50, respectively;

g) SEQ ID NOs: 34, 42, and 51, respectively;

h) SEQ ID NOs: 34, 43, and 51, respectively;

i) SEQ ID NOs: 34, 44, and 51, respectively; or

j) SEQ ID NOs: 35, 42, and 51, respectively.

11) The immunoconjugate of embodiment 9 or 10, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88.

12) The immunoconjugate of embodiment 11, wherein the isolated protein comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

13) The immunoconjugate of any one of embodiments 9-12, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

14) The immunoconjugate of embodiment 13, wherein the isolated protein is the Fab.

15) The immunoconjugate of embodiment 13, wherein the isolated protein is the VHH.

16) The immunoconjugate of embodiment 13, wherein the isolated protein is the scFv.

17) The immunoconjugate of embodiment 16, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

18) The immunoconjugate of embodiment 17, wherein the L1 comprises

a) about 5-50 amino acids;
b) about 5-40 amino acids;
c) about 10-30 amino acids; or
d) about 10-20 amino acids.

19) The immunoconjugate of embodiment 18, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

20) The immunoconjugate of embodiment 19, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

21) The immunoconjugate of any one of embodiments 9-20, wherein the isolated protein comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

22) The immunoconjugate of embodiment 21, wherein the isolated protein comprises:
    a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
    b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
    c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
    d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
    e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
    f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
    g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
    h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
    i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
    j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

23) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, and wherein the isolated protein comprises
    a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
    b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
    c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
    d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
    e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
    f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
    g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
    h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
    i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
    j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

24) The immunoconjugate of embodiment 23, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

25) The immunoconjugate of embodiment 24, wherein the isolated protein is the Fab.

26) The immunoconjugate of embodiment 24, wherein the isolated protein is the VHH.

27) The immunoconjugate of embodiment 24, wherein the isolated protein is the scFv.

28) The immunoconjugate of embodiment 27, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

29) The immunoconjugate of embodiment 28, wherein the L1 comprises
    a) about 5-50 amino acids;
    b) about 5-40 amino acids;
    c) about 10-30 amino acids; or

d) about 10-20 amino acids.

30) The immunoconjugate of embodiment 29, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

31) The immunoconjugate of embodiment 30, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

32) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, and wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

33) The immunoconjugate of embodiment 32, comprising the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 89, 90, and 92, respectively;

b) SEQ ID NOs: 89, 90, and 93, respectively;

c) SEQ ID NOs: 33, 91, and 94, respectively

d) SEQ ID NOs: 167, 168, and 169, respectively;

e) SEQ ID NOs: 172, 173, and 174, respectively;

f) SEQ ID NOs: 176, 177, and 178, respectively;

g) SEQ ID NOs: 89, 90, and 180, respectively; or

h) SEQ ID NOs: 89, 90, and 93, respectively.

34) The immunoconjugate of embodiment 32 or 33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

35) The immunoconjugate of embodiment 34, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

36) The immunoconjugate of any one of embodiments 32-35, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

37) The immunoconjugate of embodiment 36, wherein the isolated protein is the Fab.

38) The immunoconjugate of embodiment 36, wherein the isolated protein is the VHH.

39) The immunoconjugate of embodiment 36, wherein the isolated protein is the scFv.

40) The immunoconjugate of embodiment 39, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

41) The immunoconjugate of embodiment 40, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

42) The immunoconjugate of embodiment 41, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

43) The immunoconjugate of embodiment 42, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

44) The immunoconjugate of any one of embodiments 32-43, wherein the isolated protein comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

45) The immunoconjugate of embodiment 44, wherein the isolated protein comprises:

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

46) An immunoconjugate comprising an isolated protein conjugated to an agent, wherein the isolated protein binds CD33, and wherein the isolated protein comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

47) The immunoconjugate of embodiment 46, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

48) The immunoconjugate of embodiment 47, wherein the isolated protein is the Fab.

49) The immunoconjugate of embodiment 47, wherein the isolated protein is the VHH.

50) The immunoconjugate of embodiment 47, wherein the isolated protein is the scFv.

51) The immunoconjugate of embodiment 50, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

52) The immunoconjugate of embodiment 51, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

53) The immunoconjugate of embodiment 52, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

54) The immunoconjugate of embodiment 53, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

55) The immunoconjugate of any one of embodiments 1-54, wherein the isolated protein is conjugated to a half-life extending moiety.

56) The immunoconjugate of embodiment 55, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol.

57) The immunoconjugate of any one of embodiments 1-56, wherein the isolated protein is a monospecific protein.

58) The immunoconjugate of any one of embodiments 1-57, wherein the isolated protein is a multispecific protein.

59) The immunoconjugate of embodiment 58, wherein the multispecific protein is a bispecific protein.

60) The immunoconjugate of embodiment 59, wherein the multispecific protein is a trispecific protein.

61) The immunoconjugate of any one of embodiments 1-60, further comprising an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof.

62) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises a Fc region.

63) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises a CH2 domain.

64) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises a CH3 domain.

65) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.

66) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

67) The immunoconjugate of embodiment 61, wherein the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain.

68) The immunoconjugate of any one of embodiments 61-67, wherein the isolated protein is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

69) The immunoconjugate of any one of embodiments 61-67, wherein the isolated protein is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

70) The immunoconjugate of any one of embodiments 61-69, wherein the isolated protein is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

71) The immunoconjugate of embodiment 70, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs:108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

72) The immunoconjugate of any one of embodiments 58-71, wherein the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte.

73) The immunoconjugate of embodiment 72, wherein the lymphocyte is a T cell.

74) The immunoconjugate of embodiment 72, wherein the T cell is a $CD8^+$ T cell

75) The immunoconjugate of embodiment 72, wherein the lymphocyte is a natural killer (NK) cell.

76) The immunoconjugate of any one of embodiments 58-75, wherein the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

77) The immunoconjugate of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds CD3ε.

78) The immunoconjugate of embodiment 77, wherein the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

79) The immunoconjugate of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds TRGV9.

80) The immunoconjugate of embodiment 77, wherein the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

81) The immunoconjugate of any one of embodiments 61-80, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype.

82) The immunoconjugate of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR).

83) The immunoconjugate of embodiment 82, wherein the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S,

S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

84) The immunoconjugate of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR.

85) The immunoconjugate of embodiment 84, wherein the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

86) The immunoconjugate of any one of embodiments 80-85, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

87) The immunoconjugate of any one of embodiments 61-86, wherein the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein.

88) The immunoconjugate of embodiment 87, wherein the at least one mutation that modulates the half-life of the protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

89) The immunoconjugate of any one of the embodiments 61-88, wherein the isolated protein comprises at least one mutation in a CH3 domain of the Ig constant region.

90) The immunoconjugate of embodiment 89, wherein the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

91) The immunoconjugate of any one of embodiments 1-54, wherein the isolated protein is a chimeric antigen receptor (CAR).

92) The immunoconjugate of embodiment 91, wherein the CAR comprises

a) an extracellular domain comprising the antigen binding domain that binds CD33 as defined in any one of embodiments 1-54;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

93) The immunoconjugate of embodiment 91 or 92, wherein the CAR further comprises a CD8a-hinge region.

94) The immunoconjugate of any one of embodiments 91-93, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

95) The immunoconjugate of any one of embodiments 91-93, wherein

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163 and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

96) The immunoconjugate of embodiment 95, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, or any combinations thereof.

97) The immunoconjugate of any one of embodiments 1-96, wherein the agent is a therapeutic agent or an imaging agent.

98) A pharmaceutical composition comprising the immunoconjugate of any one of embodiments 1-96 and a pharmaceutically acceptable carrier.

99) A polynucleotide encoding the isolated protein of the immunoconjugate of any one of embodiments 1-96.

100) A vector comprising the polynucleotide of embodiment 99.

101) A host cell comprising the vector of embodiment 100.

102) A method of producing the immunoconjugate of any one of embodiments 1-96, comprising culturing the host cell of embodiment 101 in conditions that the protein is expressed, recovering the

protein produced by the host cell, and conjugating the protein produced by the host cell with the agent.

103) An immunoconjugate comprising an isolated multispecific protein conjugated to an agent, wherein the isolated multispecific protein comprises a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen.

104) The immunoconjugate of embodiment 103, wherein the lymphocyte antigen is a T cell antigen.

105) The immunoconjugate protein of embodiment 103, wherein the T cell antigen is a $CD8^+$ T cell antigen.

106) The immunoconjugate of embodiment 103, wherein the lymphocyte antigen is a NK cell antigen.

107) The immunoconjugate of any one of embodiments 103-106, wherein the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

108) The immunoconjugate of embodiment 107, wherein the lymphocyte antigen is CD3ε.

109) The immunoconjugate of any one of embodiments 103-108, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

110) The immunoconjugate of embodiment 109, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab.

111) The immunoconjugate of embodiment 109, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH.

112) The immunoconjugate of embodiment 110, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv.

113) The immunoconjugate of embodiment 112, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

114) The immunoconjugate of embodiment 113, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

115) The immunoconjugate of embodiment 114, wherein the L1 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

116) The immunoconjugate of embodiment 115, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

117) The immunoconjugate of any one of embodiments 103-116, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17.

118) The immunoconjugate of any one of 103-117, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR1, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;
b) SEQ ID NOs: 2, 7, and 13, respectively;
c) SEQ ID NOs: 1, 8, and 14, respectively;
d) SEQ ID NOs: 3, 9, and 13, respectively;
e) SEQ ID NOs: 4, 10, and 15, respectively;
f) SEQ ID NOs: 5, 11, and 16, respectively; or
g) SEQ ID NOs: 5, 11, and 17, respectively.

119) The immunoconjugate of any one of embodiments 103-118, wherein the first antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

120) The immunoconjugate of any one of embodiments 103-116, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, or 35, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, or 44, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, or 51, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, or 68, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, or 74, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, or 80.

121) The immunoconjugate of any one of embodiments 103-116 and 120, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

122) The immunoconjugate of any one of embodiments 103-116, 120, and 121, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

123) The immunoconjugate of any one of embodiments 103-116 and 120-122, wherein the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;
e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

124) The immunoconjugate of any one of embodiments 103-116, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199.

125) The immunoconjugate of any one of embodiments 103-116 and 124, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;
g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;
h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or
i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

126) The immunoconjugate of any one of embodiments 103-116, 124 and 125, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, or 179 and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202.

127) The immunoconjugate of any one of embodiments 103-116 and 124-126, wherein the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

128) The immunoconjugate of any one of embodiments 103-127, wherein the second antigen binding domain that binds the lymphocyte antigen comprises

a) the HCDR1 of SEQ ID NO: 141, the HCDR2 of SEQ ID NO: 142, the HCDR3 of SEQ ID NO: 143, the LCDR1 of SEQ ID NO: 144, the LCDR2 of SEQ ID NO: 145 and the LCDR3 of SEQ ID NO: 146;
b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;
c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or
d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

129) The immunoconjugate of any one of embodiments 101-128, wherein the second antigen binding domain that binds the lymphocyte antigen comprises

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;
b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

130) The immunoconjugate of any one of embodiments 103-129, wherein the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

131) The immunoconjugate of embodiment 130, further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

132) The immunoconjugate of embodiment 131, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

133) The immunoconjugate of any one of embodiments 130-132, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

134) The immunoconjugate of any one of embodiments 130-133, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR.

135) The immunoconjugate of embodiment 134, wherein the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S,

F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

136) The immunoconjugate of any one of embodiments 130-133, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

137) The immunoconjugate of embodiment 136, wherein the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

138) The immunoconjugate of any one of embodiments 134-137, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

139) The immunoconjugate of any one of embodiments 130-138, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

140) The immunoconjugate of embodiment 139, wherein the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

141) The immunoconjugate of any one of the embodiments 130-140, wherein the protein comprises at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

142) The immunoconjugate of embodiment 141, wherein the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A,Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A,

T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

143) The immunoconjugate of any one of embodiments 130-142, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

a) L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

b) L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

144) The immunoconjugate of any one of embodiments 103-143, wherein the agent is a therapeutic agent or an imaging agent.

145) A pharmaceutical composition comprising the immunoconjugate of any one of embodiments 103-143 and a pharmaceutically acceptable carrier.

146) A polynucleotide encoding the isolated multispecific protein of the immunoconjugate of any one of embodiments 103-143.

147) A vector comprising the polynucleotide of embodiment 146.

148) A host cell comprising the vector of embodiment 147.

149) A method of producing the immunoconjugate of any one of embodiments 103-143, comprising culturing the host cell of embodiment 148 in conditions that the multispecific protein is expressed, recovering the multispecific protein produced by the host cell, and conjugating the multispecific protein to an agent.

150) The immunoconjugate of any one of embodiments 1-96 and 103-143, wherein the agent is a radioactive agent.

151) The immunoconjugate of embodiment 150, wherein the radioactive agent comprises a radiometal ion.

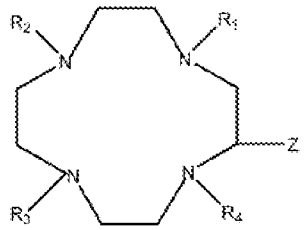
152) The immunoconjugate of embodiment 151, wherein the radiometal ion is ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{225}Ac, ^{255}Fm, ^{227}Th, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, or ^{111}In.

153) The immunoconjugate of embodiment 151, wherein the radiometal ion is ^{225}Ac, ^{111}In or ^{89}Zr.

154) The immunoconjugate of embodiment 151, wherein the radiometal ion is ^{225}Ac.

155) The immunoconjugate of any one of embodiments 151 to 154, wherein the radiometal ion is conjugated to a chelating moiety.

156) The immunoconjugate of embodiment 155, wherein the chelating moiety comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1-C_4 alkyl; and

Z is $(CH2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, Z is hydrogen; and

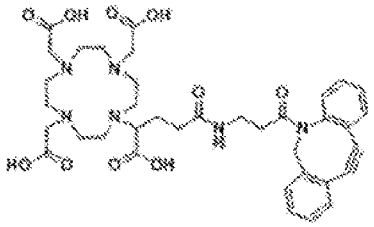
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

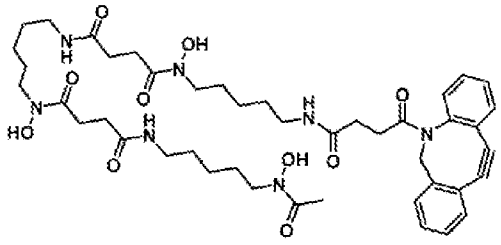
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand.

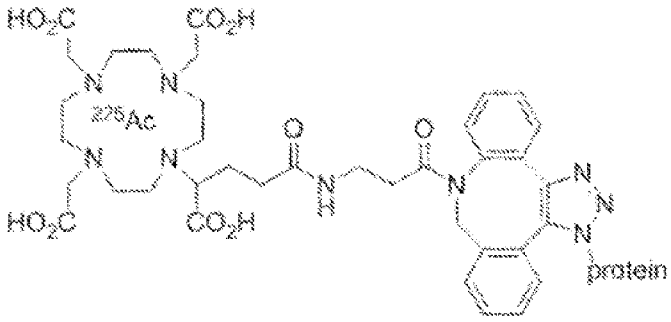
157) The immunoconjugate of embodiment 155, wherein the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



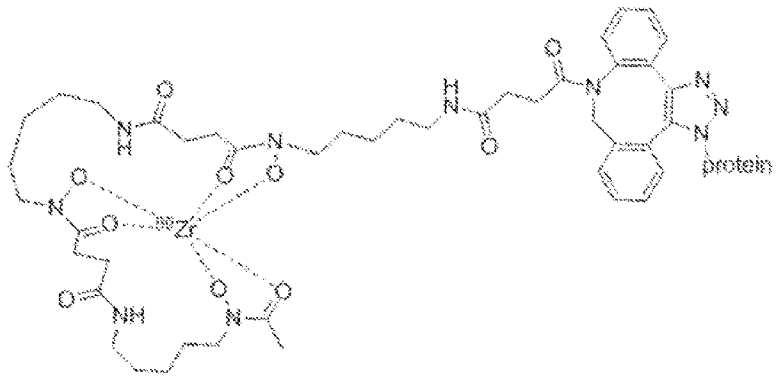
158) An immunoconjugate of formula (IV),



formula (IV)

wherein the protein is the isolated protein as defined in any one of embodiments 1-96.

159) An immunoconjugate of formula (V),



formula (V)

wherein the protein is the isolated protein as defined in any one of embodiments 1-96.

160) A pharmaceutical composition comprising a means for treating a CD33 expressing cancer in a subject.

161) A pharmaceutical composition comprising a means for reducing the amount of CD33 expressing tumor cells in a subject.

162) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject.

163) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer.

List of numbered embodiments (D):

1) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated protein binds CD33, and wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 18;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 19;
c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 20;
d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 21;
e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 22;
f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 23;
g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 24;
h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 25;
i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 26; or
j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 27,

or a CD33 binding fragment thereof.

2) The method of embodiment 1, wherein the isolated protein comprises the HCDR1, the HCDR2, the HCDR3 of
   a) SEQ ID NOs: 1, 6, and 12, respectively;
   b) SEQ ID NOs: 2, 7, and 13, respectively;
   c) SEQ ID NOs: 1, 8, and 14, respectively;
   d) SEQ ID NOs: 3, 9, and 13, respectively;
   e) SEQ ID NOs: 4, 10, and 15, respectively;
   f) SEQ ID NOs: 5, 11, and 16, respectively; or
   g) SEQ ID NOs: 5, 11, and 17, respectively.
3) The method of embodiments 1 or 2, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.
4) The method of embodiment 3, wherein the isolated protein is the VHH.
5) The method of embodiment 3, wherein the isolated protein is the Fab.
6) The method of embodiment 3, wherein the isolated protein is the scFv.
7) The method of any of embodiments 1-6, wherein the isolated protein comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.
8) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated protein binds CD33, and wherein the isolated protein comprises a VHH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.
9) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated protein binds CD33, wherein the antigen binding domain that binds CD33 comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52;
b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53;
c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54;
d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55;
e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;
f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57;
g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58;
h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;
i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60; or
j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61,
or a CD33 binding fragment thereof.

10) The method of embodiment 9, wherein the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of
a) SEQ ID NOs: 28, 36, and 45, respectively;
b) SEQ ID NOs: 29, 37, and 46, respectively;
c) SEQ ID NOs: 30, 38, and 47, respectively;
d) SEQ ID NOs: 31, 39, and 48, respectively;
e) SEQ ID NOs: 32, 40, and 49, respectively;
f) SEQ ID NOs: 33, 41, and 50, respectively;
g) SEQ ID NOs: 34, 42, and 51, respectively;
h) SEQ ID NOs: 34, 43, and 51, respectively;
i) SEQ ID NOs: 34, 44, and 51, respectively; or
j) SEQ ID NOs: 35, 42, and 51, respectively.

11) The method of embodiment 9 or 10, wherein the isolated protein comprises
a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 52 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 81;
b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;
c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 54 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 83;
d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 55 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 84;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 57 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 86;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 58 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 60 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87; or

j) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 61 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 88.

12) The method of embodiment 11, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;

b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;

d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;

e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;

g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;

h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or

j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

13) The method of any one of embodiments 9-12, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

14) The method of embodiment 13, wherein the isolated protein is the Fab.

15) The method of embodiment 13, wherein the isolated protein is the VHH.

16) The method of embodiment 13, wherein the isolated protein is the scFv.

17) The method of embodiment 16, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

18) The method of embodiment 17, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

19) The method of embodiment 18, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

20) The method of embodiment 19, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

21) The method of any one of embodiments 9-20, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61, and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

22) The method of embodiment 21, wherein the isolated protein comprises:

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

23) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein isolated protein binds CD33, and wherein the isolated protein comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

24) The method of embodiment 23, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

25) The method of embodiment 24, wherein the isolated protein is the Fab.

26) The method of embodiment 24, wherein the isolated protein is the VHH.

27) The method of embodiment 24, wherein the isolated protein is the scFv.

