(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 August 2020 (27.08.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/172571 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2020/019291

(22) International Filing Date:
21 February 2020 (21.02.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/808,646 21 February 2019 (21.02.2019) US

(71) Applicant: **ELSTAR THERAPEUTICS, INC.** [US/US]; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US).

(72) Inventors: **LOEW, Andreas**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **MALHOTRA, Nidhi**; 840 Memorial Drive, 4th Floor Cambridge, Cambridge, MA 02139 (US). **KATRAGADDA, Madan**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **TAN, Seng-lai**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **HSU, Jonathan**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **VASH, Brian, Edward**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **MAIOCCO, Stephanie, J.**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **MAREK, Peter**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US). **GUNTAS, Gurkan**; 840 Memorial Drive, 4th Floor, Cambridge, MA 02139 (US).

(74) Agent: **COLLAZO, Diana, M.** et al.; Lando & Anastasi, LLP, 60 State Street, 23th Floor, Boston, MA 02109 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: MULTIFUNCTIONAL MOLECULES THAT BIND TO T CELL RELATED CANCER CELLS AND USES THEREOF

(57) Abstract: Multifunctional molecules that include i) an antigen binding domain that binds to a T cell receptor beta chain constant domain 1 or T cell receptor beta chain constant domain 2; and one, two or all of: (ii) an immune cell engager (e.g., chosen from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager); (iii) a cytokine molecule or cytokine inhibitor molecule; (iv) a death receptor signal enhancer; and/or (v) a stromal modifying moiety are disclosed. Additionally disclosed are nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a cancer using the aforesaid molecules.

WO 2020/172571 A1

MULTIFUNCTIONAL MOLECULES THAT BIND TO T CELL RELATED CANCER CELLS AND USES THEREOF

RELATED APPLICATION

This application claims priority to U.S. Serial No. 62/808,646 filed February 21, 2019, the contents of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 19, 2020, is named E2070-7022WO_SL.txt and is 810,998 bytes in size.

BACKGROUND

Lymphomas are cancers that arise from lymphocytes. T cell lymphoma (TCL) is a lymphoma that arises from T cells; these account for approximately 7% of all non-Hodgkin's lymphomas in the United States. Common subtypes of TCL include: Peripheral T Cell Lymphoma, Not Otherwise Specified (PTCLNOS), Anaplastic Large Cell Lymphoma (ALCL), Angioimmunoblastic T Cell Lymphoma (AITL), and Cutaneous T Cell Lymphoma (CTCL). Each type of TCL has its own pathology and symptoms. Given the ongoing need for improved treatment of lymphomas such as TCLs, new compositions and treatments targeting lymphomas, e.g., TCLs, are highly desirable.

SUMMARY OF THE INVENTION

The disclosure relates, inter alia, to novel multispecific or multifunctional molecules that include (i) an antigen binding domain that binds to a tumor antigen on a lymphoma cell (e.g., a T cell), e.g., a T cell receptor comprising T cell receptor beta chain constant domain 1 (TRBC1) or a T cell receptor comprising T cell receptor beta chain constant domain 2 (TRBC2); and one, two or all of: (ii) an immune cell engager (e.g., chosen from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager); (iii) a cytokine molecule;

and/or (iv) a stromal modifying moiety. The terms "multispecific" or "multifunctional" are used interchangeably herein.

Without wishing to be bound by theory, the multispecific or multifunctional molecules disclosed herein are expected to target (e.g., localize, bridge and/or activate) an immune cell (e.g., an immune effector cell chosen from an NK cell, a T cell, a B cell, a dendritic cell or a macrophage), at a target cell, e.g., a cancer cell (e.g., a lymphoma cell), expressing a T cell receptor comprising TRBC1 or TRBC2, and/or alter the tumor stroma, e.g., alter the tumor microenvironment near the cancer site. Increasing the proximity and/or activity of the immune cell using the multispecific molecules described herein is expected to enhance an immune response against the target cell (e.g., the cancer cell, e.g., lymphoma cell), thereby providing a more effective therapy (e.g., a more effective cancer therapy). Without being bound by theory, a targeted, localized immune response against the target cell (e.g., the cancer cell) is believed to reduce the effects of systemic toxicity of the multispecific molecules described herein. Furthermore, in the case where the target cancer cell is a T cell (e.g., a T cell expressing a T cell receptor comprising TRBC1 or TRBC2), a targeted immune response against the cancerous T cell population that targets non-cancerous T cells to a lesser degree (e.g., does not target non-cancerous T cells) is believed to have fewer deleterious effects than systemic ablation of all T cells.

Without wishing to be bound by theory, clonally derived T cell lymphomas are positive for either TRBC1 or TRBC2, but not both. In the case of TRBC1+ T cell malignancies, an anti-TRBC1 molecule disclosed herein (e.g., a multifunctional molecule that binds to TRBC1 and NKp30) may deplete TRBC1+ cells while sparing TRBC2+ non-malignant T cells. Similarly, in the case of TRBC2+ T cell malignancies, an anti-TRBC2 molecule disclosed herein (e.g., a multifunctional molecule that binds to TRBC2 and NKp30) may deplete TRBC2+ cells while sparing TRBC1+ non-malignant T cells.

Without wising to be bound by theory, in some embodiments, a multifunctional molecule disclosed herein (e.g., anti-TRBC1/NKp30 antibody) only activates NK cells in the presence of a TRBC1-expressing cell. Without wising to be bound by theory, in some embodiments, a multifunctional molecule disclosed herein (e.g., anti-TRBC2/NKp30 antibody) only activates NK cells in the presence of a TRBC2-expressing cell.

Accordingly, provided herein are, *inter alia*, multispecific molecules (e.g., multispecific or multifunctional antibody molecules) that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a cancer using the aforesaid molecules.

In one aspect, provided herein is a multifunctional molecule comprising (i) a first antigen binding domain that binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2), and (ii) a second antigen binding domain that binds to NKp30.

In some embodiments, the first antigen binding domain binds to TRBC1. In some embodiments, the first antigen binding domain comprises one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the first antigen binding domain comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3, wherein the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of: SEQ ID NOs: 7346, 7355, and 202, respectively; SEQ ID NOs: 7346, 201, and 202, respectively; SEQ ID NOs: 7354, 201, and 202, respectively; or SEQ ID NOs: 7354, 7355, and 202, respectively. In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of: SEQ ID NOs: 223, 224, and 225, respectively; SEQ ID NOs: 7367, 224, and 225, respectively; SEQ ID NOs: 223, 7368, and 225, respectively; SEQ ID NOs: 223, 224, and 7369, respectively; or SEQ ID NOs: 7367, 7368, and 7369, respectively. In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of: SEQ ID NOs: 7346, 7355, 202, 223, 224, and 225, respectively; SEQ ID NOs: 7346, 201, 202, 223, 224, and 225, respectively; SEQ ID NOs: 7346, 7355, 202, 7367, 224, and 225, respectively; SEQ ID NOs: 7346, 7355, 202, 223, 7368, and 225, respectively; SEQ ID NOs: 7346, 7355, 202, 223, 224, and 7369, respectively; SEQ ID NOs: 7346, 7355, 202, 7367, 7368, and 7369, respectively; SEQ ID NOs: 7346, 201, 202, 7367, 224, and 225, respectively; SEQ ID

NOs: 7346, 201, 202, 223, 7368, and 225, respectively; SEQ ID NOs: 7346, 201, 202, 223, 224, and 7369, respectively; SEQ ID NOs: 7346, 201, 202, 7367, 7368, and 7369, respectively; SEQ ID NOs: 7354, 201, 202, 223, 224, and 225, respectively; SEQ ID NOs: 7354, 201, 202, 7367, 224, and 225, respectively; SEQ ID NOs: 7354, 201, 202, 223, 7368, and 225, respectively; SEQ ID NOs: 7354, 201, 202, 223, 224, and 7369, respectively; SEQ ID NOs: 7354, 201, 202, 7367, 7368, and 7369, respectively; SEQ ID NOs: 7354, 7355, 202, 223, 224, and 225, respectively; SEQ ID NOs: 7354, 7355, 202, 7367, 224, and 225, respectively; SEQ ID NOs: 7354, 7355, 202, 223, 7368, and 225, respectively; SEQ ID NOs: 7354, 7355, 202, 223, 224, and 7369, respectively; or SEQ ID NOs: 7354, 7355, 202, 7367, 7368, and 7369, respectively. In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7351, 253, 250-252, 254, 7343, 7344, 7350, and 7352 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 258, 255-257, 259, 260, and 7357-7360 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of: SEQ ID NOs: 7351 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or SEQ ID NOs: 253 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors not comprising TRBC1, optionally wherein the KD for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the KD for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC1. In some embodiments, the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors comprising TRBC2, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC2. In some embodiments, binding of the first antigen binding domain to TRBC1 on a lymphoma cell or lymphocyte, e.g., T cell, does not appreciably activate the lymphoma cell or lymphocyte, e.g., T cell, (e.g., as measured by T cell proliferation, expression of a T cell activation marker (e.g., CD69 or CD25), and/or expression of a cytokine (e.g., TNFα

and IFNγ). In some embodiments, the multifunctional molecule does not activate NK cells or does not substantially activate NK cells in the absence of a TRBC1-expressing cell.

In some embodiments, the first antigen binding domain binds to TRBC2. In some embodiments, the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than for T cell receptors not comprising TRBC2, optionally wherein the KD for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the KD for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC2. In some embodiments, the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than for T cell receptors comprising TRBC1, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC1. In some embodiments, binding of the first antigen binding domain to TRBC2 on a lymphoma cell or lymphocyte, e.g., T cell, does not appreciably activate the lymphoma cell or lymphocyte, e.g., T cell, (e.g., as measured by T cell proliferation, expression of a T cell activation marker (e.g., CD69 or CD25), and/or expression of a cytokine (e.g., TNFα and IFNγ). In some embodiments, the multifunctional molecule does not activate NK cells or does not substantially activate NK cells in the absence of a TRBC2-expressing cell.

In some embodiments, the second antigen binding domain comprises one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 7-10, 18, and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the second antigen binding domain comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3, wherein the VHCDR1, VHCDR2, and VHCDR3 of the second antigen binding domain comprise the amino acid sequences of: SEQ ID NOs: 7313, 6001, and 7315, respectively; SEQ ID NOs: 7313, 6001, and 6002, respectively; SEQ ID NOs: 7313, 6008, and 6009, respectively; SEQ ID NOs: 7313, 7385, and 7315, respectively; or SEQ ID NOs: 7313, 7318, and 6009, respectively. In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 of the second antigen binding domain comprise the amino acid sequences of: SEQ ID NOs: 7326,

7327, and 7329, respectively; SEQ ID NOs: 6063, 6064, and 7293, respectively; SEQ ID NOs: 6070, 6071, and 6072, respectively; or SEQ ID NOs: 6070, 6064, and 7321, respectively. In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 of the second antigen binding domain comprise the amino acid sequences of: SEQ ID NOs: 7313, 6001, 7315, 7326, 7327, and 7329, respectively; SEQ ID NOs: 7313, 6001, 6002, 6063, 6064, and 7293, respectively; SEQ ID NOs: 7313, 6008, 6009, 6070, 6071, and 6072, respectively; SEQ ID NOs: 7313, 7385, 7315, 6070, 6064, and 7321, respectively; or SEQ ID NOs: 7313, 7318, 6009, 6070, 6064, and 7321, respectively. In some embodiments, the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7302, 7298, 7300, 7301, 7303, and 7304 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7309, 7305, 7299, 7306-7308 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6121 or 6123-6128 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7294 or 6137-6141 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6122 or 6129-6134 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6136 or 6142-6147 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL of the second antigen binding domain comprise the amino acid sequences of: SEQ ID NOs: 7302 and 7309, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or SEQ ID NOs: 7302 and 7305, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the second antigen binding domain comprise the amino acid sequences of: SEQ ID NO: 7311 or 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NO: 6187

or 6188 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or SEQ ID NO: 6189 or 6190 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the multifunctional molecule binds to TRBC1 or TRBC2 monovalently. In some embodiments, the multifunctional molecule comprises a configuration shown in any of FIGs. 29A-29D, optionally wherein: (i) the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29A; (ii) the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 29B; (iii) the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC1 scFv, e.g., comprises a configuration shown in FIG. 29C; or (iv) the multifunctional antibody molecule comprises an anti-TRBC1 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29D. In some embodiments, the multifunctional molecule comprises a configuration shown in any of FIGs. 30A-30D, optionally wherein: (i) the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30A; (ii) the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 30B; (iii) the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC2 scFv, e.g., comprises a configuration shown in FIG. 30C; or (iv) the multifunctional antibody molecule comprises an anti-TRBC2 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30D.

In some embodiments, a multifunctional molecule disclosed herein further comprises a dimerization module comprising one or more immunoglobulin chain constant regions (e.g., Fc regions) comprising one or more of: a paired cavity-protuberance ("knob-in-a hole"), an electrostatic interaction, or a strand-exchange.

In some embodiments, the multifunctional molecule comprises an anti-TRBC1 amino acid sequence disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto, and/or an anti-NKp30 amino acid sequence disclosed in any of Tables 7-10, 18, and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the multifunctional molecule comprises: (i) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity

thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); (ii) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or (iii) SEQ ID NOs: 7382, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the multifunctional molecule comprises: (i) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); (ii) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or (iii) SEQ ID NOs: 7379, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the multifunctional molecule comprises: (i) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); (ii) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or (iii) SEQ ID NOs: 7382, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the multifunctional molecule comprises: (i) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%,

95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); (ii) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or (iii) SEQ ID NOs: 7379, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the multifunctional molecule comprises: a heavy chain constant region variant, e.g., an Fc region variant, that comprises one or more mutations that result in reduced or ablated affinity for at least one Fc receptor, optionally wherein the one or more mutations result in reduced or ablated antibody dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP), or complement dependent cytotoxicity (CDC). In some embodiments, the Fc region variant comprises one or more mutations disclosed in Table 20, optionally wherein the Fc region variant comprises an N297A mutation.

In one aspect, provided herein is an antibody molecule that binds to TRBC1, comprising one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

In one aspect, provided herein is an antibody molecule that binds to NKp30, comprising one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 7-10, 18, and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

In some embodiments, the antibody molecule comprises a heavy chain constant region variant, e.g., an Fc region variant, that comprises one or more mutations that result in reduced or ablated affinity for at least one Fc receptor, optionally wherein the one or more mutations result in reduced or ablated antibody dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP), or complement dependent cytotoxicity (CDC). In

some embodiments, the Fc region variant comprises one or more mutations disclosed in Table 20, optionally wherein the Fc region variant comprises an N297A mutation.

In one aspect, provide herein is a nucleic acid molecule encoding a multifunctional molecule disclosed herein or an antibody molecule disclosed herein. In one aspect, provide herein is a vector, e.g., an expression vector, comprising a nucleic acid molecule disclosed herein. In one aspect, provide herein is a cell comprising a nucleic acid molecule disclosed herein or a vector disclosed herein. In one aspect, provide herein is a pharmaceutical composition comprising a multifunctional molecule disclosed herein or an antibody molecule disclosed herein and a pharmaceutically acceptable carrier, excipient, or stabilizer.

In one aspect, provide herein is a method of making, e.g., producing, a multifunctional molecule disclosed herein or an antibody molecule disclosed herein, comprising culturing a cell disclosed herein, under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

In one aspect, provide herein is a method of treating a cancer, comprising administering to a subject in need thereof a multifunctional molecule disclosed herein or an antibody molecule disclosed herein, wherein the multifunctional molecule or antibody molecule is administered in an amount effective to treat the cancer. In some embodiments, the method further comprises identifying, evaluating, or selecting a subject in need of treatment, wherein identifying, evaluating, or selecting comprises determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2. In some embodiments, the method further comprises: responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC1: optionally, selecting the subject for treatment with a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1, and administering a multifunctional molecule disclosed herein comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1. In some embodiments, the method further comprises: responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC2: optionally, selecting the subject

for treatment with a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC2, and administering a multifunctional molecule disclosed herein comprising an antigen binding domain that binds to a T cell receptor comprising TRBC2.

In one aspect, provide herein is a method of treating a cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, comprising: responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC1, administering to the subject a multifunctional molecule disclosed herein, wherein the first antigen binding domain of the multifunctional molecule binds to TRBC1, wherein the multifunctional molecule is administered in an amount effective to treat the cancer. In one aspect, provide herein is a method of treating a cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, comprising: responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC2, administering to the subject a multifunctional molecule disclosed herein, wherein the first antigen binding domain of the multifunctional molecule binds to TRBC2, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

In one aspect, provide herein is a method of identifying a subject in need of treatment for cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, using a multifunctional molecule disclosed herein, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2, wherein:

responsive to a determination that the subject has cancer cells that express a T cell receptor comprising TRBC1, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, or

responsive to a determination that the subject has cancer cells that express a T cell receptor comprising TRBC2, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1.

In some embodiments, the method further comprises:

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, or

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC2.

In some embodiments of the aforementioned methods, the cancer is leukemia or lymphoma. In some embodiments, the cancer is selected from Acquired immune deficiency syndrome (AIDS)-associated lymphoma, Angioimmunoblastic T-cell lymphoma, Adult T-cell leukemia/lymphoma, Burkitt lymphoma, Central nervous system (CNS) lymphoma, Diffuse large B-cell lymphoma (DLBCL), Lymphoblastic lymphoma, Mantle cell lymphoma (MCL), Peripheral T-cell lymphoma (PTCL) (e.g., Hepatosplenic T-cell lymphoma (HSGDTCL), Subcutaneous paniculitis-like T-cell lymphoma, or Enteropathy-associated T-cell lymphoma), Transformed follicular and transformed mucosa-associated lymphoid tissue (MALT) lymphomas, Cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome), Follicular lymphoma, Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, Marginal zone B-cell lymphoma, Gastric mucosa-associated lymphoid tissue (MALT) lymphoma, Chronic lymphocytic leukemia/small-cell lymphocytic lymphoma (CLL/SLL), Extranodal T-/NK-cell lymphoma (nasal type), and Anaplastic large-cell lymphoma (e.g., primary cutaneous anaplastic large-cell lymphoma or systemic anaplastic large-cell lymphoma). In some embodiments, the cancer is lymphoma is Peripheral T-cell lymphoma (PTCL).

In one aspect, this invention provides a composition comprising a multifunctional molecule or an antibody molecule disclosed herein for use in a method of treating a subject having cancer.

Accordingly, in one aspect, the disclosure features multifunctional molecule, comprising:

(i) a first antigen binding domain that selectively binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2),

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), T cell engager (e.g., that binds to a T cell antigen other than CD3), a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(d) a stromal modifying moiety.

In another aspect, the disclosure features a multifunctional molecule, comprising:

(i) a first antigen binding domain that selectively targets lymphocytes expressing (e.g., on their surface, e.g., displaying) a T cell receptor comprising T cell receptor beta chain constant domain 1 (TRBC1), TRBC1, a T cell receptor comprising T cell receptor beta chain constant domain 2 (TRBC2), or TRBC2,

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a T cell engager (e.g., that binds to a T cell antigen other than CD3), a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(c) a stromal modifying moiety.

In another aspect, the disclosure features a multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to a tumor antigen on a lymphoma cell (e.g., T cell), e.g., a T cell receptor comprising T cell receptor beta chain constant domain 1 (TRBC1), TRBC1, a T cell receptor comprising T cell receptor beta chain constant domain 2 (TRBC2), or TRBC2,

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a T cell engager (e.g., that binds to a T cell antigen other than CD3), a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(d) a stromal modifying moiety.

In another aspect, the disclosure features an antibody molecule, e.g., an IgM antibody molecule, comprising:

(i) a first antigen binding domain that selectively binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2),

and

(ii) a complement activating domain that activates the complement pathway, e.g., by binding C1q.

In another aspect, the disclosure features an antibody molecule comprising:

a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 218 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and

a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 241 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

In some embodiments, the antibody molecule or fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

In another aspect, the disclosure features a nucleic acid molecule encoding a multifunctional molecule disclosed herein.

In another aspect, the disclosure features a vector, e.g., an expression vector, comprising the nucleic acid molecules disclosed herein.

In another aspect, the disclosure features a host cell comprising a nucleic acid molecule or vector disclosed herein.

In another aspect, the disclosure features a method of making, e.g., producing, a multifunctional molecule disclosed herein, comprising culturing a host cell disclosed herein under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

In another aspect, the disclosure features a pharmaceutical composition comprising a multifunctional molecule disclosed herein.

In another aspect, the disclosure features a method of treating a cancer, comprising administering to a subject in need thereof a multifunctional molecule disclosed herein, wherein

the multifunctional molecule is administered in an amount effective to treat the cancer. In some embodiments, the cancer is a T cell malignancy, e.g., a T cell lymphoma or a T cell leukemia. In some embodiments, the cancer is chosen from: anaplastic large cell lymphoma (ALCL); angioimmunoblastic T cell lymphoma; peripheral T cell lymphoma (PTCL), not otherwise specified (NOS); cutaneous T-cell lymphoma (CTCL); NKT cell lymphoma; Sézary syndrome; T acute lymphoblastic leukemia or lymphoma; adult T cell leukemia or lymphoma; T prolymphocytic leukemia; and T large granular leukemia. In some embodiments, the cancer is PTCL. In some embodiments, TRBC subtype expression is analyzed by flow cytometry analysis of, e.g., fresh tumor tissue. In some embodiments, the multifunctional molecule is used in combination with a second agent. In some embodiments, the second agent is a histone deacetylases (HDAC) inhibitor, e.g., romidepsin or belinostat. In some embodiments, the second agent is a kinase or enzyme inhibitor. In some embodiments, the second agent is a PI3K inhibitor, e.g., duvelisib. In some embodiments, the second agent is a farnesyltransferase inhibitor, e.g., tipifarnib. In some embodiments, the second agent is a SYK/JAK inhibitor, e.g., cerdulatinib. In some embodiments, the second agent is a chemotherapy.

In some embodiments, the second agent is

In another aspect, the disclosure features a method of identifying a subject in need of treatment for cancer using a multifunctional molecule disclosed herein, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2, wherein:

responsive to determining that the subject has cancer cells that express a T cell receptor comprising TRBC1, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and

responsive to determining that the subject has cancer cells that express a T cell receptor comprising TRBC2, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and optionally not as a

candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1.

In another aspect, the disclosure features a method of evaluating a subject in need of treatment for cancer, e.g., a lymphoma, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2.

Additional features of any of the aforesaid multifunctional molecules, nucleic acids, vectors, host cells, or methods include one or more of the following enumerated embodiments.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

Enumerated Embodiments

1. A multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to a tumor antigen on a lymphoma cell (e.g., T cell), wherein the tumor antigen is T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2),

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(d) a stromal modifying moiety.

1A. A multifunctional molecule, comprising:

(i) a first antigen binding domain that selectively binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2),

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a T cell engager that binds to a T cell antigen other than CD3, a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(d) a stromal modifying moiety.

2. A multifunctional molecule, comprising:

(i) a first antigen binding domain that selectively targets lymphocytes expressing T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2),

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule or cytokine inhibitor molecule;

(c) a death receptor signal engager; and

(d) a stromal modifying moiety.

3. The multifunctional molecule of any preceding embodiment, wherein the multifunctional molecule:

(i) binds specifically to an epitope of TRBC1 or TRBC2, *e.g.*, the same or similar epitope as the epitope recognized by an anti-TRBC1 or anti-TRBC2 antibody molecule as described herein;

(ii) shows the same or similar binding affinity or specificity, or both, as an anti-TRBC1 or anti-TRBC2 antibody molecule as described herein;

(iii) inhibits, *e.g.*, competitively inhibits, the binding of an anti-TRBC1 or anti-TRBC2 antibody molecule as described herein;

(iv) binds the same or an overlapping epitope with an anti-TRBC1 or anti-TRBC2 antibody molecule as described herein; or

(v) competes for binding, and/or binds the same epitope, with an anti-TRBC1 or anti-TRBC2 antibody molecule as described herein.

4. The multifunctional molecule of embodiment 3, wherein the anti-TRBC1 or anti-TRBC2 antibody molecule comprises one or more CDRs, framework regions, variable domains, heavy or light chains, or an antigen binding domain chosen from Tables 2-5, or a sequence substantially identical thereto.

5. The multifunctional molecule of any of embodiments 1-4, wherein the antigen or tumor antigen is TRBC1.

6. The multifunctional molecule of any of embodiments 1-4, wherein the antigen or tumor antigen is TRBC2.

7. The multifunctional molecule of any of embodiments 1-5, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 200 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 201 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 202 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 223 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 224 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 225 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

8. The multifunctional molecule of embodiment 7, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 200, a VHCDR2 amino acid sequence of SEQ ID NO: 201, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 202, and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 223, a VLCDR2 amino acid sequence of SEQ ID NO: 224, and/or a VLCDR3 amino acid sequence of SEQ ID NO: 225.

9. The multifunctional molecule of any of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 203 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 204 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 205 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 206 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or

(2) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 226 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 227 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 228 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 229 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

10. The multifunctional molecule of embodiment 9, wherein the first antigen binding domain comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 203, a VHFWR2 amino acid sequence of SEQ ID NO: 204, a VHFWR3 amino acid sequence of SEQ ID NO: 205, or a VHFWR4 amino acid sequence of SEQ ID NO: 206, and/or

(2) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 226, a VLFWR2 amino acid sequence of SEQ ID NO: 227, a VLFWR3 amino acid sequence of SEQ ID NO: 228, or a VLFWR4 amino acid sequence of SEQ ID NO: 229.

11. The multifunctional molecule of any one of embodiments 1-5 or 7-10, wherein the first antigen binding domain comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 250 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 250), and/or

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 255 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 255).

12. The multifunctional molecule of any of embodiments 1-5 or 7-11, wherein the first antigen binding domain comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 6154, 6155, 6167, or 6168 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6154, 6155, 6167, or 6168).

13. The multifunctional molecule of any of embodiments 1-5 or 7-12, wherein the first antigen binding domain comprises a light chain comprising the amino acid sequence of SEQ ID NOs: 6156 or 6169 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6156 or 6169).

14. The multifunctional molecule of any of embodiments 1-5, 7 or 8, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 207 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 208 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 209 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 210 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

15. The multifunctional molecule of embodiment 14, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 207, a VHFWR2 amino acid sequence of SEQ ID NO: 208, a VHFWR3 amino acid sequence of SEQ ID NO: 209, or a VHFWR4 amino acid sequence of SEQ ID NO: 210.

16. The multifunctional molecule of any of embodiments 1-5, 7 or 8, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 211 (or a sequence with

no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 212 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 213 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 214 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

17. The multifunctional molecule of embodiment 16, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 211, a VHFWR2 amino acid sequence of SEQ ID NO: 212, a VHFWR3 amino acid sequence of SEQ ID NO: 213, or a VHFWR4 amino acid sequence of SEQ ID NO: 214.

18. The multifunctional molecule of any of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 218 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

19. The multifunctional molecule of embodiment 18, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215, a VHFWR2 amino acid sequence of SEQ ID NO: 216, a VHFWR3 amino acid sequence of SEQ ID NO: 217, or a VHFWR4 amino acid sequence of SEQ ID NO: 218.

20. The multifunctional molecule of any of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 219 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 220 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 221 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 222 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

21. The multifunctional molecule of embodiment 20, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 219, a VHFWR2 amino acid sequence of SEQ ID NO: 220, a VHFWR3 amino acid sequence of SEQ ID NO: 221, or a VHFWR4 amino acid sequence of SEQ ID NO: 222.

22. The multifunctional molecule of any of embodiments 1-5, 7, 8, or 14-21, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 230 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 231 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 232 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 233 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

23. The multifunctional molecule of embodiment 22, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region

1 (VLFWR1) amino acid sequence of SEQ ID NO: 230, a VLFWR2 amino acid sequence of SEQ ID NO: 231, a VLFWR3 amino acid sequence of SEQ ID NO: 232, or a VLFWR4 amino acid sequence of SEQ ID NO: 233.

24. The multifunctional molecule of any of embodiments 1-5, 7, 8, or 14-21, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 234 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 235 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 236 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 237 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

25. The multifunctional molecule of embodiment 24, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 234, a VLFWR2 amino acid sequence of SEQ ID NO: 235, a VLFWR3 amino acid sequence of SEQ ID NO: 236, or a VLFWR4 amino acid sequence of SEQ ID NO: 237.

26. The multifunctional molecule of any of embodiments 1-5, 7, 8, or 14-21, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino

acid sequence of SEQ ID NO: 241 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

27. The multifunctional molecule of embodiment 26, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238, a VLFWR2 amino acid sequence of SEQ ID NO: 239, a VLFWR3 amino acid sequence of SEQ ID NO: 240, or a VLFWR4 amino acid sequence of SEQ ID NO: 241.

28. The multifunctional molecule of any of embodiments 1-5, 7, 8, or 14-21, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 242 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 243 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 244 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 245 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

29. The multifunctional molecule of embodiment 28, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 242, a VLFWR2 amino acid sequence of SEQ ID NO: 243, a VLFWR3 amino acid sequence of SEQ ID NO: 244, or a VLFWR4 amino acid sequence of SEQ ID NO: 245.

30. The multifunctional molecule of any of embodiments 1-5, 7, 8, or 14-21, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 246 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions,

therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 247 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 248 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 249 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

31. The multifunctional molecule of embodiment 30, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 246, a VLFWR2 amino acid sequence of SEQ ID NO: 247, a VLFWR3 amino acid sequence of SEQ ID NO: 248, or a VLFWR4 amino acid sequence of SEQ ID NO: 249.

32. The multifunctional molecule of any one of embodiments 1-5, 7, or 8 wherein the first antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 251 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

33. The multifunctional molecule of any one of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 252 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

34. The multifunctional molecule of any one of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

35. The multifunctional molecule of any one of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID

NO: 254 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

36. The multifunctional molecule of any one of embodiments 1-5, 7, 8, or 32-35, wherein the first antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 256 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

37. The multifunctional molecule of any one of embodiments 1-5, 7, 8, or 32-35, wherein the first antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 257 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

38. The multifunctional molecule of any one of embodiments 1-5, 7, 8, or 32-35, wherein the first antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

39. The multifunctional molecule of any one of embodiments 1-5, 7, 8, or 32-35, wherein the first antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 259 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

40 The multifunctional molecule of any one of embodiments 1-5, 7, 8, or 32-35, wherein the first antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 260 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

41. The multifunctional molecule of any of embodiments 1-5, 7, or 8, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 218 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and

(ii) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 241 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

42. The multifunctional molecule of embodiment 41, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215, a VHFWR2 amino acid sequence of SEQ ID NO: 216, a VHFWR3 amino acid sequence of SEQ ID NO: 217, or a VHFWR4 amino acid sequence of SEQ ID NO: 218, and

(ii) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238, a VLFWR2 amino acid sequence of SEQ ID NO: 239, a VLFWR3 amino acid sequence of SEQ ID NO: 240, or a VLFWR4 amino acid sequence of SEQ ID NO: 241.

43. The multifunctional molecule of either of embodiments 41 or 42, wherein the first antigen binding domain comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

44. The multifunctional molecule of any one of embodiments 1-43, wherein the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors not comprising TRBC1, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC1.

45. The multifunctional molecule of any one of embodiments 1-4 or 6, wherein the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than for T cell receptors not comprising TRBC2, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC2.

46. The multifunctional molecule of any one of embodiments 1-44, wherein the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors comprising TRBC2, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC2.

47. The multifunctional molecule of any one of embodiments 1-4, 6, or 45, wherein the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than

for T cell receptors comprising TRBC1, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC1.

48. The multifunctional molecule of any preceding embodiment, wherein binding of the first antigen binding domain to TRBC1 or TRBC2 on a lymphoma cell or lymphocyte (e.g., T cell) or the tumor antigen on the lymphoma cell (e.g., T cell) does not activate the lymphoma cell or lymphocyte, e.g., T cell.

49. The multifunctional molecule of any preceding embodiment, wherein binding of the first antigen binding domain to TRBC1 or TRBC2 on a lymphoma cell or lymphocyte (e.g., T cell) or the tumor antigen on the lymphoma cell e.g., T cell) does not appreciably activate the lymphoma cell or lymphocyte, e.g., T cell, (e.g., as measured by T cell proliferation, expression of a T cell activation marker (e.g., CD69 or CD25), and/or expression of a cytokine (e.g., TNFα and IFNγ).

50. The multifunctional molecule of any one of embodiments 1 or 3-49, wherein the multifunctional molecule preferentially binds to a lymphoma cell over a non-lymphoma cell, optionally wherein the binding between the multifunctional molecule and the lymphoma cell is more than 10, 20, 30, 40, or 50-fold greater than the binding between the multifunctional molecule and a non-lymphoma cell.

51. The multifunctional molecule of any one of embodiments 2-47, wherein:

(i) the binding between the multifunctional molecule and the lymphocyte expressing TRBC1 is more than 10, 20, 30, 40, or 50-fold greater than the binding between the multifunctional molecule and a lymphocyte that does not express TRBC1, or

(ii) the binding between the multifunctional molecule and the lymphocyte expressing TRBC2 is more than 10, 20, 30, 40, or 50-fold greater than the binding between the multifunctional molecule and a lymphocyte that does not express TRBC2.

52. The multifunctional molecule of any one of embodiments 1-51, wherein the multifunctional molecule comprises an immune cell engager chosen from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager.

53. The multifunctional molecule of embodiment 52, wherein the immune cell engager binds to and activates an immune cell, e.g., an effector cell.

54. The multifunctional molecule of embodiment 52, wherein the immune cell engager binds to, but does not activate, an immune cell, e.g., an effector cell.

55. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager is a T cell engager, e.g., a T cell engager that mediates binding to and activation of a T cell, or a T cell engager that mediates binding to but not activation of a T cell.

56. The multifunctional molecule of embodiment 55, wherein the T cell engager binds to TCRα, TCRβ, TCRγ, TCRζ, ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226, e.g., the T cell engager is an anti-TCRβ antibody molecule.

57. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager is an NK cell engager, e.g., an NK cell engager that mediates binding to and activation of an NK cell, or an NK cell engager that mediates binding to but not activation of an NK cell.

58. The multifunctional molecule of embodiment 57, wherein the NK cell engager is chosen from an antibody molecule, e.g., an antigen binding domain, or ligand that binds to (e.g., activates): NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16 (e.g., CD16a, CD16b, or both), CRTAM, CD27, PSGL1, CD96, CD100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS1, KIR2DS3,

KIR2DS5, KIR2DS1, CD94, NKG2C, NKG2E, or CD160, e.g., the NK cell engager is an antibody molecule or ligand that binds to (e.g., activates) NKp30.

59. The multifunctional molecule of embodiment 57, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain.

60. The multifunctional molecule of either of embodiments 58 or 59, wherein the NK cell engager is capable of engaging an NK cell.

61. The multifunctional molecule of any one of embodiments 57-60, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to NKp30, NKp46, NKG2D, or CD16.

62. The multifunctional molecule of any preceding embodiment, wherein the multifunctional molecule:

(i) binds specifically to an epitope of NKp30, NKp46, NKG2D, or CD16, *e.g.*, the same or similar epitope as the epitope recognized by an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule as described herein;

(ii) shows the same or similar binding affinity or specificity, or both, as an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule as described herein;

(iii) inhibits, *e.g.*, competitively inhibits, the binding of an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule as described herein;

(iv) binds the same or an overlapping epitope with an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule as described herein; or

(v) competes for binding, and/or binds the same epitope, with an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 molecule as described herein.

63. The multifunctional molecule of any of embodiments 57-62, wherein the anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule comprises one or more CDRs,

framework regions, variable domains, heavy or light chains, or an antigen binding domain chosen from Tables 7-10 or 15, or a sequence substantially identical thereto.

64. The multifunctional molecule of any of embodiments 57-63, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to NKp30.

65. The multifunctional molecule of any of embodiments 57-64, wherein lysis of the lymphoma cell or lymphocyte is mediated by NKp30.

66. The multifunctional molecule of any of embodiments 57-65, wherein the multifunctional molecule does not activate the NK cell when incubated with the NK cell in the absence of the tumor antigen on the lymphoma cell or TRBC1 or TRBC2 on the lymphocyte.

67. The multifunctional molecule of any of embodiments 57-66, wherein the multifunctional molecule activates the NK cell when the NK cell is a NKp30 expressing NK cell and either: (1) the tumor antigen on the lymphoma cell is also present or (2) TRBC1 or TRBC2 on the lymphocyte is also present.

68. The multifunctional molecule of any of embodiments 57-67, wherein the multifunctional molecule does not activate the NK cell when the NK cell is not a NKp30 expressing NK cell and either: (1) the tumor antigen on the lymphoma cell is also present or (2) TRBC1 or TRBC2 on the lymphocyte is also present.

69. The multifunctional molecule of any of embodiments 57-68, wherein the NK cell engager comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6001 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of

SEQ ID NO: 6002 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6064 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 7293 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

70. The multifunctional molecule of embodiment 69, wherein the NK cell engager comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000, a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002, and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063, a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and/or a VLCDR3 amino acid sequence of SEQ ID NO: 7293.

71. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6003 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6004 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6005 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of

SEQ ID NO: 6006 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or

(2) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6066 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6067 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 7292 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6069 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

72. The multifunctional molecule of embodiment 71, wherein the NK cell engager comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6003, a VHFWR2 amino acid sequence of SEQ ID NO: 6004, a VHFWR3 amino acid sequence of SEQ ID NO: 6005, or a VHFWR4 amino acid sequence of SEQ ID NO: 6006, and

(3) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6066, a VLFWR2 amino acid sequence of SEQ ID NO: 6067, a VLFWR3 amino acid sequence of SEQ ID NO: 7292, or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

73. The multifunctional molecule of any one of embodiments 57-72, wherein the NK cell engager comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 6121 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6121), and/or

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 7294 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 7294).

74. The multifunctional molecule of either of embodiments 57-73, wherein the NK cell engager comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 6148 or 6149 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6148 or 6149).

75. The multifunctional molecule of either of embodiments 57-74, wherein the NK cell engager comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6150 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6150).

76. The multifunctional molecule of either of embodiments 57-75, wherein the NK cell engager comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 6148 or 6149 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6148 or 6149), and a light chain comprising the amino acid sequence of SEQ ID NO: 6150 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6150).

77. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6014 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6015 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6016 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6017 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

78. The multifunctional molecule of embodiment 77, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6014, a VHFWR2 amino acid sequence of SEQ ID NO: 6015, a VHFWR3 amino acid sequence of SEQ ID NO: 6016, or a VHFWR4 amino acid sequence of SEQ ID NO: 6017.

79. The multifunctional molecule of embodiment 78, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6123 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6123).

80. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6018 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6019 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6020 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6021 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

81. The multifunctional molecule of embodiment 80, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6018, a VHFWR2 amino acid sequence of SEQ ID NO: 6019, a VHFWR3 amino acid sequence of SEQ ID NO: 6020, or a VHFWR4 amino acid sequence of SEQ ID NO: 6021.

82. The multifunctional molecule of embodiment 81, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6124 (or an amino acid

sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6124).

83. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6022 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6023 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6024 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6025 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

84. The multifunctional molecule of embodiment 83, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6022, a VHFWR2 amino acid sequence of SEQ ID NO: 6023, a VHFWR3 amino acid sequence of SEQ ID NO: 6024, or a VHFWR4 amino acid sequence of SEQ ID NO: 6025.

85. The multifunctional molecule of embodiment 84, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6125 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6125).

86. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6026 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6027 (or a sequence with no more than 1, 2, 3,

4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6028 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6029 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

87. The multifunctional molecule of embodiment 86, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6026, a VHFWR2 amino acid sequence of SEQ ID NO: 6027, a VHFWR3 amino acid sequence of SEQ ID NO: 6028, or a VHFWR4 amino acid sequence of SEQ ID NO: 6029.

88. The multifunctional molecule of embodiment 87, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6126 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6126).

89. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6030 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6032 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6033 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6034 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

90. The multifunctional molecule of embodiment 89, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1

(VHFWR1) amino acid sequence of SEQ ID NO: 6030, a VHFWR2 amino acid sequence of SEQ ID NO: 6032, a VHFWR3 amino acid sequence of SEQ ID NO: 6033, or a VHFWR4 amino acid sequence of SEQ ID NO: 6034.

91. The multifunctional molecule of embodiment 90, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6127 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6127).

92. The multifunctional molecule of any of embodiments 57-70, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6035 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6036 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6037 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6038 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

93. The multifunctional molecule of embodiment 92, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6035, a VHFWR2 amino acid sequence of SEQ ID NO: 6036, a VHFWR3 amino acid sequence of SEQ ID NO: 6037, or a VHFWR4 amino acid sequence of SEQ ID NO: 6038.

94. The multifunctional molecule of embodiment 93, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6128 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6128).

95. The multifunctional molecule of any of embodiments 57-70 or 77-94, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6077 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6078 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6079 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6080 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

96. The multifunctional molecule of embodiment 95, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6077, a VLFWR2 amino acid sequence of SEQ ID NO: 6078, a VLFWR3 amino acid sequence of SEQ ID NO: 6079, or a VLFWR4 amino acid sequence of SEQ ID NO: 6080.

97. The multifunctional molecule of embodiment 96, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6137 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6137).

98. The multifunctional molecule of any of embodiments 57-70 or 77-94, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6081 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6082 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6083 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of

SEQ ID NO: 6084 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

99. The multifunctional molecule of embodiment 98, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6081, a VLFWR2 amino acid sequence of SEQ ID NO: 6082, a VLFWR3 amino acid sequence of SEQ ID NO: 6083, or a VLFWR4 amino acid sequence of SEQ ID NO: 6084.

100. The multifunctional molecule of embodiment 99, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6138 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6138).

101. The multifunctional molecule of any of embodiments 57-70 or 77-94, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6085 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6086 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6087 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6088 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

102. The multifunctional molecule of embodiment 101, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6085, a VLFWR2 amino acid sequence of SEQ ID NO: 6086, a VLFWR3 amino acid sequence of SEQ ID NO: 6087, or a VLFWR4 amino acid sequence of SEQ ID NO: 6088.

103. The multifunctional molecule of embodiment 102, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6139 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6139).

104. The multifunctional molecule of any of embodiments 57-70 or 77-94, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6089 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6090 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6091 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6092 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

105. The multifunctional molecule of embodiment 104, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6089, a VLFWR2 amino acid sequence of SEQ ID NO: 6090, a VLFWR3 amino acid sequence of SEQ ID NO: 6091, or a VLFWR4 amino acid sequence of SEQ ID NO: 6092.

106. The multifunctional molecule of embodiment 105, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6140 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6140).

107. The multifunctional molecule of any of embodiments 57-70 or 77-94, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6093 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6094 (or a sequence with no more than 1, 2, 3, 4,

5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6095 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6096 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

108. The multifunctional molecule of embodiment 107, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6093, a VLFWR2 amino acid sequence of SEQ ID NO: 6094, a VLFWR3 amino acid sequence of SEQ ID NO: 6095, or a VLFWR4 amino acid sequence of SEQ ID NO: 6096.

109. The multifunctional molecule of embodiment 108, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6141 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6141).

110. The multifunctional molecule of any of embodiments 57-68, wherein the NK cell engager comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6008 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6070 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6071 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of

SEQ ID NO: 6072 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

111. The multifunctional molecule of embodiment 110, wherein the NK cell engager comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007, a VHCDR2 amino acid sequence of SEQ ID NO: 6008, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009, and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6070, a VLCDR2 amino acid sequence of SEQ ID NO: 6071, and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6072.

112. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6010 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6011 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6012 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6013 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or

(2) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6073 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6074 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid

sequence of SEQ ID NO: 6075 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6076 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

113. The multifunctional molecule of embodiment 112, wherein the NK cell engager comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6010, a VHFWR2 amino acid sequence of SEQ ID NO: 6011, a VHFWR3 amino acid sequence of SEQ ID NO: 6012, or a VHFWR4 amino acid sequence of SEQ ID NO: 6013, and

(3) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6073, a VLFWR2 amino acid sequence of SEQ ID NO: 6074, a VLFWR3 amino acid sequence of SEQ ID NO: 6075, or a VLFWR4 amino acid sequence of SEQ ID NO: 6076.

114. The multifunctional molecule of any one of embodiments 57-68 or 110-113, wherein the NK cell engager comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 6122 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6122), and/or

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 6136 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6136).

115. The multifunctional molecule of any of embodiments 57-68 or 110-114, wherein the NK cell engager comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 6151 or 6152 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6151 or 6152).

116. The multifunctional molecule of any of embodiments 57-68 or 110-115, wherein the NK cell engager comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6153 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6153).

117. The multifunctional molecule of any of embodiments 57-68 or 110-116, wherein the NK cell engager comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 6151 or 6152 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 6151 or 6152), and a light chain comprising the amino acid sequence of SEQ ID NO: 6153 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6153).

118. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6039 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6040 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6041 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6042 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

119. The multifunctional molecule of embodiment 118, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6039, a VHFWR2 amino acid sequence of SEQ ID NO: 6040, a VHFWR3 amino acid sequence of SEQ ID NO: 6041, or a VHFWR4 amino acid sequence of SEQ ID NO: 6042.

120. The multifunctional molecule of embodiment 119, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6129 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6129).

121. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6043 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6044 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6045 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6046 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

122. The multifunctional molecule of embodiment 121, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6043, a VHFWR2 amino acid sequence of SEQ ID NO: 6044, a VHFWR3 amino acid sequence of SEQ ID NO: 6045, or a VHFWR4 amino acid sequence of SEQ ID NO: 6046.

123. The multifunctional molecule of embodiment 122, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6130 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6130).

124. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6047 (or a sequence with

no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6048 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6049 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6050 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

125. The multifunctional molecule of embodiment 124, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6047, a VHFWR2 amino acid sequence of SEQ ID NO: 6048, a VHFWR3 amino acid sequence of SEQ ID NO: 6049, or a VHFWR4 amino acid sequence of SEQ ID NO: 6050.

126. The multifunctional molecule of embodiment 125, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6131 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6131).

127. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6051 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6052 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6053 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6054 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

128. The multifunctional molecule of embodiment 127, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6051, a VHFWR2 amino acid sequence of SEQ ID NO: 6052, a VHFWR3 amino acid sequence of SEQ ID NO: 6053, or a VHFWR4 amino acid sequence of SEQ ID NO: 6054.

129. The multifunctional molecule of embodiment 128, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6132 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6132).

130. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6055 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6056 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6057 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6058 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

131. The multifunctional molecule of embodiment 130, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6055, a VHFWR2 amino acid sequence of SEQ ID NO: 6056, a VHFWR3 amino acid sequence of SEQ ID NO: 6057, or a VHFWR4 amino acid sequence of SEQ ID NO: 6058.

132. The multifunctional molecule of embodiment 131, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6133 (or an amino acid

sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6133).

133. The multifunctional molecule of any of embodiments 57-68, 110, or 111, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6059 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6060 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6061 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 6062 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

134. The multifunctional molecule of embodiment 133, wherein the NK cell engager comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6059, a VHFWR2 amino acid sequence of SEQ ID NO: 6060, a VHFWR3 amino acid sequence of SEQ ID NO: 6061, or a VHFWR4 amino acid sequence of SEQ ID NO: 6062.

135. The multifunctional molecule of embodiment 134, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6134 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6134).

136. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6097 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6098 (or a sequence with no more

than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6099 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6100 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

137. The multifunctional molecule of embodiment 136, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6097, a VLFWR2 amino acid sequence of SEQ ID NO: 6098, a VLFWR3 amino acid sequence of SEQ ID NO: 6099, or a VLFWR4 amino acid sequence of SEQ ID NO: 6100.

138. The multifunctional molecule of embodiment 137, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6142 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6142).

139. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6101 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6102 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6103 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6104 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

140. The multifunctional molecule of embodiment 139, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6101, a VLFWR2 amino acid sequence of SEQ

ID NO: 6102, a VLFWR3 amino acid sequence of SEQ ID NO: 6103, or a VLFWR4 amino acid sequence of SEQ ID NO: 6104.

141. The multifunctional molecule of embodiment 140, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6143 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6143).

142. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6105 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6106 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6107 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6108 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

143. The multifunctional molecule of embodiment 142, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6105, a VLFWR2 amino acid sequence of SEQ ID NO: 6106, a VLFWR3 amino acid sequence of SEQ ID NO: 6107, or a VLFWR4 amino acid sequence of SEQ ID NO: 6108.

144. The multifunctional molecule of embodiment 143, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6144 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6144).

145. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light

chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6109 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6110 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6111 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6112 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

146. The multifunctional molecule of embodiment 145, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6109, a VLFWR2 amino acid sequence of SEQ ID NO: 6110, a VLFWR3 amino acid sequence of SEQ ID NO: 6111, or a VLFWR4 amino acid sequence of SEQ ID NO: 6112.

147. The multifunctional molecule of embodiments 146, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6145 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6145).

148. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6113 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6114 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6115 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6116 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

149. The multifunctional molecule of embodiment 148, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6113, a VLFWR2 amino acid sequence of SEQ ID NO: 6114, a VLFWR3 amino acid sequence of SEQ ID NO: 6115, or a VLFWR4 amino acid sequence of SEQ ID NO: 6116.

150. The multifunctional molecule of embodiment 149, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6146 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6146).

151. The multifunctional molecule of any of embodiments 57-68, 110, 111, or 118-135, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6117 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 6118 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 6119 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 6120 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

152. The multifunctional molecule of embodiment 151, wherein the NK cell engager comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6117, a VLFWR2 amino acid sequence of SEQ ID NO: 6118, a VLFWR3 amino acid sequence of SEQ ID NO: 6119, or a VLFWR4 amino acid sequence of SEQ ID NO: 6120.

153. The multifunctional molecule of embodiment 152, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6147 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6147).

154. The multifunctional molecule of any of embodiments 57-60, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to NKp46.

155. The multifunctional molecule of embodiment 154, wherein lysis of the lymphoma cell is mediated by NKp46.

156. The multifunctional molecule of either of embodiments 154 or 155, wherein the multifunctional molecule does not activate the NK cell when incubated with the NK cell in the absence of the tumor antigen on the lymphoma cell.

157. The multifunctional molecule of any one of embodiments 154-156, wherein the multifunctional molecule activates the NK cell when the NK cell is a NKp46 expressing NK cell and the tumor antigen on the lymphoma cell is also present.

158. The multifunctional molecule of any one of embodiments 154-157, wherein the multifunctional molecule does not activate the NK cell when the NK cell is not a NKp46 expressing NK cell and the tumor antigen on the lymphoma cell is also present.

159. The multifunctional molecule of any one of embodiments 154-158, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6182 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6182).

160. The multifunctional molecule of any one of embodiments 154-159, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6183 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6183).

161. The multifunctional molecule of 154-160, wherein the NK cell engager comprises an scFV comprising the amino acid sequence of SEQ ID NO: 6181(or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6181).

162. The multifunctional molecule of any of embodiments 57-60, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to NKG2D.

163. The multifunctional molecule of embodiment 162, wherein lysis of the lymphoma cell is mediated by NKG2D.

164. The multifunctional molecule of either of embodiments 162 or 163, wherein the multifunctional molecule does not activate the NK cell when incubated with the NK cell in the absence of the tumor antigen on the lymphoma cell.

165. The multifunctional molecule of any one of embodiments 162-164, wherein the multifunctional molecule activates the NK cell when the NK cell is a NKG2D expressing NK cell and the tumor antigen on the lymphoma cell is also present.

166. The multifunctional molecule of any one of embodiments 162-165, wherein the multifunctional molecule does not activate the NK cell when the NK cell is not a NKG2D expressing NK cell and the tumor antigen on the lymphoma cell is also present.

167. The multifunctional molecule of any one of embodiments 162-166, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6176 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6176).

168. The multifunctional molecule of any one of embodiments 162-167, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6177 (or an

amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6177).

169. The multifunctional molecule of any of embodiments 162-168, wherein the NK cell engager comprises an scFV comprising the amino acid sequence of SEQ ID NO: 6175(or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6175).

170. The multifunctional molecule of any one of embodiments 162-166, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6179 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6179).

171. The multifunctional molecule of any one of embodiments 162-166 or 170 wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6180 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6180).

172. The multifunctional molecule of any of embodiments 162-166, 170, or 171, wherein the NK cell engager comprises an scFV comprising the amino acid sequence of SEQ ID NO: 6178(or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6178).

173. The multifunctional molecule of any of embodiments 57-60, wherein the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to CD16.

174. The multifunctional molecule of embodiment 162, wherein lysis of the lymphoma cell is mediated by CD16.

175. The multifunctional molecule of either of embodiments 173 or 174, wherein the multifunctional molecule does not activate the NK cell when incubated with the NK cell in the absence of the tumor antigen on the lymphoma cell.

176. The multifunctional molecule of any one of embodiments 173-175, wherein the multifunctional molecule activates the NK cell when the NK cell is a CD16 expressing NK cell and the tumor antigen on the lymphoma cell is also present.

177. The multifunctional molecule of any one of embodiments 173-176, wherein the multifunctional molecule does not activate the NK cell when the NK cell is not a CD16 expressing NK cell and the tumor antigen on the lymphoma cell is also present.

178. The multifunctional molecule of any one of embodiments 173-177, wherein the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6185 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6185).

179. The multifunctional molecule of any one of embodiments 173-178, wherein the NK cell engager comprises a VL comprising the amino acid sequence of SEQ ID NO: 6186 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6186).

180. The multifunctional molecule of any of embodiments 173-179, wherein the NK cell engager comprises an scFV comprising the amino acid sequence of SEQ ID NO: 6184(or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6184).

181. The multifunctional molecule of embodiment 57, wherein the NK cell engager is a ligand, optionally, the ligand further comprises an immunoglobulin constant region, e.g., an Fc region.

182. The multifunctional molecule of embodiment 181, wherein the NK cell engager is a ligand of NKp44 or NKp46, e.g., a viral HA.

183. The multifunctional molecule of embodiment 181, wherein the NK cell engager is a ligand of DAP10, e.g., a coreceptor for NKG2D.

184. The multifunctional molecule of embodiment 181, wherein the NK cell engager is a ligand of CD16, e.g., a CD16a/b ligand, e.g., a CD16a/b ligand further comprising an antibody Fc region.

185. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager mediates binding to, or activation of, or both of, one or more of a B cell, a macrophage, and/or a dendritic cell.

186. The multifunctional molecule of embodiment 185, wherein the immune cell engager comprises a B cell, macrophage, and/or dendritic cell engager chosen from one or more of CD40 ligand (CD40L) or a CD70 ligand; an antibody molecule that binds to CD40 or CD70; an antibody molecule to OX40; an OX40 ligand (OX40L); an agonist of a Toll-like receptor (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); a 41BB; a CD2 agonist; a CD47; or a STING agonist, or a combination thereof.

187. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager is a B cell engager, e.g., a CD40L, an OX40L, or a CD70 ligand, or an antibody molecule that binds to OX40, CD40 or CD70.

188. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager is a macrophage cell engager, e.g., a CD2 agonist; a CD40L; an OX40L; an antibody molecule that binds to OX40, CD40 or CD70; an agonist of a Toll-like receptor (TLR)

(e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); CD47; or a STING agonist.

189. The multifunctional molecule of any one of embodiments 52-54, wherein the immune cell engager is a dendritic cell engager, e.g., a CD2 agonist, an OX40 antibody, an OX40L, 41BB agonist, a Toll-like receptor agonist or a fragment thereof (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4)), CD47 agonist, or a STING agonist.

190. The multifunctional molecule of embodiment 188 or 189, wherein the STING agonist comprises a cyclic dinucleotide, e.g., a cyclic di-GMP (cdGMP), a cyclic di-AMP (cdAMP), or a combination thereof, optionally with 2',5' or 3',5' phosphate linkages, e.g., wherein the STING agonist is covalently coupled to the multifunctional molecule.

191. The multifunctional molecule of any one of embodiments 1-51, wherein the multifunctional molecule comprises a cytokine molecule.

192. The multifunctional molecule of embodiment 191, wherein the cytokine molecule is chosen from interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), interleukin-21 (IL-21), or interferon gamma, or a fragment or variant thereof, or a combination of any of the aforesaid cytokines.

192A. The multifunctional molecule of embodiment 192, wherein the cytokine molecule is interleukin-2 (IL-2).

193. The multifunctional molecule of embodiment 191 or 192, wherein the cytokine molecule is a monomer or a dimer.

194. The multifunctional molecule of any one of embodiments 191-193, wherein the cytokine molecule further comprises a receptor dimerizing domain, e.g., an IL15Ralpha dimerizing domain.

195. The multifunctional molecule of embodiment 194, wherein the cytokine molecule (e.g., IL-15) and the receptor dimerizing domain (e.g., an IL15Ralpha dimerizing domain) are not covalently linked, e.g., are non-covalently associated.

196. The multifunctional molecule of any of embodiments 1-51, wherein the multifunctional molecule comprises a cytokine inhibitor molecule.

197. The multifunctional molecule of embodiment 196, wherein the cytokine inhibitor molecule is a TGF-beta inhibitor.

198. The multifunctional molecule of either of embodiments 196 or 197, wherein the TGF-beta inhibitor inhibits (e.g., reduces the activity of): (i) TGF-beta 1; (ii) TGF-beta 2; (iii) TGF-beta 3; (iv) (i) and (ii); (v) (i) and (iii); (vi) (ii) and (iii); or (vii) (i), (ii), and (iii).

199. The multifunctional molecule of any of embodiments 196-198, wherein the TGF-beta inhibitor comprises a portion of a TGF-beta receptor (e.g., an extracellular domain of a TGF-beta receptor) that is capable of inhibiting (e.g., reducing the activity of) TGF-beta, or functional fragment or variant thereof.

200. The multifunctional molecule of embodiment 199, wherein the TGF-beta inhibitor comprises a portion of (i) TGFBR1; (ii) TGFBR2; (iii) TGFBR3; (iv) (i) and (ii); (v) (i) and (iii); (vi) (ii) and (iii); or (vii) (i), (ii), and (iii).

201. The multifunctional molecule of any of embodiments 196-200, wherein the TGF-beta inhibitor comprises an amino acid sequence selected from Table 16, or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto.

202. The multifunctional molecule of any of embodiments 1-51, wherein the multifunctional molecule comprises a death receptor signal engager chosen from a TNF-related

apoptosis-inducing ligand (TRAIL) molecule, a death receptor molecule, or an antigen binding domain that specifically binds to a death receptor.

203. The multifunctional molecule of embodiment 202, wherein the death receptor signal engager activates death receptor signaling in the lymphoma cell (e.g., T cell) or lymphocyte expressing TRBC1 or TRBC2, e.g., and induces apoptosis or cell death in said cell.

204. The multifunctional molecule of either of embodiments 202 or 203, wherein the death receptor signal engager does not activate death receptor signaling on non-lymphoma cells and lymphocytes not expressing TRBC1 or not expressing TRBC2.

205. The multifunctional molecule of any of embodiments 202-204, wherein the death receptor signal engager comprises a TRAIL molecule, e.g., one or more TRAIL polypeptides or a fragment thereof.

206. The multifunctional molecule of embodiment 205, wherein the TRAIL molecule specifically binds to Death Receptor 4 (DR4) or Death Receptor 5 (DR5).

207. The multifunctional molecule of either of embodiments 205 or 206, wherein the TRAIL molecule comprises a truncated TRAIL polypeptide, e.g., relative to a wild-type TRAIL polypeptide.

208. The multifunctional molecule of embodiment 207, wherein the TRAIL molecule comprises at least residues corresponding to amino acids 95-281 of human TRAIL, e.g., a truncated TRAIL molecule comprising residues corresponding to amino acids 95-281 of human TRAIL.

209. The multifunctional molecule of embodiment 208, wherein the TRAIL molecule comprises a truncated TRAIL polypeptide comprising amino acids 95-281 of human TRAIL, e.g., and not amino acids 1-94 of human TRAIL.

210. The multifunctional molecule of embodiment 207, wherein the TRAIL molecule comprises at least residues corresponding to amino acids 122-281 of human TRAIL, e.g., a truncated TRAIL molecule comprising residues corresponding to amino acids 122-281 of human TRAIL.

211. The multifunctional molecule of embodiment 210, wherein the TRAIL molecule comprises a truncated TRAIL polypeptide comprising amino acids 122-281 of human TRAIL, e.g., and not amino acids 1-121 of human TRAIL.

212. The multifunctional molecule of any of embodiments 205-211, wherein the death receptor signal engager comprises one, two, or three TRAIL molecules.

213. The multifunctional molecule of any of embodiments 202-204, wherein the death receptor signal engager comprises an antigen binding domain that specifically binds to a death receptor, e.g., Death Receptor 4 (DR4) or Death Receptor 5 (DR5).

214. The multifunctional molecule of embodiment 213, wherein the death receptor signal engager comprises one, two, or three antigen binding domains that specifically binds to a death receptor.

215. The multifunctional molecule of either of embodiments 213 or 214, wherein the antigen binding domain that specifically binds to a death receptor binds to DR5.

216. The multifunctional molecule of any of embodiments 213-215, wherein the antigen binding domain that specifically binds to a death receptor comprises tigatuzumab, drozitumab, or conatumumab.

217. The multifunctional molecule of any of embodiments 202-216, wherein the death receptor signal engager comprises an amino acid sequence selected from Table 11, or an amino

acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

218. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6157, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

219. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6158, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

220. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6159, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

221. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6160, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

222. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6161, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

223. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6162, or an amino

acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

224. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6163, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

225. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6164, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

226. The multifunctional molecule of any of embodiments 202-217, wherein the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6165, or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

227. The multifunctional molecule of embodiment 56, wherein the T cell engager binds to TCRβ, e.g., to TCR beta V chain (TCRBV).

228. The multifunctional molecule of embodiment 227, wherein the T cell engager comprises an antigen binding domain (e.g., an antibody molecule or fragment thereof) that binds to (e.g., and in some embodiments activates) TCRβ.

229. The multifunctional molecule of either of embodiments 227 or 228, wherein the T cell engager comprises an anti-TCRβV antibody molecule, e.g., that specifically binds to a human TCR beta V chain (TCRβV).

230. The multifunctional molecule of any of embodiments 227-229, wherein the T cell engager does not bind to the lymphoma cell or the lymphocyte expressing TRBC1 or TRBC2.

231. The multifunctional molecule of any of embodiments 227-229, wherein the T cell engager is capable of binding to or binds to the lymphoma cell or the lymphocyte expressing TRBC1 or TRBC2.

232. The multifunctional molecule of any of embodiments 227-231, wherein the T cell engager does not activate the lymphoma cell or the lymphocyte expressing TRBC1 or TRBC2.

233. The multifunctional molecule of any of embodiments 227-232, wherein the T cell engager comprises an anti-TCRβV antibody molecule that specifically binds to a TCRβV subfamily or subfamily member of Table 12.

234. The multifunctional molecule of embodiment 233, wherein the anti-TCRβV antibody molecule specifically binds to TCRβ V6, *e.g.*, a TCRβ V6 subfamily comprising: TCRβ V6-4*01, TCRβ V6-4*02, TCRβ V6-9*01, TCRβ V6-8*01, TCRβ V6-5*01, TCRβ V6-6*02, TCRβ V6-6*01, TCRβ V6-2*01, TCRβ V6-3*01 or TCRβ V6-1*01.

235. The multifunctional molecule of embodiment 234, wherein the anti-TCRβV antibody molecule comprises one or more CDRs, framework regions, or variable heavy and/or light chain regions provided in Table 13 or having at least about 93%, 95%, or 99% sequence identity thereto.

236. The multifunctional molecule of embodiment 233, wherein the anti-TCRβV antibody molecule specifically binds to TCRβ V12, *e.g.*, a TCRβ V12 subfamily comprising: TCRβ V12-4*01, TCRβ V12-3*01 or TCRβ V12-5*01.

237. The multifunctional molecule of embodiment 236, wherein the anti-TCRβV antibody molecule comprises one or more CDRs, framework regions, or variable heavy and/or

light chain regions provided in Table 14 or having at least about 93%, 95%, or 99% sequence identity thereto.

238. The multifunctional molecule of any one of embodiments 1-51, wherein the multifunctional molecule comprises a stromal modifying moiety.

239. The multifunctional molecule of embodiment 238, wherein the stromal modifying moiety causes one or more of: decreases the level or production of a stromal or extracellular matrix (ECM) component; decreases tumor fibrosis; increases interstitial tumor transport; improves tumor perfusion; expands the tumor microvasculature; decreases interstitial fluid pressure (IFP) in a tumor; or decreases or enhances penetration or diffusion of an agent, e.g., a cancer therapeutic or a cellular therapy, into a tumor or tumor vasculature.

240. The multifunctional molecule of embodiment 239, wherein the stromal or ECM component decreased is chosen from a glycosaminoglycan or an extracellular protein, or a combination thereof.

241. The multifunctional molecule of any one of embodiments 1-240, wherein the multifunctional molecule comprises:

(i) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a cytokine molecule,

(ii) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a cytokine inhibitor molecule,

(iii) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a death receptor signal engager,

(iv) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a stromal modifying moiety,

(v) a cytokine molecule and a stromal modifying moiety,

(vi) a cytokine molecule and a death receptor signal engager,

(vii) a cytokine inhibitor molecule and a stromal modifying moiety,

(viii) a cytokine inhibitor molecule and a death receptor signal engager,

(ix) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager), a cytokine molecule, a death receptor signal engager, and a stromal modifying moiety, or

(x) an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager), a cytokine inhibitor molecule, a death receptor signal engager, and a stromal modifying moiety.

242. The multifunctional molecule of any one of embodiments 1-241, wherein the multifunctional molecule comprises the following configuration:

A, B-[dimerization module]-C, -D, wherein:

(a) the dimerization module comprises an immunoglobulin constant domain, *e.g.*, a heavy chain constant domain (*e.g.*, a homodimeric or heterodimeric heavy chain constant region, *e.g.*, an Fc region), or a constant domain of an immunoglobulin variable region (*e.g.*, a Fab region); and

(b) A, B, C, and D are independently absent; (i) an antigen binding domain that preferentially binds to TRBC1 or TRBC2; (ii) an immune cell engager chosen from a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager; (iii) a cytokine molecule or cytokine inhibitor molecule; (iv) a death receptor signal engager; or (v) a stromal modifying moiety, provided that:

at least one, two, or three of A, B, C, and D comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and

any of the remaining A, B, C, and D is absent or comprises one of an immune cell engager, a cytokine molecule, a cytokine inhibitor molecule, a death receptor signal engager, or a stromal modifying moiety.

243. The multifunctional molecule of embodiment 242, wherein:

(1) A comprises an antigen binding domain that preferentially binds to a T cell receptor comprising TRBC1 or TRBC2, and B, C, or D comprises an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule;

(2) A comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30 or anti-NKp46 antibody molecule;

(3) A comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises a cytokine molecule;

(4) A comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises a cytokine inhibitor molecule;

(5) A comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises a death receptor signal engager;

(6) A comprises an antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises a stromal modifying moiety;

(7) A comprises a first antigen binding domain that binds to a TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule;

(8) A comprises a first antigen binding domain that binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule;

(9) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises a cytokine molecule;

(10) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises a cytokine inhibitor molecule;

(11) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises a death receptor signal engager;

(12) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises a stromal modifying moiety;

(13) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule;

(14) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule;

(15) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises a cytokine molecule;

(16) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises a cytokine inhibitor molecule;

(17) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises a death receptor signal engager;

(18) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises a stromal modifying moiety;

(19) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine molecule;

(20) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an

anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine inhibitor molecule;

(21) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a death receptor signal engager;

(22) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a stromal modifying moiety;

(23) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine molecule;

(24) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine inhibitor molecule;

(25) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a death receptor signal engager;

(26) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a stromal modifying moiety;

(27) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) a cytokine molecule and (b) a stromal modifying moiety;

(28) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) a cytokine molecule and (b) a death receptor signal engager;

(29) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) a cytokine inhibitor molecule and (b) a stromal modifying moiety;

(30) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) a cytokine inhibitor molecule and (b) a death receptor signal engager;

(31) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B, C, or D comprises (a) a death receptor signal engager and (b) a stromal modifying moiety;

(32) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine molecule;

(33) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine inhibitor molecule;

(34) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a death receptor signal engager;

(35) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a stromal modifying moiety;

(36) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine molecule;

(37) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine inhibitor molecule;

(38) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a death receptor signal engager;

(39) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a stromal modifying moiety;

(40) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) a cytokine molecule and (b) a stromal modifying moiety;

(41) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) a cytokine molecule and (b) a death receptor signal engager;

(42) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) a cytokine inhibitor molecule and (b) a stromal modifying moiety;

(43) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or

TRBC2, and C or D comprises (a) a cytokine inhibitor molecule and (b) a death receptor signal engager;

(44) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, B comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and C or D comprises (a) a stromal modifying moiety and (b) a death receptor signal engager;

(45) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine molecule;

(46) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a cytokine inhibitor molecule;

(47) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a death receptor signal engager;

(48) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., an NK cell engager, e.g., an anti-NKp30, anti-NKp46, anti-NKG2D, or anti-CD16 antibody molecule, and (b) a stromal modifying moiety;

(49) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine molecule;

(50) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a cytokine inhibitor molecule;

(51) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a death receptor signal engager;

(52) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-TCRβV antibody molecule, and (b) a stromal modifying moiety;

(53) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) a cytokine molecule and (b) a stromal modifying moiety;

(54) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) a cytokine molecule and (b) a death receptor signal engager;

(55) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) a cytokine inhibitor molecule and (b) a stromal modifying moiety;

(56) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) a cytokine inhibitor molecule and (b) a death receptor signal engager; or

(57) A comprises a first antigen binding domain that preferentially binds to TRBC1 or TRBC2, C comprises a second antigen binding domain that preferentially binds to TRBC1 or TRBC2, and B or D comprises (a) a stromal modifying moiety and (b) a death receptor signal engager.

244. The multifunctional molecule of embodiment 242 or 243, wherein the dimerization module comprises one or more immunoglobulin chain constant regions (e.g., Fc regions) comprising one or more of: a paired cavity-protuberance ("knob-in-a hole"), an electrostatic interaction, or a strand-exchange.

245. The multifunctional molecule of embodiment 244, wherein the one or more immunoglobulin chain constant regions (e.g., Fc regions) comprise an amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, e.g., of the Fc region of human IgG1, optionally wherein the one or more immunoglobulin chain constant regions (e.g., Fc regions) comprise an amino acid substitution chosen from: T366S, L368A, or Y407V (e.g., corresponding to a cavity or hole), or T366W (e.g., corresponding to a protuberance or knob), or a combination thereof.

246. The multifunctional molecule of any one of embodiments 1-245, further comprising a linker, e.g., a linker between one or more of: the antigen binding domain and the immune cell engager, the antigen binding domain and the cytokine molecule, the antigen binding domain and the stromal modifying moiety, the immune cell engager and the cytokine molecule, the immune cell engager and the stromal modifying moiety, the cytokine molecule and the stromal modifying moiety, the antigen binding domain and the dimerization module, the immune cell engager and the dimerization module, the cytokine molecule and the dimerization module, or the stromal modifying moiety and the dimerization module.

247. The multifunctional molecule of embodiment 246, wherein the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker.

248. The multifunctional molecule of embodiment 246 or 247, wherein the linker is a peptide linker.

249. The multifunctional molecule of 248, wherein the peptide linker comprises Gly and Ser.

250. The multifunctional molecule of 249, wherein the peptide linker comprises an amino acid sequence chosen from SEQ ID NOs: 7249-7252 or 75-78.

251. A multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to TRBC1, and

(ii) an NK cell engager, e.g., an anti-NKp30 antibody molecule, anti-NKp46 antibody molecule, an anti-NKG2D antibody molecule, or an anti-CD16 antibody molecule.

252. The multifunctional molecule of embodiment 251, wherein the NK cell engager comprises an anti-NKp30 antibody molecule.

254. The multifunctional molecule of embodiment 251, wherein the NK cell engager comprises an anti-NKp46 antibody molecule.

255. The multifunctional molecule of claim 251, wherein the NK cell engager comprises an anti-NKG2D antibody molecule.

255A. The multifunctional molecule of claim 251, wherein the NK cell engager comprises an anti-CD16 antibody molecule.

256. A multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to TRBC1, and

(ii) a death receptor signal engager.

257. A multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to TRBC1, and

(ii) a T cell engager, e.g., an antigen binding domain that binds to TCR beta V chain (TCRBV).

259. A multifunctional molecule, comprising:

(i) a first antigen binding domain that preferentially binds to TRBC1, and

(ii) a cytokine inhibitor molecule, e.g., TGF-beta inhibitor.

262. The multifunctional molecule of any of embodiments 1 or 3-261, wherein the multifunctional molecule binds to TRBC1, TRBC2, or the tumor antigen monovalently.

263. The multifunctional molecule of any one of embodiments 1 or 3-261, wherein the multifunctional molecule binds to TRBC1, TRBC2, or the tumor antigen multivalently, e.g., di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, or deca-valently.

264. The multifunctional molecule of any of embodiments 2-261, wherein the multifunctional molecule binds to TRBC1, TRBC2, or the lymphocyte expressing TRBC1 or TRBC2 monovalently.

265. The multifunctional molecule of any one of embodiments 2-261, wherein the multifunctional molecule binds to the lymphocyte expressing TRBC1 or TRBC2 multivalently, e.g., di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, or deca-valently.

266. The multifunctional molecule of any preceding embodiment, wherein the multifunctional molecule binds, e.g., via the immune cell engager, to the immune cell monovalently.

267. The multifunctional molecule of any one of embodiments 1-265, wherein the multifunctional molecule binds, e.g., via the immune cell engager, to the immune cell multivalently, e.g., di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, or deca-valently.

268. The multifunctional molecule of any preceding embodiment, further comprising a heavy chain constant region, e.g., an Fc region, that mediates antibody dependent cellular cytotoxicity (ADCC).

268A. The multifunctional molecule of any preceding embodiment, further comprising a heavy chain constant region, e.g., an Fc region, that mediates antibody dependent cellular phagocytosis (ADCP).

