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, t~~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~~examples, 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 examples~~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 examples~~embodiments~~, the isolated protein is the VHH. In some examples~~embodiments~~, the isolated

protein is the Fab. In some examples~~embodiments~~, the isolated protein is the scFv. In some examples~~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~~example, 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 example~~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 examples~~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 example~~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 one aspect, the disclosure provides an isolated protein that binds CD33, wherein the isolated protein 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 some examples~~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 examples~~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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein is the Fab. In some ~~embodiment~~examples, the isolated protein is the VHH. In some ~~embodiment~~examples, the isolated protein is the scFv. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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;

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~~example, 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;

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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein is the Fab. In some ~~embodiment~~examples, the isolated protein is the VHH. In some ~~embodiment~~examples, the isolated protein is the scFv. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some ~~embodiment~~examples, 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 example ~~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 ~~embodiment~~examples, 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 example ~~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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein is the Fab.

In some ~~embodiment~~examples, the isolated protein is the VHH.

In some ~~embodiment~~examples, the isolated protein is the scFv.

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

In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 example ~~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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein is the Fab. In some ~~embodiment~~examples, the isolated protein is the VHH. In some ~~embodiment~~examples, the isolated protein is the scFv. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein is a monospecific protein. In some ~~embodiment~~examples, the isolated protein is a multispecific protein. In some ~~embodiment~~examples, the multispecific protein is a bispecific protein. In some ~~embodiment~~examples, the multispecific protein is a trispecific protein. In some ~~embodiment~~examples, the multispecific protein further comprises an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises a Fc region. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises a CH2 domain. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises a CH3 domain. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain. In some ~~embodiment~~examples, the fragment of the Ig constant region comprises a hinge, the CH2 domain and the CH3 domain. In some ~~embodiment~~examples, the isolated protein is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region. In some ~~embodiment~~examples, the isolated protein is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte. In some ~~embodiment~~examples, the lymphocyte is a T cell. In some ~~embodiment~~examples, the T cell is a $CD8^+$ T cell. In some ~~embodiment~~examples, the lymphocyte is a natural killer (NK) cell. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds CD3ε. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds TRGV9. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the protein comprises at least one mutation in a CH3 domain of the Ig constant region. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the CAR further comprises a CD8a-hinge region. In some ~~embodiment~~examples,

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 ~~embodiment~~examples,

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 ~~embodiment~~examples, 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 example~~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 ~~embodiment~~examples, the lymphocyte antigen is a T cell antigen. In some ~~embodiment~~examples, the T cell antigen is a $CD8^+$ T cell antigen. In some ~~embodiment~~examples, the lymphocyte antigen is a NK cell antigen. In some ~~embodiment~~examples, the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, TRGV9, or NKG2C. In certain ~~embodiment~~examples, the lymphocyte antigen is CD3ε. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the L1 comprises the amino acid sequence of SEQ ID NO: 108. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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. In another aspect, the ~~The~~ disclosure provides a pharmaceutical composition comprising any of the above isolated proteins and a pharmaceutically acceptable carrier.

In another aspect, the ~~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. In another aspect, the disclosure provides a vector comprising the polynucleotide of the above. 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 host cell comprising the vector of the above or polynucleotide of the above.

In another example ~~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 example ~~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 example ~~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 example ~~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 the isolated protein of the above, for use in treating a CD33 expressing cancer in a subject, wherein the CD33 expressing cancer is a hematologic cancer selected from 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).

In another aspect, the disclosure provides use of the isolated protein of the above in the manufacture of a medicament for the treatment of a a CD33 expressing cancer, wherein the CD33 expressing cancer is a hematologic cancer selected from 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).

In another example ~~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 ~~embodiment~~examples, the CD33 expressing cells are cancerous cells. In some ~~embodiment~~examples, the CD33 expressing cells are not cancerous cells.

In another example ~~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 example ~~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 ~~embodiment~~examples of the above ~~three aspects~~, the CD33 expressing cancer is a hematologic cancer. In some ~~embodiment~~examples, the hematologic cancer is a leukemia, a lymphoma, or a multiple myeloma. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the isolated protein or the isolated multispecific protein is administered in combination with a second therapeutic agent. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the CAR further comprises a CD8a-hinge region.

a) In some ~~embodiment~~examples, 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 ~~embodiment~~examples,

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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the antigen binding domain that binds CD33 is a VHH.

In some ~~embodiment~~examples, the antigen binding domain that binds CD33 is a scFv.