28) The method of embodiment 27, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

29) The method of embodiment 28, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

30) The method of embodiment 29, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

31) The method of embodiment 30, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

32) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated protein binds CD33, and wherein isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171;

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175;

g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179;

h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181;

i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96.

33) The method of embodiment 32, comprising the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 89, 90, and 92, respectively;

b) SEQ ID NOs: 89, 90, and 93, respectively;

c) SEQ ID NOs: 33, 91, and 94, respectively
d) SEQ ID NOs: 167, 168, and 169, respectively;
e) SEQ ID NOs: 172, 173, and 174, respectively;
f) SEQ ID NOs: 176, 177, and 178, respectively;
g) SEQ ID NOs: 89, 90, and 180, respectively; or
h) SEQ ID NOs: 89, 90, and 93, respectively.

34) The method of embodiment 32 or 33, wherein the isolated protein comprises
a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 95 and a light chain complementarity determining region (LCDR) 1, an LCDR2 and an LCDR3 of a light chain variable region (VL) of SEQ ID NO: 105;
b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 106;
c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 97 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 107;
d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 170 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 185;
e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 171 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 188;
f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 175 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 192;
g) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 179 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 196;
h) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 181 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 200; or
i) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 96 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 202.

35) The method of embodiment 34, wherein the isolated protein comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively;

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

36) The method of any one of embodiments 32-35, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

37) The method of embodiment 36, wherein the isolated protein is the Fab.

38) The method of embodiment 36, wherein the isolated protein is the VHH.

39) The method of embodiment 36, wherein the isolated protein is the scFv.

40) The method of embodiment 39, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

41) The method of embodiment 40, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

42) The method of embodiment 41, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

43) The method of embodiment 42, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

44) The method of any one of embodiments 32-43, wherein the isolated protein comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs: 105, 106, 107, 185, 188, 192, 196, 200, or 202.

45) The method of embodiment 44, wherein the isolated protein comprises:

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;

b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;

c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;

d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;

e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;

f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;

g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

46) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated protein binds CD33, wherein the isolated protein comprises
    a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
    b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
    c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
    d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
    e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
    f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
    g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
    h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
    i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.
47) The method of embodiment 46, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.
48) The method of embodiment 47, wherein the isolated protein is the Fab.
49) The method of embodiment 47, wherein the isolated protein is the VHH.
50) The method of embodiment 47, wherein the isolated protein is the scFv.
51) The method of embodiment 50, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).
52) The method of embodiment 51, wherein the L1 comprises
    a) about 5-50 amino acids;
    b) about 5-40 amino acids;
    c) about 10-30 amino acids; or
    d) about 10-20 amino acids.
53) The method of embodiment 52, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.
54) The method of embodiment 53, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.
55) The method of any one of embodiments 1-54, wherein the isolated protein is conjugated to a half-life extending moiety.

56) The method of embodiment 55, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol.

57) The method of any one of embodiments 1-56, wherein the isolated protein is a monospecific protein.

58) The method of any one of embodiments 1-57, wherein the isolated protein is a multispecific protein.

59) The method of embodiment 58, wherein the multispecific protein is a bispecific protein.

60) The method of embodiment 59, wherein the multispecific protein is a trispecific protein.

61) The method of any one of embodiments 1-60, further comprising an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof.

62) The method of embodiment 61, wherein the fragment of the Ig constant region comprises a Fc region.

63) The method of embodiment 61, wherein the fragment of the Ig constant region comprises a CH2 domain.

64) The method of embodiment 61, wherein the fragment of the Ig constant region comprises a CH3 domain.

65) The method of embodiment 61, wherein the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.

66) The method of embodiment 61, wherein the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

67) The method of embodiment 61, wherein the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain.

68) The method of any one of embodiments 61-67, wherein the isolated protein is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

69) The method of any one of embodiments 61-67, wherein the isolated protein is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

70) The method of any one of embodiments 61-69, wherein the isolated protein is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

71) The method of embodiment 70, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs:108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

72) The method of any one of embodiments 58-71, wherein the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte.

73) The method of embodiment 72, wherein the lymphocyte is a T cell.

74) The method of embodiment 72, wherein the T cell is a $CD8^+$ T cell

75) The method of embodiment 72, wherein the lymphocyte is a natural killer (NK) cell.

76) The method of any one of embodiments 58-75, wherein the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

77) The method of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds CD3ε.

78) The method of embodiment 77, wherein the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

79) The isolated protein of embodiment 76, wherein the multispecific protein comprises an antigen binding domain that binds TRGV9.

80) The isolated protein of embodiment 79, wherein the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining

region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

81) The method of any one of embodiments 61-80, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype.

82) The method of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR).

83) The method of embodiment 82, wherein the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

84) The method of any one of embodiments 61-81, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR.

85) The method of embodiment 84, wherein the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

86) The method of any one of embodiments 82-85, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

87) The method of any one of embodiments 61-86, wherein the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein.

88) The method of embodiment 87, wherein the at least one mutation that modulates the half-life of the protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

89) The method of any one of the embodiments 61-88, wherein the isolated protein comprises at least one mutation in a CH3 domain of the Ig constant region.

90) The method of embodiment 89, wherein the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

91) The method of any one of embodiments 1-54, wherein the isolated protein is a chimeric antigen receptor (CAR).

92) The method of embodiment 91, wherein the CAR comprises

a) an extracellular domain comprising the antigen binding domain that binds CD33 as defined in any one of embodiments 1-54;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

93) The method of embodiment 91 or 92, wherein the CAR further comprises a CD8a-hinge region.

94) The method of any one of embodiments 91-93, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

95) The method of any one of embodiments 91-93, wherein

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163 and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

96) The method of embodiment 95, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of

differentiation (CD27) component, a cluster of differentiation superfamily member component, or any combinations thereof.

97) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an isolated multispecific protein to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the isolated multispecific protein comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen.

98) The method of embodiment 97, wherein the lymphocyte antigen is a T cell antigen.

99) The method of embodiment 97, wherein the T cell antigen is a $CD8^+$ T cell antigen.

100) The method of embodiment 97, wherein the lymphocyte antigen is a NK cell antigen.

101) The method of any one of embodiments 97-100, wherein the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

102) The method of embodiment 101, wherein the lymphocyte antigen is CD3ε.

103) The method of any one of embodiments 97-102, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

104) The method of embodiment 103, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab.

105) The method of embodiment 103, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH.

106) The method of embodiment 103, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv.

107) The method of embodiment 106, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

108) The method of embodiment 107, wherein the L1 comprises

a) about 5-50 amino acids;

b) about 5-40 amino acids;

c) about 10-30 amino acids; or

d) about 10-20 amino acids.

109) The method of embodiment 108, wherein the L1 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

110) The method of embodiment 109, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

111) The method of any one of embodiments 97-110, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17.

112) The method of any one of 97-111, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3 of

a) SEQ ID NOs: 1, 6, and 12, respectively;
b) SEQ ID NOs: 2, 7, and 13, respectively;
c) SEQ ID NOs: 1, 8, and 14, respectively;
d) SEQ ID NOs: 3, 9, and 13, respectively;
e) SEQ ID NOs: 4, 10, and 15, respectively;
f) SEQ ID NOs: 5, 11, and 16, respectively; or
g) SEQ ID NOs: 5, 11, and 17, respectively.

113) The method of any one of embodiments 97-112, wherein the first antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

114) The method of any one of embodiments 97-110, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, or 35, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, or 44, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, or 51, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, or 68, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, or 74, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, or 80.

115) The method of any one of embodiments 97-110 and 114, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or

j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

116) The method of any one of embodiments 97-110, 114, and 115, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

117) The method of any one of embodiments 97-110 and 114-116, wherein the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;

b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;

d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;

g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;

h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or

j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

118) The method of any one of embodiments 97-110, wherein the first antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, or 201, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199.

119) The method of any one of embodiments 97-110 and 118, wherein the first antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;

b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;

c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;

d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;

e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;

f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;

g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;

h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or

i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

120) The method of any one of embodiments 97-110, 118 and 119, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, 181, or 96, and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202.

121) The method of any one of embodiments 97-110 and 118-120, wherein the first antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;
h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or
i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

122) The method of any one of embodiments 97-121, wherein the second antigen binding domain that binds the lymphocyte antigen comprises

a) the HCDR1 of SEQ ID NO: 141, the HCDR2 of SEQ ID NO: 142, the HCDR3 of SEQ ID NO: 143, the LCDR1 of SEQ ID NO: 144, the LCDR2 of SEQ ID NO: 145 and the LCDR3 of SEQ ID NO: 146;
b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;
c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or
d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

123) The method of any one of embodiments 95-119, wherein the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;
b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

124) The method of any one of embodiments 97-123, wherein the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

125) The method of embodiment 124, further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

126) The method of embodiment 125, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

127) The method of any one of embodiments 124-136, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

128) The method of any one of embodiments 124-127, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR.

129) The method of embodiment 128, wherein the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S,

F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

130) The method of any one of embodiments 124-127, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

131) The method of embodiment 130, wherein the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

132) The method of any one of embodiments 128-131, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

133) The method of any one of embodiments 124-132, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

134) The method of embodiment 133, wherein the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

135) The method of any one of the embodiments 124-134, comprising at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

136) The method of embodiment 135, wherein the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A,Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W,

L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

137) The method of any one of embodiments 124-136, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

a) L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

b) L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

138) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of an immunoconjugate to the subject for a time sufficient to treat the CD33 expressing cancer, wherein the immunoconjugate comprises an isolated protein as defined in any one of embodiments 1-137 conjugated to an agent.

139) The method of embodiment 138, wherein the agent is a radioactive agent.

140) The immunoconjugate of embodiment 139, wherein the radioactive agent comprises a radiometal ion.

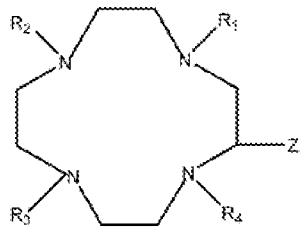
141) The immunoconjugate of embodiment 140, wherein the radiometal ion is ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{225}Ac, ^{255}Fm, ^{227}Th, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, or ^{111}In.

142) The immunoconjugate of embodiment 140, wherein the radiometal ion is ^{225}Ac, ^{111}In or ^{89}Zr.

143) The immunoconjugate of embodiment 140, wherein the radiometal ion is ^{225}Ac.

144) The immunoconjugate of any one of embodiments 140 to 143, wherein the radiometal ion is conjugated to a chelating moiety.

145) The immunoconjugate of embodiment 144, wherein the chelating moiety comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1-C_4 alkyl; and

Z is $(CH2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner; alternatively, Z is hydrogen; and

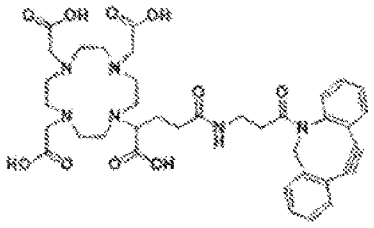
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

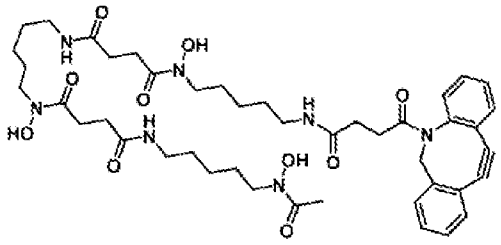
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand.

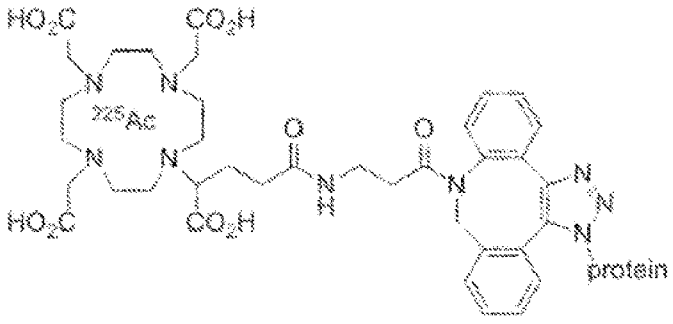
146) The immunoconjugate of embodiment 144, wherein the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



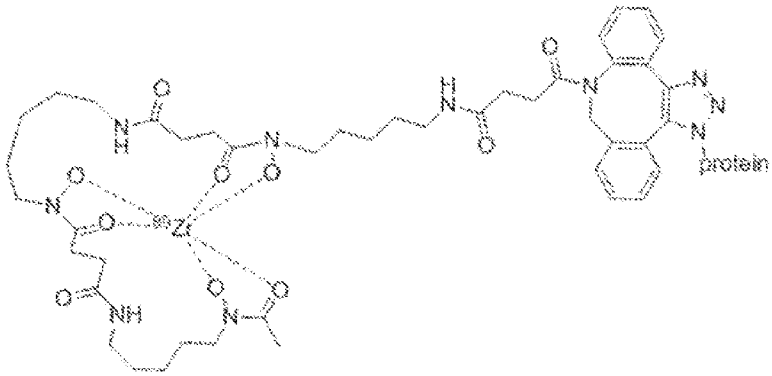
147) An immunoconjugate of formula (IV),



formula (IV)

wherein the protein is the isolated protein as defined in any one of embodiments 1-96.

148) An immunoconjugate of formula (V),



formula (V)

wherein the protein is the isolated protein as defined in any one of embodiments 1-96.

149) The method of any one of embodiments 1 to 148, wherein the method is effective to reduce the amount of CD33 expressing cells in a subject, wherein the CD33 expressing cells are cancerous cells or wherein the CD33 expressing cells are non-cancerous cells.

150) The method of any one of embodiments 1 to 149, wherein the method is effective to prevent establishment of a CD33 expressing cancer in the subject.

151) The method of any one of embodiments 148-150, wherein the CD33 expressing cancer is a hematologic cancer.

152) The method of embodiment 151, wherein the hematologic cancer is a leukemia, a lymphoma, or a multiple myeloma.

153) The method of embodiment 151, wherein the hematologic 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 (DPDCN).

154) The method of any one of embodiments 148-153, wherein the isolated protein or the isolated multispecific protein is administered in combination with a second therapeutic agent.

155) The method of embodiment 154, wherein the second therapeutic agent is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.

156) A method of treating a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cancer cell in the subject.

157) A method of reducing the amount of CD33 expressing tumor cells in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to one or more of the CD33 expressing tumor cells in the subject.

158) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

159) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

List of numbered embodiments (E):

1) A chimeric antigen receptor (CAR) comprising:
   a) an extracellular domain comprising an antigen binding domain that binds CD33;
   b) a transmembrane domain; and
   c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.
2) The CAR of embodiment 1, further comprising a CD8a-hinge region.
3) The CAR of embodiment 1 or 2, wherein
   a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and
   b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.
4) The CAR of embodiment 2 or 3, wherein
   a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;
   b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or
   c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163, and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.
5) The CAR of any one of embodiments 1-4, wherein the antigen binding domain that binds CD33 comprises the HCDR1 of any one of SEQ ID NOs: 1, 2, 3, 4, or 5, the HCDR2 of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11, and the HCDR3 of any one of SEQ ID NOs: 12, 13, 14, 15, 16, or 17.
6) The CAR of any one of 1-5, wherein the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR1, the HCDR3 of
   a) SEQ ID NOs: 1, 6, and 12, respectively;

b) SEQ ID NOs: 2, 7, and 13, respectively;
c) SEQ ID NOs: 1, 8, and 14, respectively;
d) SEQ ID NOs: 3, 9, and 13, respectively;
e) SEQ ID NOs: 4, 10, and 15, respectively;
f) SEQ ID NOs: 5, 11, and 16, respectively; or
g) SEQ ID NOs: 5, 11, and 17, respectively.

7) The CAR of any one of embodiments 1-6, wherein the antigen binding domain that binds CD33 comprises the VH of any one of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27.

8) The CAR of any one of embodiments 1-4, wherein the antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 28, 29, 30, 31, 32, 33, 34, or 35, the HCDR2 of SEQ ID NOs: 36, 37, 38, 39, 40, 41, 42, 43, or 44, the HCDR3 of SEQ ID NOs: 45, 46, 47, 48, 49, 50, or 51, the LCDR1 of SEQ ID NOs: 62, 63, 64, 65, 66, 67, or 68, the LCDR2 of SEQ ID NOs: 69, 70, 71, 72, 73, or 74, and the LCDR3 of SEQ ID NOs: 75, 76, 77, 78, 79, or 80.

9) The CAR of any one of embodiments 1-4 and 8, wherein the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
a) SEQ ID NOs: 28, 36, 45, 62, 69, and 75, respectively;
b) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;
c) SEQ ID NOs: 30, 38, 47, 64, 71, and 77, respectively;
d) SEQ ID NOs: 31, 39, 48, 65, 72, and 78, respectively;
e) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;
f) SEQ ID NOs: 33, 41, 50, 67, 73, and 79, respectively;
g) SEQ ID NOs: 34, 42, 51, 68, 74, and 80, respectively;
h) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;
i) SEQ ID NOs: 34, 44, 51, 68, 74, and 80, respectively; or
j) SEQ ID NOs: 35, 42, 51, 68, 74, and 80, respectively.

10) The CAR of any one of embodiments 1-4, 8, and 9, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61 and the VL of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, or 88.

11) The CAR of any one of embodiments 1-4 and 8-10, wherein the antigen binding domain that binds CD33 comprises
a) the VH of SEQ ID NO: 52 and the VL of SEQ ID NO: 81;
b) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
c) the VH of SEQ ID NO: 54 and the VL of SEQ ID NO: 83;
d) the VH of SEQ ID NO: 55 and the VL of SEQ ID NO: 84;

e) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
f) the VH of SEQ ID NO: 57 and the VL of SEQ ID NO: 86;
g) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 87;
h) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
i) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 87; or
j) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 88.

12) The CAR of any one of embodiments 1-4, wherein the antigen binding domain that binds CD33 comprises the HCDR1 of SEQ ID NOs: 33, 89, 167, 172 or 176, the HCDR2 of SEQ ID NOs: 90, 91, 168, 173, or 177, the HCDR3 of SEQ ID NOs: 92, 93, 94, 169, 174, 178, or 180, the LCDR1 of SEQ ID NOs: 98, 99, 100, 182, 186, 189, 193, 197, the LCDR2 of SEQ ID NOs: 101, 102, 103, 183, 187, 190, 194, or 198, and the LCDR3 of SEQ ID NO: 104, 184, 191, 195, or 199.

13) The CAR of any one of embodiments 1-4 and 12, wherein the antigen binding domain that binds CD33 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 89, 90, 92, 98, 101, and 104, respectively;
b) SEQ ID NOs: 89, 90, 93, 99, 102, and 104, respectively;
c) SEQ ID NOs: 33, 91, 94, 100, 103, and 104, respectively;
d) SEQ ID NOs: 167, 168, 169, 182, 183, and 184, respectively;
e) SEQ ID NOs: 33, 91, 94, 186, 187, and 104, respectively;
f) SEQ ID NOs: 172, 173, 174, 189, 190, and 191, respectively;
g) SEQ ID NOs: 176, 177, 178, 193, 194, and 195, respectively;
h) SEQ ID NOs: 89, 90, 180, 197, 198, and 199, respectively; or
i) SEQ ID NOs: 89, 90, 93, 201, 102, and 104, respectively.

14) The CAR of any one of embodiments 1-4, 12 and 13, wherein the antigen binding domain that binds CD33 comprises the VH of SEQ ID NOs: 95, 96, 97, 170, 171, 175, 179, or 181 and the VL of SEQ ID NOs 105, 106, 107, 185, 188, 192, 196, 200, or 202.