268B. The multifunctional molecule of embodiment 268A, wherein the first antigen binding domain that binds TRBC1 or TRBC2 comprises an IgG2 heavy chain constant region or the immune cell engager, cytokine inhibitor molecule, or death receptor signal engager comprise an IgG2 heavy chain constant region.

269. The multifunctional molecule of any preceding embodiment, further comprising a heavy chain constant region, e.g., an Fc region, that mediates complement dependent cytotoxicity (e.g., via C1q).

269A. An antibody molecule that binds TRBC1, comprising one or more CDRs, framework regions, variable domains, heavy or light chains, or an antigen binding domain chosen from Tables 2-5, or a sequence substantially identical thereto.

269B. The antibody molecule of embodiment 269A, comprising a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or

deletions, therefrom), or a VHFWR4 amino acid sequence of SEQ ID NO: 218 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

269C. The antibody molecule of either of embodiments 269A or 269B, comprising a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VLFWR4 amino acid sequence of SEQ ID NO: 241 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

269D. The antibody molecule of any of embodiments 269A-269C, wherein the antibody molecule comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

269E. The antibody molecule of any of embodiments 269A-269D, wherein the antibody molecule comprises a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

270. A nucleic acid molecule encoding the multifunctional molecule or antibody molecule of any one of embodiments 1-269E.

271. A vector, e.g., an expression vector, comprising the nucleic acid molecules of embodiment 270.

272. A host cell comprising the nucleic acid molecule of embodiment 270 or the vector of embodiment 271.

273. A method of making, e.g., producing, the multifunctional molecule or antibody molecule of any one of embodiments 1-269E, comprising culturing the host cell of embodiment 272, under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

274. A pharmaceutical composition comprising the multifunctional molecule of any one of embodiments 1-269 and a pharmaceutically acceptable carrier, excipient, or stabilizer.

275. A method of treating a cancer, comprising administering to a subject in need thereof the multifunctional molecule of any one of embodiments 1-269, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

276. The method of embodiment 275, further comprising identifying, evaluating, or selecting a subject in need of treatment, wherein identifying, evaluating, or selecting comprises determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2.

277. The method of embodiment 276, further comprising, responsive to determining that a subject has cancer cells that express a T cell receptor comprising TRBC1:

optionally, selecting the subject for treatment with a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1, and

administering a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1.

278. The method of embodiment 277, further comprising not administering a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC2.

278A. A method of treating a cancer, e.g., a lymphoma or leukemia, comprising:

responsive to determining that a subject has cancer cells that express a T cell receptor comprising TRBC1, administering to a subject in need thereof the multifunctional molecule of any one of claims 1-269, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

279. The method of embodiment 276, further comprising, responsive to determining that a subject has cancer cells that express a T cell receptor comprising TRBC2:

optionally, selecting the subject for treatment with a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC2, and

administering a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC2.

280. The method of embodiment 279, further comprising not administering a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1.

281. The method of any of embodiments 275-278, wherein the subject has cancer cells that express a T cell receptor comprising TRBC1.

282. The method of any of embodiments 275, 276, 279, or 280, wherein the subject has cancer cells that express a T cell receptor comprising TRBC2.

283. A method of identifying a subject in need of treatment for cancer using a multifunctional molecule or antibody molecule of any of embodiments 1-269E, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2, wherein:

responsive to determining that the subject has cancer cells that express a T cell receptor comprising TRBC1, identifying the subject as a candidate for treatment using a multifunctional

molecule comprising an antigen binding domain that binds to TRBC1, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and

responsive to determining that the subject has cancer cells that express a T cell receptor comprising TRBC2, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1.

284. The method of embodiment 283, further comprising:

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, or

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC2.

285. A method of evaluating a subject in need of treatment for cancer, e.g., a lymphoma, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2.

286. The method of embodiment 285, further comprising responsive to the evaluation, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC1 or a multifunctional molecule comprising an antigen binding domain that binds to TRBC2.

287. The method of any one of embodiments 275-286, wherein the cancer is a hematological cancer.

288. The method of embodiment 287, wherein the hematological cancer is leukemia or lymphoma.

289. The method of embodiment 288, wherein the hematological cancer is selected from leukemia (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, acute monocytic leukemia (AMoL), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), or large granular lymphocytic leukemia), lymphoma (*e.g.,* AIDS-related lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma (*e.g.,* classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma), mycosis fungoides, non-Hodgkin lymphoma (*e.g.,* B-cell non-Hodgkin lymphoma (*e.g.,* Burkitt lymphoma, small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, or mantle cell lymphoma) or T-cell non-Hodgkin lymphoma (mycosis fungoides, anaplastic large cell lymphoma, or precursor T-lymphoblastic lymphoma)), primary central nervous system lymphoma, Sézary syndrome, Waldenström macroglobulinemia), chronic myeloproliferative neoplasm, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, or myelodysplastic/myeloproliferative neoplasm.

290. The method of embodiment 288, wherein the lymphoma is selected from Acquired immune deficiency syndrome (AIDS)-associated lymphoma, Angioimmunoblastic T-cell lymphoma, Adult T-cell leukemia/lymphoma, Burkitt lymphoma, Central nervous system (CNS) lymphoma, Diffuse large B-cell lymphoma (DLBCL), Lymphoblastic lymphoma, Mantle cell lymphoma (MCL), Peripheral T-cell lymphoma (PTCL) (e.g., Hepatosplenic T-cell lymphoma (HSGDTCL), Subcutaneous paniculitis-like T-cell lymphoma, or Enteropathy-associated T-cell lymphoma), Transformed follicular and transformed mucosa-associated lymphoid tissue (MALT) lymphomas, Cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome),

Follicular lymphoma, Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, Marginal zone B-cell lymphoma, Gastric mucosa-associated lymphoid tissue (MALT) lymphoma, Chronic lymphocytic leukemia/small-cell lymphocytic lymphoma (CLL/SLL), Extranodal T-/NK-cell lymphoma (nasal type), or Anaplastic large-cell lymphoma (e.g., primary cutaneous anaplastic large-cell lymphoma or systemic anaplastic large-cell lymphoma).

291. The method of any one of embodiments 275-286, the cancer is a solid tumor cancer.

292. The method of any of embodiments 275-291, further comprising administering a second therapeutic treatment.

293. The method of embodiment 292, wherein the second therapeutic treatment comprises a therapeutic agent (e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery.

294. The method of embodiment 293, wherein the therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1D are schematic representations of exemplary formats and configurations of multispecific antibodies (e.g., bispecific antibodies) that bind to TRBC1 and NKp30. FIG. 1A depicts an anti-TRBC1 antibody fused to an anti-NKp30 scFv. The anti-TRBC1 antibody comprises two heavy chains and two light chains. The anti-NKp30 scFv is fused to the N-terminus of one heavy chain of the anti-TRBC1 antibody. FIG. 1B depicts an antibody molecule comprising an anti-TRBC1 Fab, an anti-NKp30 scFv, and an Fc dimer. The Fc dimer comprises two Fc chains. The C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain. The anti-NKp30 scFv is fused to the N-terminus of the other Fc chain. FIGs. 1C and 1D depict an anti-TRBC1 antibody fused to two anti-NKp30 scFvs. The anti-TRBC1 antibody comprises two heavy chains and two light chains. In FIG. 1C, the two anti-NKp30 scFvs are fused to the C-terminus of the two light chains of the anti-TRBC1 antibody, respectively. In FIG. 1D, the two anti-NKp30 scFvs are fused to the N-terminus of the two heavy chains of the anti-TRBC1 antibody, respectively.

FIGs. 2A-2F are schematic representations of exemplary formats and configurations of antibody molecules that comprises a moiety that binds to TRBC1 and a TRAIL molecule (e.g., a trimeric, dimeric, or monomeric TRAIL molecule). FIGs. 2A and 2D depict an antibody molecule comprising an anti-TRBC1 Fab, a trimeric TRAIL molecule, and an Fc dimer. FIGs. 2B and 2E depict an antibody molecule comprising an anti-TRBC1 Fab, a dimeric TRAIL molecule, and an Fc dimer. FIGs. 2C and 2F depict an antibody molecule comprising an anti-TRBC1 Fab, a monomeric TRAIL molecule, and an Fc dimer. The Fc dimer comprises two Fc chains. The C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain. The trimeric, dimeric, or monomeric TRAIL molecule is fused to the N-terminus of the other Fc chain. In some embodiments, the antibody molecule depicted in FIG. 2A comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6159. In some embodiments, the antibody molecule depicted in FIG. 2B comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6158. In some embodiments, the antibody molecule depicted in FIG. 2C comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6157. In some embodiments, then antibody molecule depicted in FIG. 2D comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6162. In some embodiments, then antibody molecule depicted in FIG. 2E comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6161. In some

embodiments, then antibody molecule depicted in FIG. 2F comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6160.

FIGs. 3A and 3B are schematic representations of exemplary formats and configurations of multispecific antibodies (e.g., bispecific antibodies) that bind to TRBC1 and DR5. FIG. 3A depicts a multispecific antibody (e.g., a bispecific antibody) comprising an anti-TRBC1 Fab, an anti-DR5 scFv, and an Fc dimer. The Fc dimer comprises two Fc chains. The C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain. The anti-DR5 scFv is fused to the N-terminus of the other Fc chain. FIG. 3B depicts an anti-TRBC1 antibody fused to two anti-DR5 scFvs. The anti-TRBC1 antibody comprises two heavy chains and two light chains. The two anti-DR5 scFvs are fused to the C-terminus of the two light chains of the anti-TRBC1 antibody, respectively. In some embodiments, the multispecific antibody depicted in FIG. 3A comprises the amino acid sequences of SEQ ID NOs: 6169, 6167, and 6163. In some embodiments, the multispecific antibody depicted in FIG. 3B comprises the amino acid sequences of SEQ ID NOs: 6170 and 6168.

FIGs. 4A-4B shows the alignment of the H131 source mouse VH and VL framework 1, CDR 1, framework 2, CDR 2, framework 3, CDR3, and framework 4 regions with their respective humanized sequences. Kabat CDRs are shown in bold, Chothia CDRs are shown in italics, and combined CDRs are shown in boxes. The framework positions that were back mutated are double underlined. **FIG. 4A** shows VH sequences for murine H131 (SEQ ID NO: 1) and humanized H131 (SEQ ID NO: 9). **FIG. 4B** shows VL sequences for murine H131 (SEQ ID NO: 2) and humanized H131 (SEQ ID NO: 10 and SEQ ID NO: 11).

FIGs. 5A-5B shows the alignment of the 16G8 source mouse VH and VL framework 1, CDR 1, framework 2, CDR 2, framework 3, CDR3, and framework 4 regions with their respective humanized sequences. Kabat CDRs are shown in bold, Chothia CDRs are shown in italics, and combined CDRs are shown in boxes. The framework positions that were back mutated are double underlined. **FIG. 5A** shows VH sequences for murine 16G8 (SEQ ID NO: 15) and humanized 16G8 (SEQ ID NOs: 23-25). **FIG. 5B** shows VL sequences for murine 16G8 (SEQ ID NO: 16) and humanized 16G8 (SEQ ID NOs: 26-30).

FIG. 6 depicts the phylogenetic tree of TCRBV gene family and subfamilies with corresponding antibodies mapped. Subfamily identities are as follows: Subfamily A: TCRβ V6;

Subfamily B: TCRβ V10; Subfamily C: TCRβ V12; Subfamily D: TCRβ V5; Subfamily E: TCRβ V7; Subfamily F: TCRβ V11; Subfamily G: TCRβ V14; Subfamily H: TCRβ V16; Subfamily I:TCRβ V18; Subfamily J:TCRβ V9; Subfamily K: TCRβ V13; Subfamily L: TCRβ V4; Subfamily M:TCRβ V3; Subfamily N:TCRβ V2; Subfamily O:TCRβ V15; Subfamily P: TCRβ V30; Subfamily Q: TCRβ V19; Subfamily R:TCRβ V27; Subfamily S:TCRβ V28; Subfamily T: TCRβ V24; Subfamily U: TCRβ V20; Subfamily V: TCRβ V25; and Subfamily W:TCRβ V29 subfamily. Subfamily members are described in detail herein in the Section titled "TCR beta V (TCRβV)".

FIG. 7 is a graph showing binding of JOVI.1 and humanized JOVI.1 to human TRBC1.

FIG. 8 is a set of graphs showing binding of JOVI.1 Fab (left) and humanized JOVI.1 Fab to human TRBC1 (right).

FIG. 9 is a graph showing binding of NKp30 antibodies to NK92 cells. Data was calculated as the percent-AF747 positive population.

FIG. 10 is a graph showing activation of NK92 cells by NKp30 antibodies. Data were generated using hamster anti-NKp30 mAbs.

FIGs. 11A-11E are schematic representations of anti-TRBC1/NKp30 antibodies and control molecules.

FIGs. 12A-12B are graphs showing binding of antibodies to Fcγ receptor-expressing THP1 cells.

FIGs. 13A-13D are graphs showing T cell activation after incubation with the indicated antibodies. FIG. 13A is a graph showing % CD4+ divided. FIG. 13B is a graph showing % CD8+ divided. FIG. 13C is a graph showing % CD69-CD25+ of CD4+. FIG. 13D is a graph showing % CD69-CD25+ of CD8+.

FIGs. 14A-14D are schematic representations of anti-TRBC1/NKp30 antibodies. In FIGs. 14B and 14D, "460" indicates a Fab based on BIM0460; "578" indicates a Fab based on BJM0578; "407" indicates a scFv (FIG. 18B) or a Fab (FIG. 14D) based on BJM0407; "411" indicates a scFv (FIG. 18B) or a Fab (FIG. 14D) based on BJM0411; and "N297A" indicates that the antibody comprises an N297A mutation in the Fc region.

FIGs. 15A-15D are graphs showing binding of the indicated antibodies to NK cell line KHYG-1 (FIG. 15A) and TRBC1+ Jurkat cells (FIG. 15B). FIG. 15C is a table providing

information on the antibodies tested. FIG. 15D is a table providing EC50 for binding to KHYG-1 cells or TRBC1+ Jurkat cells.

FIGs. 16A-16C are graphs showing killing of TRBC1+ target cells in the presence of NK-92 effector cells. The target cells are TRBC1+ Jurkat cells (FIG. 16A) or H9 cells (FIG. 16B). TRBC2+ HPB-ALL cells were used as a control (FIG. 16C).

FIGs. 17A-17C are graphs showing killing of TRBC1+ target cells in the presence of primary NK cells. The target cells are TRBC1+ Jurkat cells (FIG. 17A) or H9 cells (FIG. 17B). TRBC2+ HPB-ALL cells were used as a control (FIG. 17C).

FIGs. 18A-18C are graphs showing activation of NK cells after co-culture with TRBC1+ Jurkat cells in the presence of anti-TRBC1/NKp30 antibodies. FIG. 18A shows % CD69+CD107a+ NK cells. FIG. 18B shows the level of IFNγ. FIG. 18C shows the level of TNFα.

FIGs. 19A-19B are graphs showing cytokine levels produced by NK cells in the presence or absence of TRBC1+ Jurkat cells. FIG. 19A shows the level of IFNγ. FIG. 19B shows the level of TNFα.

FIG. 20 is a graph showing % NK cell death induced by the indicated antibodies in the presence of TRBC1+ Jurkat cells.

FIGs. 21A and 21B are schematic representations of a single arm anti-TRBC1 antibody and a bispecific anti-TRBC1/NKp30 antibody, respectively.

FIGs. 22A-22D are graphs showing NK cell-mediated killing of TRBC1+ PDX in the presence of the indicated antibodies.

FIG. 23 is a panel of figures showing killing of TRBC1+ Jurkat cells in the presence of the indicated antibodies. The NK cells tested were isolated from healthy donors (upper panel) or from PTCL patients (lower panel).

FIG. 24 is a panel of figures showing activation of NK cells during the killing assay shown in FIG. 23. The NK cells tested were isolated from healthy donors (upper panel) or from PTCL patients (lower panel).

FIGs. 25A and 25B are a panel of figures showing IFNγ (FIG. 25A) or TNFα (FIG. 25B) secretion levels of NK cells when co-cultured with Jurkat cells in the presence of the indicated

antibodies. The NK cells tested were isolated from healthy donors (upper panel) or from PTCL patients (lower panel).

FIGs. 26A-26C are graphs measuring binding to NKp30 in ELISA. FIG. 26A shows binding of B7-H6 to NKp30. FIG. 26B shows binding of BJM1042 to NKp30. FIG. 26C shows binding of B7-H6 to NKp30 in the presence of varying concentrations of the indicated antibodies.

FIGs. 27A-27C are graphs from an in vivo TRBC1+ tumor study. FIG. 27A shows the study design. FIG. 27B shows tumor volume under the indicated treatments. FIG. 27C is a water plot showing % change in tumor volume on Day 3 post treatment. The following treatment groups are shown in FIG. 27C from left to right: No NK, PBS; No NK, TRBC1 x NKp30; NK, PBS; NK, TRBC1; NK, NKp30; and NK + 1mpk BJM1042.

FIGs. 28A-28C are graphs from an in vivo TRBC2+ tumor study. FIG. 28A shows the study design. FIG. 28B shows tumor volume under the indicated treatments.

FIGs. 29A-29C are schematic representations of anti-TRBC1/NKp30 antibodies.

FIGs. 30A-30C are schematic representations of anti-TRBC1/NKp30 antibodies.

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are multifunctional molecules (also referred to herein as "multispecific molecules") that include a plurality of (e.g., two or more) functionalities (or binding specificities), comprising (i) an antigen binding domain that preferentially binds to TRBC1 or a TRBC2, and (ii) one, two, or all of: (a) an immune cell engager chosen from a T cell engager, an NK cell engager (e.g., a molecule that binds to NKp30, NKp46, NKG2D, or CD16), a B cell engager, a dendritic cell engager, or a macrophage cell engager; (b) a cytokine molecule; and (c) a stromal modifying moiety. Also disclosed herein are antibody molecules comprising an

antigen binding domain that preferentially binds to TRBC1 or TRBC2. In some embodiments, the antigen binding domain that binds to TRBC1 or TRBC2 comprises a sequence or part of a sequence found in Tables 2-5. In some embodiments, the immune cell engager comprises an NK cell engager comprising a sequence or part of a sequence found in Tables 7-10. In some embodiments, the antigen binding domain comprises a sequence or part of a sequence found in Tables 2-5 and the immune cell engager comprises an NK cell engager comprising a sequence or part of a sequence found in Tables 7-10.

In an embodiment, the multispecific or multifunctional molecule is a bispecific (or bifunctional) molecule, a trispecific (or trifunctional) molecule, or a tetraspecific (or tetrafunctional) molecule.

In some embodiments, the multifunctional molecule comprises an antigen binding domain that binds a tumor antigen on the surface of a T cell receptor comprising TRBC1 targets immune cells (e.g., via the immune cell engager) to lymphoma cells (e.g., T cells) that exhibit T cell receptors comprising TRBC1

Without being bound by theory, the multispecific or multifunctional molecules disclosed herein are expected to localize (e.g., bridge) and/or activate an immune cell (e.g., an immune effector cell chosen from a T cell, an NK cell, a B cell, a dendritic cell or a macrophage), in the presence of a cell (e.g., a cancer cell, e.g., lymphoma cell, e.g., T cell) expressing a T cell receptor comprising TRBC1 or TRBC2, e.g., on the surface. Increasing the proximity and/or activity of the immune cell, in the presence of the cell (e.g., cancer cell, e.g., lymphoma cell, e.g., T cell) expressing a T cell receptor comprising TRBC1 or TRBC2, using the multispecific or multifunctional molecules described herein is expected to enhance an immune response against the target cell, thereby providing a more effective therapy.

Without being bound by theory, it is thought that T cells do not typically express T cell receptors comprising TRBC1 and T cell receptors comprising TRBC2. By utilizing, in some embodiments, a multispecific or multifunctional molecule specific for a T cell receptor comprising TRBC1 or a T cell receptor comprising TRBC2, but not with specificity for both types of T cell receptors, it is expected that the deleterious effects of increasing the proximity or

activity of immune cells toward T cells generally may be mitigated. In this way, it is thought that use of the multispecific or multifunctional molecules disclosed herein may increase the proximity or activity of immune cells toward cancer cells (e.g., lymphoma cells, e.g., T cells) without necessarily increasing proximity or activity of immune cells toward T cells generally.

Novel multifunctional, e.g., multispecific, molecules that include (i) a stromal modifying moiety and (ii) an antigen binding domain that preferentially binds to tumor antigen on a lymphoma cell (e.g., T cell), e.g., a T cell receptor comprising TRBC1 or a T cell receptor comprising TRBC2 are disclosed. Without being bound by theory, the multifunctional molecules disclosed herein are believed to inter alia target (e.g., localize to) a cancer site, and alter the tumor stroma, e.g., alter the tumor microenvironment near the cancer site. The multifunctional molecules can further include one or both of: an immune cell engager (e.g., chosen from one, two, three, or all of a T cell engager, NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager); and/or a cytokine molecule. Accordingly, provided herein are, inter alia, multifunctional, e.g., multispecific molecules, that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a cancer using the aforesaid molecules.

Accordingly, provided herein are, *inter alia*, multispecific or multifunctional molecules (e.g., multispecific or multifunctional antibody molecules) that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a disease or disorder, e.g., cancer, using the aforesaid molecules.

Definitions

In some embodiments, the multifunctional molecule includes an immune cell engager. "An immune cell engager" refers to one or more binding specificities that bind and/or activate an immune cell, e.g., a cell involved in an immune response. In embodiments, the immune cell is chosen from a T cell, an NK cell, a B cell, a dendritic cell, and/or the macrophage cell. The immune cell engager can be an antibody molecule, a receptor molecule (e.g., a full length receptor, receptor fragment, or fusion thereof (e.g., a receptor-Fc fusion)), or a ligand molecule (e.g., a full length ligand, ligand fragment, or fusion thereof (e.g., a ligand-Fc fusion)) that binds to the immune cell antigen (e.g., the T cell, the NK cell antigen, the B cell antigen, the dendritic

cell antigen, and/or the macrophage cell antigen). In embodiments, the immune cell engager specifically binds to the target immune cell, e.g., binds preferentially to the target immune cell. For example, when the immune cell engager is an antibody molecule, it binds to an immune cell antigen (e.g., a T cell antigen, an NK cell antigen, a B cell antigen, a dendritic cell antigen, and/or a macrophage cell antigen) with a dissociation constant of less than about 10 nM.

In some embodiments, the multifunctional molecule includes a cytokine molecule. As used herein, a "cytokine molecule" refers to full length, a fragment or a variant of a cytokine; a cytokine further comprising a receptor domain, e.g., a cytokine receptor dimerizing domain; or an agonist of a cytokine receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to a cytokine receptor, that elicits at least one activity of a naturally-occurring cytokine. In some embodiments the cytokine molecule is chosen from interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), interleukin-21 (IL-21), or interferon gamma, or a fragment or variant thereof, or a combination of any of the aforesaid cytokines. The cytokine molecule can be a monomer or a dimer. In embodiments, the cytokine molecule can further include a cytokine receptor dimerizing domain. In other embodiments, the cytokine molecule is an agonist of a cytokine receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to a cytokine receptor chosen from an IL-15Ra or IL-21R.

As used herein, the term "molecule" as used in, e.g., antibody molecule, cytokine molecule, receptor molecule, includes full-length, naturally-occurring molecules, as well as variants, e.g., functional variants (e.g., truncations, fragments, mutated (e.g., substantially similar sequences) or derivatized form thereof), so long as at least one function and/or activity of the unmodified (e.g., naturally-occurring) molecule remains.

In some embodiments, the multifunctional molecule includes a stromal modifying moiety. A "stromal modifying moiety," as used herein refers to an agent, e.g., a protein (e.g., an enzyme), that is capable of altering, e.g., degrading a component of, the stroma. In embodiments, the component of the stroma is chosen from, e.g., an ECM component, e.g., a glycosaminoglycan, e.g., hyaluronan (also known as hyaluronic acid or HA), chondroitin sulfate, chondroitin, dermatan sulfate, heparin sulfate, heparin, entactin, tenascin, aggrecan and keratin sulfate; or an extracellular protein, e.g., collagen, laminin, elastin, fibrinogen, fibronectin, and vitronectin.

Certain terms are defined below.

As used herein, the articles “a” and “an” refer to one or more than one, *e.g.*, to at least one, of the grammatical object of the article. The use of the words "a" or "an" when used in conjunction with the term "comprising" herein may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

As used herein, “about” and “approximately” generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given range of values.

“Antibody molecule” as used herein refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. An antibody molecule encompasses antibodies (e.g., full-length antibodies) and antibody fragments. In an embodiment, an antibody molecule comprises an antigen binding or functional fragment of a full length antibody, or a full length immunoglobulin chain. For example, a full-length antibody is an immunoglobulin (Ig) molecule (e.g., an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes). In embodiments, an antibody molecule refers to an immunologically active, antigen-binding portion of an immunoglobulin molecule, such as an antibody fragment. An antibody fragment, e.g., functional fragment, is a portion of an antibody, e.g., Fab, Fab′, $F(ab')_2$, $F(ab)_2$, variable fragment (Fv), domain antibody (dAb), or single chain variable fragment (scFv). A functional antibody fragment binds to the same antigen as that recognized by the intact (e.g., full-length) antibody. The terms “antibody fragment” or “functional fragment” also include isolated fragments consisting of the variable regions, such as the “Fv” fragments consisting of the variable regions of the heavy and light chains or recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (“scFv proteins”). In some embodiments, an antibody fragment does not include portions of antibodies without antigen binding activity, such as Fc fragments or single amino acid residues. Exemplary antibody molecules include full length antibodies and antibody fragments, e.g., dAb (domain antibody), single chain, Fab, Fab’, and $F(ab')_2$ fragments, and single chain variable fragments (scFvs).

As used herein, an "immunoglobulin variable domain sequence" refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may or may not include one, two, or more N- or C-terminal amino acids, or may include other alterations that are compatible with formation of the protein structure.

In embodiments, an antibody molecule is monospecific, e.g., it comprises binding specificity for a single epitope. In some embodiments, an antibody molecule is multispecific, e.g., it comprises a plurality of immunoglobulin variable domain sequences, where a first immunoglobulin variable domain sequence has binding specificity for a first epitope and a second immunoglobulin variable domain sequence has binding specificity for a second epitope. In some embodiments, an antibody molecule is a bispecific antibody molecule. "Bispecific antibody molecule" as used herein refers to an antibody molecule that has specificity for more than one (e.g., two, three, four, or more) epitope and/or antigen.

"Antigen" (Ag) as used herein refers to a molecule that can provoke an immune response, e.g., involving activation of certain immune cells and/or antibody generation. Any macromolecule, including almost all proteins or peptides, can be an antigen. Antigens can also be derived from genomic recombinant or DNA. For example, any DNA comprising a nucleotide sequence or a partial nucleotide sequence that encodes a protein capable of eliciting an immune response encodes an "antigen." In embodiments, an antigen does not need to be encoded solely by a full length nucleotide sequence of a gene, nor does an antigen need to be encoded by a gene at all. In embodiments, an antigen can be synthesized or can be derived from a biological sample, e.g., a tissue sample, a tumor sample, a cell, or a fluid with other biological components. As used, herein a "tumor antigen" or interchangeably, a "cancer antigen" includes any molecule present on, or associated with, a cancer, e.g., a cancer cell or a tumor microenvironment that can provoke an immune response. As used, herein an "immune cell antigen" includes any molecule present on, or associated with, an immune cell that can provoke an immune response.

The "antigen-binding site," or "binding portion" of an antibody molecule refers to the part of an antibody molecule, e.g., an immunoglobulin (Ig) molecule, that participates in antigen binding. In embodiments, the antigen binding site is formed by amino acid residues of the

variable (V) regions of the heavy (H) and light (L) chains. Three highly divergent stretches within the variable regions of the heavy and light chains, referred to as hypervariable regions, are disposed between more conserved flanking stretches called "framework regions," (FRs). FRs are amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In embodiments, in an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface, which is complementary to the three-dimensional surface of a bound antigen. The three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." The framework region and CDRs have been defined and described, e.g., in Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917. Each variable chain (e.g., variable heavy chain and variable light chain) is typically made up of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the amino acid order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

"Cancer" as used herein can encompass all types of oncogenic processes and/or cancerous growths. In embodiments, cancer includes primary tumors as well as metastatic tissues or malignantly transformed cells, tissues, or organs. In embodiments, cancer encompasses all histopathologies and stages, e.g., stages of invasiveness/severity, of a cancer. In embodiments, cancer includes relapsed and/or resistant cancer. The terms "cancer" and "tumor" can be used interchangeably. For example, both terms encompass solid and liquid tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

As used herein, an "immune cell" refers to any of various cells that function in the immune system, e.g., to protect against agents of infection and foreign matter. In embodiments, this term includes leukocytes, e.g., neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Innate leukocytes include phagocytes (e.g., macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. Innate leukocytes identify and eliminate pathogens, either by attacking larger pathogens through contact or by engulfing and then killing microorganisms, and are mediators in the activation of an adaptive immune

response. The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are important types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response. The term "immune cell" includes immune effector cells.

"Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include, but are not limited to, T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NK T) cells, and mast cells.

The term "effector function" or "effector response" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, *e.g.*, sequences at least 80%, 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 80%, 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

The term "variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence. In some embodiments, the variant is a functional variant.

The term "functional variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence, and is capable of having one or more activities of the reference amino acid sequence.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology").

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a

length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) *CABIOS*, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

It is understood that the molecules of the present invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

The term "amino acid" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs

having variant side chains; and all stereoisomers of any of any of the foregoing. As used herein the term "amino acid" includes both the D- or L- optical isomers and peptidomimetics.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.,* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

The terms "polypeptide", "peptide" and "protein" (if single chain) are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. The polypeptide can be isolated from natural sources, can be a produced by recombinant techniques from a eukaryotic or prokaryotic host, or can be a product of synthetic procedures.

The terms "nucleic acid," "nucleic acid sequence," "nucleotide sequence," or "polynucleotide sequence," and "polynucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The polynucleotide may be either single-stranded or double-stranded, and if single-stranded may be the coding strand or non-coding (antisense) strand. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acid may be a recombinant polynucleotide, or a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

The term "isolated," as used herein, refers to material that is removed from its original or native environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature.

Various aspects of the invention are described in further detail below. Additional definitions are set out throughout the specification.

Antibody Molecules

In one embodiment, the antibody molecule binds to a cancer antigen, e.g., a tumor antigen or a stromal antigen. In some embodiments, the cancer antigen is, *e.g.*, a mammalian, e.g., a human, cancer antigen. In other embodiments, the antibody molecule binds to an immune cell antigen, *e.g.*, a mammalian, e.g., a human, immune cell antigen. For example, the antibody molecule binds specifically to an epitope, *e.g.*, linear or conformational epitope, on the cancer antigen or the immune cell antigen.

In an embodiment, an antibody molecule is a monospecific antibody molecule and binds a single epitope. *E.g.*, a monospecific antibody molecule having a plurality of immunoglobulin variable domain sequences, each of which binds the same epitope.

In an embodiment an antibody molecule is a multispecific or multifunctional antibody molecule, *e.g.*, it comprises a plurality of immunoglobulin variable domains sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a multispecific antibody

molecule comprises a third, fourth or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv or a Fab, or fragment thereof, have binding specificity for a first epitope and a scFv or a Fab, or fragment thereof, have binding specificity for a second epitope.

In an embodiment, an antibody molecule comprises a diabody, and a single-chain molecule, as well as an antigen-binding fragment of an antibody (*e.g.,* Fab, F(ab')$_2$, and Fv). For example, an antibody molecule can include a heavy (H) chain variable domain sequence (abbreviated herein as VH), and a light (L) chain variable domain sequence (abbreviated herein as VL). In an embodiment an antibody molecule comprises or consists of a heavy chain and a light chain (referred to herein as a half antibody. In another example, an antibody molecule includes two heavy (H) chain variable domain sequences and two light (L) chain variable domain

sequence, thereby forming two antigen binding sites, such as Fab, Fab', $F(ab')_2$, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable domain antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (*e.g.*, humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments retain the ability to selectively bind with their respective antigen or receptor. Antibodies and antibody fragments can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (*e.g.*, IgG1, IgG2, IgG3, and IgG4) of antibodies. The a preparation of antibody molecules can be monoclonal or polyclonal. An antibody molecule can also be a human, humanized, CDR-grafted, or *in vitro* generated antibody. The antibody can have a heavy chain constant region chosen from, *e.g.*, IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, *e.g.*, kappa or lambda. The term "immunoglobulin" (Ig) is used interchangeably with the term "antibody" herein.

Examples of antigen-binding fragments of an antibody molecule include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable domain; (vii) a single chain Fv (scFv), *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883); (viii) a single domain antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

Antibody molecules include intact molecules as well as functional fragments thereof. Constant regions of the antibody molecules can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function).

Antibody molecules can also be single domain antibodies. Single domain antibodies can include antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies

naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. According to another aspect of the invention, a single domain antibody is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. Such single domain antibodies are disclosed in WO 9404678, for example. For clarity reasons, this variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the invention.

The VH and VL regions can be subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR), interspersed with regions that are more conserved, termed "framework regions" (FR or FW).

The extent of the framework region and CDRs has been precisely defined by a number of methods (*see*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917; and the AbM definition used by Oxford Molecular's AbM antibody modeling software. *See*, generally, *e.g.*, *Protein Sequence and Structure Analysis of Antibody Variable Domains.* In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg).

The terms “complementarity determining region,” and “CDR,” as used herein refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of known schemes, including those described by Kabat *et al.* (1991),

"Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani *et al.*, (1997) *JMB* 273,927-948 ("Chothia" numbering scheme). As used herein, the CDRs defined according the "Chothia" number scheme are also sometimes referred to as "hypervariable loops."

For example, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3).

Each VH and VL typically includes three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The antibody molecule can be a polyclonal or a monoclonal antibody.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. A monoclonal antibody can be made by hybridoma technology or by methods that do not use hybridoma technology (*e.g.*, recombinant methods).

The antibody can be recombinantly produced, *e.g.,* produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating antibodies are known in the art (as described in, *e.g.,* Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* International Publication No. WO 92/18619; Dower *et al.* International Publication No. WO 91/17271; Winter *et al.* International Publication WO 92/20791; Markland *et al.* International Publication No. WO 92/15679; Breitling *et al.* International Publication WO 93/01288; McCafferty *et al.* International Publication No. WO 92/01047; Garrard *et al.* International Publication No. WO 92/09690; Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.*

(1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

In one embodiment, the antibody is a fully human antibody (*e.g.*, an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, *e.g.,* a rodent (mouse or rat), goat, primate (*e.g.*, monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Methods of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, *e.g.*, Wood *et al.* International Application WO 91/00906, Kucherlapati *et al.* PCT publication WO 91/10741; Lonberg *et al.* International Application WO 92/03918; Kay *et al.* International Application 92/03917; Lonberg, N. *et al.* 1994 *Nature* 368:856-859; Green, L.L. *et al.* 1994 *Nature Genet.* 7:13-21; Morrison, S.L. *et al.* 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman *et al.* 1993 *Year Immunol* 7:33-40; Tuaillon *et al.* 1993 *PNAS* 90:3720-3724; Bruggeman *et al.* 1991 *Eur J Immunol* 21:1323-1326).

An antibody molecule can be one in which the variable region, or a portion thereof, *e.g.*, the CDRs, are generated in a non-human organism, *e.g.*, a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibody molecules generated in a non-human organism, *e.g.*, a rat or mouse, and then modified, *e.g.*, in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

An "effectively human" protein is a protein that does substantially not evoke a neutralizing antibody response, *e.g.*, the human anti-murine antibody (HAMA) response. HAMA can be problematic in a number of circumstances, *e.g.*, if the antibody molecule is administered repeatedly, *e.g.*, in treatment of a chronic or recurrent disease condition. A HAMA response can make repeated antibody administration potentially ineffective because of an increased antibody clearance from the serum (*see, e.g.,* Saleh *et al.*, *Cancer Immunol.*

Immunother., 32:180-190 (1990)) and also because of potential allergic reactions (*see, e.g.,* LoBuglio *et al., Hybridoma*, 5:5117-5123 (1986)).

Chimeric antibodies can be produced by recombinant DNA techniques known in the art (see Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.,* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.,* European Patent Application 173,494; Neuberger *et al.*, International Application WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988 *Science* 240:1041-1043); Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.*, 1987, *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl Cancer Inst.* 80:1553-1559).

A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDRs (of heavy and or light immuoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding to the antigen. Preferably, the donor will be a rodent antibody, *e.g.,* a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDRs is called the "donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the donor immunoglobulin is a non-human (*e.g.,* rodent). The acceptor framework is a naturally-occurring (*e.g.,* a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See *e.g.*, Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

An antibody molecule can be humanized by methods known in the art (*see e.g.*, Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi *et al.*, 1986, *BioTechniques* 4:214, and by Queen *et al.* US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference).

Humanized or CDR-grafted antibody molecules can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. *See e.g.*, U.S. Patent 5,225,539; Jones *et al.* 1986 *Nature* 321:552-525; Verhoeyan *et al.* 1988 *Science* 239:1534; Beidler *et al.* 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibody molecules in which specific amino acids have been substituted, deleted or added. Criteria for selecting amino acids from the donor are described in US 5,585,089, *e.g.*, columns 12-16 of US 5,585,089, *e.g.*, columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan *et al.* EP 519596 A1, published on December 23, 1992.