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

In certain ~~embodiment~~examples, 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 ~~embodiment~~examples, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In some ~~embodiment~~examples, the extracellular domain of the CAR comprising the antigen binding domain that binds CD33 further comprises a signal polypeptide. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the CD137 component comprises the amino acid sequence of SEQ ID NO: 163. In some ~~embodiment~~examples, the CD3z component comprises an

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

In some ~~embodiment~~examples, the CAR is a multispecific protein. In some ~~embodiment~~examples, the multispecific protein is a bispecific protein. In some ~~embodiment~~examples, the multispecific protein is a trispecific protein. In some ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds an antigen on a lymphocyte. In some ~~embodiment~~examples, the lymphocyte is a T cell. In some ~~embodiment~~examples, the T cell is a $CD8^+$ T cell. In some ~~embodiment~~examples, the lymphocyte is a natural killer (NK) cell. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds CD3ε. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the multispecific protein comprises an antigen binding domain that binds TRGV9. In some ~~embodiment~~examples, 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 exampleaspect, the disclosure provides an isolated lymphocyte expressing any of the CARs above. In some embodimentexamples, the lymphocyte is a T lymphocyte. In some embodimentexamples, the lymphocyte is a natural killer (NK) cell. In another exampleaspect, 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 exampleaspect, 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 embodimentexamples, the CD33 expressing cancer is a hematologic cancer. In some embodimentexamples, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodimentexamples, 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 example 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 embodimentexamples, the cancer cell is a hematologic cancer. In some embodimentexamples, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some embodimentexamples, 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).

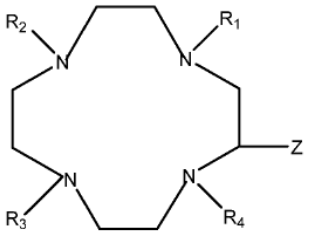
In another example ~~aspect~~ is provided a method of detecting the presence of a cancer in a subject, comprising:

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 ~~embodiment~~examples, the cancer cell is a hematologic cancer. In some ~~embodiment~~examples, the CD33 expressing cancer is a leukemia, a lymphoma, or a multiple myeloma. In some ~~embodiment~~examples, 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 example ~~aspect~~ is provided an immunoconjugate comprising any of the above isolated proteins conjugated to a radioactive agent. In some ~~embodiment~~examples, the radioactive agent comprises a radiometal ion. In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the radiometal ion is ^{225}Ac, ^{111}In or ^{89}Zr. In certain ~~embodiment~~examples, the radiometal ion is ^{225}Ac. In some ~~embodiment~~examples, the radiometal ion of the immunoconjugate is conjugated to a chelating moiety. In some ~~embodiment~~examples, 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

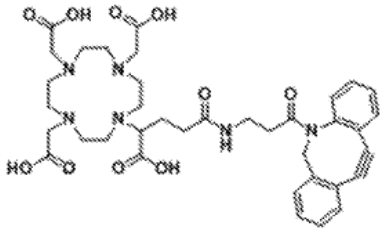
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

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

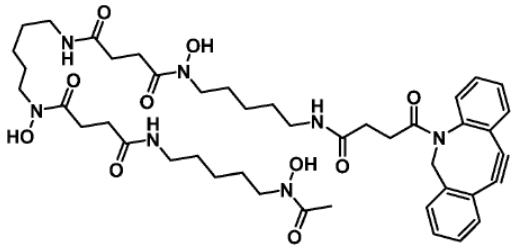
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand.

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

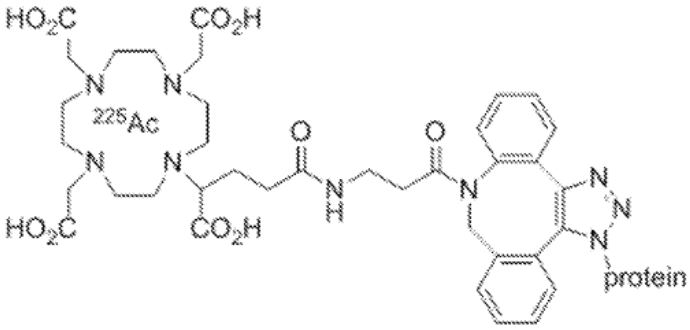


or a structure of formula (III):



.

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),

CLAIMS

1) An isolated protein that binds CD33, wherein the isolated protein 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.

~~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.

62) The isolated protein of ~~any one of claims 1-5~~claim 1, 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~~3) The isolated protein of any one of claims 1-~~7~~2, 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~~4) The isolated protein of claim ~~8~~3, wherein the isolated protein is a scFv comprising ~~scFv comprises~~, from the N- to C-terminus, (i) ~~a~~the VH, a first linker (L1), and ~~a~~the VL (VH-L1-VL), or (ii) the VL, the L1, and the VH (VL-L1-VH); and optionally 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; and/or

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

~~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~~5) The isolated protein of claim ~~10~~4, wherein the isolated protein is a scFv comprising the amino acid sequence of SEQ ID NO: 213~~, 216, 219, 274, 275 or 276~~.

~~12~~6) The isolated protein of any one of claims 1-~~11~~4, 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~~7) The isolated protein of claim~~s~~ ~~12~~6, 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~~8) The isolated protein of any one of claims 1-~~13~~7, wherein the isolated protein is a monospecific protein, a multispecific protein, a bispecific protein, or a trispecific protein; optionally 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; optionally 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.

~~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~~9) The isolated protein of ~~any one of claims 15-16~~claim 8, 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~~10) The isolated protein of any one of claims ~~12-17~~6-9, 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; optionally wherein the fragment of the Ig constant region has the sequence of SEQ ID NO: 277 or 278.