15) The CAR of any one of embodiments 1-4 and 12-14, wherein the antigen binding domain that binds CD33 comprises

a) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 105;
b) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 106;
c) the VH of SEQ ID NO: 97 and the VL of SEQ ID NO: 107;
d) the VH of SEQ ID NO: 170 and the VL of SEQ ID NO: 185;
e) the VH of SEQ ID NO: 171 and the VL of SEQ ID NO: 188;
f) the VH of SEQ ID NO: 175 and the VL of SEQ ID NO: 192;
g) the VH of SEQ ID NO: 179 and the VL of SEQ ID NO: 196;

h) the VH of SEQ ID NO: 181 and the VL of SEQ ID NO: 200; or

i) the VH of SEQ ID NO: 96 and the VL of SEQ ID NO: 202.

16) The CAR of any one of embodiments 1-15, wherein the antigen binding domain that binds CD33 is a VHH.

17) The CAR of any one of embodiments 1-16, wherein the antigen binding domain that binds CD33 is a scFv.

18) The CAR of embodiment 17, wherein the scFv comprises a first linker (L1) between the VL and the VH.

19) The CAR of embodiment 18, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

20) The CAR of embodiment 19, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

21) The CAR of any of embodiments 1-20, wherein the extracellular domain comprising the antigen binding domain that binds CD33 further comprises a signal polypeptide.

22) The CAR of embodiment 21, wherein the signal polypeptide comprises the amino acid sequence of SEQ ID NO: 166.

23) The CAR of any one of embodiments 1-22, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, and a combination thereof.

24) The CAR of any one of embodiments 3-23, wherein the CD137 component comprises the amino acid sequence of SEQ ID NO: 163.

25) The CAR of any one of embodiments 3-24, wherein the CD3z component comprises an amino acid sequence of SEQ ID NO: 164.

26) The CAR of any one of embodiments 3-25, wherein the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 165.

27) The CAR of any one of embodiments 3-26, wherein the CD8a-TM polypeptide comprises an amino acid sequence of SEQ ID NO: 162.

28) The CAR of any one of embodiments 3-27, wherein the CD8a-hinge region comprises an amino acid sequence of SEQ ID NO: 157.

29) The CAR of any one of embodiments 1-28, wherein the CAR is a multispecific protein.

30) The CAR of embodiment 29, wherein the multispecific protein is a bispecific protein.

31) The CAR of embodiment 30, wherein the multispecific protein is a trispecific protein.

32) The CAR of any one of embodiments 29-31, wherein the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte.

33) The CAR of embodiment 32, wherein the lymphocyte is a T cell.

34) The CAR of embodiment 32, wherein the T cell is a $CD8^+$ T cell

35) The CAR of embodiment 32, wherein the lymphocyte is a natural killer (NK) cell.

36) The CAR of any one of embodiments 29-35, wherein the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

37) The CAR of embodiment 36, wherein the multispecific protein comprises an antigen binding domain that binds CD3ε.

38) The CAR of embodiment 37, wherein the antigen binding domain that binds CD3ε comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 141, a HCDR2 of SEQ ID NO: 142, a HCDR3 of SEQ ID NO: 143, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 144, a LCDR2 of SEQ ID NO: 145 and a LCDR3 of SEQ ID NO: 146;

b) the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148;

c) the HCDR1 of SEQ ID NO: 149, the HCDR2 of SEQ ID NO: 150, the HCDR3 of SEQ ID NO: 151, the LCDR1 of SEQ ID NO: 152, the LCDR2 of SEQ ID NO: 153 and the LCDR3 of SEQ ID NO: 154; or

d) the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156.

39) The CAR of embodiment 36, wherein the multispecific protein comprises an antigen binding domain that binds TRGV9.

40) The CAR of embodiment 39, wherein the antigen binding domain that binds TRGV9 comprises:

a) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 238, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

b) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 242, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

c) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 243, a light chain complementarity determining

region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

d) a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 236, a HCDR2 of SEQ ID NO: 237, a HCDR3 of SEQ ID NO: 244, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 239, a LCDR2 of SEQ ID NO: 240 and a LCDR3 of SEQ ID NO: 241;

e) the VH of SEQ ID NO: 245 and the VL of SEQ ID NO: 249;

f) the VH of SEQ ID NO: 246 and the VL of SEQ ID NO: 249;

g) the VH of SEQ ID NO: 247 and the VL of SEQ ID NO: 249; or

h) the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249.

41) An isolated lymphocyte expressing the CAR of any one of embodiments 1-40.

42) The isolated lymphocyte of embodiment 41, wherein the lymphocyte is a T lymphocyte.

43) The isolated lymphocyte of embodiment 41, wherein the lymphocyte is a natural killer (NK) cell.

44) An isolated polynucleotide encoding the CAR of any one of embodiments 1-40.

45) A vector comprising the polynucleotide of embodiment 44.

46) A host cell comprising the polynucleotide of embodiment 44 or the vector of embodiment 45.

47) A pharmaceutical composition comprising the lymphocyte of any one of embodiments 41-43 and a pharmaceutically acceptable excipient.

48) A method of treating a subject having a CD33 expressing cancer, the method comprising: administering a therapeutically effective amount of the lymphocyte of any one of embodiments 41-43 to a subject in need thereof, whereby the lymphocyte mediates killing of the CD33 expressing cancer in the subject.

49) The method of embodiment 48, wherein the CD33 expressing cancer is a hematologic cancer.

50) The method of embodiment 49, wherein the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma.

51) The method of embodiment 50, wherein the CD33 expressing 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 (DPDCN).

52) A method of targeted killing of a cancer cell, the method comprising: contacting the cancer cell with the lymphocyte of any one of embodiments 41-43, whereby the lymphocyte induces targeted killing of the cancer cell.

53) The method of embodiment 52, wherein the cancer cell is a hematologic cancer.

54) The method of embodiment 53, wherein the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma.

55) The method of embodiment 54, wherein the CD33 expressing 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 (DPDCN).

56) A method of detecting the presence of a cancer in a subject, comprising:

a) contacting a cell sample obtained from the subject with the CAR of any one of embodiments 1-40, thereby forming a CAR-cell complex, and

b) detecting the CAR-cell complex, wherein detection of the CAR-cell complex is indicative of the presence of the cancer in the subject.

57) The method of embodiment 56, wherein the cancer cell is a hematologic cancer.

58) The method of embodiment 57, wherein the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma.

59) The method of embodiment 58, wherein the CD33 expressing 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 (DPDCN).

60) A kit comprising the CAR of any one of embodiments 1-40.

61) A pharmaceutical composition comprising a means for treating a CD33 expressing cancer in a subject.

62) A pharmaceutical composition comprising a means for reducing the amount of CD33 expressing tumor cells in a subject.

63) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject.

64) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer.

65) A method of treating a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cancer cell in the subject.

66) A method of reducing the amount of CD33 expressing tumor cells in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to one or more of the CD33 expressing tumor cells in the subject.

67) A pharmaceutical composition comprising a means for preventing establishment of a CD33 expressing cancer in a subject, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

68) A pharmaceutical composition comprising a means for treating a noncancerous condition in a subject at risk of developing a CD33 expressing cancer, the method comprising administering to the subject a means for targeting a therapeutic agent to a CD33 expressing cell in the subject.

List of numbered embodiments (F):

1) An isolated protein that binds CD33, wherein the isolated protein comprises
   a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 53;
   b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;
   c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;
   d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;
   e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or
   f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

2) The isolated protein of claim 1, comprising the HCDR1, the HCDR2, and the HCDR3 of
   a) SEQ ID NOs: 29, 37, and 46, respectively;
   b) SEQ ID NOs: 32, 40, and 49, respectively;
   c) SEQ ID NOs: 34, 43, and 51, respectively;
   d) SEQ ID NOs: 253, 256, and 259, respectively;
   e) SEQ ID NOs: 254, 257, and 260, respectively; or
   f) SEQ ID NOs: 255, 258, and 261, respectively.

3) An isolated protein that binds CD33, wherein the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of
   a) SEQ ID NOs: 29, 37, and 46, respectively;
   b) SEQ ID NOs: 32, 40, and 49, respectively;
   c) SEQ ID NOs: 34, 43, and 51, respectively;
   d) SEQ ID NOs: 253, 256, and 259, respectively;
   e) SEQ ID NOs: 254, 257, and 260, respectively; or
   f) SEQ ID NOs: 255, 258, and 261, respectively.

4) The isolated protein of claim 1 or 2, wherein the isolated protein comprises
   a) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273;.

5) The isolated protein of claim 3 or 4, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

b) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

c) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

d) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively

e) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively; or

f) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

6) The isolated protein of any one of claims 1-5, wherein the isolated protein comprises the VH of SEQ ID NOs: 53, 56, 59, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382, and the VL of SEQ ID NOs: 82, 85, 87, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

7) The isolated protein of claim 6, wherein the isolated protein comprises:

a) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

b) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

c) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

d) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

e) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;

f) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;

g) the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;

h) the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;
i) the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;
j) the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;
k) the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;
m) the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;
n) the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;
o) the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;
p) the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;
q) the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;
r) the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;
s) the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;
t) the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;
u) the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;
v) the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;
w) the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;
x) the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;
y) the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271;
z) the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271;
aa) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;
ab) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.
ac) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;
ad) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;
ae) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
af) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
ag) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;
ah) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
ai) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
aj) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
ak) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
al) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
am) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
an) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
ao) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;

ap) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
aq) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
ar) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
as) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
at) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
au) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;
av) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
aw) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
ax) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
ay) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
az) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
ba) the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
bb) the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;
bc) the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
bd) the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
be) the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
bf) the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
bg) the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
bh) the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
bi) the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
bj) the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;
bk) the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
bl) the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;
bm) the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
bn) the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
bo) the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272;
bp) the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.
bq) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
br) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
bs) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
bt) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
bu) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
bv) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
bw) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;

bx) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
by) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
bz) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
ca) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
cb) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
cc) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;
cd) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
ce) the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
cf) the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
cg) the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
ch) the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
ci) the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
cj) the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;
ck) the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
cl) the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
cm) the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
cn) the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
co) the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
cp) the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
cq) the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
cr) the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;
cs) the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85;
ct) the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85;
cu) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
cv) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
cw) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;
cx) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
cy) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
cz) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
da) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
db) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
dc) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
dd) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;

df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;
di) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or
dj) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

8) An isolated protein that binds CD33, wherein the isolated protein comprises
a) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;
b) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;
c) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;
d) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;
e) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;
f) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;
g) the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;
h) the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;
i) the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;
j) the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;
k) the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;
l) the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;
m) the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;
n) the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;
o) the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;
p) the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;
q) the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;
r) the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;
s) the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;
t) the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;
u) the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;
v) the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;
w) the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;
x) the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;
y) the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271;
z) the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271;
aa) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;
ab) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.

ac) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;
ad) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;
ae) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
af) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
ag) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;
ah) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
ai) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
aj) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
ak) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
al) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
am) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
an) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
ao) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;
ap) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
aq) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
ar) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
as) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
at) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
au) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;
av) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
aw) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
ax) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
ay) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
az) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
ba) the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
bb) the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;
bc) the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
bd) the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
be) the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
bf) the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
bg) the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
bh) the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
bi) the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
bj) the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;

bk) the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
bl) the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;
bm)the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
bn) the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
bo) the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272;
bp) the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.
bq) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
br) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
bs) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
bt) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
bu) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
bv) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
bw)the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;
bx) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
by) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
bz) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
ca) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
cb) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
cc) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;
cd) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
ce) the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
cf) the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
cg) the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
ch) the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
ci) the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
cj) the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;
ck) the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
cl) the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
cm)the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
cn) the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
co) the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
cp) the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
cq) the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
cr) the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;

cs) the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85;
ct) the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85;
cu) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
cv) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
cw) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;
cx) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
cy) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
cz) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
da) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
db) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
dc) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
dd) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;
di) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or
dj) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

9) The isolated protein of any one of claims 1-8, wherein the isolated protein is a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

10) The isolated protein of claim 9, wherein the isolated protein is the Fab.

11) The isolated protein of claim 9, wherein the isolated protein is the scFv.

12) The isolated protein of claim 11, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

13) The isolated protein of claim 12, wherein the L1 comprises
a) about 5-50 amino acids;
b) about 5-40 amino acids;
c) about 10-30 amino acids; or
d) about 10-20 amino acids.

14) The isolated protein of claim 13, wherein the L1 comprises an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

15) The isolated protein of claim 14, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

16) The isolated protein of claim 15, wherein the isolated protein the amino acid sequence of SEQ ID NO: 213, 216, 219, 274, 275 or 276.

17) The isolated protein of any one of claims 1-16, wherein the protein is conjugated to a half-life extending moiety.

18) The isolated protein of claim 17, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol.

19) The isolated protein of any one of claims 1-18, wherein the isolated protein is a monospecific protein.

20) The isolated protein of any one of claims 1-19, wherein the isolated protein is a multispecific protein.

21) The isolated protein of claim 20, wherein the multispecific protein is a bispecific protein.

22) The isolated protein of claim 20, wherein the multispecific protein is a trispecific protein.

23) The isolated protein of any one of claims 1-22, further comprising an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof.

24) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises a Fc region.

25) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises a CH2 domain.

26) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises a CH3 domain.

27) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.

28) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

29) The isolated protein of claim 23, wherein the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain.

30) The isolated protein of any one of claims 23-29, comprising an antigen binding domain that binds CD33 that is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

31) The isolated protein of any one of claims 23-29, comprising an antigen binding domain that binds CD33 that is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

32) The isolated protein of any one of claims 23-31, comprising an antigen binding domain that binds CD33 that is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

33) The isolated protein of claim 32, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs:108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

34) The isolated protein of any one of claims 20-33, wherein the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte.

35) The isolated protein of claim 34, wherein the lymphocyte is a T cell.

36) The isolated protein of claim 35, wherein the T cell is a $CD8^+$ T cell

37) The isolated protein of claim 34, wherein the lymphocyte is a natural killer (NK) cell.

38) The isolated protein of any one of claims 20-37, wherein the multispecific protein comprises an antigen binding domain that binds CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

39) The isolated protein of claim 38, wherein the multispecific protein comprises an antigen binding domain that binds CD3ε.

40) The isolated protein of claim 38, wherein the multispecific protein comprises an antigen binding domain that binds TRGV9.

41) The isolated protein of any one of claims 34-40, wherein a first antigen binding domain that binds CD33 and/or a second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a (scFv)2, a Fv, a Fab, a F(ab')2, a Fd, a dAb or a VHH.

42) The isolated protein of claim 41, wherein

a) the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab;

b) the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH;

c) the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv; or

d) the first antigen binding domain that binds CD33 comprises the scFv and the second antigen binding domain that binds the lymphocyte antigen comprises the VHH.

43) The isolated protein of any one of claims 23-42, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype, wherein optionally the Ig constant region or the fragment of the Ig constant region is an IgG1.

44) The isolated protein of claim 43, wherein the fragment of the Ig constant region has the sequence of SEQ ID NO: 277 or 278.

45) The isolated protein of any one of claims 23-43, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR).

46) The isolated protein of claim 45, wherein the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

47) The isolated protein of any one of claims 23-43, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR.

48) The isolated protein of claim 47, wherein the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

49) The isolated protein of any one of claims 45-48, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

50) The isolated protein of any one of claims 23-49, wherein the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein.

51) The isolated protein of claim 50, wherein the at least one mutation that modulates the half-life of the protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

52) The isolated protein of any one of the claims 23-51, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region.

53) The isolated protein of claim 52, wherein the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F,

L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

54) An isolated multispecific protein comprising a first antigen binding domain that binds CD33 and a second antigen binding domain that binds a lymphocyte antigen, wherein the first antigen binding domain that binds CD33 comprises the binding domain as defined in any one of claims 1-16.

55) The isolated multispecific protein of claim 54, wherein the lymphocyte antigen is a T cell antigen.

56) The isolated multispecific protein of claim 55, wherein the T cell antigen is a CD8+ T cell antigen.

57) The isolated multispecific protein of claim 54, wherein the lymphocyte antigen is a NK cell antigen.

58) The isolated multispecific protein of any one of claims 54-57, wherein the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C.

59) The isolated multispecific protein of claim 58, wherein the lymphocyte antigen is CD3ε.

60) The isolated multispecific protein of any one of claims 54-59, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a (scFv)2, a Fv, a Fab, a F(ab')2, a Fd, a dAb or a VHH.

61) The isolated multispecific protein of claim 60, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab.

62) The isolated multispecific protein of claim 60, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH.

63) The isolated multispecific protein of claim 60, wherein the first antigen binding domain that binds CD33 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv.

64) The isolated multispecific protein of claim 60 or 63, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

65) The isolated multispecific protein of claim 64, wherein the L1 comprises

about 5-50 amino acids;

about 5-40 amino acids;

about 10-30 amino acids; or

about 10-20 amino acids.

66) The isolated multispecific protein of claim 65, wherein the L1 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

67) The isolated multispecific protein of claim 66, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 108.

68) The isolated multispecific protein of any one of claims 54-67, wherein the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

69) The isolated multispecific protein of claim 68, further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

70) The isolated multispecific protein of claim 69 wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

71) The isolated multispecific protein of any one of claims 68-70, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

72) The isolated multispecific protein of claim 68-71, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR.

73) The isolated multispecific protein of claim 72, wherein the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

74) The isolated multispecific protein of any one of claims 68-71, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

75) The isolated multispecific protein of claim 74, wherein the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

76) The isolated multispecific protein of any one of claims 72-75, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

77) The isolated multispecific protein of any one of claims 67-76, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

78) The isolated multispecific protein of claim 77, wherein the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

79) The isolated multispecific protein of any one of the claims 68-78, comprising at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

80) The isolated multispecific protein of claim 79, wherein the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A,Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

81) The isolated multispecific protein of any one of claims 68-80, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

82) The isolated protein of any one of claims 1-16, wherein the protein is a chimeric antigen receptor (CAR).

83) The isolated protein of claim 82, wherein the CAR comprises

a) an extracellular domain comprising the antigen binding domain that binds CD33 of any one of claims 1-16;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

84) The isolated protein of claim 82 or 83, wherein the CAR further comprises a CD8a-hinge region.

85) The isolated protein of any one of claims 82-84, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

86) The isolated protein of any one of claims 82-84, wherein

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163 and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

87) The isolated protein of claim 86, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, or any combinations thereof.

88) A chimeric antigen receptor (CAR) comprising:

a) an extracellular domain comprising an antigen binding domain that binds CD33;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain,

wherein the antigen binding domain that binds CD33 comprises the binding domain as defined in any one of claims 1-16, 20-22 or 34-40.

89) The CAR of claim 88, further comprising a CD8a-hinge region.

90) The CAR of claim 88 or 89, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

91) The CAR of claim 89 or 90, wherein

d) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

e) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

f) the intracellular signaling domain comprises a co-stimulatory domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163, and a primary signaling domain having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164.

92) The CAR of any one of claims 88-91, wherein the extracellular domain comprising the antigen binding domain that binds CD33 further comprises a signal polypeptide.

93) The CAR of claim 92, wherein the signal polypeptide comprises the amino acid sequence of SEQ ID NO: 166.

94) The CAR of any one of claims 88-93, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, and a combination thereof.

95) The CAR of any one of claims 90-94, wherein the CD137 component comprises the amino acid sequence of SEQ ID NO: 163.

96) The CAR of any one of claims 90-95, wherein the CD3z component comprises an amino acid sequence of SEQ ID NO: 164.

97) The CAR of any one of claims 90-96, wherein the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 165.

98) The CAR of any one of claims 90-97, wherein the CD8a-TM polypeptide comprises an amino acid sequence of SEQ ID NO: 162.

99) The CAR of any one of claims 90-98, wherein the CD8a-hinge region comprises an amino acid sequence of SEQ ID NO: 157.

100) An isolated lymphocyte expressing the CAR of any one of claims 90-99.

101) The isolated lymphocyte of claim 100, wherein the lymphocyte is a T lymphocyte.

102) The isolated lymphocyte of claim 100, wherein the lymphocyte is a natural killer (NK) cell.

103) An immunoconjugate comprising the isolated protein of any one of claims 1-53, or isolated multispecific protein of any one of claims 54-81, conjugated to an agent.