The antibody molecule can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. *et al.* (1999) *Ann N Y Acad Sci* 880:263-80; and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target protein.

In yet other embodiments, the antibody molecule has a heavy chain constant region chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, the (*e.g.*, human) heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In another embodiment, the antibody molecule has a light chain constant region chosen from, *e.*g., the (*e.g.*, human) light chain constant regions of kappa or lambda. The constant region can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody

glycosylation, the number of cysteine residues, effector cell function, and/or complement function). In one embodiment the antibody has: effector function; and can fix complement. In other embodiments the antibody does not; recruit effector cells; or fix complement. In another embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example, it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, *e.g.*, it has a mutagenized or deleted Fc receptor binding region.

Methods for altering an antibody constant region are known in the art. Antibodies with altered function, *e.g.* altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (*see e.g.,* EP 388,151 A1, U.S. Pat. No. 5,624,821 and U.S. Pat. No. 5,648,260, the contents of all of which are hereby incorporated by reference). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

An antibody molecule can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). As used herein, a "derivatized" antibody molecule is one that has been modified. Methods of derivatization include but are not limited to the addition of a fluorescent moiety, a radionucleotide, a toxin, an enzyme or an affinity ligand such as biotin. Accordingly, the antibody molecules of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody molecule can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody molecule is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.,* to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or

homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

Multispecific or multifunctional antibody molecules

Exemplary structures of multispecific and multifunctional molecules defined herein are described throughout. Exemplary structures are further described in: Weidle U et al. (2013) The Intriguing Options of Multispecific Antibody Formats for Treatment of Cancer. *Cancer Genomics & Proteomics* 10: 1-18 (2013); and Spiess C et al. (2015) Alternative molecular formats and therapeutic applications for bispecific antibodies. *Molecular Immunology* 67: 95-106; the full contents of each of which is incorporated by reference herein).

In embodiments, multispecific antibody molecules can comprise more than one antigen-binding site, where different sites are specific for different antigens. In embodiments, multispecific antibody molecules can bind more than one (e.g., two or more) epitopes on the same antigen. In embodiments, multispecific antibody molecules comprise an antigen-binding site specific for a target cell (e.g., cancer cell) and a different antigen-binding site specific for an immune effector cell. In one embodiment, the multispecific antibody molecule is a bispecific antibody molecule. Bispecific antibody molecules can be classified into five different structural groups: (i) bispecific immunoglobulin G (BsIgG); (ii) IgG appended with an additional antigen-binding moiety; (iii) bispecific antibody fragments; (iv) bispecific fusion proteins; and (v) bispecific antibody conjugates.

BsIgG is a format that is monovalent for each antigen. Exemplary BsIgG formats include but are not limited to crossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair, Fab-arm exchange, SEEDbody, triomab, LUZ-Y, Fcab, κλ-body, orthogonal Fab. *See* Spiess et al. Mol. Immunol. 67(2015):95-106. Exemplary BsIgGs include catumaxomab (Fresenius Biotech, Trion Pharma, Neopharm), which contains an anti-CD3 arm and an anti-EpCAM arm; and ertumaxomab (Neovii Biotech, Fresenius Biotech), which targets CD3 and HER2. In some embodiments, BsIgG comprises heavy chains that are engineered for heterodimerization. For example, heavy chains can be engineered for heterodimerization using a "knobs-into-holes" strategy, a SEED platform, a common heavy chain (e.g., in κλ-bodies), and use of heterodimeric Fc regions. *See*

Spiess et al. Mol. Immunol. 67(2015):95-106. Strategies that have been used to avoid heavy chain pairing of homodimers in BsIgG include knobs-in-holes, duobody, azymetric, charge pair, HA-TF, SEEDbody, and differential protein A affinity. *See Id.* BsIgG can be produced by separate expression of the component antibodies in different host cells and subsequent purification/assembly into a BsIgG. BsIgG can also be produced by expression of the component antibodies in a single host cell. BsIgG can be purified using affinity chromatography, e.g., using protein A and sequential pH elution.

IgG appended with an additional antigen-binding moiety is another format of bispecific antibody molecules. For example, monospecific IgG can be engineered to have bispecificity by appending an additional antigen-binding unit onto the monospecific IgG, e.g., at the N- or C-terminus of either the heavy or light chain. Exemplary additional antigen-binding units include single domain antibodies (e.g., variable heavy chain or variable light chain), engineered protein scaffolds, and paired antibody variable domains (e.g., single chain variable fragments or variable fragments). *See Id.* Examples of appended IgG formats include dual variable domain IgG (DVD-Ig), IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, zybody, and DVI-IgG (four-in-one). *See* Spiess et al. Mol. Immunol. 67(2015):95-106. An example of an IgG-scFv is MM-141 (Merrimack Pharmaceuticals), which binds IGF-1R and HER3. Examples of DVD-Ig include ABT-981 (AbbVie), which binds IL-1α and IL-1β; and ABT-122 (AbbVie), which binds TNF and IL-17A.

Bispecific antibody fragments (BsAb) are a format of bispecific antibody molecules that lack some or all of the antibody constant domains. For example, some BsAb lack an Fc region. In embodiments, bispecific antibody fragments include heavy and light chain regions that are connected by a peptide linker that permits efficient expression of the BsAb in a single host cell. Exemplary bispecific antibody fragments include but are not limited to nanobody, nanobody-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, triple body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')2, F(ab')2-scFv2, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, Diabody-Fc, tandem scFv-Fc, and intrabody. *See Id.* For example, the BiTE format comprises tandem scFvs, where the component scFvs bind to CD3 on T cells and a surface antigen on cancer cells

Bispecific fusion proteins include antibody fragments linked to other proteins, e.g., to add additional specificity and/or functionality. An example of a bispecific fusion protein is an immTAC, which comprises an anti-CD3 scFv linked to an affinity-matured T-cell receptor that recognizes HLA-presented peptides. In embodiments, the dock-and-lock (DNL) method can be used to generate bispecific antibody molecules with higher valency. Also, fusions to albumin binding proteins or human serum albumin can be extend the serum half-life of antibody fragments. *See Id.*

In embodiments, chemical conjugation, e.g., chemical conjugation of antibodies and/or antibody fragments, can be used to create BsAb molecules. *See Id.* An exemplary bispecific antibody conjugate includes the CovX-body format, in which a low molecular weight drug is conjugated site-specifically to a single reactive lysine in each Fab arm or an antibody or fragment thereof. In embodiments, the conjugation improves the serum half-life of the low molecular weight drug. An exemplary CovX-body is CVX-241 (NCT01004822), which comprises an antibody conjugated to two short peptides inhibiting either VEGF or Ang2. *See Id.*

The antibody molecules can be produced by recombinant expression, e.g., of at least one or more component, in a host system. Exemplary host systems include eukaryotic cells (e.g., mammalian cells, e.g., CHO cells, or insect cells, e.g., SF9 or S2 cells) and prokaryotic cells (e.g., *E. coli*). Bispecific antibody molecules can be produced by separate expression of the components in different host cells and subsequent purification/assembly. Alternatively, the antibody molecules can be produced by expression of the components in a single host cell. Purification of bispecific antibody molecules can be performed by various methods such as affinity chromatography, e.g., using protein A and sequential pH elution. In other embodiments, affinity tags can be used for purification, e.g., histidine-containing tag, myc tag, or streptavidin tag.

CDR-grafted scaffolds

In embodiments, the antibody molecule is a CDR-grafted scaffold domain. In embodiments, the scaffold domain is based on a fibronectin domain, e.g., fibronectin type III domain. The overall fold of the fibronectin type III (Fn3) domain is closely related to that of the smallest functional antibody fragment, the variable domain of the antibody heavy chain. There

are three loops at the end of Fn3; the positions of BC, DE and FG loops approximately correspond to those of CDR1, 2 and 3 of the VH domain of an antibody. Fn3 does not have disulfide bonds; and therefore Fn3 is stable under reducing conditions, unlike antibodies and their fragments (see, e.g., WO 98/56915; WO 01/64942; WO 00/34784). An Fn3 domain can be modified (e.g., using CDRs or hypervariable loops described herein) or varied, e.g., to select domains that bind to an antigen/marker/cell described herein.

In embodiments, a scaffold domain, e.g., a folded domain, is based on an antibody, e.g., a "minibody" scaffold created by deleting three beta strands from a heavy chain variable domain of a monoclonal antibody (see, e.g., Tramontano et al., 1994, J Mol. Recognit. 7:9; and Martin et al., 1994, EMBO J. 13:5303-5309). The "minibody" can be used to present two hypervariable loops. In embodiments, the scaffold domain is a V-like domain (see, e.g., Coia et al. WO 99/45110) or a domain derived from tendamistatin, which is a 74 residue, six-strand beta sheet sandwich held together by two disulfide bonds (see, e.g., McConnell and Hoess, 1995, J Mol. Biol. 250:460). For example, the loops of tendamistatin can be modified (e.g., using CDRs or hypervariable loops) or varied, e.g., to select domains that bind to a marker/antigen/cell described herein. Another exemplary scaffold domain is a beta-sandwich structure derived from the extracellular domain of CTLA-4 (see, e.g., WO 00/60070).

Other exemplary scaffold domains include but are not limited to T-cell receptors; MHC proteins; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g., thioredoxin, and heat shock proteins; and intracellular signaling domains (such as SH2 and SH3 domains). See, e.g., US 20040009530 and US 7,501,121, incorporated herein by reference.

In embodiments, a scaffold domain is evaluated and chosen, e.g., by one or more of the following criteria: (1) amino acid sequence, (2) sequences of several homologous domains, (3) 3-dimensional structure, and/or (4) stability data over a range of pH, temperature, salinity, organic solvent, oxidant concentration. In embodiments, the scaffold domain is a small, stable protein

domain, e.g., a protein of less than 100, 70, 50, 40 or 30 amino acids. The domain may include one or more disulfide bonds or may chelate a metal, e.g., zinc.

Antibody-Based Fusions

A variety of formats can be generated which contain additional binding entities attached to the N or C terminus of antibodies. These fusions with single chain or disulfide stabilized Fvs or Fabs result in the generation of tetravalent molecules with bivalent binding specificity for each antigen. Combinations of scFvs and scFabs with IgGs enable the production of molecules which can recognize three or more different antigens.

Antibody-Fab Fusion

Antibody-Fab fusions are bispecific antibodies comprising a traditional antibody to a first target and a Fab to a second target fused to the C terminus of the antibody heavy chain. Commonly the antibody and the Fab will have a common light chain. Antibody fusions can be produced by *(1)* engineering the DNA sequence of the target fusion, and *(2)* transfecting the target DNA into a suitable host cell to express the fusion protein. It seems like the antibody-scFv fusion may be linked by a (Gly)-Ser linker between the C-terminus of the CH3 domain and the N-terminus of the scFv, as described by Coloma, J. *et al.* (1997) *Nature Biotech* 15:159.

Antibody-scFv Fusion

*Antibody-scFv Fusion*s are bispecific antibodies comprising a traditional antibody and a scFv of unique specificity fused to the C terminus of the antibody heavy chain. The scFv can be fused to the C terminus through the Heavy Chain of the scFv either directly or through a linker peptide. Antibody fusions can be produced by *(1)* engineering the DNA sequence of the target fusion, and *(2)* transfecting the target DNA into a suitable host cell to express the fusion protein. It seems like the antibody-scFv fusion may be linked by a (Gly)-Ser linker between the C-terminus of the CH3 domain and the N-terminus of the scFv, as described by Coloma, J. *et al.* (1997) *Nature Biotech* 15:159.

Variable Domain Immunoglobulin DVD

A related format is the dual variable domain immunoglobulin (DVD), which are composed of VH and VL domains of a second specificity place upon the N termini of the V domains by shorter linker sequences.

Other exemplary multispecific antibody formats include, e.g., those described in the following US20160114057A1, US20130243775A1, US20140051833, US20130022601, US20150017187A1, US20120201746A1, US20150133638A1, US20130266568A1, US20160145340A1, WO2015127158A1, US20150203591A1, US20140322221A1, US20130303396A1, US20110293613, US20130017200A1, US20160102135A1, WO2015197598A2, WO2015197582A1, US9359437, US20150018529, WO2016115274A1, WO2016087416A1, US20080069820A1, US9145588B, US7919257, and US20150232560A1. Exemplary multispecific molecules utilizing a full antibody-Fab/scFab format include those described in the following, US9382323B2, US20140072581A1, US20140308285A1, US20130165638A1, US20130267686A1, US20140377269A1, US7741446B2, and WO1995009917A1. Exemplary multispecific molecules utilizing a domain exchange format include those described in the following, US20150315296A1, WO2016087650A1, US20160075785A1, WO2016016299A1, US20160130347A1, US20150166670, US8703132B2, US20100316645, US8227577B2, US20130078249.

Fc-containing entities (mini-antibodies)

Fc-containing entities, also known as mini-antibodies, can be generated by fusing scFv to the C-termini of constant heavy region domain 3 (CH3-scFv) and/or to the hinge region (scFv-hinge-Fc) of an antibody with a different specificity. Trivalent entities can also be made which have disulfide stabilized variable domains (without peptide linker) fused to the C-terminus of CH3 domains of IgGs.

Fc-containing multispecific molecules

In some embodiments, the multispecific molecules disclosed herein includes an immunoglobulin constant region (e.g., an Fc region). Exemplary Fc regions can be chosen from

the heavy chain constant regions of IgG1, IgG2, IgG3 or IgG4; more particularly, the heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the immunoglobulin chain constant region (e.g., the Fc region) is altered, e.g., mutated, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function.

In other embodiments, an interface of a first and second immunoglobulin chain constant regions (e.g., a first and a second Fc region) is altered, e.g., mutated, to increase or decrease dimerization, e.g., relative to a non-engineered interface, e.g., a naturally-occurring interface. For example, dimerization of the immunoglobulin chain constant region (e.g., the Fc region) can be enhanced by providing an Fc interface of a first and a second Fc region with one or more of: a paired protuberance-cavity ("knob-in-a hole"), an electrostatic interaction, or a strand-exchange, such that a greater ratio of heteromultimer to homomultimer forms, e.g., relative to a non-engineered interface.

In some embodiments, the multispecific molecules include a paired amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, e.g., of the Fc region of human IgG1 For example, the immunoglobulin chain constant region (e.g., Fc region) can include a paired an amino acid substitution chosen from: T366S, L368A, or Y407V (e.g., corresponding to a cavity or hole), and T366W (e.g., corresponding to a protuberance or knob).

In other embodiments, the multifunctional molecule includes a half-life extender, e.g., a human serum albumin or an antibody molecule to human serum albumin.

Heterodimerized Antibody Molecules & Methods of Making

Various methods of producing multispecific antibodies have been disclosed to address the problem of incorrect heavy chain pairing. Exemplary methods are described below. Exemplary multispecific antibody formats and methods of making said multispecific antibodies are also disclosed in e.g., Speiss et al. Molecular Immunology 67 (2015) 95–106; and Klein et al mAbs 4:6, 653–663; November/December 2012; the entire contents of each of which are incorporated by reference herein.

Heterodimerized bispecific antibodies are based on the natural IgG structure, wherein the two binding arms recognize different antigens. IgG derived formats that enable defined monovalent (and simultaneous) antigen binding are generated by forced heavy chain heterodimerization, combined with technologies that minimize light chain mispairing (e.g., common light chain). Forced heavy chain heterodimerization can be obtained using, e.g., knob-in-hole OR strand exchange engineered domains (SEED).

Knob-in-Hole

Knob-in-Hole as described in US 5,731,116, US 7,476,724 and Ridgway, J. *et al.* (1996) *Prot. Engineering* 9(7): 617-621, broadly involves: *(1)* mutating the CH3 domain of one or both antibodies to promote heterodimerization; and (2) combining the mutated antibodies under conditions that promote heterodimerization. "Knobs" or "protuberances" are typically created by replacing a small amino acid in a parental antibody with a larger amino acid (e.g., T366Y or T366W); "Holes" or "cavities" are created by replacing a larger residue in a parental antibody with a smaller amino acid (e.g., Y407T, T366S, L368A and/or Y407V).

For bispecific antibodies including an Fc domain, introduction of specific mutations into the constant region of the heavy chains to promote the correct heterodimerization of the Fc portion can be utilized. Several such techniques are reviewed in Klein et al. (mAbs (2012) 4:6, 1-11), the contents of which are incorporated herein by reference in their entirety. These techniques include the "knobs-into-holes" (KiH) approach which involves the introduction of a bulky residue into one of the CH3 domains of one of the antibody heavy chains. This bulky residue fits into a complementary "hole" in the other CH3 domain of the paired heavy chain so as to promote correct pairing of heavy chains (see e.g., US7642228).

Exemplary KiH mutations include S354C, T366W in the "knob" heavy chain and Y349C, T366S, L368A, Y407V in the "hole" heavy chain. Other exemplary KiH mutations are provided in Table 1, with additional optional stabilizing Fc cysteine mutations.

Table 1. Exemplary Fc KiH mutations and optional Cysteine mutations

Position	**Knob Mutation**	**Hole Mutation**
T366	T366W	T366S

L368	-	L368A
Y407	-	Y407V
Additional Cysteine Mutations to form a stabilizing disulfide bridge		
Position	**Knob CH3**	**Hole CH3**
S354	S354C	-
Y349	-	Y349C

Other Fc mutations are provided by Igawa and Tsunoda who identified 3 negatively charged residues in the CH3 domain of one chain that pair with three positively charged residues in the CH3 domain of the other chain. These specific charged residue pairs are: E356-K439, E357-K370, D399-K409 and vice versa. By introducing at least two of the following three mutations in chain A: E356K, E357K and D399K, as well as K370E, K409D, K439E in chain B, alone or in combination with newly identified disulfide bridges, they were able to favor very efficient heterodimerization while suppressing homodimerization at the same time (Martens T et al. A novel one-armed antic- Met antibody inhibits glioblastoma growth in vivo. Clin Cancer Res 2006; 12:6144-52; PMID:17062691). Xencor defined 41 variant pairs based on combining structural calculations and sequence information that were subsequently screened for maximal heterodimerization, defining the combination of S364H, F405A (HA) on chain A and Y349T, T394F on chain B (TF) (Moore GL et al. A novel bispecific antibody format enables simultaneous bivalent and monovalent co-engagement of distinct target antigens. MAbs 2011; 3:546-57; PMID: 22123055).

Other exemplary Fc mutations to promote heterodimerization of multispecific antibodies include those described in the following references, the contents of each of which is incorporated by reference herein, WO2016071377A1, US20140079689A1, US20160194389A1, US20160257763, WO2016071376A2, WO2015107026A1, WO2015107025A1, WO2015107015A1, US20150353636A1, US20140199294A1, US7750128B2, US20160229915A1, US20150344570A1, US8003774A1, US20150337049A1, US20150175707A1, US20140242075A1, US20130195849A1, US20120149876A1,

US20140200331A1, US9309311B2, US8586713, US20140037621A1, US20130178605A1, US20140363426A1, US20140051835A1 and US20110054151A1.

Stabilizing cysteine mutations have also been used in combination with KiH and other Fc heterodimerization promoting variants, see e.g., US7183076. Other exemplary cysteine modifications include, e.g., those disclosed in US20140348839A1, US7855275B2, and US9000130B2.

Strand Exchange Engineered Domains (SEED)

Heterodimeric Fc platform that support the design of bispecific and asymmetric fusion proteins by devising strand-exchange engineered domain (SEED) C(H)3 heterodimers are known. These derivatives of human IgG and IgA C(H)3 domains create complementary human SEED C(H)3 heterodimers that are composed of alternating segments of human IgA and IgG C(H)3 sequences. The resulting pair of SEED C(H)3 domains preferentially associates to form heterodimers when expressed in mammalian cells. SEEDbody (Sb) fusion proteins consist of [IgG1 hinge]-C(H)2-[SEED C(H)3], that may be genetically linked to one or more fusion partners (see e.g., Davis JH et al. SEEDbodies: fusion proteins based on strand exchange engineered domain (SEED) CH3 heterodimers in an Fc analogue platform for asymmetric binders or immunofusions and bispecific antibodies. Protein Eng Des Sel 2010; 23:195-202; PMID:20299542 and US8871912. The contents of each of which are incorporated by reference herein).

Duobody

"Duobody" technology to produce bispecific antibodies with correct heavy chain pairing are known. The DuoBody technology involves three basic steps to generate stable bispecific human IgG1antibodies in a post-production exchange reaction. In a first step, two IgG1s, each containing single matched mutations in the third constant (CH3) domain, are produced separately using standard mammalian recombinant cell lines. Subsequently, these IgG1 antibodies are purified according to standard processes for recovery and purification. After production and purification (post-production), the two antibodies are recombined under tailored laboratory conditions resulting in a bispecific antibody product with a very high yield (typically >95%) (see

e.g., Labrijn et al, PNAS 2013;110(13):5145-5150 and Labrijn et al. Nature Protocols 2014;9(10):2450-63, the contents of each of which are incorporated by reference herein).

Electrostatic Interactions

Methods of making multispecific antibodies using CH3 amino acid changes with charged amino acids such that homodimer formation is electrostatically unfavorable are disclosed. EP1870459 and WO 2009089004 describe other strategies for favoring heterodimer formation upon co-expression of different antibody domains in a host cell. In these methods, one or more residues that make up the heavy chain constant domain 3 (CH3), CH3-CH3 interfaces in both CH3 domains are replaced with a charged amino acid such that homodimer formation is electrostatically unfavorable and heterodimerization is electrostatically favorable. Additional methods of making multispecific molecules using electrostatic interactions are described in the following references, the contents of each of which is incorporated by reference herein, include US20100015133, US8592562B2, US9200060B2, US20140154254A1, and US9358286A1.

Common Light Chain

Light chain mispairing needs to be avoided to generate homogenous preparations of bispecific IgGs. One way to achieve this is through the use of the common light chain principle, i.e. combining two binders that share one light chain but still have separate specificities. An exemplary method of enhancing the formation of a desired bispecific antibody from a mixture of monomers is by providing a common variable light chain to interact with each of the heteromeric variable heavy chain regions of the bispecific antibody. Compositions and methods of producing bispecific antibodies with a common light chain as disclosed in, e.g., US7183076B2, US20110177073A1, EP2847231A1, WO2016079081A1, and EP3055329A1, the contents of each of which is incorporated by reference herein.

CrossMab

Another option to reduce light chain mispairing is the CrossMab technology which avoids non-specific L chain mispairing by exchanging CH1 and CL domains in the Fab of one half of the bispecific antibody. Such crossover variants retain binding specificity and affinity, but

make the two arms so different that L chain mispairing is prevented. The CrossMab technology (as reviewed in Klein et al. *Supra*) involves domain swapping between heavy and light chains so as to promote the formation of the correct pairings. Briefly, to construct a bispecific IgG-like CrossMab antibody that could bind to two antigens by using two distinct light chain–heavy chain pairs, a two-step modification process is applied. First, a dimerization interface is engineered into the C-terminus of each heavy chain using a heterodimerization approach, e.g., Knob-into-hole (KiH) technology, to ensure that only a heterodimer of two distinct heavy chains from one antibody (e.g., Antibody A) and a second antibody (e.g., Antibody B) is efficiently formed. Next, the constant heavy 1 (CH1) and constant light (CL) domains of one antibody are exchanged (Antibody A), keeping the variable heavy (VH) and variable light (VL) domains consistent. The exchange of the CH1 and CL domains ensured that the modified antibody (Antibody A) light chain would only efficiently dimerize with the modified antibody (antibody A) heavy chain, while the unmodified antibody (Antibody B) light chain would only efficiently dimerize with the unmodified antibody (Antibody B) heavy chain; and thus only the desired bispecific CrossMab would be efficiently formed (see e.g., Cain, C. SciBX 4(28); doi:10.1038/scibx.2011.783, the contents of which are incorporated by reference herein).

Common Heavy Chain

An exemplary method of enhancing the formation of a desired bispecific antibody from a mixture of monomers is by providing a common variable heavy chain to interact with each of the heteromeric variable light chain regions of the bispecific antibody. Compositions and methods of producing bispecific antibodies with a common heavy chain are disclosed in, e.g., US20120184716, US20130317200, and US20160264685A1, the contents of each of which is incorporated by reference herein.

Amino Acid Modifications

Alternative compositions and methods of producing multispecific antibodies with correct light chain pairing include various amino acid modifications. For example, Zymeworks describes heterodimers with one or more amino acid modifications in the CH1 and/or CL domains, one or more amino acid modifications in the VH and/or VL domains, or a combination thereof, which

are part of the interface between the light chain and heavy chain and create preferential pairing between each heavy chain and a desired light chain such that when the two heavy chains and two light chains of the heterodimer pair are co-expressed in a cell, the heavy chain of the first heterodimer preferentially pairs with one of the light chains rather than the other (see e.g., WO2015181805). Other exemplary methods are described in WO2016026943 (Argen-X), US20150211001, US20140072581A1, US20160039947A1, and US20150368352.

Lambda/Kappa Formats

Multispecific molecules (*e.g.*, multispecific antibody molecules) that include the lambda light chain polypeptide and a kappa light chain polypeptides, can be used to allow for heterodimerization. Methods for generating bispecific antibody molecules comprising the lambda light chain polypeptide and a kappa light chain polypeptides are disclosed in PCT/US17/53053 filed on September 22, 2017, incorporated herein by reference in its entirety.

In embodiments, the multispecific molecules includes a multispecific antibody molecule, e.g., an antibody molecule comprising two binding specificities, e.g., a bispecific antibody molecule. The multispecific antibody molecule includes:

a lambda light chain polypeptide 1 (LLCP1) specific for a first epitope;

a heavy chain polypeptide 1 (HCP1) specific for the first epitope;

a kappa light chain polypeptide 2 (KLCP2) specific for a second epitope; and

a heavy chain polypeptide 2 (HCP2) specific for the second epitope.

"Lambda light chain polypeptide 1 (LLCP1)", as that term is used herein, refers to a polypeptide comprising sufficient light chain (LC) sequence, such that when combined with a cognate heavy chain variable region, can mediate specific binding to its epitope and complex with an HCP1. In an embodiment it comprises all or a fragment of a CH1 region. In an embodiment, an LLCP1 comprises LC-CDR1, LC-CDR2, LC-CDR3, FR1, FR2, FR3, FR4, and CH1, or sufficient sequence therefrom to mediate specific binding of its epitope and complex with an HCP1. LLCP1, together with its HCP1, provide specificity for a first epitope (while KLCP2, together with its HCP2, provide specificity for a second epitope). As described elsewhere herein, LLCP1 has a higher affinity for HCP1 than for HCP2.

"Kappa light chain polypeptide 2 (KLCP2)", as that term is used herein, refers to a polypeptide comprising sufficient light chain (LC) sequence, such that when combined with a cognate heavy chain variable region, can mediate specific binding to its epitope and complex with an HCP2. In an embodiments it comprises all or a fragment of a CH1 region. In an embodiment, a KLCP2 comprises LC-CDR1, LC-CDR2, LC-CDR3, FR1, FR2, FR3, FR4, and CH1, or sufficient sequence therefrom to mediate specific binding of its epitope and complex with an HCP2. KLCP2, together with its HCP2, provide specificity for a second epitope (while LLCP1, together with its HCP1, provide specificity for a first epitope).

"Heavy chain polypeptide 1 (HCP1)", as that term is used herein, refers to a polypeptide comprising sufficient heavy chain (HC) sequence, e.g., HC variable region sequence, such that when combined with a cognate LLCP1, can mediate specific binding to its epitope and complex with an HCP1. In an embodiments it comprises all or a fragment of a CH1region. In an embodiment, it comprises all or a fragment of a CH2 and/or CH3 region. In an embodiment an HCP1 comprises HC-CDR1, HC-CDR2, HC-CDR3, FR1, FR2, FR3, FR4, CH1, CH2, and CH3, or sufficient sequence therefrom to: (i) mediate specific binding of its epitope and complex with an LLCP1, (ii) to complex preferentially, as described herein to LLCP1 as opposed to KLCP2; and (iii) to complex preferentially, as described herein, to an HCP2, as opposed to another molecule of HCP1. HCP1, together with its LLCP1, provide specificity for a first epitope (while KLCP2, together with its HCP2, provide specificity for a second epitope).

"Heavy chain polypeptide 2 (HCP2)", as that term is used herein, refers to a polypeptide comprising sufficient heavy chain (HC) sequence, e.g., HC variable region sequence, such that when combined with a cognate LLCP1, can mediate specific binding to its epitope and complex with an HCP1. In an embodiments it comprises all or a fragment of a CH1region. In an embodiments it comprises all or a fragment of a CH2 and/or CH3 region. In an embodiment an HCP1 comprises HC-CDR1, HC-CDR2, HC-CDR3, FR1, FR2, FR3, FR4, CH1, CH2, and CH3, or sufficient sequence therefrom to: (i) mediate specific binding of its epitope and complex with an KLCP2, (ii) to complex preferentially, as described herein to KLCP2 as opposed to LLCP1; and (iii) to complex preferentially, as described herein, to an HCP1, as opposed to another molecule of HCP2. HCP2, together with its KLCP2, provide specificity for a second epitope (while LLCP1, together with its HCP1, provide specificity for a first epitope).

In some embodiments of the multispecific antibody molecule disclosed herein:

LLCP1 has a higher affinity for HCP1 than for HCP2; and/or

KLCP2 has a higher affinity for HCP2 than for HCP1.

In embodiments, the affinity of LLCP1 for HCP1 is sufficiently greater than its affinity for HCP2, such that under preselected conditions, e.g., in aqueous buffer, e.g., at pH 7, in saline, e.g., at pH 7, or under physiological conditions, at least 75, 80, 90, 95, 98, 99, 99.5, or 99.9 % of the multispecific antibody molecule molecules have a LLCP1complexed, or interfaced with, a HCP1.

In some embodiments of the multispecific antibody molecule disclosed herein:

the HCP1 has a greater affinity for HCP2, than for a second molecule of HCP1; and/or

the HCP2 has a greater affinity for HCP1, than for a second molecule of HCP2.

In embodiments, the affinity of HCP1 for HCP2 is sufficiently greater than its affinity for a second molecule of HCP1, such that under preselected conditions, e.g., in aqueous buffer, e.g., at pH 7, in saline, e.g., at pH 7, or under physiological conditions, at least 75%, 80, 90, 95, 98, 99 99.5 or 99.9 % of the multispecific antibody molecule molecules have a HCP1complexed, or interfaced with, a HCP2.

In another aspect, disclosed herein is a method for making, or producing, a multispecific antibody molecule. The method includes:

(i) providing a first heavy chain polypeptide (e.g., a heavy chain polypeptide comprising one, two, three or all of a first heavy chain variable region (first VH), a first CH1, a first heavy chain constant region (e.g., a first CH2, a first CH3, or both));

(ii) providing a second heavy chain polypeptide (e.g., a heavy chain polypeptide comprising one, two, three or all of a second heavy chain variable region (second VH), a second CH1, a second heavy chain constant region (e.g., a second CH2, a second CH3, or both));

(iii) providing a lambda chain polypeptide (e.g., a lambda light variable region (VLλ), a lambda light constant chain (VLλ), or both) that preferentially associates with the first heavy chain polypeptide (e.g., the first VH); and

(iv) providing a kappa chain polypeptide (e.g., a lambda light variable region (VLκ), a lambda light constant chain (VLκ), or both) that preferentially associates with the second heavy chain polypeptide (e.g., the second VH),

under conditions where (i)-(iv) associate.

In embodiments, the first and second heavy chain polypeptides form an Fc interface that enhances heterodimerization.

In embodiments, (i)-(iv) (e.g., nucleic acid encoding (i)-(iv)) are introduced in a single cell, e.g., a single mammalian cell, e.g., a CHO cell. In embodiments, (i)-(iv) are expressed in the cell.

In embodiments, (i)-(iv) (e.g., nucleic acid encoding (i)-(iv)) are introduced in different cells, e.g., different mammalian cells, e.g., two or more CHO cell. In embodiments, (i)-(iv) are expressed in the cells.

In one embodiments, the method further comprises purifying a cell-expressed antibody molecule, e.g., using a lambda- and/or- kappa-specific purification, e.g., affinity chromatography.

In embodiments, the method further comprises evaluating the cell-expressed multispecific antibody molecule. For example, the purified cell-expressed multispecific antibody molecule can be analyzed by techniques known in the art, include mass spectrometry. In one embodiment, the purified cell-expressed antibody molecule is cleaved, e.g., digested with papain to yield the Fab moieties and evaluated using mass spectrometry.

In embodiments, the method produces correctly paired kappa/lambda multispecific, e.g., bispecific, antibody molecules in a high yield, e.g., at least 75%, 80, 90, 95, 98, 99 99.5 or 99.9 %.

In other embodiments, the multispecific, e.g., a bispecific, antibody molecule that includes:

(i) a first heavy chain polypeptide (HCP1) (e.g., a heavy chain polypeptide comprising one, two, three or all of a first heavy chain variable region (first VH), a first CH1, a first heavy chain constant region (e.g., a first CH2, a first CH3, or both)), e.g., wherein the HCP1 binds to a first epitope;

(ii) a second heavy chain polypeptide (HCP2) (e.g., a heavy chain polypeptide comprising one, two, three or all of a second heavy chain variable region (second VH), a second CH1, a second heavy chain constant region (e.g., a second CH2, a second CH3, or both)), e.g., wherein the HCP2 binds to a second epitope;

(iii) a lambda light chain polypeptide (LLCP1) (e.g., a lambda light variable region (VLl), a lambda light constant chain (VLl), or both) that preferentially associates with the first heavy chain polypeptide (e.g., the first VH), e.g., wherein the LLCP1 binds to a first epitope; and

(iv) a kappa light chain polypeptide (KLCP2) (e.g., a lambda light variable region (VLk), a lambda light constant chain (VLk), or both) that preferentially associates with the second heavy chain polypeptide (e.g., the second VH), e.g., wherein the KLCP2 binds to a second epitope.

In embodiments, the first and second heavy chain polypeptides form an Fc interface that enhances heterodimerization. In embodiments, the multispecific antibody molecule has a first binding specificity that includes a hybrid VLl-CLl heterodimerized to a first heavy chain variable region connected to the Fc constant, CH2-CH3 domain (having a knob modification) and a second binding specificity that includes a hybrid VLk-CLk heterodimerized to a second heavy chain variable region connected to the Fc constant, CH2-CH3 domain (having a hole modification).

TRBC1 and TRBC2 Antigen Binding Domains

The present disclosure provides, *inter alia*, antibody molecules, e.g., multispecific (e.g., bi-, tri-, tetra- specific) or multifunctional molecules, that include, e.g., are engineered to contain, one or more antigen binding domains that bind to a tumor antigen on a lymphoma cell (e.g., T cell). In some embodiments, the tumor antigen comprises a T cell receptor comprising TRBC1 or TRBC2. In some embodiments, the antigen binding domain preferentially binds to a T cell receptor comprising TRBC1 (e.g., relative to a T cell receptor comprising TRBC2). In some embodiments, the antigen binding domain preferentially binds to a T cell receptor comprising TRBC2 (e.g., relative to a T cell receptor comprising TRBC1). In some embodiments, the multifunctional molecules include, e.g., are engineered to contain, one or more antigen binding domains that selectively target lymphocytes expressing TRBC1 or TRBC2. In some

embodiments, the antigen binding domain selectively targets lymphocytes expressing a T cell receptor comprising TRBC1 or a T cell receptor comprising TRBC2.

T cell receptors (TCRs) are receptors found on the surface of lymphocytes, specifically on T lymphocytes (T cells). TCRs are responsible for recognizing antigen fragments presented by major histocompatibility complex (MHC) molecules on other immune cells (e.g., B cells) by signaling through associated CD3 and activating the T cell. The vast majority of TCRs in humans are heterodimers comprising an alpha chain and a beta chain. Both alpha and beta chains of TCR comprise variable and constant regions. The variable regions of the alpha and beta chain are encoded by distinct DNA elements (V, D, and J elements for beta chain; V and J elements for the alpha chain). Recombination between these elements produces in large part the variation in antigen binding specificity of TCRs. The TCR beta chain constant region is selected from two different domains, beta constant domain 1 and beta constant domain 2. Without wishing to be bound by theory, it is thought that the majority of TCRs comprising a beta chain comprise a beta chain comprising beta constant domain 1 or beta constant domain 2, but not both constant domain 1 and constant domain 2.

In some embodiments, the multifunctional or multispecific molecules or antibody molecules of the present application comprise an antigen binding domain that binds to a tumor antigen on a lymphoma cell (e.g., a T cell), e.g., a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2. In some embodiments, the multifunctional or multispecific molecules or antibody molecules of the present application comprise an antigen binding domain that selectively targets lymphocytes expressing a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2. While it is most typical for a lymphocyte or lymphoma cell presenting a T cell receptor comprising TRBC1 or TRBC2 to be a T cell, cancer causes many disruptions in non-disease expression patterns. Thus, in some embodiments, the lymphoma cell or lymphocyte may not be a T cell. In some embodiments, the lymphoma cell or lymphocyte is a B cell. In some embodiments, the lymphoma cell or lymphocyte is a natural killer cell.