~~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.

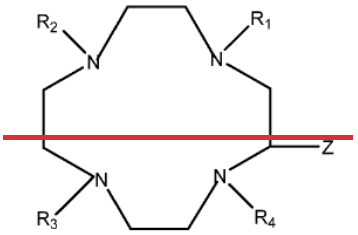
39) The isolated lymphocyte of claim 38, wherein the lymphocyte is a T lymphocyte or a natural killer (NK) cell.

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

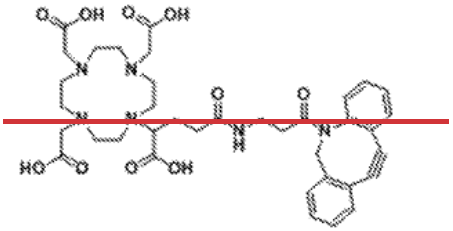
each of R_1, R_2, R_3 and R_4 is independently $CHQCO_2X$, wherein

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

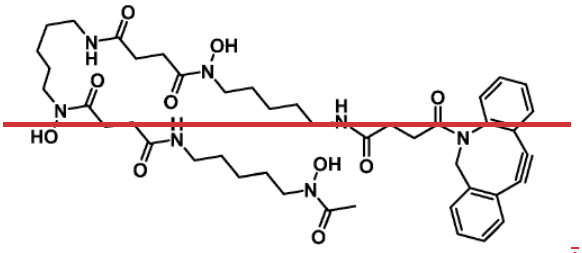
X is independently hydrogen, benzyl, C_1-C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, the chelant comprises an open chain ligand; or

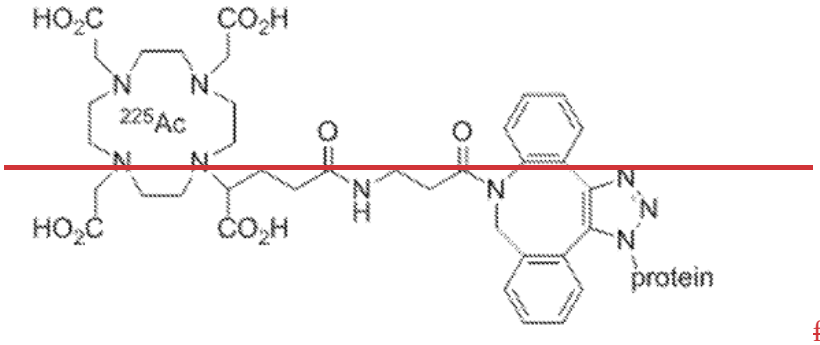
(b) wherein the chelating moiety comprises a macrocycle having the structure of formula (II):



or a structure of formula (III):



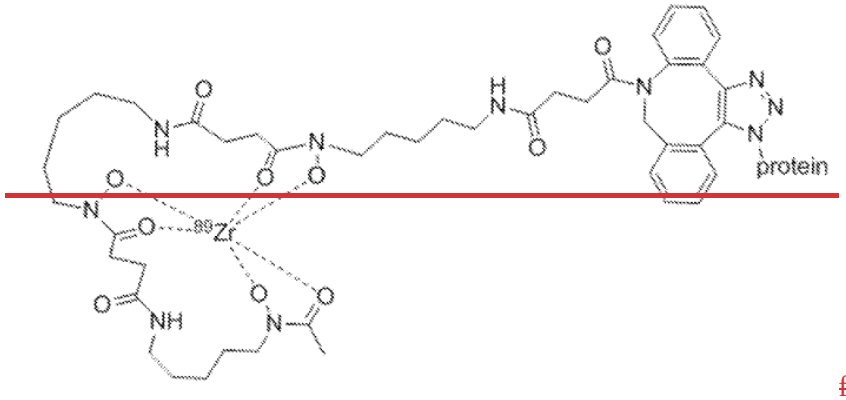
43) An immunoconjugate of formula (IV),



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.

4511) A pharmaceutical composition comprising the isolated protein of any one of claims 1-2910, 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.

4612) A polynucleotide encoding the isolated protein of any one of claims 1-2910, the CAR of any one of claims 30-37, or the immunoconjugate of any one of claims 40-44.

4713) A vector comprising the polynucleotide of claim 4612.

4814) A host cell comprising the vector of claim 47 13 or polynucleotide of claim 4612.

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~~15) The isolated protein of any one of claims 1-~~29~~10, ~~or the immunoconjugate of any one of claims 40-44,~~ for use in ~~a method of~~ treating a CD33 expressing cancer in a subject, wherein the CD33 expressing cancer is a hematologic cancer selected from 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)~~comprising administering a therapeutically effective amount of the to the subject for a time sufficient to treat the CD33 expressing cancer~~.

16) Use of the isolated protein of any one of claims 1-10 in the manufacture of a medicament for the treatment of a a CD33 expressing cancer, wherein the CD33 expressing cancer is a hematologic cancer selected from 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).

~~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.