104) The immunoconjugate of claim 103, wherein the agent is a therapeutic agent or an imaging agent.

105) The immunoconjugate of claim 103, wherein the agent is a radioactive agent.

106) The immunoconjugate of claim 105, wherein the radioactive agent comprises a radiometal ion.

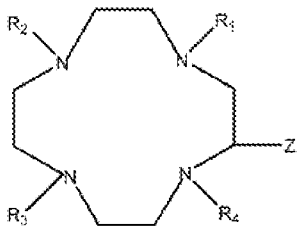
107) The immunoconjugate of claim 106, wherein the radiometal ion is ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{225}Ac, ^{255}Fm, ^{227}Th, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, or ^{111}In.

108) The immunoconjugate of claim 107, wherein the radiometal ion is ^{225}Ac, ^{111}In or ^{89}Zr.

109) The immunoconjugate of claim 107, wherein the radiometal ion is ^{225}Ac.

110) The immunoconjugate of any one of claims 106 to 109, wherein the radiometal ion is conjugated to a chelating moiety.

111) The immunoconjugate of claim 110, wherein the chelating moiety comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1-C_4 alkyl; and

Z is $(CH2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, Z is hydrogen; and

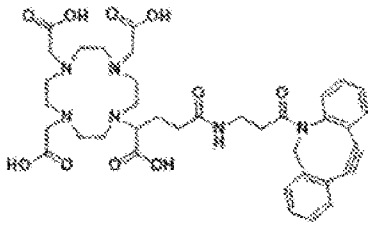
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

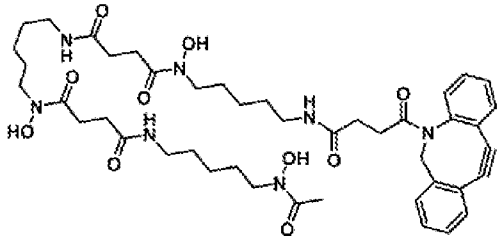
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand.

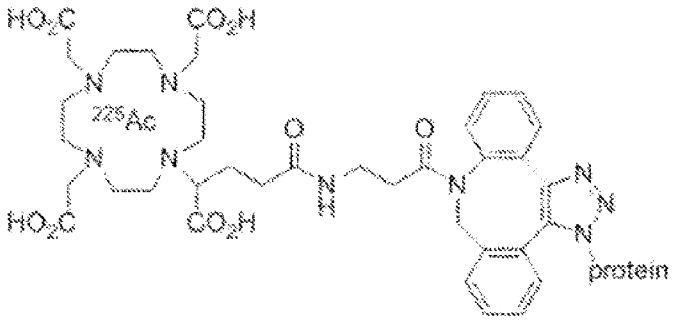
112) The immunoconjugate of claim 110, wherein the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



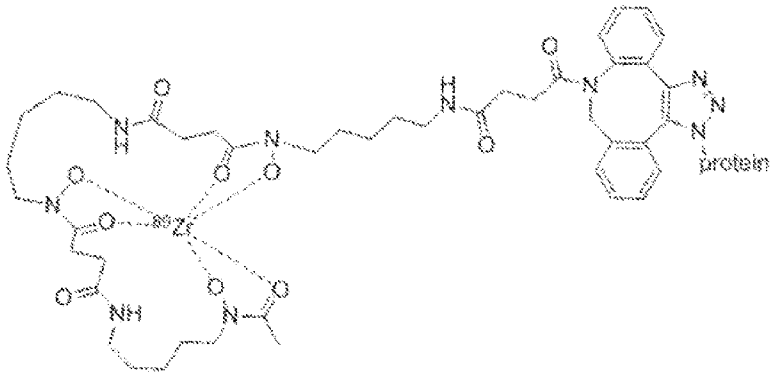
113) An immunoconjugate of formula (IV),



formula (IV)

wherein the protein is the isolated protein as defined in any one of claims 1-53, 82-87 or the isolated multispecific protein as defined in any one of claims 54-81.

114) An immunoconjugate of formula (V),



formula (V)

wherein the protein is the isolated protein as defined in any one of claims 1-53, 82-87 or the isolated multispecific protein as defined in any one of claims 54-81.

115) A pharmaceutical composition comprising the isolated protein of any one of claims 1-53, 82-87, the isolated multispecific protein of any one of claims 54-81, the CAR of any one of claims 88-99, the lymphocyte of any one of claims 100-102 or immunoconjugate of any one of claims 103-114, and a pharmaceutically acceptable carrier.

116) A polynucleotide encoding the isolated protein of any one of claims 1-53, 82-87, the isolated multispecific protein of any one of claims 54-81, the CAR of any one of claims 88-99, or immunoconjugate of any one of claims 103-114.

117) A vector comprising the polynucleotide of claim 116.

118) A host cell comprising the vector of claim 117 or polynucleotide of claim 116.

119) A method of producing the isolated protein of any one of claims 1-53, 82-87, or the isolated multispecific protein of any one of claims 54-81, comprising culturing the host cell of claim 118 in conditions that the protein is expressed, and recovering the protein produced by the host cell.

120) A method of producing the immunoconjugate of any one of claims 103-114, comprising culturing the host cell of claim 118 in conditions that the protein is expressed, recovering the protein produced by the host cell, and conjugating the protein produced by the host cell with the agent.

121) An anti-idiotypic antibody binding to the isolated protein of any one of claims 1-53 or 82-87.

122) A kit comprising the isolated protein of any one of claims 1-53, 82-87, or the isolated multispecific protein of any one of claims 54-81 or CAR of any one of claims 88-99.

123) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the isolated protein of any one of claims 1-53, 82-87, the isolated multispecific protein of any one of claims 54-81, or immunoconjugate of any one of claims 103-114, to the subject for a time sufficient to treat the CD33 expressing cancer.

124) The method of claim 123, wherein the method is effective to reduce the amount of CD33 expressing cells in a subject, wherein the CD33 expressing cells are cancerous cells or wherein the CD33 expressing cells are non-cancerous cells.

125) The method of claim 123 or 124, wherein the method is effective to prevent establishment of a CD33 expressing cancer in the subject.

126) A method of treating a subject having a CD33 expressing cancer, the method comprising: administering a therapeutically effective amount of the lymphocyte of any one of claims 100-102 to a subject in need thereof, whereby the lymphocyte mediates killing of the CD33 expressing cancer in the subject.

127) A method of targeted killing of a cancer cell, the method comprising: contacting the cancer cell with the lymphocyte of any one of claims 100-102, whereby the lymphocyte induces targeted killing of the cancer cell.

128) A method of detecting the presence of a cancer in a subject, comprising:

a) contacting a cell sample obtained from the subject with the CAR of any one of claims 88-99, thereby forming a CAR-cell complex, and

b) detecting the CAR-cell complex, wherein detection of the CAR-cell complex is indicative of the presence of the cancer in the subject.

129) The method of any one of claims 123-128, wherein the CD33 expressing cancer is a hematologic cancer.

130) The method of claim 129, wherein the hematologic cancer is a leukemia, a lymphoma, or a multiple myeloma.

131) The method of claim 130, wherein the hematologic 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 (DPDCN).

132) The method of claim 131, wherein the hematologic cancer is acute myeloid leukemia (AML).

133) The method of any one of claims 123-132, wherein the isolated protein, isolated multispecific protein or the immunoconjugate, is administered in combination with a second therapeutic agent.

134) The method of claim 133, wherein the second therapeutic agent is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.

Sequences

SEQ ID NO: 1 CDR1 (C33B1785; C33B1787; C33B1780; C33B1789; C33B1788)
DYAIG

SEQ ID NO: 2 CDR1 (C33B1784)
TYVMG

SEQ ID NO: 3 CDR1 (C33B1786)
FSTMD

SEQ ID NO: 4 CDR1 (C33B1781)
INAMG

SEQ ID NO: 5 CDR1 (C33B1783; C33B1782)
DYAVG

SEQ ID NO: 6 CDR2 (C33B1785; C33B1780; C33B1789; C33B1788)
CISESDGRTYLSDSVKS

SEQ ID NO: 7 CDR2 (C33B1784)
AIVSGRNPTYADSVKS

SEQ ID NO: 8 CDR2 (C33B1787)
CISSSDGSTYYVDSVKG

SEQ ID NO: 9 CDR2 (C33B1786)
CIRPRYSTTTHADSIKG

SEQ ID NO: 10 CDR2 (C33B1781)
RITGGGATDYVDSVKG

SEQ ID NO: 11 CDR2 (C33B1783; C33B1782)
CISSSDGATYYADSVKG

SEQ ID NO: 12 CDR3 (C33B1785; C33B1780; C33B1789; C33B1788)
VDQAISESSYHCEKGFGS

SEQ ID NO: 13 CDR3 (C33B1784; C33B1786)
YHYSSQY

SEQ ID NO: 14 CDR3 (C33B1787)
LIIMYGSGSERAAAACRYKYEY

SEQ ID NO: 15 CDR3 (C33B1781)
DLVTRVGSDLLYDDY

SEQ ID NO: 16 CDR3 (C33B1783)
IIVRGSGWERAAAACRYEYSY

SEQ ID NO: 17 CDR3 (C33B1782)
VIVRGTGWERAAAACRYEYAY

SEQ ID NO: 18 VHH sequence (C33B1785)
QVQLVESGGGSVQPGGSLRLSCAVFGFSLSDYAIGWFRQAPGKEREKVSCISESDGRTYLSDSVK
SRFTISRDNGKNTVYLQMNNLKPDDTGVYYCATVDQAISESSYHCEKGFGSWGQGTQVTVSS

SEQ ID NO: 19 VHH sequence (C33B1784)
EVQLVESGGGLVQAGGSLRLSCAASGRTFSTYVMGWFRQAPGQERELVAAIVSGRNPTYADSV
KSRFTISRDNAKNTIYLHMNSLQPEDTAVYYCHMYHYSSQYWGQGTQVTVSS

SEQ ID NO: 20 VHH sequence (C33B1787)
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCISSSDGSTYYVDSV
KGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCTALIIMYGSGSERAAAACRYKYEYWGQGTQV
TVSS

SEQ ID NO: 21 VHH sequence (C33B1786)

EVQVVESGGGVVQSGGSLTLSCEASESILSFSTMDWFRQAPGKEREGVSCIRPRYSTTTHADSIKG
RFKMYSDNAKNTIYLHMNSLQPEDTAVYYCHMYHYSSQYWGQGTQVTVSS

SEQ ID NO: 22 VHH sequence (C33B1781)
EVQVVESGGGLVQGGGSLRLSCAASGSIFSINAMGWYHQAPGKQRELVARITGGGATDYVDSV
KGRFTISLDSAKSTVYLQMNSLKPEDTAVYHCYADLVTRVGSDLLYDDYWGQGTQVTVSS

SEQ ID NO: 23 VHH sequence (C33B1780)
EVQLVESGGGLVQPGGSLRLSCAVFGFSLSDYAIGWFRQAPGKEREKVSCISESDGRTYLSDSVK
SRFTISRDNGKNTVYLQMNNLKPDDTGVYYCATVDQAISESSYHCEKGFGSWGQGTQVTVSS

SEQ ID NO: 24 VHH sequence (C33B1783)
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAVGWFRQVPGKEREGVSCISSSDGATYYADSV
KGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCTAIIVRGSGWERAAAACRYEYSYWGQGTQVT
VSS

SEQ ID NO: 25 VHH sequence (C33B1782)
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAVGWFRQAPGKEREGISCISSSDGATYYADSV
KGRFTISTDNAKNTAFLQMNSLKPEDTAVYYCTAVIVRGTGWERAAAACRYEYAYWGQGTQV
TVSS

SEQ ID NO: 26 VHH sequence (C33B1789)
QVQLVESGGGLVQAGGSLRLSCAVFGFSLSDYAIGWFRQAPGKEREKVSCISESDGRTYLSDSVK
SRFTISRDNGKNTVYLQMNNLKPDDTGVYYCATVDQAISESSYHCEKGFGSWGQGTQVTVSS

SEQ ID NO: 27 VHH sequence (C33B1788)
QVQLVESGGGLVQPGGSLRLSCAVFGFSLSDYAIGWFRQAPGKEREKVSCISESDGRTYLSDSVK
SRFTISRDNGKNTVYLQMNNLKPDDTGVYYCATVDQAISESSYHCEKGFGSWGQGTQVTVSS

SEQ ID NO: 28 CDR1 of VH (C33B1474)
SFGMS

SEQ ID NO: 29 CDR1 of VH (C33B1522)
SYYWG

SEQ ID NO: 30 CDR1 of VH (C33B1607)
DYNIN

SEQ ID NO: 31 CDR1 of VH (C33B1601)
NYAMS

SEQ ID NO: 32 CDR1 of VH (C33B1517)
NYYWS

SEQ ID NO: 33 CDR1 of VH (C33B1495; C33B931; C33B782)
SYWMS

SEQ ID NO: 34 CDR1 of VH (C33B1478; C33B1477; C33B1476)
VSAIH

SEQ ID NO: 35 CDR1 of VH (C33B1484)
VFAIH

SEQ ID NO: 36 CDR2 of VH (C33B1474)
NIKRDGSEKYYVDSVKG

SEQ ID NO: 37 CDR2 of VH (C33B1522)
YIYYSGSTNYNPSLKS

SEQ ID NO: 38 CDR2 of VH (C33B1607)
TINPNNGVTFYNQRFKG

SEQ ID NO: 39 CDR2 of VH (C33B1601)
GISNSGYSLYYPDTLKG

SEQ ID NO: 40 CDR2 of VH (C33B1517)
HIYSTGNIHYNPSLKS

SEQ ID NO: 41 CDR2 of VH (C33B1495)
NIKRDGSEKHYVDSVKG

SEQ ID NO: 42 CDR2 of VH (C33B1478; C33B1484)
RIRSKGNSYATAYDVSVKG

SEQ ID NO: 43 CDR2 of VH (C33B1477)
RIRSKGNSYATAYAASVKG

SEQ ID NO: 44 CDR2 of VH (C33B1476)
RIRSKGNSYATAYNVSVKG

SEQ ID NO: 45 CDR3 of VH (C33B1474)
DYGYFDF

SEQ ID NO: 46 CDR3 of VH (C33B1522)
MWEILGFDP

SEQ ID NO: 47 CDR3 of VH (C33B1607)
DGYDTYYAMDY

SEQ ID NO: 48 CDR3 of VH (C33B1601)
DGGSYPYAMDY

SEQ ID NO: 49 CDR3 of VH (C33B1517)
DNGAALFDY

SEQ ID NO: 50 CDR3 of VH (C33B1495)
DYGYFDY

SEQ ID NO: 51 CDR3 of VH (C33B1478; C33B1477; C33B1476; C33B1484)
HNDKWNYYGLDV

SEQ ID NO: 52 VH sequence (C33B1474)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVANIKRDGSEKYYVDS
VKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCVRDYGYFDFWGQGTLVTVSS

SEQ ID NO: 53 VH sequence (C33B1522)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWGWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSR
VTISVDTSKNQFSLKLSSVTAADTAVYYCARMWEILGFDPWGQGTLVTVS

SEQ ID NO: 54 VH sequence (C33B1607)
EVQLQQSGPELVKPGSSVKISCKASGYTFTDYNINWVKQSHGKNLEWIGTINPNNGVTFYNQRF
KGQATLTVDKSSSTAYMELRSLTSEDSAVYYCSRDGYDTYYAMDYWGQGTTLTVSS

SEQ ID NO: 55 VH sequence (C33B1607)
EVQLVESGGGLVRPGGSLKLSCAASGFTFSNYAMSWVRQTPEKRLEWVAGISNSGYSLYYPDTL
KGRFTISRDNARNTLYLQMISLRSEDTAMYYCARDGGSYPYAMDYWGHGTSVTVSS

SEQ ID NO: 56 VH sequence (C33B1517)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDYWGQGTLVTVSS

SEQ ID NO: 57 VH sequence (C33B1495)
EVQLVESGGGLVQPGGSLRLSCTASGFTFSSYWMSWVRQAPGKGLDWVANIKRDGSEKHYVDS
VKGRFTISRDNAKNSLYLQMNSLRAEDSAVYYCTRDYGYFDYWGQGTLVTVSS

SEQ ID NO: 58 VH sequence (C33B1478)
EVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYDV
SVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 59 VH sequence (C33B1477)
EVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYAA
SVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 60 VH sequence (C33B1476)
EVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYNV
SVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 61 VH sequence (C33B1484)
EVQLVESGGGLVQPGGSLKVSCEASGFTFSVFAIHWVRQASGKGLEWIGRIRSKGNSYATAYDV
SVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 62 CDR1 of VL (C33B1474)
QASQAISNYLN

SEQ ID NO: 63 CDR1 of VL (C33B1522)
SGSSSNIGSNPVN

SEQ ID NO: 64 CDR1 of VL (C33B1607)
KASQDVRTAEA

SEQ ID NO: 65 CDR1 of VL (C33B1601)
KASQNVGTYVA

SEQ ID NO: 66 CDR1 of VL (C33B1517; C33B1516)
SGSSSNIGSNIVN

SEQ ID NO: 67 CDR1 of VL (C33B1495)
RASQGISSYLA

SEQ ID NO: 68 CDR1 of VL (C33B1478; C33B1477; C33B1476; C33B1484)
KSSQSLLFSNGYKFLD

SEQ ID NO: 69 CDR2 of VL (C33B1474)
DASNLET

SEQ ID NO: 70 CDR2 of VL (C33B1522; C33B1517)
SNNQRPS

SEQ ID NO: 71 CDR2 of VL (C33B1607)
SASNRYT

SEQ ID NO: 72 CDR2 of VL (C33B1601)
SASYRYS

SEQ ID NO: 73 CDR2 of VL (C33B1495)
AASTLQS

SEQ ID NO: 74 CDR2 of VL (C33B1478; C33B1477; C33B1476; C33B1484; C33B1475)
LGSYRAS

SEQ ID NO: 75 CDR3 of VL (C33B1474)
QHYDNLPYT

SEQ ID NO: 76 CDR3 of VL (C33B1522; C33B1517; C33B1516)
AAWDDSLNGPV

SEQ ID NO: 77 CDR3 of VL (C33B1607)
QQYYTTPRT

SEQ ID NO: 78 CDR3 of VL (C33B1601)
QQYKSYPLT

SEQ ID NO: 79 CDR3 of VL (C33B1495)
QQLNSYPVT

SEQ ID NO: 80 CDR3 of VL (C33B1478; C33B1477; C33B1476; C33B1484)
MQALQTPPT

SEQ ID NO: 81 VL sequence (C33B1474)
DIQMTQSPSSLSASVGDRVTITCQASQAISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGS
GSGTDFTFTISSLQPEDFSTYFCQHYDNLPYTFGQGTKLEIK

SEQ ID NO: 82 VL sequence (C33B1522)

QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNGPVFGGGTKLTVL

SEQ ID NO: 83 VL sequence (C33B1607)
DIVMTQSHKFMSTSVGDRVSITCKASQDVRTAEAWYQQKPGQSPKLLIYSASNRYTGVPDRFTG
SGSGTDFTFTISSVQAEDLAVYYCQQYYTTPRTFGGGTKLEIK

SEQ ID NO: 84 VL sequence (C33B1601)
DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTYVAWYQQKPGHSPKALIYSASYRYSGVPDRFT
GSGSGTDFTLTISNVQSEDLADYFCQQYKSYPLTFGAGTKLELK

SEQ ID NO: 85 VL sequence (C33B1517)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGPGTKVTVL

SEQ ID NO: 86 VL sequence (C33B1495)
DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKVLIYAASTLQSGVPSRFSGSG
SGTEFTLTISSLQPEDFATYFCQQLNSYPVTFGGGTKVEIK

SEQ ID NO: 87 VL sequence (C33B1478; C33B1477; C33B1476)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIK

SEQ ID NO: 88 VL sequence (C33B1484)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGQGTKVEIK

SEQ ID NO: 89 CDR1 of VH (C33B933; C33B919; C33B937; C33B125)
SYGMH

SEQ ID NO: 90 CDR2 of VH (C33B933; C33B919; C33B937; C33B125)
VISYDGSNKYYADSVKG

SEQ ID NO: 91 CDR2 of VH (C33B931; C33B782)

NIKQHGSEKYYVDSVKG

SEQ ID NO: 92 CDR3 of VH (C33B933)
DFRSLDWLPPDSTSYDGMDV

SEQ ID NO: 93 CDR3 of VH (C33B933)
DFRSFDWLPPDSTSYYGMDV

SEQ ID NO: 94 CDR3 of VH (C33B931)
DRDLGYFDY

SEQ ID NO: 95 VH sequence (C33B933)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQSPGKGLEWVAVISYDGSNKYYADS
VKGRFTISRDNSKSTLYLQMNSLRAEDTAVYYCAKDFRSLDWLPPDSTSYDGMDVWGQGTTVT
VSS

SEQ ID NO: 96 VH sequence (C33B919; C33B125)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDFRSFDWLPPDSTSYYGMDVWGQGTTV
TVSS

SEQ ID NO: 97 VH sequence (C33B931)
EVQLVESGGGFVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDS
VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS

SEQ ID NO: 98 CDR1 of VL (C33B933)
SGHKLGDKYAS

SEQ ID NO: 99 CDR1 of VL (C33B919)
SGDKLGDKYVS

SEQ ID NO: 100 CDR1 of VL (C33B931)
SGDKLGSKFAS

SEQ ID NO: 101 CDR2 of VL (C33B933)
KDSKRPS

SEQ ID NO: 102 CDR2 of VL (C33B919; C33B125)
QDRKRPS

SEQ ID NO: 103 CDR2 of VL (C33B931)
QDSKRPS

SEQ ID NO: 104 CDR3 of VL (C33B933; C33B919; C33B931; C33B782; C33B125)
QAWDSSTVV

SEQ ID NO: 105 VL sequence (C33B933)
SYELTQPPSVSVSPGQTASITCSGHKLGDKYASWYQQKPGQSPVVVIYKDSKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVL

SEQ ID NO: 106 VL sequence (C33B919)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYVSWYQQKPGQSPVVVIHQDRKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVL

SEQ ID NO: 107 VL sequence (C33B931)
SYELTQPPSVSVSPGQTASITCSGDKLGSKFASWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNS
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVL

SEQ ID NO: 108 Linker sequence
GGSEGKSSGSGSESKSTGGS

SEQ ID NO: 109 Linker sequence
GGGSGGGS

SEQ ID NO: 110 Linker sequence
GGGSGGGSGGGS

SEQ ID NO: 111 Linker sequence

GGGSGGGSGGGSGGGS

SEQ ID NO: 112 Linker sequence
GGGSGGGSGGGSGGGSGGGS

SEQ ID NO: 113 Linker sequence
GGGGSGGGGSGGGGS

SEQ ID NO: 114 Linker sequence
GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 115 Linker sequence
GGGGSGGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 116 Linker sequence
GSTSGSGKPGSGEGSTKG

SEQ ID NO: 117 Linker sequence
IRPRAIGGSKPRVA

SEQ ID NO: 118 Linker sequence
GKGGSGKGGSGKGGS

SEQ ID NO: 119 Linker sequence
GGKGSGGKGSGGKGS

SEQ ID NO: 120 Linker sequence
GGGKSGGGKSGGGKS

SEQ ID NO: 121 Linker sequence
GKGKSGKGKSGKGKS

SEQ ID NO: 122 Linker sequence
GGGKSGGKGSGKGGS

SEQ ID NO: 123 Linker sequence
GKPGSGKPGSGKPGS

SEQ ID NO: 124 Linker sequence
GKPGSGKPGSGKPGSGKPGS

SEQ ID NO: 125 Linker sequence
GKGKSGKGKSGKGKSGKGKS

SEQ ID NO: 126 Linker sequence
STAGDTHLGGEDFD

SEQ ID NO: 127 Linker sequence
GEGGSGEGGSGEGGS

SEQ ID NO: 128 Linker sequence
GGEGSGGEGSGGEGS

SEQ ID NO: 129 Linker sequence
GEGESGEGESGEGES

SEQ ID NO: 130 Linker sequence
GGGESGGEGSGEGGS

SEQ ID NO: 131 Linker sequence
GEGESGEGESGEGESGEGES

SEQ ID NO: 132 Linker sequence
GSTSGSGKPGSGEGSTKG

SEQ ID NO: 133 Linker sequence
PRGASKSGSASQTGSAPGS

SEQ ID NO: 134 Linker sequence
GTAAAGAGAAGGAAAGAAG

SEQ ID NO: 135 Linker sequence
GTSGSSGSGSGGSGSGGGG

SEQ ID NO: 136 Linker sequence
GKPGSGKPGSGKPGSGKPGS

SEQ ID NO: 137 Linker sequence
GSGS

SEQ ID NO: 138 Linker sequence
APAPAPAPAP

SEQ ID NO: 139 Linker sequence
APAPAPAPAPAPAPAPAPAP

SEQ ID NO: 140 Linker sequence
AEAAAKEAAAKEAAAAKEAAAAKEAAAAKAAA

SEQ ID NO: 141 HCDR1 of antigen binding domain that binds CD3ε (CD3B219)
TYAMN

SEQ ID NO: 142 HCDR2 of antigen binding domain that binds CD3ε (CD3B219)
RIRSKYNNYATYYAASVKG

SEQ ID NO: 143 HCDR3 of antigen binding domain that binds CD3ε (CD3B219)
HGNFGNSYVSWFAY

SEQ ID NO: 144 LCDR1 of antigen binding domain that binds CD3ε (CD3B219)
RSSTGAVTTSNYAN

SEQ ID NO: 145 LCDR2 of antigen binding domain that binds CD3ε (CD3B219)

GTNKRAP

SEQ ID NO: 146 LCDR3 of antigen binding domain that binds CD3ε (CD3B219)
ALWYSNLWV

SEQ ID NO: 147 VH of antigen binding domain that binds CD3ε (CD3B219)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYY
AASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO: 148 VL of antigen binding domain that binds CD3ε (CD3B219)
QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARF
SGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO: 149 HCDR1 of antigen binding domain that binds CD3ε (CD3B376)
NNNAAWS

SEQ ID NO: 150 HCDR2 of antigen binding domain that binds CD3ε (CD3B376)
RTYYRSKWLYDYAVSVKS

SEQ ID NO: 151 HCDR3 of antigen binding domain that binds CD3ε (CD3B376)
GYSSSFDY

SEQ ID NO: 152 LCDR1 of antigen binding domain that binds CD3ε (CD3B376)
TGTSSNIGTYKFVS

SEQ ID NO: 153 LCDR2 of antigen binding domain that binds CD3ε (CD3B376)
EVSKRPS

SEQ ID NO: 154 LCDR3 of antigen binding domain that binds CD3ε (CD3B376)
VSYAGSGTLL

SEQ ID NO: 155 VH of antigen binding domain that binds CD3ε (CD3B376)

QVQLQQSGPRLVRPSQTLSLTCAISGDSVFNNNAAWSWIRQSPSRGLEWLGRTYYRSKWLYDY
AVSVKSRITVNPDTSRNQFTLQLNSVTPEDTALYYCARGYSSSFDYWGQGTLVTVSS

SEQ ID NO: 156 VL of antigen binding domain that binds CD3ε (CD3B376)
QSALTQPASVSGSPGQSITISCTGTSSNIGTYKFVSWYQQHPDKAPKVLLYEVSKRPSGVSSRFSG
SKSGNTASLTISGLQAEDQADYHCVSYAGSGTLLFGGGTKLTVL

SEQ ID NO: 157 CD8a-hinge region of CAR comprising antigen binding domain that binds CD33
TSTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

SEQ ID NO: 158 hinge region of CAR comprising antigen binding domain that binds CD33
EPKSCDKTHTCPPCP

SEQ ID NO: 159 hinge region of CAR comprising antigen binding domain that binds CD33
ERKCCVECPPCP

SEQ ID NO: 160 hinge region of CAR comprising antigen binding domain that binds CD33
ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$

SEQ ID NO: 161 hinge region of CAR comprising antigen binding domain that binds CD33
ESKYGPPCPSCP

SEQ ID NO: 162 CD8a transmembrane region of CAR comprising antigen binding domain that binds CD33
IYIWAPLAGTCGVLLLSLVITLYC

SEQ ID NO: 163 CD137 co-stimulatory domain of CAR comprising antigen binding domain that binds CD33
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 164 CD137 co-stimulatory domain of CAR comprising antigen binding domain that binds CD33
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 165 Intracellular signaling domain of CAR comprising antigen binding domain that binds CD33

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 166 hinge region of CAR comprising antigen binding domain that binds CD33

ESKYGPPCPSCP

SEQ ID NO: 167 CDR1 of VH (C33B836)

NYAMN

SEQ ID NO: 168 CDR2 of VH (C33B836)

WINTNTGNPTYAQGFTG

SEQ ID NO: 169 CDR3 of VH (C33B836)

DRDRGTDY

SEQ ID NO: 170 VH sequence (C33B836)

QVQLVQSGSELRKPGASVKVSCKASGYTFTNYAMNWVRQAPGQGLEWMGWINTNTGNPTYA
QGFTGRFVFSLDTSVSSAYLQISSLKAEDTAMYYCATDRDRGTDYWGQGTLVTVSS

SEQ ID NO: 171 VH sequence (C33B782)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDS
VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS

SEQ ID NO: 172 CDR1 of VH (C33B806)

SSSYYWG

SEQ ID NO: 173 CDR2 of VH (C33B806)

SINYSGSTYYNPSLKS

SEQ ID NO: 174 CDR3 of VH (C33B806)

LDGYESPFDY

SEQ ID NO: 175 VH sequence (C33B806)
QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLDWIGSINYSGSTYYNPSLK
SRVTISVDTSKIQFSLKLRSVTAADTAVYYCARLDGYESPFDYWGQGTLVTVSS

SEQ ID NO: 176 CDR1 of VH (C33B904)
DYAMH

SEQ ID NO: 177 CDR2 of VH (C33B904)
GIGWSGGSIVYADSVKG

SEQ ID NO: 178 CDR3 of VH (C33B904)
DSPYGDFFDY

SEQ ID NO: 179 VH sequence (C33B904)
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGIGWSGGSIVYADS
VKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSPYGDFFDYWGQGTLVTVSS

SEQ ID NO: 180 CDR3 of VH (C33B937)
DFRSFDWLPPDSASYHGMDV

SEQ ID NO: 181 VH sequence (C33B937)
QVQLVESGGGVVQPGRSLRLSCVASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEGTAVYYCAKDFRSFDWLPPDSASYHGMDVWGQGTTV
TVSS

SEQ ID NO: 182 CDR1 of VL (C33B836)
TGTSSDVGDYNYVS

SEQ ID NO: 183 CDR2 of VL (C33B836)
DVSNRPS

SEQ ID NO: 184 CDR3 of VL (C33B836)

SSYSSSSALEV

SEQ ID NO: 185 VL sequence (C33B836)
QSALTQPASVSGSPGQSITISCTGTSSDVGDYNYVSWYQQHPGKVPKLMIYDVSNRPSGVSNRFS
GSMSGNTASLTISGLQAEDEADYYCSSYSSSSALEVFGGGTKLTVL

SEQ ID NO: 186 CDR1 of VL (C33B782)
SGNKLGAKFAS

SEQ ID NO: 187 CDR2 of VL (C33B782)
QDNKRPS

SEQ ID NO: 188 VL sequence (C33B782)
SYELTQPPSVSVSPGQTASITCSGNKLGAKFASWYQQKPGQSPVLVIYQDNKRPSGIPERFSGSNS
GNTATLTISGTQAVDEADYYCQAWDSSTVVFGGGTKLTVL

SEQ ID NO: 189 CDR1 of VL (C33B806)
RASQGISSWLA

SEQ ID NO: 190 CDR2 of VL (C33B806)
AASSLQS

SEQ ID NO: 191 CDR3 of VL (C33B806)
QQANSFPFT

SEQ ID NO: 192 VL sequence (C33B806)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQANSFPFTFGPGTKVDIK

SEQ ID NO: 193 CDR1 of VL (C33B904)
KSSQTVFYSSNNKNYLA

SEQ ID NO: 194 CDR2 of VL (C33B904)
WASTRKS

SEQ ID NO: 195 CDR3 of VL (C33B904)
QHYYSTPYT

SEQ ID NO: 196 VL sequence (C33B904)
DIVMTQSPDSLAVSLGERATINCKSSQTVFYSSNNKNYLAWYQQKPGQPPKLLISWASTRKSGVP
DRFSGSGSGTDFTLTVSSLQAEDVAVYYCQHYYSTPYTFGQGTKLEIK

SEQ ID NO: 197 CDR1 of VL (C33B937)
SGDKLGDKYAS

SEQ ID NO: 198 CDR2 of VL (C33B937)
QDGKRPS

SEQ ID NO: 199 CDR3 of VL (C33B937)
QAWDRNTVV

SEQ ID NO: 200 VL sequence (C33B937)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYASWYQQKPGQSPVLVIYQDGKRPSGIPERFSGSNF
GNKATLTISGTQAMDEADYYCQAWDRNTVVFGGGTKLTVL

SEQ ID NO: 201 CDR1 of VL (C33B125)
SGDKLGDKYVC

SEQ ID NO: 202 VL sequence (C33B125)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYVCWYQQKPGQSPVVVIHQDRKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVL

SEQ ID NO: 203 scFv (C33B836)
QSALTQPASVSGSPGQSITISCTGTSSDVGDYNYVSWYQQHPGKVPKLMIYDVSNRPSGVSNRFS
GSMSGNTASLTISGLQAEDEADYYCSSYSSSSALEVFGGGTKLTVLGGSEGKSSGSGSESKSTGG
SQVQLVQSGSELRKPGASVKVSCKASGYTFTNYAMNWVRQAPGQGLEWMGWINTNTGNPTYA
QGFTGRFVFSLDTSVSSAYLQISSLKAEDTAMYYCATDRDRGTDYWGQGTLVTVSS

SEQ ID NO: 204 scFv (C33B782)

SYELTQPPSVSVSPGQTASITCSGNKLGAKFASWYQQKPGQSPVLVIYQDNKRPSGIPERFSGSNS
GNTATLTISGTQAVDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSEVQL
VESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDSVKG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS

SEQ ID NO: 205 scFv (C33B806)

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQANSFPFTFGPGTKVDIKGGSEGKSSGSGSESKSTGGSQLQL
QESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLDWIGSINYSGSTYYNPSLKSRVTI
SVDTSKIQFSLKLRSVTAADTAVYYCARLDGYESPFDYWGQGTLVTVSS

SEQ ID NO: 206 scFv (C33B904)

DIVMTQSPDSLAVSLGERATINCKSSQTVFYSSNNKNYLAWYQQKPGQPPKLLISWASTRKSGVP
DRFSGSGSGTDFTLTVSSLQAEDVAVYYCQHYYSTPYTFGQGTKLEIKGGSEGKSSGSGSESKST
GGSEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGIGWSGGSIVY
ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSPYGDFFDYWGQGTLVTVSS

SEQ ID NO: 207 scFv (C33B933)

SYELTQPPSVSVSPGQTASITCSGHKLGDKYASWYQQKPGQSPVVVIYKDSKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQL
VESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQSPGKGLEWVAVISYDGSNKYYADSVKGR
FTISRDNSKSTLYLQMNSLRAEDTAVYYCAKDFRSLDWLPPDSTSYDGMDVWGQGTTVTVSS

SEQ ID NO: 208 scFv (C33B937)

SYELTQPPSVSVSPGQTASITCSGDKLGDKYASWYQQKPGQSPVLVIYQDGKRPSGIPERFSGSNF
GNKATLTISGTQAMDEADYYCQAWDRNTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQ
LVESGGGVVQPGRSLRLSCVASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEGTAVYYCAKDFRSFDWLPPDSASYHGMDVWGQGTTVTVS
S

SEQ ID NO: 209 scFv (C33B919)

SYELTQPPSVSVSPGQTASITCSGDKLGDKYVSWYQQKPGQSPVVVIHQDRKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQL

VESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKG
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDFRSFDWLPPDSTSYYGMDVWGQGTTVTVSS

SEQ ID NO: 210 scFv (C33B125)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYVCWYQQKPGQSPVVVIHQDRKRPSGIPERFSGSNF
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQL
VESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKG
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDFRSFDWLPPDSTSYYGMDVWGQGTTVTVSS

SEQ ID NO: 211 scFv (C33B931)
SYELTQPPSVSVSPGQTASITCSGDKLGSKFASWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNS
GNTATLTISGTQAMDEADYYCQAWDSSTVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSEVQL
VESGGGFVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQHGSEKYYVDSVKG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRDLGYFDYWGQGTLVTVSS

SEQ ID NO: 212 scFv (C33B1474)
DIQMTQSPSSLSASVGDRVTITCQASQAISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGS
GSGTDFTFTISSLQPEDFSTYFCQHYDNLPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSQVQL
VESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVANIKRDGSEKYYVDSVKGR
FTISRDNAKNSLFLQMSSLRAEDTAVYYCVRDYGYFDFWGQGTLVTVSS

SEQ ID NO: 213 scFv (C33B1522)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNGPVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQ
VQLQESGPGLVKPSETLSLTCTVSGGSISSYYWGWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRV
TISVDTSKNQFSLKLSSVTAADTAVYYCARMWEILGFDPWGQGTLVTVS

SEQ ID NO: 214 scFv (C33B1607)
DIVMTQSHKFMSTSVGDRVSITCKASQDVRTAEAWYQQKPGQSPKLLIYSASNRYTGVPDRFTG
SGSGTDFTFTISSVQAEDLAVYYCQQYYTTPRTFGGGTKLEIKGGSEGKSSGSGSESKSTGGSEVQ
LQQSGPELVKPGSSVKISCKASGYTFTDYNINWVKQSHGKNLEWIGTINPNNGVTFYNQRFKGQ
ATLTVDKSSSTAYMELRSLTSEDSAVYYCSRDGYDTYYAMDYWGQGTTLTVSS

SEQ ID NO: 215 scFv (C33B1601)

DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTYVAWYQQKPGHSPKALIYSASYRYSGVPDRFT
GSGSGTDFTLTISNVQSEDLADYFCQQYKSYPLTFGAGTKLELKGGSEGKSSGSGSESKSTGGSE
VQLVESGGGLVRPGGSLKLSCAASGFTFSNYAMSWVRQTPEKRLEWVAGISNSGYSLYYPDTLK
GRFTISRDNARNTLYLQMISLRSEDTAMYYCARDGGSYPYAMDYWGHGTSVTVSS

SEQ ID NO: 216 scFv (C33B1517)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGPGTKVTVLGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDYWGQGTLVTVSS

SEQ ID NO: 217 scFv (C33B1495)
DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKVLIYAASTLQSGVPSRFSGSG
SGTEFTLTISSLQPEDFATYFCQQLNSYPVTFGGGTKVEIKGGSEGKSSGSGSESKSTGGSEVQLV
ESGGGLVQPGGSLRLSCTASGFTFSSYWMSWVRQAPGKGLDWVANIKRDGSEKHYVDSVKGRF
TISRDNAKNSLYLQMNSLRAEDSAVYYCTRDYGYFDYWGQGTLVTVSS

SEQ ID NO: 218 scFv (C33B1478)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKSTGG
SEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYD
VSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 219 scFv (C33B1477)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKSTGG
SEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYA
ASVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 220 scFv (C33B1476)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKGGSEGKSSGSGSESKSTGG
SEVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYN
VSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 221 scFv (C33B1484)

DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGQGTKVEIKGGSEGKSSGSGSESKSTGG
SEVQLVESGGGLVQPGGSLKVSCEASGFTFSVFAIHWVRQASGKGLEWIGRIRSKGNSYATAYD
VSVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSS

SEQ ID NO: 222 wild-type IgG1

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK

SEQ ID NO: 223 wild-type IgG2

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN
GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVE
WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

SEQ ID NO: 224 wild-type IgG4

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL
SLGK

SEQ ID NO: 225 CD33 antigen: HSA N-terminal Fusion; CynoCD33ECD-HSA(C34S)-6xHis

DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFREGAIVSLDSPVATNKLDQE
VQEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRP

QILIPGALDPDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIITPRPQDHGTNLTC
QVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDGSGKQGVVQGSGSGSENLYFQGVRSSSDAH
KSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSLH
TLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDN
EETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSA
KQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDR
ADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEA
KDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQ
NLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA
EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADIC
TLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA
ASQAALGLGGGSHHHHHH

SEQ ID NO: 226 CD33 antigen: HSA N-terminal Fusion; Human CD33ECD-Tev-HSA(C34S)-6xHis

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEV
QEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKI
LIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQV
KFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGSGSGSENLYFQGVRSSSDA
HKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSL
HTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHD
NEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADD
RADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAE
AKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEP
QNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPC
AEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV
AASQAALGLGGGSHHHHHH

SEQ ID NO: 227 CD33 antigen: HSA N-terminal Fusion; hCD33 Ig-like V-type domain (Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEV
QEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRDA
HKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSL

HTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHD
NEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADD
RADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAE
AKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEP
QNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPC
AEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV
AASQAALGLHHHHHH

SEQ ID NO: 228 CD33 antigen: HSA N-terminal Fusion; hCD33 Ig-like C2-type domain (Uniprot P20138, V136-H259) fused to HSA and 6xHis Tag at C-terminus

VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRP
QDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHDAHKSEV
AHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFG
DKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRL
KCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLA
KYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVF
LGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQ
NCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYL
SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSE
KERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQ
AALGLHHHHHH

SEQ ID NO: 229 CD33 antigen: hCD33_Ig-like V-Type domain

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEV
QEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRGSH
HHHHH

SEQ ID NO: 230 CD33 antigen: hCD33 Ig-like C2-type domain

VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRP
QDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGSHHHHH
H

SEQ ID NO: 231 CD33 antigen: MuIgV_Hu IgC2 chimera CD33-6xHis_v1

QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLRKGVSLHEDSPVATSDPRQLV
QKATQGRFQLLGDPQKHDCSLFIRDAQKNDTGMYFFRVVREPFVRYSYKKSQLSLHVTSLSRTP
KILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQ
VKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGSHHHHHH

SEQ ID NO: 232 CD33 antigen: MuIgV_cyno IgC2 chimera CD33-6xHis_v1

QDLEFQLVAPESVTVEEGLCVHVPCSVFYPSIKLTLGPVTGSWLRKGVSLHEDSPVATSDPRQLV
QKATQGRFQLLGDPQKHDCSLFIRDAQKNDTGMYFFRVVREPFVRYSYKKSQLSLHVTSLSRTP
QILIPGALDPDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIITPRPQDHGTNLTC
QVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDGSGRKARKQGVVQGSHHHHHH

SEQ ID NO: 233 CD33 antigen: Human CD33 ECD (D18-H259) from Uniprot P20138 with C-terminal 6xHis tag

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEV
QEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKI
LIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQV
KFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGSHHHHHH

SEQ ID NO: 234 CD33 antigen: Cyno CD33 ECD (D37-Q274) from XP_005590138.1 with C-terminal 6xHis tag

DPRVRLEVQESVTVQEGLCVLVPCTFFHPVPYHTRNSPVHGYWFREGAIVSLDSPVATNKLDQE
VQEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMEKGSTKYSYKSTQLSVHVTDLTHRP
QILIPGALDPDHSKNLTCSVPWACEQGTPPIFSWMSAAPTSLGLRTTHSSVLIITPRPQDHGTNLTC
QVKFPGAGVTTERTIQLNVSYASQNPRTDIFLGDGSGKQGVVQGSHHHHHH

SEQ ID NO: 235 CD33 antigen: Human CD33 ECD (D18-G241) from Uniprot P20138 with 6xHis tag

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEV
QEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKI
LIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQV
KFAGAGVTTERTIQLNVTYVPQNPTTGHHHHHH

SEQ ID NO: 236 HCDR1 sequence of anti-TRGV9 mAbs LP7A5_1, LP7A5_2, LP7A5_3, LP7A5_4

DHYIN

SEQ ID NO: 237 HCDR2 sequence of anti-TRGV9 mAbs LP7A5_1, LP7A5_2, LP7A5_3, LP7A5_4
QIYPGDGNTYYNQKFKG

SEQ ID NO: 238 HCDR3 sequence of anti-TRGV9 mAb LP7A5_1
NYGDYTIDF

SEQ ID NO: 239 LCDR1 sequence of anti-TRGV9 mAbs LP7A5_1, LP7A5_2, LP7A5_3, LP7A5_4
KSSQSLLYSSNQKNYLA

SEQ ID NO: 240 LCDR2 sequence of anti-TRGV9 mAbs LP7A5_1, LP7A5_2, LP7A5_3, LP7A5_4
WASTRES

SEQ ID NO: 241 LCDR3 sequence of anti-TRGV9 mAbs LP7A5_1, LP7A5_2, LP7A5_3, LP7A5_4
QQYYRYHT

SEQ ID NO: 242 HCDR3 sequence of anti-TRGV9 mAb LP7A5_2
NMGMYTIDF

SEQ ID NO: 243 HCDR3 sequence of anti-TRGV9 mAb LP7A5_3
NMGMYTLDF

SEQ ID NO: 244 HCDR3 sequence of anti-TRGV9 mAb LP7A5_4
NYGDYTLDF

SEQ ID NO: 245 Heavy Chain Sequence of LP7A5_1
EVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIGQIYPGDGNTYYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAPNYGDYTIDFWGQGTSVTVSS

SEQ ID NO: 246 Heavy Chain Sequence of LP7A5_2
EVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIGQIYPGDGNTYYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAPNMGMYTIDFWGQGTSVTVSS

SEQ ID NO: 247 Heavy Chain Sequence of LP7A5_3
EVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIGQIYPGDGNTYYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAPNMGMYTLDFWGQGTSVTVSS

SEQ ID NO: 248 Heavy Chain Sequence of LP7A5_4

EVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIGQIYPGDGNTYYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAPNMGMYTLDFWGQGTSVTVSS

SEQ ID NO: 249 Light Chain Sequence of LP7A5_1

DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGV
PDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYRYHTFGTGTKLEIK

SEQ ID NO: 250 Anti-TRGV9 hole arm sequence

MAWVWTLLFLMAAAQSIQADIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQ
QKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYRYHTFGTGTK
LEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEG
KSSGSGSESKSTGGSEVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIG
QIYPGDGNTYYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAPNYGDYTIDFWGQGTS
VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLSCAVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQ
KSLSLSLGK

SEQ ID NO: 251 Anti-CD33 knob arm sequence

MAWVWTLLFLMAAAQSIQADIVMTQSPDSLAVSLGERATINCKSSQTVFYSSNNKNYLAWYQQ
KPGQPPKLLISWASTRKSGVPDRFSGSGSGTDFTLTVSSLQAEDVAVYYCQHYYSTPYTFGQGTK
LEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEG
KSSGSGSESKSTGGSEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWV
SGIGWSGGSIVYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSPYGDFFDYWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK

SEQ ID NO: 252 Anti-RSV knob arm sequence

MAWVWTLLFLMAAAQSIQAEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAP
RLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQDYGFPWTFGQGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEGKSSGSGS
ESKSTGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWISWVRQMPGKGLEWMGIIDPSDSD
TRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGDGSTDLDYWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 253 CDR1 of VH (C33B1475)

SYWMT

SEQ ID NO: 254 CDR1 of VH (C33B1516)

NYYWS

SEQ ID NO: 255 CDR1 of VH (C33B1481)

NYWMS

SEQ ID NO: 256 CDR2 of VH (C33B1475)

NIKQDGSERYYVDSVKG

SEQ ID NO: 257 CDR2 of VH (C33B1516)

HIFSTGHINYDSSLKS

SEQ ID NO: 258 CDR2 of VH (C33B1481)

SIKRDGSDKYYVDSVKG

SEQ ID NO: 259 CDR3 of VH (C33B1475)

EVGYNWNQGGYFDY

SEQ ID NO: 260 CDR3 of VH (C33B1516)
DNGAALFDF

SEQ ID NO: 261 CDR3 of VH (C33B1481)
GEFDY

SEQ ID NO: 262 VH sequence (C33B1475)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 263 VH sequence (C33B1516)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDFWGQGTLVTVSS

SEQ ID NO: 264 VH sequence (C33B1481)
QVQLVESGGGLVQPGGSLRLSCAASGITFSNYWMSWVRQAPGKGLEWVASIKRDGSDKYYVDS
VKGRFTISRDNAKNSLSLQMHSLRAEDTAVYYCAKGEFDYWGQGTLVTVSS

SEQ ID NO: 265 CDR1 of VL (C33B1475)
RSSQSLLHSDGYNYLD

SEQ ID NO: 266 CDR1 of VL (C33B1481)
RSSQSLVYSDGNTYLN

SEQ ID NO: 267 CDR2 of VL (C33B1516)
SDNQRPS

SEQ ID NO: 268 CDR2 of VL (C33B1481)
KVSTRDS

SEQ ID NO: 269 CDR3 of VL (C33B1475)

MQVLQTPWT

SEQ ID NO: 270 CDR3 of VL (C33B1481)
LQGTHWPWT

SEQ ID NO: 271 VL sequence (C33B1475)
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKSGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTPWTFGQGTKVEIK

SEQ ID NO: 272 VL sequence (C33B1516)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVL

SEQ ID NO: 273 VL sequence (C33B1481)
DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYKVSTRDSGVPD
RFSGSGSGTDFTLKISRVEAEDVAVYYCLQGTHWPWTFGQGTKVEIK

SEQ ID NO: 274 scFv (C33B1475)
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKSGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTPWTFGQGTKVEIKGGSEGKSSGSGSESKSTGG
SEVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSERYYVD
SVKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 275 scFv (C33B1516)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVLGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDFWGQGTLVTVSS

SEQ ID NO: 276 scFv (C33B1481)
DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYKVSTRDSGVPD
RFSGSGSGTDFTLKISRVEAEDVAVYYCLQGTHWPWTFGQGTKVEIKGGSEGKSSGSGSESKSTG
GSQVQLVESGGGLVQPGGSLRLSCAASGITFSNYWMSWVRQAPGKGLEWVASIKRDGSDKYYV
DSVKGRFTISRDNAKNSLSLQMHSLRAEDTAVYYCAKGEFDYWGQGTLVTVSS

SEQ ID NO: 277 huIgG1_G1m(17)-hinge-FC_C220S|IgG1| (C33B1475; C33B1516; C33B1481; C33B1477; C33B1517; C33B1522)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO 278: huIgG1_G1m(17)
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 279 Fab HC (C33B1475)
QVQLVESGGGLVQPGGSLRLSCAASGITFSNYWMSWVRQAPGKGLEWVASIKRDGSDKYYVDS
VKGRFTISRDNAKNSLSLQMHSLRAEDTAVYYCAKGEFDYWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKV

SEQ ID NO: 280 Fab HC (C33B1516)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDFWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKV

SEQ ID NO: 281 Fab HC (C33B1481)
QVQLVESGGGLVQPGGSLRLSCAASGITFSNYWMSWVRQAPGKGLEWVASIKRDGSDKYYVDS
VKGRFTISRDNAKNSLSLQMHSLRAEDTAVYYCAKGEFDYWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKV

SEQ ID NO: 282 Fab HC (C33B1477)

EVQLVESGGGLVQPGGSLKVSCEASGFTFSVSAIHWVRQASGKGLEWIGRIRSKGNSYATAYAA
SVKGRFTISRDDSKNTAYLQMDSLKTEDTAVYYCTRHNDKWNYYGLDVWGQGTTVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKV

SEQ ID NO: 283 Fab HC (C33B1517)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNGAALFDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHKPSNTKVDKKV

SEQ ID NO: 284 Fab HC (C33B1522)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWGWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSR
VTISVDTSKNQFSLKLSSVTAADTAVYYCARMWEILGFDPWGQGTLVTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKV

SEQ ID NO: 285 Fab LC (C33B1475)
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKSGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 286 Fab LC (C33B1516)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVLGQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY
SCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 287 Fab LC (C33B1481)
DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYKVSTRDSGVPD
RFSGSGSGTDFTLKISRVEAEDVAVYYCLQGTHWPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 288 Fab LC (C33B1477)
DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSNGYKFLDWYLQRPGQSPQLLIYLGSYRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGLYYCMQALQTPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 289 Fab LC (C33B1517)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGPGTKVTVLGQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY
SCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 290 Fab LC (C33B1522)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNGPVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY
SCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 291 (C33B1475VH1-2)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQGGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 292 (C33B1475VH1-3)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQAGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 293 (C33B1475VH1-5)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQYGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 294 (C33B1475VH1-12)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQPGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 295 (C33B1475VH1-14)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQNGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 296 (C33B1475VH1-22)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQSGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 297 (C33B1475VH1-27)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQRGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 298 (C33B1475VH1-32)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQLGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 299 (C33B1475VH1-36)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQVGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 300 (C33B1475VH1-46)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQTGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 301 (C33B1475VH1-63)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQFGSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 302 (C33B1475VH1-89)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQWGSERYYVD
SVKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 303 (C33B1475VH2-12)

EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDVSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 304 (C33B1475VH2-16)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDPSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 305 (C33B1475VH2-20)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDRSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 306 (C33B1475VH2-22)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDASERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 307 (C33B1475VH2-24)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDLSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 308 (C33B1475VH2-35)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDQSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 309 (C33B1475VH2-58)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDTSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 310 (C33B1475VH2-86)
EVQLVESGGGLVQPGGSLRLSCVVSGFTFSSYWMTWVRQAPGKGLEWVANIKQDHSERYYVDS
VKGRFTISRDSAKNSLYLQMNSLRAEDTAVYYCAREVGYNWNQGGYFDYWGQGTLVTVSS

SEQ ID NO: 311 (C33B1516 VL1-1)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLVGPVFGTGTKVTVL

SEQ ID NO: 312 (C33B1516 VL1-2)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLRGPVFGTGTKVTVL

SEQ ID NO: 313 (C33B1516 VL1-4)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLPGPVFGTGTKVTVL

SEQ ID NO: 314 (C33B1516 VL1-10)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLTGPVFGTGTKVTVL

SEQ ID NO: 315 (C33B1516 VL1-11)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLGGPVFGTGTKVTVL

SEQ ID NO: 316 (C33B1516 VL1-14)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLQGPVFGTGTKVTVL

SEQ ID NO: 317 (C33B1516 VL1-16)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLSGPVFGTGTKVTVL

SEQ ID NO: 328 (C33B1516 VL1-20)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLAGPVFGTGTKVTVL

SEQ ID NO: 319 (C33B1516 VL1-22)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLLGPVFGTGTKVTVL

SEQ ID NO: 320 (C33B1516 VL1-23)

QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLYGPVFGTGTKVTVL

SEQ ID NO: 321 (C33B1516 VL1-30)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLEGPVFGTGTKVTVL

SEQ ID NO: 322 (C33B1516VL1-39)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLFGPVFGTGTKVTVL

SEQ ID NO: 323 (C33B1516VL1-41)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLDGPVFGTGTKVTVL

SEQ ID NO: 324 (C33B1516VL1-63)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLIGPVFGTGTKVTVL

SEQ ID NO: 325 (C33B1516VL1-90)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLHGPVFGTGTKVTVL

SEQ ID NO: 326 (C33B1516 VL2-3)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNVPVFGTGTKVTVL

SEQ ID NO: 327 (C33B1516 VL2-4)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNPPVFGTGTKVTVL

SEQ ID NO: 328 (C33B1516 VL2-7)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNRPVFGTGTKVTVL

SEQ ID NO: 329 (C33B1516 VL2-8)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNDPVFGTGTKVTVL

SEQ ID NO: 330 (C33B1516 VL2-10)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNEPVFGTGTKVTVL

SEQ ID NO: 331 (C33B1516 VL2-11)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNWPVFGTGTKVTVL

SEQ ID NO: 332 (C33B1516 VL2-29)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNYPVFGTGTKVTVL

SEQ ID NO: 333 (C33B1516 VL2-35)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNAPVFGTGTKVTVL

SEQ ID NO: 334 (C33B1516VL2-37)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNNPVFGTGTKVTVL

SEQ ID NO: 335 (C33B1516VL2-38)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNKPVFGTGTKVTVL

SEQ ID NO: 336 (C33B1516VL2-90)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLLYSDNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLNLPVFGTGTKVTVL

SEQ ID NO: 337 (C33B1516VH1-1)

QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDVGAALFDFWGQGTLVTVSS

SEQ ID NO: 338 (C33B1516VH1-3)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDSGAALFDFWGQGTLVTVSS

SEQ ID NO: 339 (C33B1516VH1-4)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDEGAALFDFWGQGTLVTVSS

SEQ ID NO: 340 (C33B1516VH1-6)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDHGAALFDFWGQGTLVTVSS

SEQ ID NO: 341 (C33B1516VH1-7)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDAGAALFDFWGQGTLVTVSS

SEQ ID NO: 342 (C33B1516VH1-8)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDGGAALFDFWGQGTLVTVSS

SEQ ID NO: 343 (C33B1516VH1-10)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDKGAALFDFWGQGTLVTVSS

SEQ ID NO: 344 (C33B1516VH1-13)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDTGAALFDFWGQGTLVTVSS

SEQ ID NO: 345 (C33B1516VH1-17)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDFGAALFDFWGQGTLVTVSS

SEQ ID NO: 346 (C33B1516VH1-20)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDVGAALFDFWGQGTLVTVSS

SEQ ID NO: 347 (C33B1516VH1-21)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDRGAALFDFWGQGTLVTVSS

SEQ ID NO: 348 (C33B1516VH1-27)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDLGAALFDFWGQGTLVTVSS

SEQ ID NO: 349 (C33B1516VH1-38)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDHGAALFDFWGQGTLVTVSS

SEQ ID NO: 350 (C33B1516VH1-56)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDIGAALFDFWGQGTLVTVSS

SEQ ID NO: 351 (C33B1516VH2-8)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNSAALFDFWGQGTLVTVSS

SEQ ID NO: 352 (C33B1516VH2-9)
QVQLQESGPGLVKPSETLSLTCSVSGGSIRNYYWSWIRQSAGKELEWFGHIFSTGHINYDSSLKSR
VTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNAAALFDFWGQGTLVTVSS

SEQ ID NO: 353 (C33B1517VL1-1)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLGGPVFGPGTKVTVL

SEQ ID NO: 354 (C33B1517VL1-7)

QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLDGPVFGPGTKVTVL

SEQ ID NO: 355 (C33B1517VL1-8)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLPGPVFGPGTKVTVL

SEQ ID NO: 356 (C33B1517VL1-12)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLKGPVFGPGTKVTVL

SEQ ID NO: 357 (C33B1517VL1-13)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLEGPVFGPGTKVTVL

SEQ ID NO: 358 (C33B1517VL1-16)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLAGPVFGPGTKVTVL

SEQ ID NO: 359 (C33B1517VL1-22)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLRGPVFGPGTKVTVL

SEQ ID NO: 360 (C33B1517VL1-24)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLQGPVFGPGTKVTVL

SEQ ID NO: 361 (C33B1517VL1-27)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLIGPVFGPGTKVTVL

SEQ ID NO: 362 (C33B1517VL1-35)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLVGPVFGPGTKVTVL

SEQ ID NO: 363 (C33B1517VL1-49)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLHGPVFGPGTKVTVL

SEQ ID NO: 364 (C33B1517VL1-66)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLKGPVFGPGTKVTVL

SEQ ID NO: 365 (C33B1517VL1-69)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLWGPVFGPGTKVTVL

SEQ ID NO: 366 (C33B1517VL1-71)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNIVNWYQQFPGTAPKLLIYSNNQRPSGVPDRVSGS
KSGTSASLAISGLQSEDEADYYCAAWDDSLSGPVFGPGTKVTVL

SEQ ID NO: 367 (C33B1517VH1-1)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDRGAALFDYWGQGTLVTVSS

SEQ ID NO: 368 (C33B1517VH1-3)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDPGAALFDYWGQGTLVTVSS

SEQ ID NO: 369 (C33B1517VH1-4)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDGGAALFDYWGQGTLVTVSS

SEQ ID NO: 370 (C33B1517VH1-6)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDVGAALFDYWGQGTLVTVSS

SEQ ID NO: 371 (C33B1517VH1-8)

QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDLGAALFDYWGQGTLVTVSS

SEQ ID NO: 372 (C33B1517VH1-11)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDDGAALFDYWGQGTLVTVSS

SEQ ID NO: 373 (C33B1517VH1-17)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDEGAALFDYWGQGTLVTVSS

SEQ ID NO: 374 (C33B1517VH1-33)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDAGAALFDYWGQGTLVTVSS

SEQ ID NO: 375 (C33B1517VH1-60)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDQGAALFDYWGQGTLVTVSS

SEQ ID NO: 376 (C33B1517VH1-71)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDTGAALFDYWGQGTLVTVSS

SEQ ID NO: 377 (C33B1517VH2-7)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNSAALFDYWGQGTLVTVSS

SEQ ID NO: 378 (C33B1517VH2-30)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNAAALFDYWGQGTLVTVSS

SEQ ID NO: 379 (C33B1517VH2-31)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNLAALFDYWGQGTLVTVSS

SEQ ID NO: 380 (C33B1517VH2-60)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNPAALFDYWGQGTLVTVSS

SEQ ID NO: 381 (C33B1517VH2-68)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNTAALFDYWGQGTLVTVSS

SEQ ID NO: 382 (C33B1517VH2-76)
QVQLQESGPGLVKPSETLSLTCSVSGASIRNYYWSWIRQTAGKGLEWLGHIYSTGNIHYNPSLKS
RVTMSVDTSNNQFSLKLRSVTAADTAVYYCARDNQAALFDYWGQGTLVTVSS

SEQ ID NO: 383 (C33B1522VL1-2)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLSGPVFGGGTKLTVL

SEQ ID NO: 384 (C33B1522VL1-3)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLQGPVFGGGTKLTVL

SEQ ID NO: 385 (C33B1522VL1-4)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLVGPVFGGGTKLTVL

SEQ ID NO: 386 (C33B1522VL1-5)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLGGPVFGGGTKLTVL

SEQ ID NO: 387 (C33B1522VL1-7)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLWGPVFGGGTKLTVL

SEQ ID NO: 388 (C33B1522VL1-12)

QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLTGPVFGGGTKLTVL

SEQ ID NO: 389 (C33B1522VL1-15)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLIGPVFGGGTKLTVL

SEQ ID NO: 390 (C33B1522VL1-27)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLEGPVFGGGTKLTVL

SEQ ID NO: 391 (C33B1522VL1-29)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLAGPVFGGGTKLTVL

SEQ ID NO: 392 (C33B1522VL1-32)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLRGPVFGGGTKLTVL

SEQ ID NO: 393 (C33B1522VL1-47)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLLGPVFGGGTKLTVL

SEQ ID NO: 394 (C33B1522VL2-82)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNVPVFGGGTKLTVL

SEQ ID NO: 395 (C33B1522VL2-1)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNAPVFGGGTKLTVL

SEQ ID NO: 396 (C33B1522VL2-2)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNPPVFGGGTKLTVL

SEQ ID NO: 397 (C33B1522VL2-8)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNLPVFGGGTKLTVL

SEQ ID NO: 398 (C33B1522VL2-17)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNPVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNTPVFGGGTKLTVL

SEQ ID NO: 399 HC AbM CDR1 (C33B1522)
GGSISSYYWG

SEQ ID NO: 400 HC AbM CDR1 (C33B1477)
GFTFSVSAIH

SEQ ID NO: 401 HC AbM CDR1 (C33B1475)
GFTFSSYWMT

SEQ ID NO: 402 HC AbM CDR1 (C33B1517)
GASIRNYYWS

SEQ ID NO: 403 HC AbM CDR1 (C33B1516)
GGSIRNYYWS

SEQ ID NO: 404 HC AbM CDR1 (C33B1481)
GITFSNYWMS

SEQ ID NO: 405 HC AbM CDR2 (C33B1522)
YIYYSGSTN

SEQ ID NO: 406 HC AbM CDR2 (C33B1477)
RIRSKGNSYATA

SEQ ID NO: 407 HC AbM CDR2 (C33B1475)

NIKQDGSERY

SEQ ID NO: 408 HC AbM CDR2 (C33B1517)
HIYSTGNIH

SEQ ID NO: 409 HC AbM CDR2 (C33B1516)
HIFSTGHIN

SEQ ID NO: 410 HC AbM CDR2 (C33B1481)
SIKRDGSDKY

SEQ ID NO: 411 HC AbM CDR3 (C33B1522)
MWEILGFDP

SEQ ID NO: 412 HC AbM CDR3 (C33B1477)
HNDKWNYYGLDV

SEQ ID NO: 413 HC AbM CDR3 (C33B1475)
EVGYNWNQGGYFDY

SEQ ID NO: 414 HC AbM CDR3 (C33B1517)
DNGAALFDY

SEQ ID NO: 415 HC AbM CDR3 (C33B1516)
DNGAALFDF

SEQ ID NO: 416 HC AbM CDR3 (C33B1481)
GEFDY

SEQ ID NO: 417 LC AbM CDR1 (C33B1522)
SGSSSNIGSNPVN

SEQ ID NO: 418 LC AbM CDR1 (C33B1477)
KSSQSLLFSNGYKFLD

SEQ ID NO: 419 LC AbM CDR1 (C33B1475)
RSSQSLLHSDGYNYLD

SEQ ID NO: 420 LC AbM CDR1 (C33B1517)
SGSSSNIGSNIVN

SEQ ID NO: 421 LC AbM CDR1 (C33B1516)
SGSSSNIGSNIVN

SEQ ID NO: 422 LC AbM CDR1 (C33B1481)
RSSQSLVYSDGNTYLN

SEQ ID NO: 423 LC AbM CDR2 (C33B1522)
SNNQRPS

SEQ ID NO: 424 LC AbM CDR2 (C33B1477)
LGSYRAS

SEQ ID NO: 425 LC AbM CDR2 (C33B1475)
LGSYRAS

SEQ ID NO: 426 LC AbM CDR2 (C33B1517)
SNNQRPS

SEQ ID NO: 427 LC AbM CDR2 (C33B1516)
SDNQRPS

SEQ ID NO: 428 LC AbM CDR2 (C33B1481)
KVSTRDS

SEQ ID NO: 429 LC AbM CDR3 (C33B1522)
AAWDDSLNGPV

SEQ ID NO: 430 LC AbM CDR3 (C33B1477)
MQALQTPPT

SEQ ID NO: 431 LC AbM CDR3 (C33B1475)
MQVLQTPWT

SEQ ID NO: 432 LC AbM CDR3 (C33B1517)
AAWDDSLNGPV

SEQ ID NO: 433 LC AbM CDR3 (C33B1516)
AAWDDSLNGPV

SEQ ID NO: 434 LC AbM CDR3 (C33B1481)
LQGTHWPWT

SEQ ID NO: 435 HC CHOTHIA CDR1 (C33B1522)
GGSISSY

SEQ ID NO: 436 HC CHOTHIA CDR1 (C33B1477)
GFTFSVS

SEQ ID NO: 437 HC CHOTHIA CDR1 (C33B1475)
GFTFSSY

SEQ ID NO: 438 HC CHOTHIA CDR1 (C33B1517)
GASIRNY

SEQ ID NO: 439 HC CHOTHIA CDR1 (C33B1516)
GGSIRNY

SEQ ID NO: 440 HC CHOTHIA CDR1 (C33B1481)
GITFSNY

SEQ ID NO: 441 HC CHOTHIA CDR2 (C33B1522)

YYSGS

SEQ ID NO: 442 HC CHOTHIA CDR2 (C33B1477)
RSKGNSYA

SEQ ID NO: 443 HC CHOTHIA CDR2 (C33B1475)
KQDGSE

SEQ ID NO: 444 HC CHOTHIA CDR2 (C33B1517)
YSTGN

SEQ ID NO: 445 HC CHOTHIA CDR2 (C33B1516)
FSTGH

SEQ ID NO: 446 HC CHOTHIA CDR2 (C33B1481)
KRDGSD

SEQ ID NO: 447 HC CHOTHIA CDR3 (C33B1522)
MWEILGFD

SEQ ID NO: 448 HC CHOTHIA CDR3 (C33B1477)
HNDKWNYYGLD

SEQ ID NO: 449 HC CHOTHIA CDR3 (C33B1475)
EVGYNWNQGGYFD

SEQ ID NO: 450 HC CHOTHIA CDR3 (C33B1517)
DNGAALFD

SEQ ID NO: 451 HC CHOTHIA CDR3 (C33B1516)
DNGAALFD

SEQ ID NO: 452 HC CHOTHIA CDR3 (C33B1481)
GEFD

SEQ ID NO: 453 LC CHOTHIA CDR1 (C33B1522)
SSSNIGSNP

SEQ ID NO: 454 LC CHOTHIA CDR1 (C33B1477)
SQSLLFSNGYKF

SEQ ID NO: 455 LC CHOTHIA CDR1 (C33B1475)
SQSLLHSDGYNY

SEQ ID NO: 456 LC CHOTHIA CDR1 (C33B1517)
SSSNIGSNI

SEQ ID NO: 457 LC CHOTHIA CDR1 (C33B1516)
SSSNIGSNI

SEQ ID NO: 458 LC CHOTHIA CDR1 (C33B1481)
SQSLVYSDGNTY

SEQ ID NO: 459 LC CHOTHIA CDR2 (C33B1522)
SNN

SEQ ID NO: 460 LC CHOTHIA CDR2 (C33B1477)
LGS

SEQ ID NO: 461 LC CHOTHIA CDR2 (C33B1475)
LGS

SEQ ID NO: 462 LC CHOTHIA CDR2 (C33B1517)
SNN

SEQ ID NO: 463 LC CHOTHIA CDR2 (C33B1516)
SDN

SEQ ID NO: 464 LC CHOTHIA CDR2 (C33B1481)
KVS

SEQ ID NO: 465 LC CHOTHIA CDR3 (C33B1522)
WDDSLNGP

SEQ ID NO: 466 LC CHOTHIA CDR3 (C33B1477)
ALQTPP

SEQ ID NO: 467 LC CHOTHIA CDR3 (C33B1475)
VLQTPW

SEQ ID NO: 468 LC CHOTHIA CDR3 (C33B1517)
WDDSLNGP

SEQ ID NO: 469 LC CHOTHIA CDR3 (C33B1516)
WDDSLNGP

SEQ ID NO: 470 LC CHOTHIA CDR3 (C33B1481)
GTHWPW

SEQ ID NO: 471 HC IMGT CDR1 (C33B1522)
GGSISSYY

SEQ ID NO: 472 HC IMGT CDR1 (C33B1477)
GFTFSVSA

SEQ ID NO: 473 HC IMGT CDR1 (C33B1475)
GFTFSSYW

SEQ ID NO: 474 HC IMGT CDR1 (C33B1517)
GASIRNYY

SEQ ID NO: 475 HC IMGT CDR1 (C33B1516)

GGSIRNYY

SEQ ID NO: 476 HC IMGT CDR1 (C33B1481)
GITFSNYW

SEQ ID NO: 477 HC IMGT CDR2 (C33B1522)
IYYSGST

SEQ ID NO: 478 HC IMGT CDR2 (C33B1477)
IRSKGNSYAT

SEQ ID NO: 479 HC IMGT CDR2 (C33B1475)
IKQDGSER

SEQ ID NO: 480 HC IMGT CDR2 (C33B1517)
IYSTGNI

SEQ ID NO: 481 HC IMGT CDR2 (C33B1516)
IFSTGHI

SEQ ID NO: 482 HC IMGT CDR2 (C33B1481)
IKRDGSDK

SEQ ID NO: 483 HC IMGT CDR3 (C33B1522)
ARMWEILGFDP

SEQ ID NO: 484 HC IMGT CDR3 (C33B1477)
TRHNDKWNYYGLDV

SEQ ID NO: 485 HC IMGT CDR3 (C33B1475)
AREVGYNWNQGGYFDY

SEQ ID NO: 486 HC IMGT CDR3 (C33B1517)
ARDNGAALFDY

SEQ ID NO: 487 HC IMGT CDR3 (C33B1516)
ARDNGAALFDF

SEQ ID NO: 488 HC IMGT CDR3 (C33B1481)
AKGEFDY

SEQ ID NO: 489 LC IMGT CDR1 (C33B1522)
SSNIGSNP

SEQ ID NO: 490 LC IMGT CDR1 (C33B1477)
QSLLFSNGYKF

SEQ ID NO: 491 LC IMGT CDR1 (C33B1475)
QSLLHSDGYNY

SEQ ID NO: 492 LC IMGT CDR1 (C33B1517)
SSNIGSNI

SEQ ID NO: 493 LC IMGT CDR1 (C33B1516)
SSNIGSNI

SEQ ID NO: 494 LC IMGT CDR1 (C33B1481)
QSLVYSDGNTY

SEQ ID NO: 495 LC IMGT CDR2 (C33B1522)
SNN

SEQ ID NO: 496 LC IMGT CDR2 (C33B1477)
LGS

SEQ ID NO: 497 LC IMGT CDR2 (C33B1475)
LGS

SEQ ID NO: 498 LC IMGT CDR2 (C33B1517)
SNN
SEQ ID NO: 499 LC IMGT CDR2 (C33B1516)
SDN

SEQ ID NO: 500 LC IMGT CDR2 (C33B1481)
KVS

SEQ ID NO: 501 LC IMGT CDR3 (C33B1522)
AAWDDSLNGPV

SEQ ID NO: 502 LC IMGT CDR3 (C33B1477)
MQALQTPPT

SEQ ID NO: 503 LC IMGT CDR3 (C33B1475)
MQVLQTPWT

SEQ ID NO: 504 LC IMGT CDR3 (C33B1517)
AAWDDSLNGPV

SEQ ID NO: 505 LC IMGT CDR3 (C33B1516)
AAWDDSLNGPV

SEQ ID NO: 506 LC IMGT CDR3 (C33B1481)
LQGTHWPWT

SEQ ID NO: 507 HC CONTACT CDR1 (C33B1522)
SSYYWG

SEQ ID NO: 508 HC CONTACT CDR1 (C33B1477)
SVSAIH

SEQ ID NO: 509 HC CONTACT CDR1 (C33B1475)
SSYWMT

SEQ ID NO: 510 HC CONTACT CDR1 (C33B1517)
RNYYWS

SEQ ID NO: 511 HC CONTACT CDR1 (C33B1516)
RNYYWS

SEQ ID NO: 512 HC CONTACT CDR1 (C33B1481)
SNYWMS

SEQ ID NO: 513 HC CONTACT CDR2 (C33B1522)
WIGYIYYSGSTN

SEQ ID NO: 514 HC CONTACT CDR2 (C33B1477)
WIGRIRSKGNSYATA

SEQ ID NO: 515 HC CONTACT CDR2 (C33B1475)
WVANIKQDGSERY

SEQ ID NO: 516 HC CONTACT CDR2 (C33B1517)
WLGHIYSTGNIH

SEQ ID NO: 517 HC CONTACT CDR2 (C33B1516)
WFGHIFSTGHIN

SEQ ID NO: 518 HC CONTACT CDR2 (C33B1481)
WVASIKRDGSDKY

SEQ ID NO: 519 HC CONTACT CDR3 (C33B1522)
ARMWEILGFD

SEQ ID NO: 520 HC CONTACT CDR3 (C33B1477)
TRHNDKWNYYGLD

SEQ ID NO: 521 HC CONTACT CDR3 (C33B1475)
AREVGYNWNQGGYFD

SEQ ID NO: 522 HC CONTACT CDR3 (C33B1517)
ARDNGAALFD

SEQ ID NO: 523 HC CONTACT CDR3 (C33B1516)
ARDNGAALFD

SEQ ID NO: 524 HC CONTACT CDR3 (C33B1481)
AKGEFD

SEQ ID NO: 525 LC CONTACT CDR1 (C33B1522)
IGSNPVNWY

SEQ ID NO: 526 LC CONTACT CDR1 (C33B1477)
LFSNGYKFLDWY

SEQ ID NO: 527 LC CONTACT CDR1 (C33B1475)
LHSDGYNYLDWY

SEQ ID NO: 528 LC CONTACT CDR1 (C33B1517)
IGSNIVNWY

SEQ ID NO: 529 LC CONTACT CDR1 (C33B1516)
IGSNIVNWY

SEQ ID NO: 530 LC CONTACT CDR1 (C33B1481)
VYSDGNTYLNWF

SEQ ID NO: 531 LC CONTACT CDR2 (C33B1522)
LLIYSNNQRP

SEQ ID NO: 532 LC CONTACT CDR2 (C33B1477)

LLIYLGSYRA

SEQ ID NO: 533 LC CONTACT CDR2 (C33B1475)
LLIYLGSYRA

SEQ ID NO: 534 LC CONTACT CDR2 (C33B1517)
LLIYSNNQRP

SEQ ID NO: 535 LC CONTACT CDR2 (C33B1516)
LLLYSDNQRP

SEQ ID NO: 536 LC CONTACT CDR2 (C33B1481)
RLIYKVSTRD

SEQ ID NO: 537 LC CONTACT CDR3 (C33B1522)
AAWDDSLNGP

SEQ ID NO: 538 LC CONTACT CDR3 (C33B1477)
MQALQTPP

SEQ ID NO: 539 LC CONTACT CDR3 (C33B1475)
MQVLQTPW

SEQ ID NO: 540 LC CONTACT CDR3 (C33B1517)
AAWDDSLNGP

SEQ ID NO: 541 LC CONTACT CDR3 (C33B1516)
AAWDDSLNGP

SEQ ID NO: 542 LC CONTACT CDR3 (C33B1481)
LQGTHWPW

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* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims. It is further to be understood that all values are approximate, and are provided for description.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

CLAIMS

1) An isolated protein that binds CD33, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 53;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263; or

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264.

2) The isolated protein of claim 1, comprising the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 29, 37, and 46, respectively;

b) SEQ ID NOs: 32, 40, and 49, respectively;

c) SEQ ID NOs: 34, 43, and 51, respectively;

d) SEQ ID NOs: 253, 256, and 259, respectively;

e) SEQ ID NOs: 254, 257, and 260, respectively; or

f) SEQ ID NOs: 255, 258, and 261, respectively.

3) An isolated protein that binds CD33, wherein the isolated protein comprises the HCDR1, the HCDR2, and the HCDR3 of

a) SEQ ID NOs: 29, 37, and 46, respectively;

b) SEQ ID NOs: 32, 40, and 49, respectively;

c) SEQ ID NOs: 34, 43, and 51, respectively;

d) SEQ ID NOs: 253, 256, and 259, respectively;

e) SEQ ID NOs: 254, 257, and 260, respectively; or

f) SEQ ID NOs: 255, 258, and 261, respectively.

4) The isolated protein of claim 1 or 2, wherein the isolated protein comprises

a) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 53 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 82;

b) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 56 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 85;

c) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 59 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 87;

d) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 262 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 271;

e) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 263 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 272; or

f) an HCDR1, an HCDR2 and an HCDR3 of a VH of SEQ ID NO: 264 and an LCDR1, an LCDR2 and an LCDR3 of a VL of SEQ ID NO: 273.