In some embodiments, the antigen binding domain (e.g., first antigen binding domain) comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or

variable region amino acid sequence of an anti-TRBC1 antibody known in the art. In some embodiments, CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence are selected from JOVI.1.

In some embodiments, the antigen binding domain that binds to TRBC1 comprises one or more CDRs (e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and/or VLCDR3) disclosed in Table 2, Table 6, or Table 3, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to TRBC1 comprises one or more framework regions (e.g., VHFWR1, VHFWR2, VHFWR3, VHFWR4, VLFWR1, VLFWR2, VLFWR3, and/or VLFWR4) disclosed in Table 2, Table 6, or Table 3, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to TRBC1 comprises a VH and/or a VL disclosed in Table 4, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to TRBC1 comprises an amino acid sequence disclosed in Table 5, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

In some embodiments, the antigen binding domain that binds to TRBC1 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3.

In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 7355, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 201, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7354, 201, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7354, 7355, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 7355, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 201, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of: SEQ ID NOs: 7346, 7355, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346,

201, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or SEQ ID NOs: 7354, 7355, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7351, 253, 250-252, 254, 7343, 7344, 7350, and 7352 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 258, 255-257, 259, 260, and 7357-7360 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7351 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 253 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the antigen binding domain (e.g., first antigen binding domain) that binds to a tumor antigen on a lymphoma cell (e.g., a T cell), e.g., a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2 comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region

amino acid sequence Tables 2-6. In some embodiments, the antigen binding domain (e.g., first antigen binding domain) that binds to a tumor antigen on a lymphoma cell (e.g., a T cell), e.g., a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2 comprises heavy and/or light chain amino acid sequences of Table 5. In some embodiments, the antigen binding domain (e.g., first antigen binding domain) that selectively targets lymphocytes expressing a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2 comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in Tables 2-6. In some embodiments, the antigen binding domain (e.g., first antigen binding domain) that selectively targets lymphocytes expressing a T cell receptor comprising TRBC1, TRBC1, a T cell receptor comprising TRBC2, or TRBC2 comprises heavy and/or light chain amino acid sequences of Table 5. An antigen binding domain that binds to a tumor antigen comprising TRBC1 or selectively targets lymphocytes expressing TRBC1 may be said to target TRBC1 (i.e., a TRBC1-targeting antigen binding domain). An antigen binding domain that binds to a tumor antigen comprising TRBC2 or selectively targets lymphocytes expressing TRBC2 may be said to target TRBC2 (i.e., a TRBC2-targeting antigen binding domain).

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 200 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 201 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 202 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the TRBC1 antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 200, a VHCDR2 amino acid sequence of SEQ ID NO: 201, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 202.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 223 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 224 (or a sequence

with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 225 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 223, a VLCDR2 amino acid sequence of SEQ ID NO: 224, and a VLCDR3 amino acid sequence of SEQ ID NO: 225.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 203, a VHFWR2 amino acid sequence of SEQ ID NO: 204, a VHFWR3 amino acid sequence of SEQ ID NO: 205, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 206.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 226, a VLFWR2 amino acid sequence of SEQ ID NO: 227, a VLFWR3 amino acid sequence of SEQ ID NO: 228, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 229.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 203 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 204 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 205 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 206.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 226 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 227 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 228 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 229.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 207, a VHFWR2 amino acid sequence of SEQ ID NO: 208, a VHFWR3 amino acid sequence of SEQ ID NO: 209, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 210.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 207 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 208 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 209 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 210.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 211, a VHFWR2 amino acid sequence of SEQ ID NO: 212, a VHFWR3 amino acid sequence of SEQ ID NO: 213, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 214.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 211 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 212 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 213 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 214.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO:

215, a VHFWR2 amino acid sequence of SEQ ID NO: 216, a VHFWR3 amino acid sequence of SEQ ID NO: 217, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 218.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 218.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 219, a VHFWR2 amino acid sequence of SEQ ID NO: 220, a VHFWR3 amino acid sequence of SEQ ID NO: 221, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 222.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 219 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 220 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 221 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 222.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 230, a VLFWR2 amino acid sequence of SEQ ID NO: 231, a VLFWR3 amino acid sequence of SEQ ID NO: 232, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 233.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 230 (or a sequence with no more

than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 231 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 232 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 233.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 234, a VLFWR2 amino acid sequence of SEQ ID NO: 235, a VLFWR3 amino acid sequence of SEQ ID NO: 236, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 237.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 234 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 235 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 236 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 237.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238, a VLFWR2 amino acid sequence of SEQ ID NO: 239, a VLFWR3 amino acid sequence of SEQ ID NO: 240, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 241.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 241.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 242, a VLFWR2 amino acid sequence of SEQ ID NO: 243, a VLFWR3 amino acid sequence of SEQ ID NO: 244, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 245.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 242 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 243 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 244 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 245.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 246, a VLFWR2 amino acid sequence of SEQ ID NO: 247, a VLFWR3 amino acid sequence of SEQ ID NO: 248, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 249.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 246 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 247 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 248 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 249.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 250 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 250). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 255 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 255). In some embodiments, antigen binding

domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 250. In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 255.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 250, and a VL comprising the amino acid sequence of SEQ ID NO: 255.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 251 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 251). In some embodiments, antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 251.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 252 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 252). In some embodiments, antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 252.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 253). In some embodiments, antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 253.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 254 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 254). In some embodiments, antigen binding domain that targets TRBC1 comprises a VH comprising the amino acid sequence of SEQ ID NO: 254.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 256 (or an amino acid sequence having at

least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 256). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 256.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 257 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 257). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 257.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 258). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 258.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 259 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 259). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 259.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 260 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 260). In some embodiments, the antigen binding domain that targets TRBC1 comprises a VL comprising the amino acid sequence of SEQ ID NO: 260.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6154 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6154). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6154.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6155 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6155). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6155.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6156 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6156). In some embodiments, the antigen binding domain that targets TRBC1 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6156.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6167 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6167). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6167.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6168 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6168). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6168.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6169 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6169). In some embodiments, the antigen binding domain that targets TRBC1 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 6169.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6154 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6154) and a light chain comprising the amino acid sequence of SEQ ID NO: 6156 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6156). In some

embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6154 and a light chain comprising the amino acid sequence of SEQ ID NO: 6156.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6155 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6155) and a light chain comprising the amino acid sequence of SEQ ID NO: 6156 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6156). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6155 and a light chain comprising the amino acid sequence of SEQ ID NO: 6156.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6167 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6167) and a light chain comprising the amino acid sequence of SEQ ID NO: 6169 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6169). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6167 and a light chain comprising the amino acid sequence of SEQ ID NO: 6169.

In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6168 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6168) and a light chain comprising the amino acid sequence of SEQ ID NO: 6169 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6169). In some embodiments, the antigen binding domain that targets TRBC1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 6168 and a light chain comprising the amino acid sequence of SEQ ID NO: 6169.

Table 2. Exemplary heavy chain CDRs and FWRs of TRBC1-targeting antigen binding domains derived from JOVI.1

Ab ID	VHFWR1	VHCDR1	VHFWR2	VHCDR2	VHFWR3	VHCDR3	VHFWR4

mJOVI.1-H	EVRLQQSGPDLIKPGASVKMSCKASGYT (SEQ ID NO: 203)	FTGYVMH (SEQ ID NO: 200)	WVKQRPGQGLEWIG (SEQ ID NO: 204)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	KATLTSDKSSTTAYMELSSLTSEDSAVYYCAR (SEQ ID NO: 205)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTTLTVSS (SEQ ID NO: 206)
h1JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYT (SEQ ID NO: 207)	FTGYVMH (SEQ ID NO: 200)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTMTSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 209)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 210)
h2JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYT (SEQ ID NO: 211)	FTGYVMH (SEQ ID NO: 200)	WVRQAPGQGLEWMG (SEQ ID NO: 212)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	WVTMTSDKSITTAYMELSRLRSDDTAVYYCAR (SEQ ID NO: 213)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 214)
h3JOVI.1-H	QVQLVQSGAEVKKPGSSVKVSCKASGYT (SEQ ID NO: 215)	FTGYVMH (SEQ ID NO: 200)	WVRQAPGQGLEWMG (SEQ ID NO: 216)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 218)
h4JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYT (SEQ ID NO: 219)	FTGYVMH (SEQ ID NO: 200)	WVRQAPGQRLEWMG (SEQ ID NO: 220)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSATTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 221)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 222)

Table 6. Exemplary heavy chain CDRs and FWRs of TRBC1-targeting antigen binding domains derived from JOVI.1 (according to the Kabat numbering scheme)

Ab ID	VHFWR1	VHCDR1	VHFWR2	VHCDR2	VHFWR3	VHCDR3	VHFWR4
mJOVI.1-H	EVRLQQSGPDLIKPGASVKMSCKASGYTFT (SEQ ID NO: 7370)	GYVMH (SEQ ID NO: 7346)	WVKQRPGQGLEWIG (SEQ ID NO: 204)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	KATLTSDKSSTTAYMELSSLTSEDSAVYYCAR (SEQ ID NO: 205)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTTLTVSS (SEQ ID NO: 206)
h1JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 7348)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTMTSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 209)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 210)
h2JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 212)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	WVTMTSDKSITTAYMELSRLRSDDTAVYYCAR (SEQ ID	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 214)

	NO: 7348)				NO: 213)		
h3JOVI.1-H	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT (SEQ ID NO: 7345)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 216)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 218)
h4JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 7348)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQRLEWMG (SEQ ID NO: 220)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSATTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 221)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 222)
h5JOVI.1-H	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT (SEQ ID NO: 7345)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTTVTVSS (SEQ ID NO: 7347)
h6JOVI.1-H	QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 7348)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTMTSDKSITTAYMELSRLRSDDTAVYYCAR (SEQ ID NO: 7349)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTTVTVSS (SEQ ID NO: 7347)
H1 germlined-VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFS (SEQ ID NO: 7353)	GYAIS (SEQ ID NO: 7354)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FINPYNDDIQSNERFRG (SEQ ID NO: 201)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 210)
H2 germlined-VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT (SEQ ID NO: 7345)	GYVMH (SEQ ID NO: 7346)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FIIPIFGTANYAQKFQG (SEQ ID NO: 7355)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 210)
H1/H2 germlined-VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFS (SEQ ID NO: 7353)	GYAIS (SEQ ID NO: 7354)	WVRQAPGQGLEWMG (SEQ ID NO: 208)	FIIPIFGTANYAQKFQG (SEQ ID NO: 7355)	RVTITSDKSTTTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 217)	GAGYNFDGAYRFFDF (SEQ ID NO: 202)	WGQGTLVTVSS (SEQ ID NO: 210)

Table 3. Exemplary light chain CDRs and FWRs of TRBC1-targeting antigen binding domains derived from JOVI.1

Ab ID	FWR1	CDR1	FWR2	CDR2	FWR3	CDR3	FWR4
mJOVI.1-L	DVVMTQSP LSLPVSLG DQASISC (SEQ ID NO: 226)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYLQKPGQ SPKLLIY (SEQ ID NO: 227)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDLGIYFC (SEQ ID NO: 228)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKLE IK (SEQ ID NO: 229)
h1JOVI.1-L	DVVMTQSP LSLPVTPG EPASISC (SEQ ID NO: 230)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYLQKPGQ SPQLLIY (SEQ ID NO: 231)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 232)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 233)
h2JOVI.1-L	EVVMTQSP GTLSLSPG ERATLSC (SEQ ID NO: 234)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYQQKPGQ APRLLIY (SEQ ID NO: 235)	RVSNRFP (SEQ ID NO: 224)	GIPDRFSG SGSGTDFT LTISRLEP EDFAVYFC (SEQ ID NO: 236)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 237)
h3JOVI.1-L	DVVMTQSP LSLPVTLG QPASISC (SEQ ID NO: 238)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYQQRPGQ SPRLLIY (SEQ ID NO: 239)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 240)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 241)
h4JOVI.1-L	DVVMTQTP LSLPVTPG EPASISC (SEQ ID NO: 242)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYLQKPGQ SPQLLIY (SEQ ID NO: 243)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 244)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 245)
h5JOVI.1-L	DVVMTQTP LSLSVTPG QPASISC (SEQ ID NO: 246)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYLQKPGQ SPQLLIY (SEQ ID NO: 247)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 248)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 249)
L1 germlined-VL	DVVMTQSP LSLPVTLG QPASISC (SEQ ID NO: 238)	RSSQSLVY SDGNTYH (SEQ ID NO: 7367)	WYQQRPGQ SPRLLIY (SEQ ID NO: 239)	RVSNRFP (SEQ ID NO: 224)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 232)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 233)
L2 germlined-VL	DVVMTQSP LSLPVTLG QPASISC (SEQ ID NO: 238)	RSSQRLVH SNGNTYLH (SEQ ID NO: 223)	WYQQRPGQ SPRLLIY (SEQ ID NO: 239)	KVSNRDS (SEQ ID NO: 7368)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 232)	SQSTHVPY T (SEQ ID NO: 225)	FGGGTKVE IK (SEQ ID NO: 233)
L3 germline	DVVMTQSP LSLPVTLG	RSSQRLVH SNGNTYLH	WYQQRPGQ SPRLLIY	RVSNRFP (SEQ ID	GVPDRFSG SGSGTDFT	MQSTHWPY T (SEQ	FGGGTKVE IK (SEQ

d-VL	QPASISC (SEQ ID NO: 238)	(SEQ ID NO: 223)	(SEQ ID NO: 239)	NO: 224)	LKISRVEA EDVGVYFC (SEQ ID NO: 232)	ID NO: 7369)	ID NO: 233
L1/L2/L3 germline d-VL	DVVMTQSP LSLPVTLG QPASISC (SEQ ID NO: 238)	RSSQSLVY SDGNTYH (SEQ ID NO: 7367)	WYQQRPGQ SPRLLIY (SEQ ID NO: 239)	KVSNRDS (SEQ ID NO: 7368)	GVPDRFSG SGSGTDFT LKISRVEA EDVGVYFC (SEQ ID NO: 232)	MQSTHWPY T (SEQ ID NO: 7369)	FGGGTKVE IK (SEQ ID NO: 233)

Table 4. Exemplary variable regions of TRBC1-targeting antigen binding domains

SEQ ID NO	Ab ID	Description	Sequence
SEQ ID NO: 250	mJOVI.1-H	JOVI.1 heavy chain variable region	EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVKQ RPGQGLEWIGFINPYNDDIQSNERFRGKATLTSDKSSTTAY MELSSLTSEDSAVYYCARGAGYNFDGAYRFFDFWGQGTT LTVSS
SEQ ID NO: 251	h1JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFINPYNDDIQSNERFRGRVTMTSDKSTTT AYMELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQG TLVTVSS
SEQ ID NO: 252	h2JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFINPYNDDIQSNERFRGWVTMTSDKSITT AYMELSRLRSDDTAVYYCARGAGYNFDGAYRFFDFWGQ GTLVTVSS
SEQ ID NO: 253	h3JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 3	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTA YMELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGT LVTVSS
SEQ ID NO: 254	h4JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 4	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVR QAPGQRLEWMGFINPYNDDIQSNERFRGRVTITSDKSATTA YMELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGT LVTVSS
SEQ ID NO: 7343	h5JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 5	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTA YMELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGT TVTVSS
SEQ ID NO: 7344	h6JOVI.1-H	JOVI.1 heavy chain variable region humanized variant 6	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFINPYNDDIQSNERFRGRVTMTSDKSITT AYMELSRLRSDDTAVYYCARGAGYNFDGAYRFFDFWGQ GTTVTVSS
SEQ ID NO: 7350	H1 germlined-VH	JOVI.1 heavy chain variable region humanized H1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSGYAISWVRQA PGQGLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTAYM ELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGTLV TVSS
SEQ ID NO: 7351	H2 germlined-VH	JOVI.1 heavy chain variable region humanized H2	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVR QAPGQGLEWMGFIIPIFGTANYAQKFQGRVTITSDKSTTTA YMELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGT LVTVSS
SEQ ID NO: 7352	H1/H2 germlined-VH	JOVI.1 heavy chain variable region humanized	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSGYAISWVRQA PGQGLEWMGFIIPIFGTANYAQKFQGRVTITSDKSTTTAYM ELSSLRSEDTAVYYCARGAGYNFDGAYRFFDFWGQGTLV

		H1/H2	TVSS
SEQ ID NO: 255	mJOVI.1-L	JOVI.1 light chain variable region	DVVMTQSPLSLPVSLGDQASISCRSSQRLVHSNGNTYLHW YLQKPGQSPKLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISR VEAEDLGIYFCSQSTHVPYTFGGGTKLEIK
SEQ ID NO: 256	h1JOVI.1-L	JOVI.1 light chain variable region humanized variant 1	DVVMTQSPLSLPVTPGEPASISCRSSQRLVHSNGNTYLHWY LQKPGQSPQLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 257	h2JOVI.1-L	JOVI.1 light chain variable region humanized variant 2	EVVMTQSPGTLSLSPGERATLSCRSSQRLVHSNGNTYLHW YQQKPGQAPRLLIYRVSNRFPGIPDRFSGSGSGTDFTLTISR LEPEDFAVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 258	h3JOVI.1-L	JOVI.1 light chain variable region humanized variant 3	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHW YQQRPGQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISR VEAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 259	h4JOVI.1-L	JOVI.1 light chain variable region humanized variant 4	DVVMTQTPLSLPVTPGEPASISCRSSQRLVHSNGNTYLHWY LQKPGQSPQLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 260	h5JOVI.1-L	JOVI.1 light chain variable region humanized variant 5	DVVMTQTPLSLSVTPGQPASISCRSSQRLVHSNGNTYLHW YLQKPGQSPQLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISR VEAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 7357	L1 germlined-VL	JOVI.1 light chain variable region humanized L1	DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYHWY QQRPGQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 7358	L2 germlined-VL	JOVI.1 light chain variable region humanized L2	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHW YQQRPGQSPRLLIYKVSNRDSGVPDRFSGSGSGTDFTLKISR VEAEDVGVYFCSQSTHVPYTFGGGTKVEIK
SEQ ID NO: 7359	L3 germlined-VL	JOVI.1 light chain variable region humanized L3	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHW YQQRPGQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISR VEAEDVGVYFCMQSTHWPYTFGGGTKVEIK
SEQ ID NO: 7360	L1/L2/L3 germlined-VL	JOVI.1 light chain variable region humanized L1/L2/L3	DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYHWY QQRPGQSPRLLIYKVSNRDSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCMQSTHWPYTFGGGTKVEIK

Table 5 Exemplary TRBC1-targeting antigen binding domains/antibody molecules

SEQ ID NO	Ab ID	Description	Sequence
SEQ ID NO: 6154	Ch(anti-TRBC1)HC N297A	Anti-TRBC1 heavy chain	EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVKQ RPGQGLEWIGFINPYNDDIQSNERFRGKATLTSDKSSTTAY MELSSLTSEDSAVYYCARGAGYNFDGAYRFFDFWGQGTT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREE

			MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
SEQ ID NO: 6155	Ch(anti-TRBC1)HC	Anti-TRBC1 heavy chain	EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVKQ RPGQGLEWIGFINPYNDDIQSNERFRGKATLTSDKSSTTAY MELSSLTSEDSAVYYCARGAGYNFDGAYRFFDFWGQGTT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	
SEQ ID NO: 6156	Ch(anti-TRBC1) LC	Anti-TRBC1 light chain, e.g., a LC Fab	DVVMTQSPLSLPVSLGDQASISCRSSQRLVHSNGNTYLHW YLQKPGQSPKLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISR VEAEDLGIYFCSQSTHVPYTFGGGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
SEQ ID NO: 6191	Ch(anti-TRBC1)HC	Anti-TRBC1 heavy chain, e.g., a HC Fab	EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVKQ RPGQGLEWIGFINPYNDDIQSNERFRGKATLTSDKSSTTAY MELSSLTSEDSAVYYCARGAGYNFDGAYRFFDFWGQGTT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO: 6167	a_hTRBC1_Jovi1_Hum5_VH-hCHIg_Hole_Cys-Blank	Anti-TRBC1 heavy chain	METDTLLLWVLLLWVPGSTGQVQLVQSGAEVKKPGSSVK VSCKASGYTFTGYVMHWVRQAPGQGLEWMGFINPYNDDI QSNERFRGRVTITSDKSTTTAYMELSSLRSEDTAVYYCARG AGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6168	a_hTRBC1_Jovi1_Hum5_VH-hCHIg-Blank	Anti-TRBC1 heavy chain	METDTLLLWVLLLWVPGSTGQVQLVQSGAEVKKPGSSVK VSCKASGYTFTGYVMHWVRQAPGQGLEWMGFINPYNDDI QSNERFRGRVTITSDKSTTTAYMELSSLRSEDTAVYYCARG AGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6169	a_hTRBC1_Jovi1_Hum3_VL-	Anti-TRBC1 light chain	METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTLGQPAS ISCRSSQRLVHSNGNTYLHWYQQRPGQSPRLLIYRVSNRFP GVPDRFSGSGSGTDFTLKISRVEAEDVGVYFCSQSTHVPYT

	hCLIg_vk -Blank		FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Antibody molecules that bind to TRBC1/TRBC2 and NKp30

In some embodiments, the disclosure features a multifunctional antibody molecule that binds to TRBC1 and NKp30. In some embodiments, the multifunctional antibody molecule comprises a configuration shown in any of FIGs. 29A-29D. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 Fab. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 scFv. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 Fab. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 scFv. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29A. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 29B. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC1 scFv, e.g., comprises a configuration shown in FIG. 29C. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29D. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 antigen binding domain disclosed herein. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 antigen binding domain disclosed herein.

In some embodiments, exemplary multifunctional antibody molecules that bind to TRBC1 and NKp30 are disclosed in Table 19.

In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ

ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises SEQ ID NOs: 7382, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises SEQ ID NOs: 7379, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises

SEQ ID NOs: 7382, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the anti-TRBC1/NKp30 antibody molecule comprises SEQ ID NOs: 7379, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

Table 19 Exemplary antibody molecules that bind to TRBC1 and NKp30

SEQ ID NO	Description	Sequence
BJM0772		
SEQ ID NO: 7379	anti-TRBC1 HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVRQAPGQ GLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTAYMELSSLRSE DTAVYYCARGAGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SEQ ID NO: 7380	anti-TRBC1 LC	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHWYQQRP GQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGVY FCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 7381	anti-NKp30 15E1 scFv-Fc	QIQLQESGPGLVKPSQSLSLSCSVTGFSITTTGYHWNWIRQFPGKK LEWMGYIYSSGSTSYNPSLKSRFSITRDTSKNQFFLQLNSVTTEDT ATYYCARGDWHYFDYWGPGTMVTVSSGGGGSGGGGSGGGGSG GGGSSFTLTQPPLVSVAVGQVATITCSGEKLSDKYVHWYQQKPG

		RAPVMVIYENDRRPSGIPDQFSGSNSGNIASLTISKAQAGDEADYF CQFWDSTNSAVFGGGTQLTVLDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVF SCSVMHEALHNRFTQKSLSLSPGK
BJM1042		
SEQ ID NO: 7382	anti-TRBC1 HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVRQAPGQ GLEWMGFIIPIFGTANYAQKFQGRVTITSDKSTTTAYMELSSLRSE DTAVYYCARGAGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SEQ ID NO: 7380	anti-TRBC1 LC	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHWYQQRP GQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGVY FCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 7383	anti-NKp30 humanized 15E1 scFv-Fc	EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWNWVRQAPGK GLEWVGYIYSSGSTSYNPSLKSRFTISRDTSKNTFYLQMNSLRAED TAVYYCARGDWHYFDYWGQGTMVTVSSGGGGSGGGGSGGGGS GGGGSDSVTTQSPLSLPVTLGQPASISCSGEKLSDKYVHWYQQRP GQSPRMLIYENDRRPSGVPDRFSGSNSGNDATLKISRVEAEDVGV YFCQFWDSTNSAVFGGGTKVEIKDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
BJM1052		
SEQ ID NO: 7379	anti-TRBC1 HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVRQAPGQ GLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTAYMELSSLRSE DTAVYYCARGAGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SEQ ID NO: 7380	anti-TRBC1 LC	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHWYQQRP GQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGVY

		FCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 7383	anti-NKp30 humanized 15E1 scFv-Fc	EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWNWVRQAPGK GLEWVGYIYSSGSTSYNPSLKSRFTISRDTSKNTFYLQMNSLRAED TAVYYCARGDWHYFDYWGQGTMVTVSSGGGGSGGGGSGGGGS GGGGSDSVTTQSPLSLPVTLGQPASISCSGEKLSDKYVHWYQQRP GQSPRMLIYENDRRPSGVPDRFSGSNSGNDATLKISRVEAEDVGV YFCQFWDSTNSAVFGGGTKVEIKDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
BJM1038		
SEQ ID NO: 7382	anti-TRBC1 HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVRQAPGQ GLEWMGFIIPIFGTANYAQKFQGRVTITSDKSTTTAYMELSSLRSE DTAVYYCARGAGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SEQ ID NO: 7380	anti-TRBC1 LC	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHWYQQRP GQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGVY FCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 7384	anti-NKp30 humanized 15E1 scFv-Fc	EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWNWVRQAPGK GLEWVGYIYSSGSTSYNPSLKSRFTISRDTSKNTFYLQMNSLRAED TAVYYCARGDWHYFDYWGQGTMVTVSSGGGGSGGGGSGGGGS GGGGSSSETTQPPSVSVSPGQTASITCSGEKLSDKYVHWYQQKPG QSPVMVIYENDRRPSGIPERFSGSNSGNTATLTISGTQAMDEADYF CQFWDSTNSAVFGGGTQLTVLDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK
BJM1048		
SEQ ID NO: 7379	anti-TRBC1 HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHWVRQAPGQ GLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTAYMELSSLRSE DTAVYYCARGAGYNFDGAYRFFDFWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV

		VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SEQ ID NO: 7380	anti-TRBC1 LC	DVVMTQSPLSLPVTLGQPASISCRSSQRLVHSNGNTYLHWYQQRP GQSPRLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGVY FCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 7384	anti-NKp30 humanized 15E1 scFv-Fc	EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWNWVRQAPGK GLEWVGYIYSSGSTSYNPSLKSRFTISRDTSKNTFYLQMNSLRAED TAVYYCARGDWHYFDYWGQGTMVTVSSGGGGSGGGGSGGGGS GGGGSSSETTQPPSVSVSPGQTASITCSGEKLSDKYVHWYQQKPG QSPVMVIYENDRRPSGIPERFSGSNSGNTATLTISGTQAMDEADYF CQFWDSTNSAVFGGGTQLTVLDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK

In some embodiments, the disclosure features a multifunctional antibody molecule that binds to TRBC2 and NKp30. In some embodiments, the multifunctional antibody molecule comprises a configuration shown in any of FIGs. 30A-30D. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 Fab. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 scFv. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 Fab. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 scFv. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30A. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 30B. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC2 scFv, e.g., comprises a configuration shown in FIG. 30C. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30D. In some embodiments, the multifunctional antibody molecule comprises an anti-TRBC2 antigen binding domain disclosed herein. In some embodiments, the multifunctional antibody molecule comprises an anti-NKp30 antigen binding domain disclosed herein.

Multifunctional antibody effector function and Fc variants

In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) disclosed herein comprises an Fc region, e.g., as described herein. In some embodiments, the Fc region is a wildtype Fc region, e.g., a wildtype human Fc region. In some embodiments, the Fc region comprises a variant, e.g., an Fc region comprising an addition, substitution, or deletion of at least one amino acid residue in the Fc region which results in, e.g., reduced or ablated affinity for at least one Fc receptor.

The Fc region of an antibody interacts with a number of receptors or ligands including Fc Receptors (e.g., FcγRI, FcγRIIA, FcγRIIIA), the complement protein CIq, and other molecules such as proteins A and G. These interactions are essential for a variety of effector functions and downstream signaling events including: antibody dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC).

In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprising a variant Fc region has reduced, e.g., ablated, affinity for an Fc receptor, e.g., an Fc receptor described herein. In some embodiments, the reduced affinity is compared to an otherwise similar antibody with a wildtype Fc region.

In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprising a variant Fc region has one or more of the following properties: (1) reduced effector function (e.g., reduced ADCC, ADCP and/or CDC); (2) reduced binding to one or more Fc receptors; and/or (3) reduced binding to C1q complement. In some embodiments, the reduction in any one, or all of properties (1)-(3) is compared to an otherwise similar antibody with a wildtype Fc region.

In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprising a variant Fc region has reduced affinity to a human Fc receptor, e.g., FcγR I, FcγR II and/or FcγR III. In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprising a variant Fc region comprises a human IgG1 region or a human IgG4 region.

Exemplary Fc region variants are provided in **Table 20** and also disclosed in Saunders O, (2019) *Frontiers in Immunology;* vol 10, article1296, the entire contents of which is hereby incorporated by reference.

In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprises any one or all, or any combination of Fc region variants, e.g., mutations, disclosed in **Table 20**. In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprises an Asn297Ala (N297A) mutation. In some embodiments, the multifunctional molecule (e.g., an anti-TRBC1/NKp30 antibody molecule or an anti-TRBC2/NKp30 antibody molecule) comprises a Leu234Ala/Leu235Ala (LALA) mutation.

Table 20: Exemplary Fc modifications

Modification or mutation	**Altered effector function**
Leu235Glu	ADCC;
Leu234Ala/Leu235Ala (LALA)	ADCC; ADCP; CDC
Ser228Pro/Leu235Glu	
Leu234Ala/Leu235Ala/Pro329Gly	ADCP
Pro331Ser/Leu234Glu/Leu235Phe	CDC
Asp265Ala	ADCC, ADCP
Gly237Ala	ADCP
Glu318Ala	ADCP
Glu233Pro	
Gly236Arg/Leu328Arg	ADCC
His268Gln/Val309Leu/Ala330Ser/Pro331Ser	ADCC; ADCP; CDC
Val234Ala/Gly237Ala/Pro238Ser/ His268Ala/Val309Leu/Ala330Ser/Pro331Ser	ADCC; ADCP; CDC
Leu234Ala/L235Ala/Gly237Ala/P238Ser/ His268Ala/Ala330Ser/Pro331Ser	ADCC; CDC
Ala330Leu	CDC
Asp270Ala	CDC

Lys322Ala	CDC
Pro329Ala	CDC
Pro331Ala	CDC
Val264Ala	CDC
High mannose glycosylation	CDC
Phe241Ala	CDC
Asn297Ala or Gly or Gln	ADCC; ADCP; CDC
S228P/Phe234Ala/Leu235Ala	ADCC; CDC

Antibody Molecules Targeting TRBC1

In another aspect, the present disclosure features an antibody molecule, e.g., a monoclonal antibody molecule, or fragment thereof that binds TRBC1.

In some embodiments, the antibody molecule, or fragment thereof, that binds to TRBC1 comprises one or more CDRs (e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and/or VLCDR3) disclosed in Table 2, Table 6, or Table 3, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antibody molecule, or fragment thereof, that binds to TRBC1 comprises one or more framework regions (e.g., VHFWR1, VHFWR2, VHFWR3, VHFWR4, VLFWR1, VLFWR2, VLFWR3, and/or VLFWR4) disclosed in Table 2, Table 6, or Table 3, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antibody molecule, or fragment thereof, that binds to TRBC1 comprises a VH and/or a VL disclosed in Table 4, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antibody molecule, or fragment thereof, that binds to TRBC1 comprises an amino acid sequence disclosed in Table 5, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

In some embodiments, the antibody molecule, or fragment thereof, that binds to TRBC1 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3.

In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 7355, and 202, respectively (or a sequence having at least

85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 201, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7354, 201, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7354, 7355, and 202, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 7355, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7346, 201, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of: SEQ ID NOs: 7346, 7355, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%,

90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 7355, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7346, 201, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 201, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 7367, 224, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 7368, and 225, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); SEQ ID NOs: 7354, 7355, 202, 223, 224, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or SEQ ID NOs: 7354, 7355, 202, 7367, 7368, and 7369, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7351, 253, 250-252, 254, 7343, 7344, 7350, and 7352 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 258, 255-257, 259, 260, and 7357-7360 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7351

and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 253 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the antibody molecule or fragment thereof comprises:

a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 215 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 216 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 217 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 218 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and

a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 238 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 amino acid sequence of SEQ ID NO: 239 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 amino acid sequence of SEQ ID NO: 240 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 241 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

In some embodiments, the antibody molecule or fragment thereof comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 200, a VHCDR2 amino acid sequence of SEQ ID NO: 201, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 202.

In some embodiments, the antibody molecule or fragment thereof comprises a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 223, a VLCDR2 amino acid sequence of SEQ ID NO: 224, and a VLCDR3 amino acid sequence of SEQ ID NO: 225.

In some embodiments, the antibody molecule or fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or a VL comprising the amino acid sequence of SEQ ID NO: 258 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto). In some embodiments, the antibody molecule or fragment thereof comprises a VH and/or VL substantially homologous to SEQ ID NOs: 253 and/or 258.

In another aspect, the disclosure features an antibody molecule, e.g., an IgM antibody molecule comprising: (i) a first antigen binding domain that selectively binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2), and (ii) a complement activating domain that activates the complement pathway, e.g., by binding C1q. In some embodiments, an antibody molecule, e.g., IgM antibody molecule, comprises an antigen binding domain that targets TRBC1. In some embodiments, the antibody molecule is an IgM antibody molecule, e.g., that multimerizes into tetramers, pentamers, and/or hexamers and is capable of activating complement pathway(s). In some embodiments, the IgM antibody molecule comprises an antigen binding domain that targets TRBC1 comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 6173 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6173).

METDTLLLWVLLLWVPGSTGQVQLVQSGAEVKKPGSSVKVSCKASGYTFTGYVMHW
VRQAPGQGLEWMGFINPYNDDIQSNERFRGRVTITSDKSTTTAYMELSSLRSEDTAVYY
CARGAGYNFDGAYRFFDFWGQGTLVTVSSGSASAPTLFPLVSCENSPSDTSSVAVGCLA
QDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCK
VQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWL
REGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQ
QNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVK
THTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRP
DVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQA
PGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSD
TAGTCY (SEQ ID NO: 6173).

In some embodiments, the IgM antibody molecule comprises an antigen binding domain that targets TRBC1 comprising a light chain comprising the amino acid sequence of SEQ ID NO: 6174 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6174).

MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNI RIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSAT ETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD (SEQ ID NO: 6174).

In some embodiments, the IgM antibody molecule comprises an antigen binding domain that targets TRBC1 comprising amino acid sequences of SEQ ID NO: 6173 and 6174 (or amino acid sequences having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6173 and 6174) and an amino acid sequence of a light chain sequence provided herein, e.g., in Tables 3 or 4.

In some embodiments, the complement activating domain comprises a portion of an antibody molecule capable of binding or being bound by C1q, e.g., a portion of a IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, or IgE. In some embodiments, a complement activating domain comprises a Ch2, Ch3, or Ch4 domain.

Without wishing to be bound by theory, it is thought that complement activation in proximity to a target cell (e.g., a TRBC1 or TRBC2 expressing cell, e.g., a lymphocyte expressing TRBC1 or TRBC2, e.g., a lymphoma cell expressing TRBC1 or TRBC2) may induce the death of the target cell. In some embodiments, use of an antibody molecule, e.g., IgM antibody molecule, or a multifunctional molecule in the methods described herein induces complement mediated cell death of the target cell.

In another aspect, the disclosure features a multispecific antibody molecule (e.g., a bispecific antibody molecule) that binds to TRBC1 and NKp30. In some embodiments, the multispecific antibody molecule comprises one or more moieties that bind to TRBC1, e.g., one or more Fabs that bind to TRBC1, e.g., one or two Fabs that bind to TRBC1. In some embodiments, the multispecific antibody molecule comprises one or more moieties that bind to NKp30, e.g., one or more scFvs that bind to NKp30, e.g., one or two scFvs that bind to NKp30. In some embodiments, the moiety that binds to TRBC1 comprises an anti-TRBC1 sequence disclosed herein, e.g., comprises a CDR, VH, VL, heavy chain, or light chain sequence disclosed

in Tables 2-5, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the moiety that binds to NKp30 comprises an anti-NKp30 sequence disclosed herein, e.g., comprises a CDR, VH, VL, heavy chain, or light chain sequence disclosed in Tables 7-10, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 1A. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 antibody molecule and an anti-NKp30 antibody molecule, e.g., an anti-TRBC1 antibody molecule comprising two heavy chains and two light chains, and an anti-NKp30 scFv that is fused to the N-terminus of one of the heavy chains of the anti-TRBC1 antibody. In some embodiments, the two heavy chains of the anti-TRBC1 antibody form a heterodimer, e.g., via knob-and-hole mutations. In some embodiments, the two heavy chains of the anti-TRBC1 antibody comprise the N297A mutation. In some embodiments, the two heavy chains of the anti-TRBC1 antibody do not comprise the N297A mutation. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, a third chain, and a fourth chain, wherein the first chain comprises an anti-TRBC1 light chain variable region (VL) and a light chain constant region (CL); the second chain comprises an anti-NKp30 scFv, an anti-TRBC1 heavy chain variable region (VH), a CH1, a CH2, and a CH3; the third chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3; and the fourth chain comprises an anti-TRBC1 VL and a CL.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 1B. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 antibody molecule and an anti-NKp30 antibody molecule. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 Fab, an anti-NKp30 scFv, and an Fc dimer comprising two Fc chains. In some embodiments, the C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain, and the anti-NKp30 scFv is fused to the N-terminus of the other Fc chain. In some embodiments, the two Fc chains form a heterodimer, e.g., via knob-and-hole mutations. In some embodiments, the two Fc chains comprise the N297A mutation. In some embodiments, the two Fc chains do not comprise the N297A mutation. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, and a third chain, wherein the first chain comprises an anti-TRBC1 VL

and a CL; the second chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3; and the third chain comprises an anti-NKp30 scFv, a CH2, and a CH3.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 1C. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 antibody molecule and an anti-NKp30 antibody molecule, e.g., an anti-TRBC1 antibody molecule comprising two heavy chains and two light chains, and two anti-NKp30 scFvs that are fused to the C-terminus of the two light chains of the anti-TRBC1 antibody molecule, respectively. In some embodiments, the two heavy chains of the anti-TRBC1 antibody form a homodimer. In some embodiments, the two heavy chains of the anti-TRBC1 antibody comprise the N297A mutation. In some embodiments, the two heavy chains of the anti-TRBC1 antibody do not comprise the N297A mutation. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, a third chain, and a fourth chain, wherein the first chain comprises an anti-TRBC1 VL, a CL, and an anti-NKp30 scFv; the second chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3; the third chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3; and the fourth chain comprises an anti-TRBC1 VL, a CL, and an anti-NKp30 scFv.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 1D. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 antibody molecule and an anti-NKp30 antibody molecule, e.g., an anti-TRBC1 antibody molecule comprising two heavy chains and two light chains, and two anti-NKp30 scFvs that are fused to the N-terminus of the two heavy chains of the anti-TRBC1 antibody molecule, respectively. In some embodiments, the two heavy chains of the anti-TRBC1 antibody form a homodimer. In some embodiments, the two heavy chains of the anti-TRBC1 antibody comprise the N297A mutation. In some embodiments, the two heavy chains of the anti-TRBC1 antibody do not comprise the N297A mutation. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, a third chain, and a fourth chain, wherein the first chain comprises an anti-TRBC1 VL and a CL; the second chain comprises an anti-NKp30 scFv, an anti-TRBC1 VH, a CH1, a CH2, and a CH3; the third chain comprises an anti-NKp30 scFv, an anti-TRBC1 VH, a CH1, a CH2, and a CH3; and the fourth chain comprises an anti-TRBC1 VL and a CL.

In another aspect, the disclosure features an antibody molecule that comprises a moiety that binds to TRBC1 and a TRAIL molecule (e.g., a trimeric, dimeric, or monomeric TRAIL molecule). In some embodiments, the antibody molecule comprises one or more moieties that bind to TRBC1, e.g., one or more Fabs that bind to TRBC1, e.g., one Fab that binds to TRBC1. In some embodiments, the moiety that binds to TRBC1 comprises an anti-TRBC1 sequence disclosed herein, e.g., comprises a CDR, VH, VL, heavy chain, or light chain sequence disclosed in Tables 2-5, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the antibody molecule comprises a TRAIL molecule (e.g., a trimeric, dimeric, or monomeric TRAIL molecule). In some embodiments, each monomer of TRAIL comprises amino acid residues 122-281 of human TRAIL, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, each monomer of TRAIL comprises amino acid residues 95-281 of human TRAIL, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

In some embodiments, the antibody molecule comprises a configuration shown in FIGs. 2A-2F. In some embodiments, the antibody molecule comprises a moiety that binds to TRBC1 and a trimeric, dimeric, or monomeric TRAIL molecule, e.g., comprises an anti-TRBC1 Fab, a trimeric, dimeric, or monomeric TRAIL molecule, and an Fc dimer comprising two Fc chains. In some embodiments, the two Fc chains form a heterodimer, e.g., via knob-and-hold mutations. In some embodiments, the two Fc chains comprise the N297A mutation. In some embodiments, the two Fc chains do not comprise the N297A mutation. In some embodiments, the C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain. In some embodiments, the trimeric, dimeric, or monomeric TRAIL molecule is fused to the N-terminus of the other Fc chain. In some embodiments, the antibody molecule comprises a first chain, a second chain, and a third chain. In some embodiments, the first chain comprises an anti-TRBC1 VL and a CL, e.g., comprises the amino acid sequence of SEQ ID NO: 6169, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the second chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6167, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the third chain comprises a trimeric TRAIL molecule, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6159 or 6162, or a sequence

having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the third chain comprises a dimeric TRAIL molecule, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6158 or 6161, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the third chain comprises a monomeric TRAIL molecule, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6157 or 6160, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

In another aspect, the disclosure features a multispecific antibody molecule (e.g., a bispecific antibody molecule) that binds to TRBC1 and DR5. In some embodiments, the multispecific antibody molecule comprises one or more moieties that bind to TRBC1, e.g., one or more Fabs that bind to TRBC1, e.g., one Fab that binds to TRBC1. In some embodiments, the multispecific antibody molecule comprises one or more moieties that bind to DR5, e.g., one or more scFvs that bind to DR5, e.g., one or two scFvs that bind to DR5. In some embodiments, the moiety that binds to TRBC1 comprises an anti-TRBC1 sequence disclosed herein, e.g., comprises a CDR, VH, VL, heavy chain, or light chain sequence disclosed in Tables 2-5, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the moiety that binds to DR5 comprises an anti-DR5 sequence disclosed herein, e.g., comprises a CDR, VH, VL, heavy chain, or light chain sequence disclosed in Table 11, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 3A. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 Fab, an anti-DR5 scFv, and an Fc dimer comprising two Fc chains. In some embodiments, the two Fc chains form a heterodimer, e.g., via knob-and-hold mutations. In some embodiments, the two Fc chains comprise the N297A mutation. In some embodiments, the two Fc chains do not comprise the N297A mutation. In some embodiments, the C-terminus of the heavy chain of the anti-TRBC1 Fab is fused to the N-terminus of one Fc chain. In some embodiments, the anti-DR5 scFv is fused to the N-terminus of the other Fc chain. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, and a third chain. In some embodiments, the first chain comprises an anti-TRBC1 VL and a CL, e.g., comprises the amino acid sequence of SEQ ID NO: 6169, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the second chain comprises an anti-

TRBC1 VH, a CH1, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6167, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the third chain comprises an anti-DR5 scFv, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6163, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

In some embodiments, the multispecific antibody molecule comprises a configuration shown in FIG. 3B. In some embodiments, the multispecific antibody molecule comprises an anti-TRBC1 antibody molecule and an anti-DR5 antibody molecule, e.g., an anti-TRBC1 antibody molecule comprising two heavy chains and two light chains, and two anti-DR5 scFvs that are fused to the C-terminus of the two light chains of the anti-TRBC1 antibody, respectively. In some embodiments, the two heavy chains of the anti-TRBC1 antibody comprise the N297A mutation. In some embodiments, the two heavy chains of the anti-TRBC1 antibody do not comprise the N297A mutation. In some embodiments, the multispecific antibody molecule comprises a first chain, a second chain, a third chain, and a fourth chain. In some embodiments, the first chain comprises an anti-TRBC1 VL, a CL, and an anti-DR5 scFv, e.g., comprises the amino acid sequence of SEQ ID NO: 6170, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the second chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6168, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the fourth chain comprises an anti-TRBC1 VH, a CH1, a CH2, and a CH3, e.g., comprises the amino acid sequence of SEQ ID NO: 6168, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto. In some embodiments, the first chain comprises an anti-TRBC1 VL, a CL, and an anti-DR5 scFv, e.g., comprises the amino acid sequence of SEQ ID NO: 6170, or a sequence having at least 70, 80, 90, 95, or 99% identity thereto.

Uses of the antibody molecules disclosed herein include but are not limited to methods of treating cancer (e.g., a cancer expressing TRBC1) disclosed herein; methods of identifying, evaluating, or selecting a subject in need of treatment (e.g., determining whether a subject has cancer cells that express TRBC1) disclosed herein; and methods of laboratory or diagnostic analysis (e.g., immunological assays comprising detecting the presence and/or level of TRBC1 or TRBC1 expressing cells).

Cytokine Molecules and Cytokine Inhibitor Molecules

Cytokines are generally polypeptides that influence cellular activity, for example, through signal transduction pathways. Accordingly, a cytokine of the multispecific or multifunctional polypeptide is useful and can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response within the cell. Cytokines are proteinaceous signaling compounds that are mediators of the immune response. They control many different cellular functions including proliferation, differentiation and cell survival/apoptosis; cytokines are also involved in several pathophysiological processes including viral infections and autoimmune diseases. Cytokines are synthesized under various stimuli by a variety of cells of both the innate (monocytes, macrophages, dendritic cells) and adaptive (T- and B-cells) immune systems. Cytokines can be classified into two groups: pro- and anti-inflammatory. Pro-inflammatory cytokines, including IFNγ, IL-1, IL-6 and TNF-alpha, are predominantly derived from the innate immune cells and Th1 cells. Anti-inflammatory cytokines, including IL-10, IL-4, IL-13 and IL-5, are synthesized from Th2 immune cells.

The present disclosure provides, *inter alia*, multispecific (e.g., bi-, tri-, quad- specific) or multifunctional molecules, that include, e.g., are engineered to contain, one or more cytokine molecules, e.g., immunomodulatory (e.g., proinflammatory) cytokines and variants, e.g., functional variants, thereof. Accordingly, in some embodiments, the cytokine molecule is an interleukin or a variant, e.g., a functional variant thereof. In some embodiments the interleukin is a proinflammatory interleukin. In some embodiments the interleukin is chosen from interleukin-2 (IL-2), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), interleukin-21 (IL-21), interleukin-7 (IL-7), or interferon gamma. In some embodiments, the cytokine molecule is a proinflammatory cytokine.

In certain embodiments, the cytokine is a single chain cytokine. In certain embodiments, the cytokine is a multichain cytokine (e.g., the cytokine comprises 2 or more (e.g., 2) polypeptide chains. An exemplary multichain cytokine is IL-12.

Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-21, IFN-α, IFN-β, IFN-γ, MIP-1α, MIP-1β, TGF-β, TNF-α, and TNFβ. In one embodiment the cytokine of the multispecific or

multifunctional polypeptide is a cytokine selected from the group of GM-CSF, IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, IFN-α, IFN-γ, MIP-1α, MIP-1β and TGF-β. In one embodiment the cytokine of the i the multispecific or multifunctional polypeptide is a cytokine selected from the group of IL-2, IL-7, IL-10, IL-12, IL-15, IFN-α, and IFN-γ. In certain embodiments the cytokine is mutated to remove N- and/or O-glycosylation sites. Elimination of glycosylation increases homogeneity of the product obtainable in recombinant production.

In one embodiment, the cytokine of the multispecific or multifunctional polypeptide is IL-2. In a specific embodiment, the IL-2 cytokine can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity. In another particular embodiment the IL-2 cytokine is a mutant IL-2 cytokine having reduced binding affinity to the .alpha.-subunit of the IL-2 receptor. Together with the .beta.- and .gamma.-subunits (also known as CD122 and CD132, respectively), the .alpha.-subunit (also known as CD25) forms the heterotrimeric high-affinity IL-2 receptor, while the dimeric receptor consisting only of the β- and γ-subunits is termed the intermediate-affinity IL-2 receptor. As described in PCT patent application number PCT/EP2012/051991, which is incorporated herein by reference in its entirety, a mutant IL-2 polypeptide with reduced binding to the .alpha.-subunit of the IL-2 receptor has a reduced ability to induce IL-2 signaling in regulatory T cells, induces less activation-induced cell death (AICD) in T cells, and has a reduced toxicity profile in vivo, compared to a wild-type IL-2 polypeptide. The use of such an cytokine with reduced toxicity is particularly advantageous in a multispecific or multifunctional polypeptide according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment, the mutant IL-2 cytokine of the multispecific or multifunctional polypeptide according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-2 cytokine to the .alpha.-subunit of the IL-2 receptor (CD25) but preserves the affinity of the mutant IL-2 cytokine to the intermediate-affinity IL-2 receptor (consisting of the β and γ subunits of the IL-2 receptor), compared to the non-mutated IL-2 cytokine. In one embodiment the one or more amino acid

mutations are amino acid substitutions. In a specific embodiment, the mutant IL-2 cytokine comprises one, two or three amino acid substitutions at one, two or three position(s) selected from the positions corresponding to residue 42, 45, and 72 of human IL-2. In a more specific embodiment, the mutant IL-2 cytokine comprises three amino acid substitutions at the positions corresponding to residue 42, 45 and 72 of human IL-2. In an even more specific embodiment, the mutant IL-2 cytokine is human IL-2 comprising the amino acid substitutions F42A, Y45A and L72G. In one embodiment the mutant IL-2 cytokine additionally comprises an amino acid mutation at a position corresponding to position 3 of human IL-2, which eliminates the O-glycosylation site of IL-2. Particularly, said additional amino acid mutation is an amino acid substitution replacing a threonine residue by an alanine residue. A particular mutant IL-2 cytokine useful in the invention comprises four amino acid substitutions at positions corresponding to residues 3, 42, 45 and 72 of human IL-2. Specific amino acid substitutions are T3A, F42A, Y45A and L72G. As demonstrated in PCT patent application number PCT/EP2012/051991 and in the appended Examples, said quadruple mutant IL-2 polypeptide (IL-2 qm) exhibits no detectable binding to CD25, reduced ability to induce apoptosis in T cells, reduced ability to induce IL-2 signaling in T.sub.reg cells, and a reduced toxicity profile in vivo. However, it retains ability to activate IL-2 signaling in effector cells, to induce proliferation of effector cells, and to generate IFN-γ as a secondary cytokine by NK cells.

The IL-2 or mutant IL-2 cytokine according to any of the above embodiments may comprise additional mutations that provide further advantages such as increased expression or stability. For example, the cysteine at position 125 may be replaced with a neutral amino acid such as alanine, to avoid the formation of disulfide-bridged IL-2 dimers. Thus, in certain embodiments the IL-2 or mutant IL-2 cytokine of the multispecific or multifunctional polypeptide according to the invention comprises an additional amino acid mutation at a position corresponding to residue 125 of human IL-2. In one embodiment said additional amino acid mutation is the amino acid substitution C125A.

In a specific embodiment the IL-2 cytokine of the multispecific or multifunctional polypeptide comprises the polypeptide sequence of SEQ ID NO: 7227 [APTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHL

RPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT]. In another specific embodiment the IL-2 cytokine of the multispecific or multifunctional polypeptide comprises the polypeptide sequence of SEQ ID NO: 7228 [APASSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHL RPRDLISNIN VIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT].

In another embodiment the cytokine of the multispecific or multifunctional polypeptide is IL-12. In a specific embodiment said IL-12 cytokine is a single chain IL-12 cytokine. In an even more specific embodiment the single chain IL-12 cytokine comprises the polypeptide sequence of SEQ ID NO: 7229 [IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVK EFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSA CPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEY PDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSS SWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQ KARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRK TSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFN SETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS]. In one embodiment, the IL-12 cytokine can elicit one or more of the cellular responses selected from the group consisting of: proliferation in a NK cell, differentiation in a NK cell, proliferation in a T cell, and differentiation in a T cell.

In another embodiment the cytokine of the multispecific or multifunctional polypeptide is IL-10. In a specific embodiment said IL-10 cytokine is a single chain IL-10 cytokine. In an even more specific embodiment the single chain IL-10 cytokine comprises the polypeptide sequence of SEQ ID NO: 7230 [SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKG YLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENK SKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNGGGGSGGGGSGGGGS GGGGSSPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLE

DFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLP CENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN]. In another specific embodiment the IL-10 cytokine is a monomeric IL-10 cytokine. In a more specific embodiment the monomeric IL-10 cytokine comprises the polypeptide sequence of SEQ ID NO: 7231 [SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKG YLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENG GGSGGKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN]. In one embodiment, the IL-10 cytokine can elicit one or more of the cellular responses selected from the group consisting of: inhibition of cytokine secretion, inhibition of antigen presentation by antigen presenting cells, reduction of oxygen radical release, and inhibition of T cell proliferation. A multispecific or multifunctional polypeptide according to the invention wherein the cytokine is IL-10 is particularly useful for downregulation of inflammation, e.g. in the treatment of an inflammatory disorder.

In another embodiment, the cytokine of the multispecific or multifunctional polypeptide is IL-15. In a specific embodiment said IL-15 cytokine is a mutant IL-15 cytokine having reduced binding affinity to the α-subunit of the IL-15 receptor. Without wishing to be bound by theory, a mutant IL-15 polypeptide with reduced binding to the .alpha.-subunit of the IL-15 receptor has a reduced ability to bind to fibroblasts throughout the body, resulting in improved pharmacokinetics and toxicity profile, compared to a wild-type IL-15 polypeptide. The use of an cytokine with reduced toxicity, such as the described mutant IL-2 and mutant IL-15 effector moieties, is particularly advantageous in a multispecific or multifunctional polypeptide according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment the mutant IL-15 cytokine of the multispecific or multifunctional polypeptide according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-15 cytokine to the .alpha.-subunit of the IL-15 receptor but preserves the affinity of the mutant IL-15 cytokine to the intermediate-affinity IL-15/IL-2 receptor (consisting of the .beta.- and .gamma.-subunits of the IL-15/IL-2 receptor), compared to the non-mutated IL-15 cytokine. In one embodiment the amino acid mutation is an amino acid substitution. In a specific embodiment, the mutant IL-15 cytokine comprises an amino acid

substitution at the position corresponding to residue 53 of human IL-15. In a more specific embodiment, the mutant IL-15 cytokine is human IL-15 comprising the amino acid substitution E53A. In one embodiment the mutant IL-15 cytokine additionally comprises an amino acid mutation at a position corresponding to position 79 of human IL-15, which eliminates the N-glycosylation site of IL-15. Particularly, said additional amino acid mutation is an amino acid substitution replacing an asparagine residue by an alanine residue. In an even more specific embodiment the IL-15 cytokine comprises the polypeptide sequence of SEQ ID NO: 7232 [NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLASGDASIH DTVENLIILANNSLSSNGAVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS]. In one embodiment, the IL-15 cytokine can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity.

Mutant cytokine molecules useful as effector moieties in the multispecific or multifunctional polypeptide can be prepared by deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing. Substitution or insertion may involve natural as well as non-natural amino acid residues. Amino acid modification includes well known methods of chemical modification such as the addition or removal of glycosylation sites or carbohydrate attachments, and the like.

In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is GM-CSF. In a specific embodiment, the GM-CSF cytokine can elicit proliferation and/or differentiation in a granulocyte, a monocyte or a dendritic cell. In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is IFN-α. In a specific embodiment, the IFN-α cytokine can elicit one or more of the cellular responses selected from the group consisting of: inhibiting viral replication in a virus-infected cell, and upregulating the expression of major histocompatibility

complex I (MHC I). In another specific embodiment, the IFN-α cytokine can inhibit proliferation in a tumor cell. In one embodiment the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is IFNγ. In a specific embodiment, the IFN-γ cytokine can elicit one or more of the cellular responses selected from the group of: increased macrophage activity, increased expression of MHC molecules, and increased NK cell activity. In one embodiment the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is IL-7. In a specific embodiment, the IL-7 cytokine can elicit proliferation of T and/or B lymphocytes. In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is IL-8. In a specific embodiment, the IL-8 cytokine can elicit chemotaxis in neutrophils. In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide, is MIP-1α. In a specific embodiment, the MIP-1α cytokine can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is MIP-1β. In a specific embodiment, the MIP-1β cytokine can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the cytokine, particularly a single-chain cytokine, of the multispecific or multifunctional polypeptide is TGF-β. In a specific embodiment, the TGF-β cytokine can elicit one or more of the cellular responses selected from the group consisting of: chemotaxis in monocytes, chemotaxis in macrophages, upregulation of IL-1 expression in activated macrophages, and upregulation of IgA expression in activated B cells.

In one embodiment, the multispecific or multifunctional polypeptide of the invention binds to an cytokine receptor with a dissociation constant (K_D) that is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 times greater than that for a control cytokine. In another embodiment, the multispecific or multifunctional polypeptide binds to an cytokine receptor with a K_D that is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 times greater than that for a corresponding multispecific or multifunctional polypeptide comprising two or more effector moieties. In another embodiment, the multispecific or multifunctional polypeptide binds to an cytokine receptor with a dissociation constant K_D that is about 10 times greater than that for a corresponding the multispecific or multifunctional polypeptide comprising two or more cytokines.

In some embodiments, the multispecific molecules disclosed herein include a cytokine molecule. In embodiments, the cytokine molecule includes a full length, a fragment or a variant of a cytokine; a cytokine receptor domain, e.g., a cytokine receptor dimerizing domain; or an agonist of a cytokine receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to a cytokine receptor.

In some embodiments the cytokine molecule is chosen from IL-2, IL-12, IL-15, IL-18, IL-7, IL-21, or interferon gamma, or a fragment or variant thereof, or a combination of any of the aforesaid cytokines. The cytokine molecule can be a monomer or a dimer. In embodiments, the cytokine molecule can further include a cytokine receptor dimerizing domain.

In other embodiments, the cytokine molecule is an agonist of a cytokine receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to a cytokine receptor chosen from an IL-15Ra or IL-21R.

In one embodiment, the cytokine molecule is IL-15, e.g., human IL-15 (e.g., comprising the amino acid sequence:
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 7017), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7017.

In some embodiments, the cytokine molecule comprises a receptor dimerizing domain, e.g., an IL15Ralpha dimerizing domain. In one embodiment, the IL15Ralpha dimerizing domain comprises the amino acid sequence:
MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLYSRERYICN SGFKRKAGTSSLTECVL (SEQ ID NO: 7018), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 7018. In some embodiments, the cytokine molecule (e.g., IL-15) and the receptor dimerizing domain (e.g., an IL15Ralpha dimerizing domain) of the multispecific molecule are covalently linked,

e.g., via a linker (e.g., a Gly-Ser linker, e.g., a linker comprising the amino acid sequence SGGSGGGGSGGGSGGGGSLQ (SEQ ID NO: 7019). In other embodiments, the cytokine molecule (e.g., IL-15) and the receptor dimerizing domain (e.g., an IL15Ralpha dimerizing domain) of the multispecific molecule are not covalently linked, e.g., are non-covalently associated.

In other embodiments, the cytokine molecule is IL-2, e.g., human IL-2 (e.g., comprising the amino acid sequence: APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNR WITFCQSIISTLT (SEQ ID NO: 7020), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7020).

In other embodiments, the cytokine molecule is IL-18, e.g., human IL-18 (e.g., comprising the amino acid sequence: YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSY EGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 7021), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7021).

In other embodiments, the cytokine molecule is IL-21, e.g., human IL-21 (e.g., comprising the amino acid sequence: QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSA NTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMI HQHLSSRTHGSEDS (SEQ ID NO: 7022), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7022).

In yet other embodiments, the cytokine molecule is interferon gamma, e.g., human interferon gamma (e.g., comprising the amino acid sequence: QDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFK NFKDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKLTNYSVTDLNVQRKAIHELIQVM AELSPAAKTGKRKRSQMLFRG (SEQ ID NO: 7023), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7023).

TGF-beta Inhibitors

The present disclosure further provides, *inter alia*, multispecific (e.g., bi-, tri-, quad-specific) or multifunctional molecules, that include, e.g., are engineered to contain, one or more cytokine inhibitor molecules, e.g., inhibitors of immunomodulatory (e.g., proinflammatory) cytokines and variants, e.g., functional variants, thereof. Accordingly, in some embodiments, the cytokine inhibitor molecule is a TGF-beta inhibitor. In some embodiments, the TGF-beta inhibitor binds to and inhibits TGF-beta, e.g., reduces the activity of TGF-beta. In some embodiments, the TGF-beta inhibitor inhibits (e.g., reduces the activity of) TGF-beta 1. In some embodiments, the TGF-beta inhibitor inhibits (e.g., reduces the activity of) TGF-beta 2. In some embodiments, the TGF-beta inhibitor inhibits (e.g., reduces the activity of) TGF-beta 3. In some embodiments, the TGF-beta inhibitor inhibits (e.g., reduces the activity of) TGF-beta 1 and TGF-beta 3. In some embodiments, the TGF-beta inhibitor inhibits (e.g., reduces the activity of) TGF-beta 1, TGF-beta 2, and TGF-beta 3.

In some embodiments, the TGF-beta inhibitor comprises a portion of a TGF-beta receptor (e.g., an extracellular domain of a TGF-beta receptor) that is capable of inhibiting (e.g., reducing the activity of) TGF-beta, or functional fragment or variant thereof. In some embodiments, the TGF-beta inhibitor comprises a TGFBR1 polypeptide (e.g., an extracellular domain of TGFBR1 or functional variant thereof). In some embodiments, the TGF-beta inhibitor comprises a TGFBR2 polypeptide (e.g., an extracellular domain of TGFBR2 or functional variant thereof). In some embodiments, the TGF-beta inhibitor comprises a TGFBR3 polypeptide (e.g., an

extracellular domain of TGFBR3 or functional variant thereof). In some embodiments, the TGF-beta inhibitor comprises a TGFBR1 polypeptide (e.g., an extracellular domain of TGFBR1 or functional variant thereof) and a TGFBR2 polypeptide (e.g., an extracellular domain of TGFBR2 or functional variant thereof). In some embodiments, the TGF-beta inhibitor comprises a TGFBR1 polypeptide (e.g., an extracellular domain of TGFBR1 or functional variant thereof) and a TGFBR3 polypeptide (e.g., an extracellular domain of TGFBR3 or functional variant thereof). In some embodiments, the TGF-beta inhibitor comprises a TGFBR2 polypeptide (e.g., an extracellular domain of TGFBR2 or functional variant thereof) and a TGFBR3 polypeptide (e.g., an extracellular domain of TGFBR3 or functional variant thereof).

Exemplary TGF-beta receptor polypeptides that can be used as TGF-beta inhibitors have been disclosed in US8993524, US9676863, US8658135, US20150056199, US20070184052, and WO2017037634, all of which are herein incorporated by reference in their entirety.

In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of TGFBR1 or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 95, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 96, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 97, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 104, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 105, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto).

In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of TGFBR2 or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an

extracellular domain of SEQ ID NO: 98, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 99, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 100, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 101, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 102, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 103, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto).

In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of TGFBR3 or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 106, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises an extracellular domain of SEQ ID NO: 107, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto). In some embodiments, the TGF-beta inhibitor comprises the amino acid sequence of SEQ ID NO: 108, or a sequence substantially identical thereto (e.g., a sequence that is at least 80%, 85%, 90%, or 95% identical thereto).

In some embodiments, the TGF-beta inhibitor comprises no more than one TGF-beta receptor extracellular domain. In some embodiments, the TGF-beta inhibitor comprises two or more (e.g., two, three, four, five, or more) TGF-beta receptor extracellular domains, linked together, e.g., via a linker.

Table 16. Exemplary amino acid sequences of TGF-beta polypeptides or TGF-beta receptor polypeptides

SEQ ID NO	Description	Amino acid sequence
SEQ ID NO: 92	Immature human TGF-beta 1 (P01137-1)	MPPSGLRLLLLLLPLLWLLVLTPGRPAAGLSTCKTIDMELVKRKRIE AIRGQILSKLRLASPPSQGEVPPGPLPEAVLALYNSTRDRVAGESAEP EPEPEADYYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELRE AVPEPVLLSRAELRLLRLKLKVEQHVELYQKYSNNSWRYLSNRLLA PSDSPEWLSFDVTGVVRQWLSRGGEIEGFRLSAHCSCDSRDNTLQV DINGFTTGRRGDLATIHGMNRPFLLLMATPLERAQHLQSSRHRRAL DTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGP CPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVG RKPKVEQLSNMIVRSCKCS
SEQ ID NO: 117	Human TGF-beta 1 (P01137-1)	LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEVPPGPLPEAV LALYNSTRDRVAGESAEPEPEPEADYYAKEVTRVLMVETHNEIYDK FKQSTHSIYMFFNTSELREAVPEPVLLSRAELRLLRLKLKVEQHVEL YQKYSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIE GFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHGMNRPFLLLM ATPLERAQHLQSSRHRRALDTNYCFSSTEKNCCVRQLYIDFRKDLG WKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASA APCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS
SEQ ID NO: 93	Immature human TGF-beta 2 (P61812-1)	MHYCVLSAFLILHLVTVALSLSTCSTLDMDQFMRKRIEAIRGQILSK LKLTSPPEDYPEPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSD EEYYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSAMEKNAS NLVKAEFRVFRLQNPKARVPEQRIELYQILKSKDLTSPTQRYIDSKV VKTRAEGEWLSFDVTDAVHEWLHHKDRNLGFKISLHCPCCTFVPS NNYIIPNKSEELEARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLL LMLLPSYRLESQQTNRRKKRALDAAYCFRNVQDNCCLRPLYIDFKR DLGWKWIHEPKGYNANFCAGACPYLWSSDTQHSRVLSLYNTINPE ASASPCCVSQDLEPLTILYYIGKTPKIEQLSNMIVKSCKCS
SEQ ID NO: 118	Human TGF-beta 2 (P61812-1)	LSTCSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYPEPEEVPPEVIS IYNSTRDLLQEKASRRAAACERERSDEEYYAKEVYKIDMPPFFPSEN AIPPTFYRPYFRIVRFDVSAMEKNASNLVKAEFRVFRLQNPKARVPE QRIELYQILKSKDLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHE WLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELEARFAGIDGTS TYTSGDQKTIKSTRKKNSGKTPHLLLMLLPSYRLESQQTNRRKKRA LDAAYCFRNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYNANFCAG ACPYLWSSDTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGK TPKIEQLSNMIVKSCKCS

SEQ ID NO: 94	Immature human TGF-beta 3 (P10600-1)	MKMHLQRALVVLALLNFATVSLSLSTCTTLDFGHIKKKRVEAIRGQ ILSKLRLTSPPEPTVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQ ENTESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRFNVSSVE KNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQILRPDEHIAKQRYIGG KNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHCPCHTFQPN GDILENIHEVMEIKFKGVDNEDDHGRGDLGRLKKQKDHHNPHLIL MMIPPHRLDNPGQGGQRKKRALDTNYCFRNLEENCCVRPLYIDFRQ DLGWKWVHEPKGYYANFCSGPCPYLRSADTTHSTVLGLYNTLNPE ASASPCCVPQDLEPLTILYYVGRTPKVEQLSNMVVKSCKCS
SEQ ID NO: 119	Human TGF-beta 3 (P10600-1)	LSTCTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPTVMTHVPYQVL ALYNSTRELLEEMHGEREEGCTQENTESEYYAKEIHKFDMIQGLAE HNELAVCPKGITSKVFRFNVSSVEKNRTNLFRAEFRVLRVPNPSSKR NEQRIELFQILRPDEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVRE WLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIKFKGVDNED DHGRGDLGRLKKQKDHHNPHLILMMIPPHRLDNPGQGGQRKKRAL DTNYCFRNLEENCCVRPLYIDFRQDLGWKWVHEPKGYYANFCSGP CPYLRSADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGR TPKVEQLSNMVVKSCKCS
SEQ ID NO: 95	Immature human TGFBR1 isoform 1 (P36897-1)	MEAAVAAPRPRLLLLVLAAAAAAAAALLPGATALQCFCHLCTKDN FTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTG SVTTTYCCNQDHCNKIELPTTVKSSPGLGPVELAAVIAGPVCFVCISL MLMVYICHNRTVIHHRVPNEEDPSLDRPFISEGTTLKDLIYDMTTSG SGSGLPLLVQRTIARTIVLQESIGKGRFGEVWRGKWRGEEVAVKIFS SREERSWFREAEIYQTVMLRHENILGFIAADNKDNGTWTQLWLVSD YHEHGSLFDYLNRYTVTVEGMIKLALSTASGLAHLHMEIVGTQGKP AIAHRDLKSKNILVKKNGTCCIADLGLAVRHDSATDTIDIAPNHRVG TKRYMAPEVLDDSINMKHFESFKRADIYAMGLVFWEIARRCSIGGI HEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRPNIPNRWQSCEALR VMAKIMRECWYANGAARLTALRIKKTLSQLSQQEGIKM
SEQ ID NO: 120	Human TGFBR1 isoform 1 (P36897-1)	LQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPR DRPFVCAPSSKTGSVTTTYCCNQDHCNKIELPTTVKSSPGLGPVELA AVIAGPVCFVCISLMLMVYICHNRTVIHHRVPNEEDPSLDRPFISEGT TLKDLIYDMTTSGSGSGLPLLVQRTIARTIVLQESIGKGRFGEVWRG KWRGEEVAVKIFSSREERSWFREAEIYQTVMLRHENILGFIAADNK DNGTWTQLWLVSDYHEHGSLFDYLNRYTVTVEGMIKLALSTASGL AHLHMEIVGTQGKPAIAHRDLKSKNILVKKNGTCCIADLGLAVRHD SATDTIDIAPNHRVGTKRYMAPEVLDDSINMKHFESFKRADIYAMG LVFWEIARRCSIGGIHEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRP NIPNRWQSCEALRVMAKIMRECWYANGAARLTALRIKKTLSQLSQ QEGIKM

SEQ ID NO: 96	Immature human TGFBR1 isoform 2 (P36897-2)	MEAAVAAPRPRLLLLVLAAAAAAAAALLPGATALQCFCHLCTKDN FTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTG SVTTTYCCNQDHCNKIELPTTGPFSVKSSPGLGPVELAAVIAGPVCF VCISLMLMVYICHNRTVIHHRVPNEEDPSLDRPFISEGTTLKDLIYD MTTSGSGSGLPLLVQRTIARTIVLQESIGKGRFGEVWRGKWRGEEV AVKIFSSREERSWFREAEIYQTVMLRHENILGFIAADNKDNGTWTQ LWLVSDYHEHGSLFDYLNRYTVTVEGMIKLALSTASGLAHLHMEI VGTQGKPAIAHRDLKSKNILVKKNGTCCIADLGLAVRHDSATDTIDI APNHRVGTKRYMAPEVLDDSINMKHFESFKRADIYAMGLVFWEIA RRCSIGGIHEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRPNIPNRW QSCEALRVMAKIMRECWYANGAARLTALRIKKTLSQLSQQEGIKM
SEQ ID NO: 121	Human TGFBR1 isoform 2 (P36897-2)	LQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPR DRPFVCAPSSKTGSVTTTYCCNQDHCNKIELPTTGPFSVKSSPGLGP VELAAVIAGPVCFVCISLMLMVYICHNRTVIHHRVPNEEDPSLDRPFI SEGTTLKDLIYDMTTSGSGSGLPLLVQRTIARTIVLQESIGKGRFGEV WRGKWRGEEVAVKIFSSREERSWFREAEIYQTVMLRHENILGFIAA DNKDNGTWTQLWLVSDYHEHGSLFDYLNRYTVTVEGMIKLALST ASGLAHLHMEIVGTQGKPAIAHRDLKSKNILVKKNGTCCIADLGLA VRHDSATDTIDIAPNHRVGTKRYMAPEVLDDSINMKHFESFKRADI YAMGLVFWEIARRCSIGGIHEDYQLPYYDLVPSDPSVEEMRKVVCE QKLRPNIPNRWQSCEALRVMAKIMRECWYANGAARLTALRIKKTL SQLSQQEGIKM
SEQ ID NO: 97	Immature human TGFBR1 isoform 3 (P36897-3)	MEAAVAAPRPRLLLLVLAAAAAAAAALLPGATALQCFCHLCTKDN FTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTG SVTTTYCCNQDHCNKIELPTTGLPLLVQRTIARTIVLQESIGKGRFGE VWRGKWRGEEVAVKIFSSREERSWFREAEIYQTVMLRHENILGFIA ADNKDNGTWTQLWLVSDYHEHGSLFDYLNRYTVTVEGMIKLALS TASGLAHLHMEIVGTQGKPAIAHRDLKSKNILVKKNGTCCIADLGL AVRHDSATDTIDIAPNHRVGTKRYMAPEVLDDSINMKHFESFKRAD IYAMGLVFWEIARRCSIGGIHEDYQLPYYDLVPSDPSVEEMRKVVC EQKLRPNIPNRWQSCEALRVMAKIMRECWYANGAARLTALRIKKT LSQLSQQEGIKM
SEQ ID NO: 122	Human TGFBR1 isoform 3 (P36897-3)	LQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPR DRPFVCAPSSKTGSVTTTYCCNQDHCNKIELPTTGLPLLVQRTIARTI VLQESIGKGRFGEVWRGKWRGEEVAVKIFSSREERSWFREAEIYQT VMLRHENILGFIAADNKDNGTWTQLWLVSDYHEHGSLFDYLNRYT VTVEGMIKLALSTASGLAHLHMEIVGTQGKPAIAHRDLKSKNILVK KNGTCCIADLGLAVRHDSATDTIDIAPNHRVGTKRYMAPEVLDDSI NMKHFESFKRADIYAMGLVFWEIARRCSIGGIHEDYQLPYYDLVPS DPSVEEMRKVVCEQKLRPNIPNRWQSCEALRVMAKIMRECWYAN