5) The isolated protein of claim 3 or 4, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

a) SEQ ID NOs: 29, 37, 46, 63, 70, and 76, respectively;

b) SEQ ID NOs: 32, 40, 49, 66, 70, and 76, respectively;

c) SEQ ID NOs: 34, 43, 51, 68, 74, and 80, respectively;

d) SEQ ID NOs: 253, 256, 259, 265, 74 and 269, respectively

e) SEQ ID NOs: 254, 257, 260, 66, 267 and 76, respectively; or

f) SEQ ID NOs: 255, 258, 261, 266, 268 and 270, respectively.

6) The isolated protein of any one of claims 1-5, wherein the isolated protein comprises the VH of SEQ ID NOs: 53, 56, 59, 262, 263, 264, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, or 382, and the VL of SEQ ID NOs: 82, 85, 87, 271, 272, 273, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397 or 398.

7) The isolated protein of claim 6, wherein the isolated protein comprises:

a) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 82;

b) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 85;

c) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 87;

d) the VH of SEQ ID NO: 262 and the VL of SEQ ID NO: 271;

e) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 272;

f) the VH of SEQ ID NO: 264 and the VL of SEQ ID NO: 273;

g) the VH of SEQ ID NO: 291 and the VL of SEQ ID NO: 271;

h) the VH of SEQ ID NO: 292 and the VL of SEQ ID NO: 271;

i) the VH of SEQ ID NO: 293 and the VL of SEQ ID NO: 271;

j) the VH of SEQ ID NO: 294 and the VL of SEQ ID NO: 271;

k) the VH of SEQ ID NO: 295 and the VL of SEQ ID NO: 271;

l) the VH of SEQ ID NO: 296 and the VL of SEQ ID NO: 271;

m) the VH of SEQ ID NO: 297 and the VL of SEQ ID NO: 271;

n) the VH of SEQ ID NO: 298 and the VL of SEQ ID NO: 271;
o) the VH of SEQ ID NO: 299 and the VL of SEQ ID NO: 271;
p) the VH of SEQ ID NO: 300 and the VL of SEQ ID NO: 271;
q) the VH of SEQ ID NO: 301 and the VL of SEQ ID NO: 271;
r) the VH of SEQ ID NO: 302 and the VL of SEQ ID NO: 271;
s) the VH of SEQ ID NO: 303 and the VL of SEQ ID NO: 271;
t) the VH of SEQ ID NO: 304 and the VL of SEQ ID NO: 271;
u) the VH of SEQ ID NO: 305 and the VL of SEQ ID NO: 271;
v) the VH of SEQ ID NO: 306 and the VL of SEQ ID NO: 271;
w) the VH of SEQ ID NO: 307 and the VL of SEQ ID NO: 271;
x) the VH of SEQ ID NO: 308 and the VL of SEQ ID NO: 271;
y) the VH of SEQ ID NO: 309 and the VL of SEQ ID NO: 271;
z) the VH of SEQ ID NO: 310 and the VL of SEQ ID NO: 271;
aa) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 311;
ab) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 312.
ac) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 313;
ad) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 314;
ae) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 315;
af) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 316;
ag) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 317;
ah) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 318;
ai) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 319;
aj) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 320;
ak) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 321;
al) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 322;
am) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 323;
an) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 324;
ao) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 325;
ap) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 326;
aq) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 327;
ar) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 328;
as) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 329;
at) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 330;
au) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 331;

av) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 332;
aw) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 333;
ax) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 334;
ay) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 335;
az) the VH of SEQ ID NO: 263 and the VL of SEQ ID NO: 336;
ba) the VH of SEQ ID NO: 337 and the VL of SEQ ID NO: 272;
bb) the VH of SEQ ID NO: 338 and the VL of SEQ ID NO: 272;
bc) the VH of SEQ ID NO: 339 and the VL of SEQ ID NO: 272;
bd) the VH of SEQ ID NO: 340 and the VL of SEQ ID NO: 272;
be) the VH of SEQ ID NO: 341 and the VL of SEQ ID NO: 272;
bf) the VH of SEQ ID NO: 342 and the VL of SEQ ID NO: 272;
bg) the VH of SEQ ID NO: 343 and the VL of SEQ ID NO: 272;
bh) the VH of SEQ ID NO: 344 and the VL of SEQ ID NO: 272;
bi) the VH of SEQ ID NO: 345 and the VL of SEQ ID NO: 272;
bj) the VH of SEQ ID NO: 346 and the VL of SEQ ID NO: 272;
bk) the VH of SEQ ID NO: 347 and the VL of SEQ ID NO: 272;
bl) the VH of SEQ ID NO: 348 and the VL of SEQ ID NO: 272;
bm) the VH of SEQ ID NO: 349 and the VL of SEQ ID NO: 272;
bn) the VH of SEQ ID NO: 350 and the VL of SEQ ID NO: 272;
bo) the VH of SEQ ID NO: 351 and the VL of SEQ ID NO: 272;
bp) the VH of SEQ ID NO: 352 and the VL of SEQ ID NO: 272.
bq) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 353;
br) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 354;
bs) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 355;
bt) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 356;
bu) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 357;
bv) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 358;
bw) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 359;
bx) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 360;
by) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 361;
bz) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 362;
ca) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 363;
cb) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 364;
cc) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 365;

cd) the VH of SEQ ID NO: 56 and the VL of SEQ ID NO: 366;
ce) the VH of SEQ ID NO: 367 and the VL of SEQ ID NO: 85;
cf) the VH of SEQ ID NO: 368 and the VL of SEQ ID NO: 85;
cg) the VH of SEQ ID NO: 369 and the VL of SEQ ID NO: 85;
ch) the VH of SEQ ID NO: 370 and the VL of SEQ ID NO: 85;
ci) the VH of SEQ ID NO: 371 and the VL of SEQ ID NO: 85;
cj) the VH of SEQ ID NO: 372 and the VL of SEQ ID NO: 85;
ck) the VH of SEQ ID NO: 373 and the VL of SEQ ID NO: 85;
cl) the VH of SEQ ID NO: 374 and the VL of SEQ ID NO: 85;
cm) the VH of SEQ ID NO: 375 and the VL of SEQ ID NO: 85;
cn) the VH of SEQ ID NO: 376 and the VL of SEQ ID NO: 85;
co) the VH of SEQ ID NO: 377 and the VL of SEQ ID NO: 85;
cp) the VH of SEQ ID NO: 378 and the VL of SEQ ID NO: 85;
cq) the VH of SEQ ID NO: 379 and the VL of SEQ ID NO: 85;
cr) the VH of SEQ ID NO: 380 and the VL of SEQ ID NO: 85;
cs) the VH of SEQ ID NO: 381 and the VL of SEQ ID NO: 85;
ct) the VH of SEQ ID NO: 382 and the VL of SEQ ID NO: 85;
cu) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 383;
cv) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 384;
cw) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 385;
cx) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 386;
cy) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 387;
cz) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 388;
da) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 389;
db) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 390;
dc) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 391;
dd) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 392;
de) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 393;
df) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 394;
dg) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 395;
dh) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 396;
di) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 397; or
dj) the VH of SEQ ID NO: 53 and the VL of SEQ ID NO: 398.

8) The isolated protein of any one of claims 1-7, wherein the isolated protein is a Fab, a scFv, a $(scFv)_2$, a Fv, a a $F(ab')_2$, a Fd, a dAb or a VHH.

9) The isolated protein of claim 8, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

10) The isolated protein of claim 9, wherein the L1 comprises:

a) an amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140;

b) about 5-50 amino acids; about 5-40 amino acids; about 10-30 amino acids; or about 10-20 amino acids.

11) The isolated protein of claim 10, wherein the isolated protein is a scFv comprising the amino acid sequence of SEQ ID NO: 213, 216, 219, 274, 275 or 276.

12) The isolated protein of any one of claims 1-11, wherein the protein is conjugated to a half-life-extending or -modulating moiety selected from: an immunoglobulin (Ig); a fragment of the Ig; an Ig constant region; a fragment of the Ig constant region; a Fc region; a fragment of the Ig constant region comprising a Fc region; a fragment of the Ig constant region comprising a CH2 domain; a fragment of the Ig constant region comprising a CH3 domain; a fragment of the Ig constant region comprising a CH2 domain and a CH3 domain; a fragment of the Ig constant region comprising at least a portion of a hinge, a CH2 domain and a CH3 domain; a fragment of the Ig constant region comprising a hinge, a CH2 domain and a CH3 domain; transferrin; albumin; an albumin binding domain; or polyethylene glycol.

13) The isolated protein of claims 12, comprising an antigen binding domain that binds CD33, wherein:

a) said antigen binding domain is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region;

b) said antigen binding domain is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region; or

c) said antigen binding domain is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2) comprising the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

14) The isolated protein of any one of claims 1-13, wherein the isolated protein is a monospecific protein, a multispecific protein, a bispecific protein, or a trispecific protein.

15) The isolated protein of claim 14, wherein the multispecific protein, the bispecific protein, or the trispecific protein, each independently, comprises an antigen binding domain that binds an antigen on: a lymphocyte, a T cell, a $CD8^+$ T cell, or a natural killer (NK) cell.

16) The isolated protein of claim 15, wherein the multispecific protein, the bispecific protein, or the trispecific protein, each independently, comprises an antigen binding domain that binds TRGV9, CD3 epsilon (CD3ε), CD3, CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C.

17) The isolated protein of any one of claims 15-16, wherein a first antigen binding domain that binds CD33 and/or a second antigen binding domain that binds the lymphocyte antigen comprise a Fab, a scFv, a VHH, a (scFv)2, a Fv, a F(ab')2, a Fd, or a dAb; or wherein the first antigen binding domain that binds CD33 comprises the scFv and the second antigen binding domain that binds the lymphocyte antigen comprises the VHH.

18) The isolated protein of any one of claims 12-17, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype, wherein optionally the Ig constant region or the fragment of the Ig constant region is an IgG1.

19) The isolated protein of claim 18, wherein the fragment of the Ig constant region has the sequence of SEQ ID NO: 277 or 278.

20) The isolated protein of any one of claims 12-19, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR); wherein the at least one mutation that results in reduced binding of the protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

21) The isolated protein of any one of claims 12-19, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the protein to the FcγR; wherein the at least one mutation that results in enhanced binding of the protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

22) The isolated protein of claims 20 or 21, wherein the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

23) The isolated protein of any one of claims 12-22, wherein the Ig constant region of the fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the protein selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

24) The isolated protein of any one of the claims 12-23, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

25) The isolated multispecific protein, the bispecific protein, or the trispecific protein, each independently, of any one of claims 14-24, wherein the first antigen binding domain that binds CD33 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

26) The isolated multispecific protein, the bispecific protein, or the trispecific protein, each independently, of claim 25, further comprising a second linker (L2) between the first antigen binding domain that binds CD33 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

27) The isolated multispecific protein, the bispecific protein, or the trispecific protein, each independently, of claim 26, wherein the L2 comprises the amino acid sequence of any one of SEQ ID NOs: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140.

28) The isolated bispecific protein of any one of claims 25-27, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

29) The isolated bispecific protein of any one of claims 25-28, wherein the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations:

L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

30) A chimeric antigen receptor (CAR) comprising:

a) an extracellular domain comprising the antigen binding domain that binds CD33 of any one of claims 1-11;

b) a transmembrane domain; and

c) an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

31) The CAR of claim 30, wherein the CAR further comprises a CD8a-hinge region.

32) The CAR of claims 30 or 31, wherein

a) the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide; and

b) the intracellular signaling domain comprises a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, and a combination thereof.

33) The CAR of any one of claims 30-32, wherein

a) the CD8a-hinge region comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 157;

b) the transmembrane domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 162; and/or

c) the intracellular signaling domain comprises a co-stimulatory domain having a CD137 component comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 163; a CD3z component comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 164; or an amino acid sequence of SEQ ID NO: 165.

34) The CAR of any one of claims 30-33, wherein the extracellular domain comprising the antigen binding domain that binds CD33 further comprises a signal polypeptide.

35) The CAR of claim 34, wherein the signal polypeptide comprises the amino acid sequence of SEQ ID NO: 166.

36) The CAR of any one of claims 30-35, wherein the CD8a-TM polypeptide comprises an amino acid sequence of SEQ ID NO: 162.

37) The CAR of any one of claims 30-36, wherein the CD8a-hinge region comprises an amino acid sequence of SEQ ID NO: 157.

38) An isolated lymphocyte expressing the CAR of any one of claims 30-37.

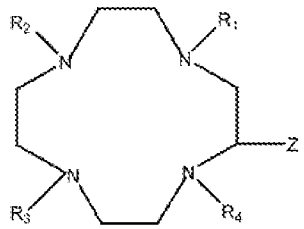
39) The isolated lymphocyte of claim 38, wherein the lymphocyte is a T lymphocyte or a natural killer (NK) cell.

40) An immunoconjugate comprising the isolated protein of any one of claims 1-29 or the CAR of any one of claims 30-37, conjugated to a therapeutic agent, an imaging agent, or a radioactive agent.

41) The immunoconjugate of claim 40, wherein the radioactive agent comprises a radiometal ion selected from ^{225}Ac, ^{111}In, ^{89}Zr, ^{32}P, ^{47}Sc, ^{67}Cu, ^{77}As, ^{89}Sr, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{131}I, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{169}Er, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{255}Fm, ^{227}Th, ^{62}Cu, ^{64}Cu, ^{67}Ga, ^{68}Ga, or ^{86}Y.

42) The immunoconjugate of claim 41, wherein the radiometal ion is conjugated to a chelating moiety,

(a) wherein the chelating moiety comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1-C_4 alkyl; and

Z is $(CH2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, Z is hydrogen; and

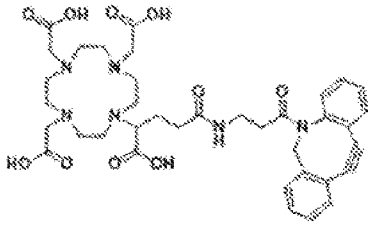
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1-C_4 alkyl or (C_1-C_2 alkyl) phenyl, and

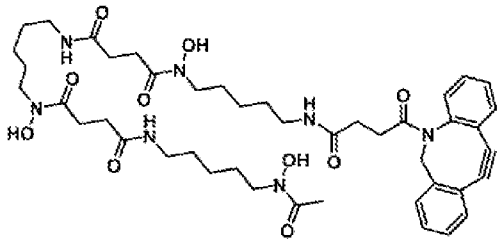
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand; or

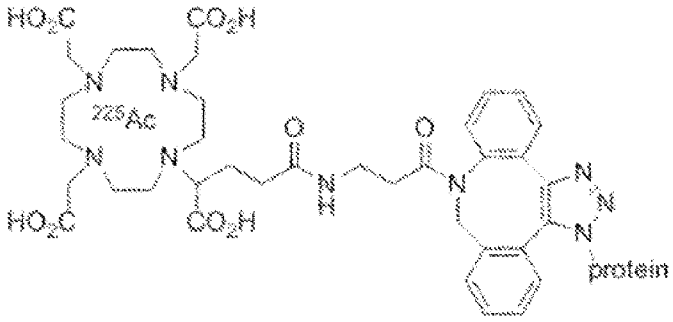
(b) wherein the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



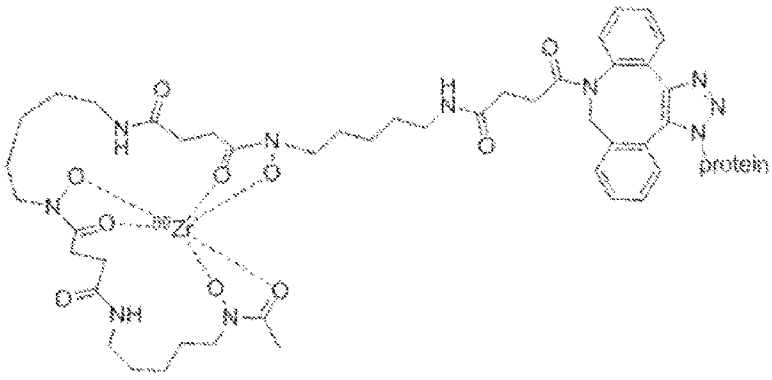
43) An immunoconjugate of formula (IV),



formula (IV)

wherein the protein is the isolated protein as defined in any one of claims 1-29, or the CAR as defined in any one of claims 30-37.

44) An immunoconjugate of formula (V),



formula (V)

wherein the protein is the isolated protein as defined in any one of claims 1-29, or the CAR as defined in any one of claims 30-37.

45) A pharmaceutical composition comprising the isolated protein of any one of claims 1-29, the CAR of any one of claims 30-37, the isolated lymphocyte of any one of claims 38-39, or the immunoconjugate of any one of claims 40-44, and a pharmaceutically acceptable carrier.

46) A polynucleotide encoding the isolated protein of any one of claims 1-29, the CAR of any one of claims 30-37, or the immunoconjugate of any one of claims 40-44.

47) A vector comprising the polynucleotide of claim 46.

48) A host cell comprising the vector of claim 47 or polynucleotide of claim 46.

49) A method of producing the isolated protein of any one of claims 1-29, comprising culturing the host cell of claim 48 in conditions that the protein is expressed, and recovering the protein produced by the host cell.

50) A method of producing the immunoconjugate of any one of claims 40-44, comprising culturing the host cell of claim 48 in conditions that the protein is expressed, recovering the protein produced by the host cell, and conjugating the protein produced by the host cell with the agent.

51) An anti-idiotypic antibody binding to the isolated protein of any one of claims 1-29.

52) A kit comprising the isolated protein of any one of claims 1-29, or the CAR of any one of claims 30-37.

53) A method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the isolated protein of any one of claims 1-29, or immunoconjugate of any one of claims 40-44, to the subject for a time sufficient to treat the CD33 expressing cancer.

54) A method of treating a subject having a CD33 expressing cancer, the method comprising: administering a therapeutically effective amount of the lymphocyte of any one of claims 38-39 to a subject in need thereof, whereby the lymphocyte mediates killing of the CD33 expressing cancer in the subject.

55) The method of any one of claims 53-54, wherein the CD33 expressing cancer is a hematologic cancer.

56) The method of claim 55, wherein the hematologic cancer is a leukemia, a lymphoma, a multiple myeloma, 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 (DPDCN).

57) The method of any one of claims 53-56, wherein the isolated protein, or the immunoconjugate, is administered in combination with a second therapeutic agent.

58) The method of claim 57, wherein the second therapeutic agent is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.

59) The isolated protein of any one of claims 1-29, or the immunoconjugate of any one of claims 40-44, for use in a method of treating a CD33 expressing cancer in a subject, comprising administering a therapeutically effective amount of the to the subject for a time sufficient to treat the CD33 expressing cancer.

60) The lymphocyte of any one of claims 38-39, for use in a method of treating a subject having a CD33 expressing cancer, the method comprising: administering a therapeutically effective amount of the lymphocyte of any one of claims 38-39 to a subject in need thereof, whereby the lymphocyte mediates killing of the CD33 expressing cancer in the subject.

61) The isolated protein, the immunoconjugate, or the lymphocyte for the use of claims 59 or 60, wherein the CD33 expressing cancer is a hematologic cancer.

62) The isolated protein, the immunoconjugate, or the lymphocyte for the use of claim 61, wherein the hematologic cancer is a leukemia, a lymphoma, a multiple myeloma, 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 (DPDCN).

63) The isolated protein, the immunoconjugate, or the lymphocyte for the use of any one of claims 59-62, wherein the isolated protein, or the immunoconjugate, is administered in combination with a second therapeutic agent.

64) The isolated protein, the immunoconjugate, or the lymphocyte for the use of claim 63, wherein the second therapeutic agent is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.

65) A method of detecting the presence of a cancer in a subject, comprising:

a) contacting a cell sample obtained from the subject with the CAR of any one of claims 30-37, thereby forming a CAR-cell complex, and

b) detecting the CAR-cell complex, wherein detection of the CAR-cell complex is indicative of the presence of the cancer in the subject.