		GAARLTALRIKKTLSQLSQQEGIKM
SEQ ID NO: 104	Human TGFBR1 fragment 1	LQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPR DRPFVCAPSSKTGSVTTTYCCNQDHCNKIELPTTVKSSPGLGPVEL
SEQ ID NO: 105	Human TGFBR1 fragment 2	ALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIP RDRPFVCAPSSKTGSVTTTYCCNQDHCNKIEL
SEQ ID NO: 98	Immature human TGFBR2 isoform B (short isoform) (P37173-1)	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAV KFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSC SSDECNDNIIFSEEYNTSNPDLLLVIFQVTGISLLPPLGVAISVIIIFYCY RVNRQQKLSSTWETGKTRKLMEFSEHCAIILEDDRSDISSTCANNIN HNTELLPIELDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYEE YASWKTEKDIFSDINLKHENILQFLTAEERKTELGKQYWLITAFHAK GNLQEYLTRHVISWEDLRKLGSSLARGIAHLHSDHTPCGRPKMPIV HRDLKSSNILVKNDLTCCLCDFGLSLRLDPTLSVDDLANSGQVGTA RYMAPEVLESRMNLENVESFKQTDVYSMALVLWEMTSRCNAVGE VKDYEPPFGSKVREHPCVESMKDNVLRDRGRPEIPSFWLNHQGIQM VCETLTECWDHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKIPE DGSLNTTK
SEQ ID NO: 123	Human TGFBR2 isoform B (short isoform) (P37173-1)	TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILE DAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLL VIFQVTGISLLPPLGVAISVIIIFYCYRVNRQQKLSSTWETGKTRKLM EFSEHCAIILEDDRSDISSTCANNINHNTELLPIELDTLVGKGRFAEVY KAKLKQNTSEQFETVAVKIFPYEEYASWKTEKDIFSDINLKHENILQ FLTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRKLGS SLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNILVKNDLTCCLCDFG LSLRLDPTLSVDDLANSGQVGTARYMAPEVLESRMNLENVESFKQT DVYSMALVLWEMTSRCNAVGEVKDYEPPFGSKVREHPCVESMKD NVLRDRGRPEIPSFWLNHQGIQMVCETLTECWDHDPEARLTAQCV AERFSELEHLDRLSGRSCSEEKIPEDGSLNTTK
SEQ ID NO: 99	Immature human TGFBR2 isoform A (long isoform) (P37173-2)	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSDVEMEAQKDEIICPSC NRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILE DAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLL VIFQVTGISLLPPLGVAISVIIIFYCYRVNRQQKLSSTWETGKTRKLM EFSEHCAIILEDDRSDISSTCANNINHNTELLPIELDTLVGKGRFAEVY KAKLKQNTSEQFETVAVKIFPYEEYASWKTEKDIFSDINLKHENILQ

		FLTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRKLGS SLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNILVKNDLTCCLCDFG LSLRLDPTLSVDDLANSGQVGTARYMAPEVLESRMNLENVESFKQT DVYSMALVLWEMTSRCNAVGEVKDYEPPFGSKVREHPCVESMKD NVLRDRGRPEIPSFWLNHQGIQMVCETLTECWDHDPEARLTAQCV AERFSELEHLDRLSGRSCSEEKIPEDGSLNTTK
SEQ ID NO: 124	Human TGFBR2 isoform A (long isoform) (P37173-2)	TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGA VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCS CSSDECNDNIIFSEEYNTSNPDLLLVIFQVTGISLLPPLGVAISVIIIFYC YRVNRQQKLSSTWETGKTRKLMEFSEHCAIILEDDRSDISSTCANNI NHNTELLPIELDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYE EYASWKTEKDIFSDINLKHENILQFLTAEERKTELGKQYWLITAFHA KGNLQEYLTRHVISWEDLRKLGSSLARGIAHLHSDHTPCGRPKMPI VHRDLKSSNILVKNDLTCCLCDFGLSLRLDPTLSVDDLANSGQVGT ARYMAPEVLESRMNLENVESFKQTDVYSMALVLWEMTSRCNAVG EVKDYEPPFGSKVREHPCVESMKDNVLRDRGRPEIPSFWLNHQGIQ MVCETLTECWDHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKI PEDGSLNTTK
SEQ ID NO: 100	Human TGFBR2 fragment 1 (ECD of human TGFBR2 isoform B)	TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILE DAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
SEQ ID NO: 101	Human TGFBR2 fragment 2	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
SEQ ID NO: 102	Human TGFBR2 fragment 3 (ECD of human TGFBR2 isoform A)	TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGA VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCS CSSDECNDNIIFSEEYNTSNPD
SEQ ID NO: 103	Human TGFBR2 fragment 4	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDEN ITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSD ECNDNIIF

SEQ ID NO: 106	Immature human TGFBR3 isoform 1 (Q03167-1)	MTSHYVIAIFALMSSCLATAGPEPGALCELSPVSASHPVQALMESFT VLSGCASRGTTGLPQEVHVLNLRTAGQGPGQLQREVTLHLNPISSV HIHHKSVVFLLNSPHPLVWHLKTERLATGVSRLFLVSEGSVVQFSSA NFSLTAETEERNFPHGNEHLLNWARKEYGAVTSFTELKIARNIYIKV GEDQVFPPKCNIGKNFLSLNYLAEYLQPKAAEGCVMSSQPQNEEVH IIELITPNSNPYSAFQVDITIDIRPSQEDLEVVKNLILILKCKKSVNWVI KSFDVKGSLKIIAPNSIGFGKESERSMTMTKSIRDDIPSTQGNLVKW ALDNGYSPITSYTMAPVANRFHLRLENNAEEMGDEEVHTIPPELRIL LDPGALPALQNPPIRGGEGQNGGLPFPFPDISRRVWNEEGEDGLPRP KDPVIPSIQLFPGLREPEEVQGSVDIALSVKCDNEKMIVAVEKDSFQ ASGYSGMDVTLLDPTCKAKMNGTHFVLESPLNGCGTRPRWSALDG VVYYNSIVIQVPALGDSSGWPDGYEDLESGDNGFPGDMDEGDASLF TRPEIVVFNCSLQQVRNPSSFQEQPHGNITFNMELYNTDLFLVPSQG VFSVPENGHVYVEVSVTKAEQELGFAIQTCFISPYSNPDRMSHYTIIE NICPKDESVKFYSPKRVHFPIPQADMDKKRFSFVFKPVFNTSLLFLQ CELTLCTKMEKHPQKLPKCVPPDEACTSLDASIIWAMMQNKKTFTK PLAVIHHEAESKEKGPSMKEPNPISPPIFHGLDTLTVMGIAFAAFVIG ALLTGALWYIYSHTGETAGRQQVPTSPPASENSSAAHSIGSTQSTPC SSSSTA
SEQ ID NO: 125	Human TGFBR3 isoform 1 (Q03167-1)	GPEPGALCELSPVSASHPVQALMESFTVLSGCASRGTTGLPQEVHVL NLRTAGQGPGQLQREVTLHLNPISSVHIHHKSVVFLLNSPHPLVWH LKTERLATGVSRLFLVSEGSVVQFSSANFSLTAETEERNFPHGNEHL LNWARKEYGAVTSFTELKIARNIYIKVGEDQVFPPKCNIGKNFLSLN YLAEYLQPKAAEGCVMSSQPQNEEVHIIELITPNSNPYSAFQVDITID IRPSQEDLEVVKNLILILKCKKSVNWVIKSFDVKGSLKIIAPNSIGFGK ESERSMTMTKSIRDDIPSTQGNLVKWALDNGYSPITSYTMAPVANR FHLRLENNAEEMGDEEVHTIPPELRILLDPGALPALQNPPIRGGEGQ NGGLPFPFPDISRRVWNEEGEDGLPRPKDPVIPSIQLFPGLREPEEVQ GSVDIALSVKCDNEKMIVAVEKDSFQASGYSGMDVTLLDPTCKAK MNGTHFVLESPLNGCGTRPRWSALDGVVYYNSIVIQVPALGDSSG WPDGYEDLESGDNGFPGDMDEGDASLFTRPEIVVFNCSLQQVRNPS SFQEQPHGNITFNMELYNTDLFLVPSQGVFSVPENGHVYVEVSVTK AEQELGFAIQTCFISPYSNPDRMSHYTIIENICPKDESVKFYSPKRVHF PIPQADMDKKRFSFVFKPVFNTSLLFLQCELTLCTKMEKHPQKLPKC VPPDEACTSLDASIIWAMMQNKKTFTKPLAVIHHEAESKEKGPSMK EPNPISPPIFHGLDTLTVMGIAFAAFVIGALLTGALWYIYSHTGETAG RQQVPTSPPASENSSAAHSIGSTQSTPCSSSSTA
SEQ ID NO: 107	Immature human TGFBR3 isoform 2	MTSHYVIAIFALMSSCLATAGPEPGALCELSPVSASHPVQALMESFT VLSGCASRGTTGLPQEVHVLNLRTAGQGPGQLQREVTLHLNPISSV HIHHKSVVFLLNSPHPLVWHLKTERLATGVSRLFLVSEGSVVQFSSA NFSLTAETEERNFPHGNEHLLNWARKEYGAVTSFTELKIARNIYIKV GEDQVFPPKCNIGKNFLSLNYLAEYLQPKAAEGCVMSSQPQNEEVH

	(Q03167-2)	IIELITPNSNPYSAFQVDITIDIRPSQEDLEVVKNLILILKCKKSVNWVI KSFDVKGSLKIIAPNSIGFGKESERSMTMTKSIRDDIPSTQGNLVKW ALDNGYSPITSYTMAPVANRFHLRLENNEEMGDEEVHTIPPELRILL DPGALPALQNPPIRGGEGQNGGLPFPFPDISRRVWNEEGEDGLPRPK DPVIPSIQLFPGLREPEEVQGSVDIALSVKCDNEKMIVAVEKDSFQAS GYSGMDVTLLDPTCKAKMNGTHFVLESPLNGCGTRPRWSALDGV VYYYNSIVIQVPALGDSSGWPDGYEDLESGDNGFPGDMDEGDASLFT RPEIVVFNCSLQQVRNPSSFQEQPHGNITFNMELYNTDLFLVPSQGV FSVPENGHVYVEVSVTKAEQELGFAIQTCFISPYSNPDRMSHYTIIEN ICPKDESVKFYSPKRVHFPIPQADMDKKRFSFVFKPVFNTSLLFLQCE LTLCTKMEKHPQKLPKCVPPDEACTSLDASIIWAMMQNKKTFTKPL AVIHHEAESKEKGPSMKEPNPISPPIFHGLDTLTVMGIAFAAFVIGAL LTGALWYIYSHTGETAGRQQVPTSPPASENSSAAHSIGSTQSTPCSSS STA
SEQ ID NO: 126	Human TGFBR3 isoform 2 (Q03167-2)	GPEPGALCELSPVSASHPVQALMESFTVLSGCASRGTTGLPQEVHVL NLRTAGQGPGQLQREVTLHLNPISSVHIHHKSVVFLLNSPHPLVWH LKTERLATGVSRLFLVSEGSVVQFSSANFSLTAETEERNFPHGNEHL LNWARKEYGAVTSFTELKIARNIYIKVGEDQVFPPKCNIGKNFLSLN YLAEYLQPKAAEGCVMSSQPQNEEVHIIELITPNSNPYSAFQVDITID IRPSQEDLEVVKNLILILKCKKSVNWVIKSFDVKGSLKIIAPNSIGFGK ESERSMTMTKSIRDDIPSTQGNLVKWALDNGYSPITSYTMAPVANR FHLRLENNEEMGDEEVHTIPPELRILLDPGALPALQNPPIRGGEGQN GGLPFPFPDISRRVWNEEGEDGLPRPKDPVIPSIQLFPGLREPEEVQG SVDIALSVKCDNEKMIVAVEKDSFQASGYSGMDVTLLDPTCKAKM NGTHFVLESPLNGCGTRPRWSALDGVVYYYNSIVIQVPALGDSSGWP DGYEDLESGDNGFPGDMDEGDASLFTRPEIVVFNCSLQQVRNPSSF QEQPHGNITFNMELYNTDLFLVPSQGVFSVPENGHVYVEVSVTKAE QELGFAIQTCFISPYSNPDRMSHYTIIENICPKDESVKFYSPKRVHFPI PQADMDKKRFSFVFKPVFNTSLLFLQCELTLCTKMEKHPQKLPKCV PPDEACTSLDASIIWAMMQNKKTFTKPLAVIHHEAESKEKGPSMKE PNPISPPIFHGLDTLTVMGIAFAAFVIGALLTGALWYIYSHTGETAGR QQVPTSPPASENSSAAHSIGSTQSTPCSSSSTA
SEQ ID NO: 108	Human TGFBR3 fragment 1	GPEPGALCELSPVSASHPVQALMESFTVLSGCASRGTTGLPQEVHVL NLRTAGQGPGQLQREVTLHLNPISSVHIHHKSVVFLLNSPHPLVWH LKTERLATGVSRLFLVSEGSVVQFSSANFSLTAETEERNFPHGNEHL LNWARKEYGAVTSFTELKIARNIYIKVGEDQVFPPKCNIGKNFLSLN YLAEYLQPKAAEGCVMSSQPQNEEVHIIELITPNSNPYSAFQVDITID IRPSQEDLEVVKNLILILKCKKSVNWVIKSFDVKGSLKIIAPNSIGFGK ESERSMTMTKSIRDDIPSTQGNLVKWALDNGYSPITSYTMAPVANR FHLRLENNAEEMGDEEVHTIPPELRILLDPGALPALQNPPIRGGEGQ NGGLPFPFPDISRRVWNEEGEDGLPRPKDPVIPSIQLFPGLREPEEVQ GSVDIALSVKCDNEKMIVAVEKDSFQASGYSGMDVTLLDPTCKAK

		MNGTHFVLESPLNGCGTRPRWSALDGVVYYNSIVIQVPALGDSSG WPDGYEDLESGDNGFPGDMDEGDASLFTRPEIVVFNCSLQQVRNPS SFQEQPHGNITFNMELYNTDLFLVPSQGVFSVPENGHVYVEVSVTK AEQELGFAIQTCFISPYSNPDRMSHYTIIENICPKDESVKFYSPKRVHF PIPQADMDKKRFSFVFKPVFNTSLLFLQCELTLCTKMEKHPQKLPKC VPPDEACTSLDASIIWAMMQNKKTFTKPLAVIHHEAESKEKGPSMK EPNPISPPIFHGLDTLTV
SEQ ID NO: 192	hCH1-hFc_Hole-3x4GS-TGFbR2	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGXGGGGSGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVK FPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND ENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCS SDECNDNIIFSEEYNTSNPD, wherein X is K or absent
SEQ ID NO: 193	hCH1-hFc_Knob-3x4GS-TGFbR2	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGXGGGGSGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVK FPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND ENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCS SDECNDNIIFSEEYNTSNPD, wherein X is K or absent
SEQ ID NO: 194	hFc_Hole-3x4GS-TGFbR2	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGXG GGGSGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC DVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETV CHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI IFSEEYNTSNPD, wherein X is K or absent
SEQ ID	hFc_Knob-3x4GS-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

NO: 195	TGFbR2	DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX GGGGSGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKF CDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLET VCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD, wherein X is K or absent
SEQ ID NO: 196	TGFbR2-3x4GS-hCH1-hFc_Hole	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGG SGGGGSGGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 197	TGFbR2-3x4GS-hCH1-hFc_Knob	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGG SGGGGSGGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 198	TGFbR2-3x4GS-hCLIg_vl	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGG SGGGGSGGGGSGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPG AVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKS HRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 199	TGFβR2-3x4GS-hCLIg_vk	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGG SGGGGSGGGGSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK

		HKVYACEVTHQGLSSPVTKSFNRGEC

Immune Cell Engagers

The immune cell engagers of the multispecific or multifunctional molecules disclosed herein can mediate binding to, and/or activation of, an immune cell, e.g., an immune effector cell. In some embodiments, the immune cell is chosen from a T cell, an NK cell, a B cell, a dendritic cell, or a macrophage cell engager, or a combination thereof. In some embodiments, the immune cell engager is chosen from one, two, three, or all of a T cell engager, NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager, or a combination thereof. The immune cell engager can be an agonist of the immune system. In some embodiments, the immune cell engager can be an antibody molecule, a ligand molecule (e.g., a ligand that further comprises an immunoglobulin constant region, e.g., an Fc region), a small molecule, a nucleotide molecule.

Natural Killer Cell Engagers

Natural Killer (NK) cells recognize and destroy tumors and virus-infected cells in an antibody-independent manner. The regulation of NK cells is mediated by activating and inhibiting receptors on the NK cell surface. One family of activating receptors is the natural cytotoxicity receptors (NCRs) which include NKp30, NKp44 and NKp46. The NCRs initiate tumor targeting by recognition of heparan sulfate on cancer cells. NKG2D is a receptor that provides both stimulatory and costimulatory innate immune responses on activated killer (NK) cells, leading to cytotoxic activity. DNAM1 is a receptor involved in intercellular adhesion, lymphocyte signaling, cytotoxicity and lymphokine secretion mediated by cytotoxic T-lymphocyte (CTL) and NK cell. DAP10 (also known as HCST) is a transmembrane adapter protein which associates with KLRK1 to form an activation receptor KLRK1-HCST in lymphoid and myeloid cells; this receptor plays a major role in triggering cytotoxicity against target cells expressing cell surface ligands such as MHC class I chain-related MICA and MICB, and U(optionally L1)6-binding proteins (ULBPs); it KLRK1-HCST receptor plays a role in immune surveillance against tumors and is required for cytolysis of tumors cells; indeed, melanoma cells

that do not express KLRK1 ligands escape from immune surveillance mediated by NK cells. CD16 is a receptor for the Fc region of IgG, which binds complexed or aggregated IgG and also monomeric IgG and thereby mediates antibody-dependent cellular cytotoxicity (ADCC) and other antibody-dependent responses, such as phagocytosis.

The present disclosure provides, *inter alia*, multispecific (e.g., bi-, tri-, quad- specific) or multifunctional molecules, that are engineered to contain one or more NK cell engagers that mediate binding to and/or activation of an NK cell. Accordingly, in some embodiments, the NK cell engager is selected from an antigen binding domain or ligand that binds to (e.g., activates): NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16 (e.g., CD16a, CD16b, or both), CRTAM, CD27, PSGL1, CD96, CD100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS1, KIR2DS3, KIR2DS5, KIR2DS1, CD94, NKG2C, NKG2E, or CD160.

In some embodiments, the NK cell engager is an antigen binding domain that binds to NKp30 (e.g., NKp30 present, e.g., expressed or displayed, on the surface of an NK cell) and comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in Tables 7-10. In some embodiments, the NK cell engager is an antigen binding domain that binds to NKp30 (e.g., NKp30 present, e.g., expressed or displayed, on the surface of an NK cell) and comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in U.S. Patent No. 6,979,546, U.S. Patent No. 9,447,185, PCT Application No. WO2015121383A1, PCT Application No. WO2016110468A1, PCT Application No. WO2004056392A1, or U.S. Application Publication No. US20070231322A1, the sequences of which are hereby incorporated by reference. In some embodiments, binding of the NK cell engager, e.g., antigen binding domain that binds to NKp30, to the NK cell activates the NK cell. An antigen binding domain that binds to NKp30 (e.g., NKp30 present, e.g., expressed or displayed, on the surface of an NK cell) may be said to target NKp30, the NK cell, or both.

In some embodiments, the antigen binding domain that binds to NKp30 comprises one or more CDRs (e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and/or VLCDR3)

disclosed in Table 7, Table 18, or Table 8, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to NKp30 comprises one or more framework regions (e.g., VHFWR1, VHFWR2, VHFWR3, VHFWR4, VLFWR1, VLFWR2, VLFWR3, and/or VLFWR4) disclosed in Table 7, Table 18, or Table 8, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to NKp30 comprises a VH and/or a VL disclosed in Table 9, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain that binds to NKp30 comprises an amino acid sequence disclosed in Table 10, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

In some embodiments, the antigen binding domain that binds to NKp30 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3.

In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6001, and 7315, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6001, and 6002, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6008, and 6009, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 7385, and 7315, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 7318, and 6009, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7326, 7327, and 7329, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 6063, 6064, and 7293,

respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 6070, 6071, and 6072, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 6070, 6064, and 7321, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6001, 7315, 7326, 7327, and 7329, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6001, 6002, 6063, 6064, and 7293, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 6008, 6009, 6070, 6071, and 6072, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 7385, 7315, 6070, 6064, and 7321, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of SEQ ID NOs: 7313, 7318, 6009, 6070, 6064, and 7321, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7298 or 7300-7304 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7299 or 7305-7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7302 and 7305, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7302 and 7309, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6121 or 6123-6128 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7294 or 6137-6141 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6122 or 6129-6134 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6136 or 6142-6147 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7295 and 7296, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 7297 and 7296, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the VH and VL comprise the amino acid sequences of SEQ ID NOs: 6122 and 6136, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the antigen binding domain that binds to NKp30 comprises the amino acid sequence of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the antigen binding domain that binds to NKp30 comprises the amino acid sequence of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto). In some embodiments, the antigen binding domain that binds to NKp30 comprises the amino acid sequence of SEQ ID NO: 6187, 6188, 6189 or 6190 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6001 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the

NKp30 antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6000, a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6064 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 7293 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6063, a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and a VLCDR3 amino acid sequence of SEQ ID NO: 7293.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6001 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and a VL comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6064 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 7293 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the NKp30 antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6000, a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6063,

a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and a VLCDR3 amino acid sequence of SEQ ID NO: 7293.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6008 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the NKp30 antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6007, a VHCDR2 amino acid sequence of SEQ ID NO: 6008, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6070 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6071 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6072 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6070, a VLCDR2 amino acid sequence of SEQ ID NO: 6071, and a VLCDR3 amino acid sequence of SEQ ID NO: 6072.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6008 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and a VL comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO:

6070 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6071 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6072 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some embodiments, the NKp30 antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6007, a VHCDR2 amino acid sequence of SEQ ID NO: 6008, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6009, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6070, a VLCDR2 amino acid sequence of SEQ ID NO: 6071, and a VLCDR3 amino acid sequence of SEQ ID NO: 6072.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6003, a VHFWR2 amino acid sequence of SEQ ID NO: 6004, a VHFWR3 amino acid sequence of SEQ ID NO: 6005, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6006.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6066, a VLFWR2 amino acid sequence of SEQ ID NO: 6067, a VLFWR3 amino acid sequence of SEQ ID NO: 7292, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6003, a VHFWR2 amino acid sequence of SEQ ID NO: 6004, a VHFWR3 amino acid sequence of SEQ ID NO: 6005, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6006, and a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6066, a VLFWR2 amino acid sequence of SEQ ID NO: 6067, a VLFWR3 amino acid sequence of SEQ ID NO: 7292, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6003 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6004 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino

acid sequence of SEQ ID NO: 6005 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6006.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6066 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6067 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 7292 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6003 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6004 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6005 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6006, and a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6066 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6067 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 7292 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6010, a VHFWR2 amino acid sequence of SEQ ID NO: 6011, a VHFWR3 amino acid sequence of SEQ ID NO: 6012, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6013.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO:

6073, a VLFWR2 amino acid sequence of SEQ ID NO: 6074, a VLFWR3 amino acid sequence of SEQ ID NO: 6075, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6076.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6010, a VHFWR2 amino acid sequence of SEQ ID NO: 6011, a VHFWR3 amino acid sequence of SEQ ID NO: 6012, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6013, and a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6073, a VLFWR2 amino acid sequence of SEQ ID NO: 6074, a VLFWR3 amino acid sequence of SEQ ID NO: 6075, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6076.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6010 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6011 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6012 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6013.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6073 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6074 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6075 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6076.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6010 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6011 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6012 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6013, and a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6073 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6074 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6075 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6076.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6014, a VHFWR2 amino acid sequence of SEQ ID NO: 6015, a VHFWR3 amino acid sequence of SEQ ID NO: 6016, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6017.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6014 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6015 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6016 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6017.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6077, a VLFWR2 amino acid sequence of SEQ ID NO: 6078, a VLFWR3 amino acid sequence of SEQ ID NO: 6079, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6080.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6077 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6078 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6079 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6080.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6018, a VHFWR2 amino acid sequence of SEQ ID NO: 6019, a VHFWR3 amino acid sequence of SEQ ID NO: 6020, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6021.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6018 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6019 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6020 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6021.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6081, a VLFWR2 amino acid sequence of SEQ ID NO: 6082, a VLFWR3 amino acid sequence of SEQ ID NO: 6083, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6084.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6081 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6082 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6083 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6084.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6022, a VHFWR2 amino acid sequence of SEQ ID NO: 6023, a VHFWR3 amino acid sequence of SEQ ID NO: 6024, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6025.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6022 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a

VHFWR2 amino acid sequence of SEQ ID NO: 6023 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6024 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6025.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6085, a VLFWR2 amino acid sequence of SEQ ID NO: 6086, a VLFWR3 amino acid sequence of SEQ ID NO: 6087, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6088.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6085 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6086 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6087 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6088.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6026, a VHFWR2 amino acid sequence of SEQ ID NO: 6027, a VHFWR3 amino acid sequence of SEQ ID NO: 6028, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6029.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6026 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6027 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6028 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6029.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO:

6089, a VLFWR2 amino acid sequence of SEQ ID NO: 6090, a VLFWR3 amino acid sequence of SEQ ID NO: 6091, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6092.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6089 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6090 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6091 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6092.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6030, a VHFWR2 amino acid sequence of SEQ ID NO: 6032, a VHFWR3 amino acid sequence of SEQ ID NO: 6033, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6034.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6030 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6032 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6033 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6034.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6093, a VLFWR2 amino acid sequence of SEQ ID NO: 6094, a VLFWR3 amino acid sequence of SEQ ID NO: 6095, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6096.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6093 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6094 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6095 (or a sequence with

no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6096.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6035, a VHFWR2 amino acid sequence of SEQ ID NO: 6036, a VHFWR3 amino acid sequence of SEQ ID NO: 6037, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6038.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6035 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6036 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6037 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6038.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6039, a VHFWR2 amino acid sequence of SEQ ID NO: 6040, a VHFWR3 amino acid sequence of SEQ ID NO: 6041, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6042.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6039 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6040 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6041 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6042.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6097, a VLFWR2 amino acid sequence of SEQ ID NO: 6098, a VLFWR3 amino acid sequence of SEQ ID NO: 6099, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6100.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6097 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6098 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6099 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6100.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6043, a VHFWR2 amino acid sequence of SEQ ID NO: 6044, a VHFWR3 amino acid sequence of SEQ ID NO: 6045, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6046.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6043 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6044 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6045 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6046.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6101, a VLFWR2 amino acid sequence of SEQ ID NO: 6102, a VLFWR3 amino acid sequence of SEQ ID NO: 6103, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6104.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6101 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6102 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6103 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6104.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6047, a VHFWR2 amino acid sequence of SEQ ID NO: 6048, a VHFWR3 amino acid sequence of SEQ ID NO: 6049, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6050.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6047 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6048 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6049 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6050.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6105, a VLFWR2 amino acid sequence of SEQ ID NO: 6106, a VLFWR3 amino acid sequence of SEQ ID NO: 6107, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6108.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6105 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6106 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6107 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6108.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6051, a VHFWR2 amino acid sequence of SEQ ID NO: 6052, a VHFWR3 amino acid sequence of SEQ ID NO: 6053, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6054.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6051 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a

VHFWR2 amino acid sequence of SEQ ID NO: 6052 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6053 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6054.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6109, a VLFWR2 amino acid sequence of SEQ ID NO: 6110, a VLFWR3 amino acid sequence of SEQ ID NO: 6111, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6112.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6109 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6110 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6111 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6112.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6055, a VHFWR2 amino acid sequence of SEQ ID NO: 6056, a VHFWR3 amino acid sequence of SEQ ID NO: 6057, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6058.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6055 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6056 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6057 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6058.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6113, a VLFWR2 amino acid sequence of SEQ ID NO: 6114, a VLFWR3 amino acid sequence of SEQ ID NO: 6115, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6116.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6113 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6114 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6115 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6116.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6059, a VHFWR2 amino acid sequence of SEQ ID NO: 6060, a VHFWR3 amino acid sequence of SEQ ID NO: 6061, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6062.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6059 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 amino acid sequence of SEQ ID NO: 6060 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 amino acid sequence of SEQ ID NO: 6061 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6062.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6117, a VLFWR2 amino acid sequence of SEQ ID NO: 6118, a VLFWR3 amino acid sequence of SEQ ID NO: 6119, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6120.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6117 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid

sequence of SEQ ID NO: 6118 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6119 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6120.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6148 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6148). In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6149 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6149). In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising the amino acid sequence of SEQ ID NO: 6150 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6150). In some embodiments, antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6148. In some embodiments, antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6149. In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising the amino acid sequence of SEQ ID NO: 6150.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6148, and a VL comprising the amino acid sequence of SEQ ID NO: 6150. In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6149, and a VL comprising the amino acid sequence of SEQ ID NO: 6150.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6151 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6151). In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6152 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6152). In some

embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising the amino acid sequence of SEQ ID NO: 6153 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6153). In some embodiments, antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6151. In some embodiments, antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6152. In some embodiments, the antigen binding domain that targets NKp30 comprises a VL comprising the amino acid sequence of SEQ ID NO: 6153.

In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6151, and a VL comprising the amino acid sequence of SEQ ID NO: 6153. In some embodiments, the antigen binding domain that targets NKp30 comprises a VH comprising the amino acid sequence of SEQ ID NO: 6152, and a VL comprising the amino acid sequence of SEQ ID NO: 6153.

In some embodiments, the antigen binding domain that targets NKp30 comprises an scFv. In some embodiments, the scFv comprises an amino acid sequence selected from SEQ ID NOs: 6187-6190, or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto.

Table 7. Exemplary heavy chain CDRs and FWRs of NKp30-targeting antigen binding domains

Ab ID	VHFWR1	VHCDR1	VHFWR2	VHCDR2	VHFWR3	VHCDR3	VHFWR4
9G1-HC	QIQLQESGPGLVKPSQSLSLTCSVTGFSIN (SEQ ID NO: 6003)	TGGYHWN (SEQ ID NO: 6000)	WIRQFPGKKLEWMG (SEQ ID NO: 6004)	YIYSSGSTSYNPSLKS (SEQ ID NO: 6001)	RISITRDTSKNQFFLQLNSVTTEDTATYYCAR (SEQ ID NO: 6005)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVTVSS (SEQ ID NO: 6006)
15H6-HC	QIQLQESGPGLVKPSQSLSLTCSVTGFSIN (SEQ ID NO: 6010)	TGGYHWN (SEQ ID NO: 6007)	WIRQFPGKKLEWMG (SEQ ID NO: 6011)	YIYSSGTTRYNPSLKS (SEQ ID NO: 6008)	RISITRDTSKNQFFLQLNSVTPEDTATYYCTR (SEQ ID NO: 6012)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVAVSS (SEQ ID NO: 6013)
9G1-HC_1	QIQLQESGPGLVKPSETLSLTCTVSGFSIN (SEQ ID NO:	TGGYHWN (SEQ ID NO: 6000)	WIRQPAGKGLEWIG (SEQ ID NO: 6015)	YIYSSGSTSYNPSLKS (SEQ ID NO: 6001)	RVTMSRDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVTVSS (SEQ ID NO: 6017)

	6014)				6016)		
9G1-HC_2	QIQLQESG PGLVKPSQ TLSLTCTV SGFSIN (SEQ ID NO: 6018)	TGGYHWN (SEQ ID NO: 6000)	WIRQHPGK GLEWIG (SEQ ID NO: 6019)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	LVTISRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6020)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6021)
9G1-HC_3	EIQLLESG GGLVQPGG SLRLSCAV SGFSIN (SEQ ID NO: 6022)	TGGYHWN (SEQ ID NO: 6000)	WVRQAPGK GLEWVG (SEQ ID NO: 6023)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT SKNTFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6024)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6025)
9G1-HC_4	QIQLVQSG AEVKKPGS SVKVSCKV SGFSIN (SEQ ID NO: 6026)	TGGYHWN (SEQ ID NO: 6000)	WVRQAPGQ GLEWMG (SEQ ID NO: 6027)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTITRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6028)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6029)
9G1-HC_5	EIQLVESG GGLVQPGG SLRLSCAV SGFSIN (SEQ ID NO: 6030)	TGGYHWN (SEQ ID NO: 6000)	WVRQAPGK GLEWVG (SEQ ID NOL 6032)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT AKNSFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6033)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6034)
9G1-HC_6	QIQLVQSG AEVKKPGA SVKVSCKV SGFSIN (SEQ ID NO: 6035)	TGGYHWN (SEQ ID NO: 6000)	WVRQAPGQ GLEWMG (SEQ ID NO: 6036)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTMTRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6037)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6038)
15H6-HC_1	QIQLQESG PGLVKPSQ TLSLTCTV SGFSIN (SEQ ID NO: 6039)	TGGYHWN (SEQ ID NO: 6007)	WIRQHPGK GLEWIG (SEQ ID NO: 6040)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	LVTISRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6041)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6042)
15H6-HC_2	QIQLQESG PGLVKPSE TLSLTCTV SGFSIN (SEQ ID NO: 6043)	TGGYHWN (SEQ ID NO: 6007)	WIRQPAGK GLEWIG (SEQ ID NO: 6044)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTMSRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6045)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6046)
15H6-HC_3	EIQLLESG GGLVQPGG SLRLSCAV SGFSIN (SEQ ID	TGGYHWN (SEQ ID NO: 6007)	WVRQAPGK GLEWVG (SEQ ID NO: 6048)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RFTISRDT SKNTFYLQ MNSLRAED TAVYYCAR (SEQ ID	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6050)

	NO: 6047)				NO: 6049)		
15H6-HC_4	QIQLVESG GGLVKPGG SLRLSCAV SGFSIN (SEQ ID NO: 6051)	TGGYHWN (SEQ ID NO: 6007)	WIRQAPGK GLEWVG (SEQ ID NO: 6052)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RFTISRDT AKNSFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6053)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6054)
15H6-HC_5	QIQLVQSG AEVKKPGA SVKVSCKV SGFSIN (SEQ ID NO: 6055)	TGGYHWN (SEQ ID NO: 6007)	WVRQAPGQ GLEWMG (SEQ ID NO: 6056)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTMTRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6057)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6058)
15H6-HC_6	EIQLVQSG AEVKKPGA TVKISCKV SGFSIN (SEQ ID NO: 6059)	TGGYHWN (SEQ ID NO: 6007)	WVQQAPGK GLEWMG (SEQ ID NO: 6060)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTITRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6061)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6062)

Table 18. Exemplary heavy chain CDRs and FWRs of NKp30-targeting antigen binding domains (according to the Kabat numbering scheme)

Ab ID	VHFWR1	VHCDR1	VHFWR2	VHCDR2	VHFWR3	VHCDR3	VHFWR4
9G1-HC	QIQLQESG PGLVKPSQ SLSLTCSV TGFSINTG (SEQ ID NO: 7317)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KLEWMG (SEQ ID NO: 6004)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RISITRDT SKNQFFLQ LNSVTTED TATYYCAR (SEQ ID NO: 6005)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6006)
15H6-HC	QIQLQESG PGLVKPSQ SLSLTCSV TGFSINTG (SEQ ID NO: 7317)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KLEWMG (SEQ ID NO: 6011)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RISITRDT SKNQFFLQ LNSVTPED TATYYCTR (SEQ ID NO: 6012)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVA VSS (SEQ ID NO: 6013)
9G1-HC_1	QIQLQESG PGLVKPSE TLSLTCTV SGFSINTG (SEQ ID NO: 7371)	GYHWN (SEQ ID NO: 7313)	WIRQPAGK GLEWIG (SEQ ID NO: 6015)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTMSRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6016)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6017)
9G1-HC_2	QIQLQESG PGLVKPSQ TLSLTCTV SGFSINTG	GYHWN (SEQ ID NO: 7313)	WIRQHPGK GLEWIG (SEQ ID NO:	YIYSSGST SYNPSLKS (SEQ ID NO:	LVTISRDT SKNQFSLK LSSVTAAD TAVYYCAR	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6021)

	(SEQ ID NO: 7372)		6019)	6001)	(SEQ ID NO: 6020)		
9G1-HC_3	EIQLLESG GGLVQPGG SLRLSCAV SGFSINTG (SEQ ID NO: 7373)	GYHWN (SEQ ID NO: 7313)	WVRQAPGK GLEWVG (SEQ ID NO: 6023)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT SKNTFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6024)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6025)
9G1-HC_4	QIQLVQSG AEVKKPGS SVKVSCKV SGFSINTG (SEQ ID NO: 7374)	GYHWN (SEQ ID NO: 7313)	WVRQAPGQ GLEWMG (SEQ ID NO: 6027)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTITRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6028)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6029)
9G1-HC_5	EIQLVESG GGLVQPGG SLRLSCAV SGFSINTG (SEQ ID NO: 7375)	GYHWN (SEQ ID NO: 7313)	WVRQAPGK GLEWVG (SEQ ID NOL 6032)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT AKNSFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6033)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6034)
9G1-HC_6	QIQLVQSG AEVKKPGA SVKVSCKV SGFSINTG (SEQ ID NO: 7376)	GYHWN (SEQ ID NO: 7313)	WVRQAPGQ GLEWMG (SEQ ID NO: 6036)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTMTRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6037)	GNWHYFDF (SEQ ID NO: 6002)	WGQGTMVT VSS (SEQ ID NO: 6038)
15H6-HC_1	QIQLQESG PGLVKPSQ TLSLTCTV SGFSINTG (SEQ ID NO: 7372)	GYHWN (SEQ ID NO: 7313)	WIRQHPGK GLEWIG (SEQ ID NO: 6040)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	LVTISRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6041)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6042)
15H6-HC_2	QIQLQESG PGLVKPSE TLSLTCTV SGFSINTG (SEQ ID NO: 7371)	GYHWN (SEQ ID NO: 7313)	WIRQPAGK GLEWIG (SEQ ID NO: 6044)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTMSRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6045)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6046)
15H6-HC_3	EIQLLESG GGLVQPGG SLRLSCAV SGFSINTG (SEQ ID NO: 7373)	GYHWN (SEQ ID NO: 7313)	WVRQAPGK GLEWVG (SEQ ID NO: 6048)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RFTISRDT SKNTFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6049)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6050)
15H6-HC_4	QIQLVESG GGLVKPGG SLRLSCAV	GYHWN (SEQ ID NO:	WIRQAPGK GLEWVG (SEQ ID	YIYSSGTT RYNPSLKS (SEQ ID	RFTISRDT AKNSFYLQ MNSLRAED	GNWHYFDY (SEQ ID NO:	WGQGTLVT VSS (SEQ ID NO:

	SGFSINTG (SEQ ID NO: 7377)	7313)	NO: 6052)	NO: 6008)	TAVYYCAR (SEQ ID NO: 6053)	6009)	6054)
15H6-HC_5	QIQLVQSG AEVKKPGA SVKVSCKV SGFSINTG (SEQ ID NO: 7376)	GYHWN (SEQ ID NO: 7313)	WVRQAPGQ GLEWMG (SEQ ID NO: 6056)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTMTRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6057)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6058)
15H6-HC_6	EIQLVQSG AEVKKPGA TVKISCKV SGFSINTG (SEQ ID NO: 7378)	GYHWN (SEQ ID NO: 7313)	WVQQAPGK GLEWMG (SEQ ID NO: 6060)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RVTITRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6061)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVT VSS (SEQ ID NO: 6062)
9D9-HC	QIQLQESG PGLVKPSQ SLSLSCSV TGFSINTG (SEQ ID NO: 7312)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KVEWMG (SEQ ID NO: 7314)	YIYSSGTT KYNPSLKS (SEQ ID NO: 7385)	RISITRDT SKNQFFLQ LNSVTTED TATYYCAR (SEQ ID NO: 6005)	GDWHYFDY (SEQ ID NO: 7315)	WGQGTMVA VSS (SEQ ID NO: 7316)
3A12-HC	QIQLQESG PGLVKPSQ SLSLTCSV TGFSINTG (SEQ ID NO: 7317)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KLEWMG (SEQ ID NO: 6004)	YIYSSGST RYNPSLKS (SEQ ID NO: 7318)	RFSITRDT SKNQFFLQ LNSVTTED TATYYCTR (SEQ ID NO: 7319)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVA VSS (SEQ ID NO: 6013)
12D10-HC	QIQLQESG PGLVKPSQ SLSLTCSV TGFSINTG (SEQ ID NO: 7317)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KLEWMG (SEQ ID NO: 6004)	YIYSSGTT RYNPSLKS (SEQ ID NO: 6008)	RISITRDT SKNQFFLQ LNSVTPED TATYYCTR (SEQ ID NO: 6012)	GNWHYFDY (SEQ ID NO: 6009)	WGQGTLVA VSS (SEQ ID NO: 6013)
15E1-HC	QIQLQESG PGLVKPSQ SLSLSCSV TGFSITTT (SEQ ID NO: 7322)	GYHWN (SEQ ID NO: 7313)	WIRQFPGK KLEWMG (SEQ ID NO: 6004)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFSITRDT SKNQFFLQ LNSVTTED TATYYCAR (SEQ ID NO: 7323)	GDWHYFDY (SEQ ID NO: 7315)	WGPGTMVT VSS (SEQ ID NO: 7324)
15E1_Humanized variant_VH1	QIQLQESG PGLVKPSQ TLSLTCTV SGFSITTT (SEQ ID NO: 7330)	GYHWN (SEQ ID NO: 7313)	WIRQHPGK GLEWIG (SEQ ID NO: 6019)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	LVTISRDT SKNQFSLK LSSVTAAD TAVYYCAR (SEQ ID NO: 6020)	GDWHYFDY (SEQ ID NO: 7315)	WGQGTMVT VSS (SEQ ID NO: 6006)
15E1_Humanized	QIQLVESG GGLVKPGG	GYHWN (SEQ ID	WIRQAPGK GLEWVG	YIYSSGST SYNPSLKS	RFTISRDT AKNSFYLQ	GDWHYFDY (SEQ ID	WGQGTMVT VSS (SEQ

variant_VH 2	SLRLSCAV SGFSITTT (SEQ ID NO: 7331)	NO: 7313)	(SEQ ID NO: 6052)	(SEQ ID NO: 6001)	MNSLRAED TAVYYCAR (SEQ ID NO: 6033)	NO: 7315)	ID NO: 6006)
15E1_Humanized variant_VH 3	EIQLLESG GGLVQPGG SLRLSCAV SGFSITTT (SEQ ID NO: 7332)	GYHWN (SEQ ID NO: 7313)	WVRQAPGK GLEWVG (SEQ ID NO: 6023)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT SKNTFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6024)	GDWHYFDY (SEQ ID NO: 7315)	WGQGTMVT VSS (SEQ ID NO: 6006)
15E1_Humanized variant_VH 4	EIQLVESG GGLVQPGG SLRLSCAV SGFSITTT (SEQ ID NO: 7333)	GYHWN (SEQ ID NO: 7313)	WVRQAPGK GLEWVG (SEQ ID NO: 6023)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RFTISRDT AKNSFYLQ MNSLRAED TAVYYCAR (SEQ ID NO: 6033)	GDWHYFDY (SEQ ID NO: 7315)	WGQGTMVT VSS (SEQ ID NO: 6006)
15E1_Humanized variant_VH 5	QIQLVQSG AEVKKPGA SVKVSCKV SGFSITTT (SEQ ID NO: 7334)	GYHWN (SEQ ID NO: 7313)	WVRQAPGQ GLEWMG (SEQ ID NO: 6027)	YIYSSGST SYNPSLKS (SEQ ID NO: 6001)	RVTMTRDT STNTFYME LSSLRSED TAVYYCAR (SEQ ID NO: 6037)	GDWHYFDY (SEQ ID NO: 7315)	WGQGTMVT VSS (SEQ ID NO: 6006)

Table 8. Exemplary light chain CDRs and FWRs of NKp30-targeting antigen binding domains

Ab ID	VLFWR1	VLCDR1	VLFWR2	VLCDR2	VLFWR3	VLCDR3	VLFWR4
9G1-LC	SYTLTQPP LLSVALG HKATITC (SEQ ID NO: 6066)	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQKP GRAPVM VIY (SEQ ID NO: 6067)	ENDKRPS (SEQ ID NO: 6064)	GIPDQFSG SNSGNIAT LTISKAQ AGYEADY YC (SEQ ID NO: 7292)	QSWDSTN SAV (SEQ ID NO: 7293)	FGSGTQL TVL (SEQ ID NO: 6069)
15H6-LC	SYTLTQPP SLSVAPG QKATIIC (SEQ ID NO: 6073)	SGENLSD KYVH (SEQ ID NO: 6070)	WYQQKP GRAPVM VIY (SEQ ID NO: 6074)	ENEKRPS (SEQ ID NO: 6071)	GIPDQFSG SNSGNIAT LTISKAQP GSEADYY C (SEQ ID NO: 6075)	HYWESIN SVV (SEQ ID NO: 6072)	FGSGTHL TVL (SEQ ID NO: 6076)
9G1-LC_1	QSVTTQP PSVSGAP GQRVTIS C (SEQ ID NO: 6077)	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQLP GTAPKML IY (SEQ ID NO: 6078)	ENDKRPS (SEQ ID NO: 6064)	GVPDRFS GSNSGNS ASLAITGL QAEDEAD YYC (SEQ ID NO: 6079)	QSWDSTN SAV (SEQ ID NO: 7293)	FGGGTQL TVL (SEQ ID NO: 6080)
9G1-LC_2	QSVTTQP PSASGTP GQRVTIS C (SEQ ID	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQLP GTAPKML IY (SEQ ID NO: 6082)	ENDKRPS (SEQ ID NO: 6064)	GVPDRFS GSNSGNS ASLAISGL QSEDEAD	QSWDSTN SAV (SEQ ID NO: 7293)	FGGGTQL TVL (SEQ ID NO: 6084)

	NO: 6081)				YYC (SEQ ID NO: 6083)		
9G1-LC_3	QSVTTQP PSASGTP GQRVTIS C (SEQ ID NO: 6085)	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQLP GTAPKML IY (SEQ ID NO: 6086)	ENDKRPS (SEQ ID NO: 6064)	GVPDRFS GSNSGNS ASLAISGL RSEDEAD YYC (SEQ ID NO: 6087)	QSWDSTN SAV (SEQ ID NO: 7293)	FGGGTQL TVL (SEQ ID NO: 6088)
9G1-LC_4	SSETTQPH SVSVATA QMARITC (SEQ ID NO: 6089)	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQKP GQDPVM VIY (SEQ ID NO: 6090)	ENDKRPS (SEQ ID NO: 6064)	GIPERFSG SNPGNTA TLTISRIE AGDEADY YC (SEQ ID NO: 6091)	QSWDSTN SAV (SEQ ID NO: 7293)	FGGGTQL TVL (SEQ ID NO: 6092)
9G1-LC_5	DIQMTQS PSTLSASV GDRVTIT C (SEQ ID NO: 6093)	SGERLSD KYVH (SEQ ID NO: 6063)	WYQQKP GKAPKML IY (SEQ ID NO: 6094)	ENDKRPS (SEQ ID NO: 6064)	GVPSRFS GSNSGNE ATLTISSL QPDDFAT YYC (SEQ ID NO: 6095)	QSWDSTN SAV (SEQ ID NO: 7293)	FGQGTKV EIK (SEQ ID NO: 6096)
15H6-LC_1	QYVLTQP PSASGTP GQRVTIS C (SEQ ID NO: 6097)	SGENLSD KYVH (SEQ ID NO: 6070)	WYQQLP GTAPKML IY (SEQ ID NO: 6098)	ENEKRPS (SEQ ID NO: 6071)	GVPDRFS GSNSGNS ASLAISGL QSEDEAD YYC (SEQ ID NO: 6099)	HYWESIN SVV (SEQ ID NO: 6072)	FGEGTEL TVL (SEQ ID NO: 6100)
15H6-LC_2	QYVLTQP PSASGTP GQRVTIS C (SEQ ID NO: 6101)	SGENLSD KYVH (SEQ ID NO: 6070)	WYQQLP GTAPKML IY (SEQ ID NO: 6102)	ENEKRPS (SEQ ID NO: 6071)	GVPDRFS GSNSGNS ASLAISGL RSEDEAD YYC (SEQ ID NO: 6103)	HYWESIN SVV (SEQ ID NO: 6072)	FGEGTEL TVL (SEQ ID NO: 6104)
15H6-LC_3	SYELTQPP SVSVSPG QTASITC (SEQ ID NO: 6105)	SGENLSD KYVH (SEQ ID NO: 6070)	WYQQKP GQSPVMV IY (SEQ ID NO: 6106)	ENEKRPS (SEQ ID NO: 6071)	GIPERFSG SNSGNTA TLTISGTQ AMDEAD YYC (SEQ ID NO: 6107)	HYWESIN SVV (SEQ ID NO: 6072)	FGEGTEL TVL (SEQ ID NO: 6108)
15H6-LC_4	DYVLTQS PLSLPVTP GEPASISC (SEQ ID NO: 6109)	SGENLSD KYVH (SEQ ID NO: 6070)	WYLQKP GQSPQML IY (SEQ ID NO: 6110)	ENEKRPS (SEQ ID NO: 6071)	GVPDRFS GSNSGND ATLKISRV EAEDVGV YYC (SEQ ID NO: 6111)	HYWESIN SVV (SEQ ID NO: 6072)	FGQGTKV EIK (SEQ ID NO: 6112)
15H6-LC_5	AYQLTQS PSSLSASV	SGENLSD KYVH	WYQQKP GKAPKML	ENEKRPS (SEQ ID	GVPSRFS GSNSGND	HYWESIN SVV (SEQ	FGQGTKV EIK (SEQ

	GDRVTITC (SEQ ID NO: 6113)	(SEQ ID NO: 6070)	IY (SEQ ID NO: 6114)	NO: 6071)	ATLTISSLQPEDFATYYC (SEQ ID NO: 6115)	ID NO: 6072)	ID NO: 6116)
15H6-LC_6	EYVLTQSPATLSVSPGERATLSC (SEQ ID NO: 6117)	SGENLSDKYVH (SEQ ID NO: 6070)	WYQQKPGQAPRMLIY (SEQ ID NO: 6118)	ENEKRPS (SEQ ID NO: 6071)	GIPARFSGSNSGNEATLTISSLQSEDFAVYYC (SEQ ID NO: 6119)	HYWESINSVV (SEQ ID NO: 6072)	FGQGTKVEIK (SEQ ID NO: 6120)
9D9-LC	SYTLTQPPLVSVALGQKATIIC (SEQ ID NO: 7320)	SGENLSDKYVH (SEQ ID NO: 6070)	WYQQKPGRAPVMVIY (SEQ ID NO: 6067)	ENDKRPS (SEQ ID NO: 6064)	GIPDQFSGSNSGNIATLTISKAQAGYEADYYC (SEQ ID NO: 7292)	HCWDSTNSAV (SEQ ID NO: 7321)	FGSGTHLTVL (SEQ ID NO: 6076)
3A12-LC	SYTLTQPPLVSVALGQKATIIC (SEQ ID NO: 7320)	SGENLSDKYVH (SEQ ID NO: 6070)	WYQQKPGRAPVMVIY (SEQ ID NO: 6067)	ENDKRPS (SEQ ID NO: 6064)	GIPDQFSGSNSGNIATLTISKAQAGYEADYYC (SEQ ID NO: 7292)	HCWDSTNSAV (SEQ ID NO: 7321)	FGSGTHLTVL (SEQ ID NO: 6076)
12D10-LC	SYTLTQPPSLSVAPGQKATIIC (SEQ ID NO: 6073)	SGENLSDKYVH (SEQ ID NO: 6070)	WYQQKPGRAPVMVIY (SEQ ID NO: 6074)	ENEKRPS (SEQ ID NO: 6071)	GIPDQFSGSNSGNIATLTISKAQPGSEADYYC (SEQ ID NO: 6075)	HYWESINSVV (SEQ ID NO: 6072)	FGSGTHLTVL (SEQ ID NO: 6076)
15E1-LC	SFTLTQPPLVSVAVGQVATITC (SEQ ID NO: 7325)	SGEKLSDKYVH (SEQ ID NO: 7326)	WYQQKPGRAPVMVIY (SEQ ID NO: 6067)	ENDRRPS (SEQ ID NO: 7327)	GIPDQFSGSNSGNIASLTISKAQAGDEADYFC (SEQ ID NO: 7328)	QFWDSTNSAV (SEQ ID NO: 7329)	FGGGTQLTVL (SEQ ID NO: 6080)
15E1_Humanized variant_VL1	SSETTQPPSVSVSPGQTASITC (SEQ ID NO: 7335)	SGEKLSDKYVH (SEQ ID NO: 7326)	WYQQKPGQSPVMVIY (SEQ ID NO: 6106)	ENDRRPS (SEQ ID NO: 7327)	GIPERFSGSNSGNTATLTISGTQAMDEADYFC (SEQ ID NO: 7336)	QFWDSTNSAV (SEQ ID NO: 7329)	FGGGTQLTVL (SEQ ID NO: 6080)
15E1_Humanized variant_VL2	SSETTQPHSVSVATAQMARITC (SEQ ID NO: 6089)	SGEKLSDKYVH (SEQ ID NO: 7326)	WYQQKPGQDPVMVIY (SEQ ID NO: 6090)	ENDRRPS (SEQ ID NO: 7327)	GIPERFSGSNPGNTATLTISRIEAGDEADYFC (SEQ ID NO: 7337)	QFWDSTNSAV (SEQ ID NO: 7329)	FGGGTQLTVL (SEQ ID NO: 6080)
15E1_Hum	QSVTTQP	SGEKLSD	WYQQLP	ENDRRPS	GVPDRFS	QFWDSTN	FGGGTQL

anized variant_VL 3	PSASGTP GQRVTIS C (SEQ ID NO: 6081)	KYVH (SEQ ID NO: 7326)	GTAPKML IY (SEQ ID NO: 6078)	(SEQ ID NO: 7327)	GSNSGNS ASLAISGL RSEDEAD YFC (SEQ ID NO: 7338)	SAV (SEQ ID NO: 7329)	TVL (SEQ ID NO: 6080)
15E1_Hum anized variant_VL 4	QSVTTQP PSVSGAP GQRVTIS C (SEQ ID NO: 6077)	SGEKLSD KYVH (SEQ ID NO: 7326)	WYQQLP GTAPKML IY (SEQ ID NO: 6078)	ENDRRPS (SEQ ID NO: 7327)	GVPDRFS GSNSGNS ASLAITGL QAEDEAD YFC (SEQ ID NO: 7339)	QFWDSTN SAV (SEQ ID NO: 7329)	FGGGTQL TVL (SEQ ID NO: 6080)
15E1_Hum anized variant_VL 5	DSVTTQS PLSLPVTL GQPASISC (SEQ ID NO: 7340)	SGEKLSD KYVH (SEQ ID NO: 7326)	WYQQRP GQSPRML IY (SEQ ID NO: 7341)	ENDRRPS (SEQ ID NO: 7327)	GVPDRFS GSNSGND ATLKISRV EAEDVGV YFC (SEQ ID NO: 7342)	QFWDSTN SAV (SEQ ID NO: 7329)	FGGGTKV EIK (SEQ ID NO: 233)

Table 9. Exemplary variable regions of NKp30-targeting antigen binding domains

SEQ ID NO	Ab ID	Description	Sequence
SEQ ID NO: 6121	9G1-HC	9G1 heavy chain variable region	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGY HWNWIRQFPGKKLEWMGYIYSSGSTSYNPSLK SRISITRDTSKNQFFLQLNSVTTEDTATYYCAR GNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6122	15H6-HC	15H6 heavy chain variable region	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGY HWNWIRQFPGKKLEWMGYIYSSGTTRYNPSLK SRISITRDTSKNQFFLQLNSVTPEDTATYYCTRG NWHYFDYWGQGTLVAVSS
SEQ ID NO: 6123	9G1-HC_1	9G1 heavy chain variable region humanized variant 1	QIQLQESGPGLVKPSETLSLTCTVSGFSINTGGY HWNWIRQPAGKGLEWIGYIYSSGSTSYNPSLKS RVTMSRDTSKNQFSLKLSSVTAADTAVYYCAR GNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6124	9G1-HC_2	9G1 heavy chain variable region humanized variant 2	QIQLQESGPGLVKPSQTLSLTCTVSGFSINTGGY HWNWIRQHPGKGLEWIGYIYSSGSTSYNPSLKS LVTISRDTSKNQFSLKLSSVTAADTAVYYCARG NWHYFDFWGQGTMVTVSS
SEQ ID NO: 6125	9G1-HC_3	9G1 heavy chain variable region humanized variant 3	EIQLLESGGGLVQPGGSLRLSCAVSGFSINTGG YHWNWVRQAPGKGLEWVGYIYSSGSTSYNPS LKSRFTISRDTSKNTFYLQMNSLRAEDTAVYYC ARGNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6126	9G1-HC_4	9G1 heavy chain variable region humanized variant 4	QIQLVQSGAEVKKPGSSVKVSCKVSGFSINTGG YHWNWVRQAPGQGLEWMGYIYSSGSTSYNPS LKSRVTITRDTSTNTFYMELSSLRSEDTAVYYC ARGNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6127	9G1-HC_5	9G1 heavy chain variable region humanized	EIQLVESGGGLVQPGGSLRLSCAVSGFSINTGG YHWNWVRQAPGKGLEWVGYIYSSGSTSYNPS LKSRFTISRDTAKNSFYLQMNSLRAEDTAVYY

		variant 5	CARGNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6128	9G1-HC_6	9G1 heavy chain variable region humanized variant 6	QIQLVQSGAEVKKPGASVKVSCKVSGFSINTGG YHWNWVRQAPGQGLEWMGYIYSSGSTSYNPS LKSRVTMTRDTSTNTFYMELSSLRSEDTAVYY CARGNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6129	15H6-HC_1	15H6 heavy chain variable region humanized variant 1	QIQLQESGPGLVKPSQTLSLTCTVSGFSINTGGY HWNWIRQHPGKGLEWIGYIYSSGTTRYNPSLK SLVTISRDTSKNQFSLKLSSVTAADTAVYYCAR GNWHYFDYWGQGTLVTVSS
SEQ ID NO: 6130	15H6-HC_2	15H6 heavy chain variable region humanized variant 2	QIQLQESGPGLVKPSETLSLTCTVSGFSINTGGY HWNWIRQPAGKGLEWIGYIYSSGTTRYNPSLK SRVTMSRDTSKNQFSLKLSSVTAADTAVYYCA RGNWHYFDYWGQGTLVTVSS
SEQ ID NO: 6131	15H6-HC_3	15H6 heavy chain variable region humanized variant 3	EIQLLESGGGLVQPGGSLRLSCAVSGFSINTGG YHWNWVRQAPGKGLEWVGYIYSSGTTRYNPS LKSRFTISRDTSKNTFYLQMNSLRAEDTAVYYC ARGNWHYFDYWGQGTLVTVSS
SEQ ID NO: 6132	15H6-HC_4	15H6 heavy chain variable region humanized variant 4	QIQLVESGGGLVKPGGSLRLSCAVSGFSINTGG YHWNWIRQAPGKGLEWVGYIYSSGTTRYNPSL KSRFTISRDTAKNSFYLQMNSLRAEDTAVYYC ARGNWHYFDYWGQGTLVTVSS
SEQ ID NO: 6133	15H6-HC_5	15H6 heavy chain variable region humanized variant 5	QIQLVQSGAEVKKPGASVKVSCKVSGFSINTGG YHWNWVRQAPGQGLEWMGYIYSSGTTRYNPS LKSRVTMTRDTSTNTFYMELSSLRSEDTAVYY CARGNWHYFDYWGQGTLVTVSS
SEQ ID NO: 6134	15H6-HC_6	15H6 heavy chain variable region humanized variant 6	EIQLVQSGAEVKKPGATVKISCKVSGFSINTGG YHWNWVQQAPGKGLEWMGYIYSSGTTRYNPS LKSRVTITRDTSTNTFYMELSSLRSEDTAVYYC ARGNWHYFDYWGQGTLVTVSS
SEQ ID NO: 7294	9G1-LC	9G1 light chain variable region	SYTLTQPPLLSVALGHKATITCSGERLSDKYVH WYQQKPGRAPVMVIYENDKRPSGIPDQFSGSN SGNIATLTISKAQAGYEADYYCQSWDSTNSAV FGSGTQLTVL
SEQ ID NO: 6136	15H6-LC	15H6 light chain variable region	SYTLTQPPSLSVAPGQKATIICSGENLSDKYVH WYQQKPGRAPVMVIYENEKRPSGIPDQFSGSN SGNIATLTISKAQPGSEADYYCHYWESINSVVF GSGTHLTVL
SEQ ID NO: 6137	9G1-LC_1	9G1 light chain variable region humanized variant 1	QSVTTQPPSVSGAPGQRVTISCSGERLSDKYVH WYQQLPGTAPKMLIYENDKRPSGVPDRFSGSN SGNSASLAITGLQAEDEADYYCQSWDSTNSAV FGGGTQLTVL
SEQ ID NO: 6138	9G1-LC_2	9G1 light chain variable region humanized variant 2	QSVTTQPPSASGTPGQRVTISCSGERLSDKYVH WYQQLPGTAPKMLIYENDKRPSGVPDRFSGSN SGNSASLAISGLQSEDEADYYCQSWDSTNSAV FGGGTQLTVL

SEQ ID NO: 6139	9G1-LC_3	9G1 light chain variable region humanized variant 3	QSVTTQPPSASGTPGQRVTISCSGERLSDKYVH WYQQLPGTAPKMLIYENDKRPSGVPDRFSGSN SGNSASLAISGLRSEDEADYYCQSWDSTNSAVF GGGTQLTVL
SEQ ID NO: 6140	9G1-LC_4	9G1 light chain variable region humanized variant 4	SSETTQPHSVSVATAQMARITCSGERLSDKYVH WYQQKPGQDPVMVIYENDKRPSGIPERFSGSN PGNTATLTISRIEAGDEADYYCQSWDSTNSAVF GGGTQLTVL
SEQ ID NO: 6141	9G1-LC_5	9G1 light chain variable region humanized variant 5	DIQMTQSPSTLSASVGDRVTITCSGERLSDKYV HWYQQKPGKAPKMLIYENDKRPSGVPSRFSGS NSGNEATLTISSLQPDDFATYYCQSWDSTNSAV FGQGTKVEIK
SEQ ID NO: 6142	15H6-LC_1	15H6 light chain variable region humanized variant 1	QYVLTQPPSASGTPGQRVTISCSGENLSDKYVH WYQQLPGTAPKMLIYENEKRPSGVPDRFSGSN SGNSASLAISGLQSEDEADYYCHYWESINSVVF GEGTELTVL
SEQ ID NO: 6143	15H6-LC_2	15H6 light chain variable region humanized variant 2	QYVLTQPPSASGTPGQRVTISCSGENLSDKYVH WYQQLPGTAPKMLIYENEKRPSGVPDRFSGSN SGNSASLAISGLRSEDEADYYCHYWESINSVVF GEGTELTVL
SEQ ID NO: 6144	15H6-LC_3	15H6 light chain variable region humanized variant 3	SYELTQPPSVSVSPGQTASITCSGENLSDKYVH WYQQKPGQSPVMVIYENEKRPSGIPERFSGSNS GNTATLTISGTQAMDEADYYCHYWESINSVVF GEGTELTVL
SEQ ID NO: 6145	15H6-LC_4	15H6 light chain variable region humanized variant 4	DYVLTQSPLSLPVTPGEPASISCSGENLSDKYV HWYLQKPGQSPQMLIYENEKRPSGVPDRFSGS NSGNDATLKISRVEAEDVGVYYCHYWESINSV VFGQGTKVEIK
SEQ ID NO: 6146	15H6-LC_5	15H6 light chain variable region humanized variant 5	AYQLTQSPSSLSASVGDRVTITCSGENLSDKYV HWYQQKPGKAPKMLIYENEKRPSGVPSRFSGS NSGNDATLTISSLQPEDFATYYCHYWESINSVV FGQGTKVEIK
SEQ ID NO: 6147	15H6-LC_6	15H6 light chain variable region humanized variant 6	EYVLTQSPATLSVSPGERATLSCSGENLSDKYV HWYQQKPGQAPRMLIYENEKRPSGIPARFSGS NSGNEATLTISSLQSEDFAVYYCHYWESINSVV FGQGTKVEIK
SEQ ID NO: 7295	9D9-HC	9D9 heavy chain variable region	QIQLQESGPGLVKPSQSLSLSCSVTGFSINTGGY HWNWIRQFPGKKVEWMGYIYSSGTTKYNPSL KSRISITRDTSKNQFFLQLNSVTTEDTATYYCA RGDWHYFDYWGQGTMVAVSS
SEQ ID NO: 7296	9D9-LC	9D9 light chain variable region	SYTLTQPPLVSVALGQKATIICSGENLSDKYVH WYQQKPGRAPVMVIYENDKRPSGIPDQFSGSN SGNIATLTISKAQAGYEADYYCHCWDSTNSAV FGSGTHLTVL
SEQ ID NO: 7297	3A12-HC	3A12 heavy chain variable region	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGY HWNWIRQFPGKKLEWMGYIYSSGSTRYNPSLK SRFSITRDTSKNQFFLQLNSVTTEDTATYYCTR GNWHYFDYWGQGTLVAVSS

SEQ ID NO: 7296	3A12-LC	3A12 light chain variable region	SYTLTQPPLVSVALGQKATIICSGENLSDKYVH WYQQKPGRAPVMVIYENDKRPSGIPDQFSGSN SGNIATLTISKAQAGYEADYYCHCWDSTNSAV FGSGTHLTVL
SEQ ID NO: 6122	12D10-HC	12D10 heavy chain variable region	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGY HWNWIRQFPGKKLEWMGYIYSSGTTRYNPSLK SRISITRDTSKNQFFLQLNSVTPEDTATYYCTRG NWHYFDYWGQGTLVAVSS
SEQ ID NO: 6136	12D10-LC	12D10 light chain variable region	SYTLTQPPSLSVAPGQKATIICSGENLSDKYVH WYQQKPGRAPVMVIYENEKRPSGIPDQFSGSN SGNIATLTISKAQPGSEADYYCHYWESINSVVF GSGTHLTVL
SEQ ID NO: 7298	15E1-HC	15E1 heavy chain variable region	QIQLQESGPGLVKPSQSLSLSCSVTGFSITTTGY HWNWIRQFPGKKLEWMGYIYSSGSTSYNPSLK SRFSITRDTSKNQFFLQLNSVTTEDTATYYCAR GDWHYFDYWGPGTMVTVSS
SEQ ID NO: 7299	15E1-LC	15E1 light chain variable region	SFTLTQPPLVSVAVGQVATITCSGEKLSDKYVH WYQQKPGRAPVMVIYENDRRPSGIPDQFSGSN SGNIASLTISKAQAGDEADYFCQFWDSTNSAVF GGGTQLTVL
SEQ ID NO: 7300	15E1_Humaniz ed variant_VH1	15E1 heavy chain variable region humanized variant 1	QIQLQESGPGLVKPSQTLSLTCTVSGFSITTTGY HWNWIRQHPGKGLEWIGYIYSSGSTSYNPSLKS LVTISRDTSKNQFSLKLSSVTAADTAVYYCARG DWHYFDYWGQGTMVTVSS
SEQ ID NO: 7301	15E1_Humaniz ed variant_VH2	15E1 heavy chain variable region humanized variant 2	QIQLVESGGGLVKPGGSLRLSCAVSGFSITTTG YHWNWIRQAPGKGLEWVGYIYSSGSTSYNPSL KSRFTISRDTAKNSFYLQMNSLRAEDTAVYYC ARGDWHYFDYWGQGTMVTVSS
SEQ ID NO: 7302	15E1_Humaniz ed variant_VH3 (BJM0407 VH and BJM0411 VH)	15E1 heavy chain variable region humanized variant 3	EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGY HWNWVRQAPGKGLEWVGYIYSSGSTSYNPSL KSRFTISRDTSKNTFYLQMNSLRAEDTAVYYC ARGDWHYFDYWGQGTMVTVSS
SEQ ID NO: 7303	15E1_Humaniz ed variant_VH4	15E1 heavy chain variable region humanized variant 4	EIQLVESGGGLVQPGGSLRLSCAVSGFSITTTGY HWNWVRQAPGKGLEWVGYIYSSGSTSYNPSL KSRFTISRDTAKNSFYLQMNSLRAEDTAVYYC ARGDWHYFDYWGQGTMVTVSS
SEQ ID NO: 7304	15E1_Humaniz ed variant_VH5	15E1 heavy chain variable region humanized variant 5	QIQLVQSGAEVKKPGASVKVSCKVSGFSITTTG YHWNWVRQAPGQGLEWMGYIYSSGSTSYNPS LKSRVTMTRDTSTNTFYMELSSLRSEDTAVYY CARGDWHYFDYWGQGTMVTVSS
SEQ ID NO: 7305	15E1_Humaniz ed variant_VL1 (BJM0407 VL)	15E1 light chain variable region humanized variant 1	SSETTQPPSVSVSPGQTASITCSGEKLSDKYVH WYQQKPGQSPVMVIYENDRRPSGIPERFSGSNS GNTATLTISGTQAMDEADYFCQFWDSTNSAVF GGGTQLTVL
SEQ ID NO: 7306	15E1_Humaniz ed variant_VL2	15E1 light chain variable region humanized variant 2	SSETTQPHSVSVATAQMARITCSGEKLSDKYV HWYQQKPGQDPVMVIYENDRRPSGIPERFSGS NPGNTATLTISRIEAGDEADYFCQFWDSTNSAV FGGGTQLTVL

SEQ ID NO: 7307	15E1_Humanized variant_VL3	15E1 light chain variable region humanized variant 3	QSVTTQPPSASGTPGQRVTISCSGEKLSDKYVH WYQQLPGTAPKMLIYENDRRPSGVPDRFSGSN SGNSASLAISGLRSEDEADYFCQFWDSTNSAVF GGGTQLTVL
SEQ ID NO: 7308	15E1_Humanized variant_VL4	15E1 light chain variable region humanized variant 4	QSVTTQPPSVSGAPGQRVTISCSGEKLSDKYVH WYQQLPGTAPKMLIYENDRRPSGVPDRFSGSN SGNSASLAITGLQAEDEADYFCQFWDSTNSAV FGGGTQLTVL
SEQ ID NO: 7309	15E1_Humanized variant_VL5 (BJM0411 VL)	15E1 light chain variable region humanized variant 5	DSVTTQSPLSLPVTLGQPASISCSGEKLSDKYV HWYQQRPGQSPRMLIYENDRRPSGVPDRFSGS NSGNDATLKISRVEAEDVGVYFCQFWDSTNSA VFGGGTKVEIK

Table 10. Exemplary NKp30-targeting antigen binding domains/antibody molecules

SEQ ID NO	Ab ID	Description	Sequence
SEQ ID NO: 6148	Ch(anti-NKp30 9G1)HC N297A	9G1 heavy chain	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGSTSYNPSLKSRISITRDT SKNQFFLQLNSVTTEDTATYYCARGNWHYFDFWGQ GTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
SEQ ID NO: 6149	Ch(anti-NKp30 9G1)HC	9G1 heavy chain	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGSTSYNPSLKSRISITRDT SKNQFFLQLNSVTTEDTATYYCARGNWHYFDFWGQ GTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
SEQ ID NO: 6150	Ch(anti-NKp30 9G1)LC	9G1 light chain	SYTLTQPPLLSVALGHKATITCSGERLSDKYVHWYQQ KPGRAPVMVIYENDKRPSGIPDQFSGSNSGNIATLTIS KAQAGYEADYYCQSWDSTNSAVFGSGTQLTVLGQP KANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTP EQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 6151	Ch(anti-NKp30 15H6)HC N297A	15H6 heavy chain	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGTTRYNPSLKSRISITRDT SKNQFFLQLNSVTPEDTATYYCTRGNWHYFDYWGQ GTLVAVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
SEQ ID NO: 6152	Ch(anti-NKp30 15H6)HC (hole)	15H6 heavy chain	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGTTRYNPSLKSRISITRDT SKNQFFLQLNSVTPEDTATYYCTRGNWHYFDYWGQ GTLVAVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
SEQ ID NO: 6153	Ch(anti-NKp30 15H6)LC	15H6 light chain	SYTLTQPPSLSVAPGQKATIICSGENLSDKYVHWYQQ KPGRAPVMVIYENEKRPSGIPDQFSGSNSGNIATLTIS KAQPGSEADYYCHYWESINSVVFGSGTHLTVLGQPK ANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVA WKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 6187	anti-NKp30 9G1 scFv (VH-VL)	Hamster anti-NKp30 scFv of 9G1 in VH to VL orientation	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGSTSYNPSLKSRISITRDT SKNQFFLQLNSVTTEDTATYYCARGNWHYFDFWGQ GTMVTVSSGGGGSGGGGSGGGGSGGGGSSYTLTQPP LLSVALGHKATITCSGERLSDKYVHWYQQKPGRAPV MVIYENDKRPSGIPDQFSGSNSGNIATLTISKAQAGYE ADYYCQSWDSTNSAVFGSGTQLTVL
SEQ ID NO: 6188	anti-NKp30 9G1 scFv (VL-VH)	Hamster anti-NKp30 scFv of 9G1 in VL to VH orientation	SYTLTQPPLLSVALGHKATITCSGERLSDKYVHWYQQ KPGRAPVMVIYENDKRPSGIPDQFSGSNSGNIATLTIS KAQAGYEADYYCQSWDSTNSAVFGSGTQLTVLGGG GSGGGGSGGGGSGGGGSQIQLQESGPGLVKPSQSLSL TCSVTGFSINTGGYHWNWIRQFPGKKLEWMGYIYSS GSTSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTAT YYCARGNWHYFDFWGQGTMVTVSS
SEQ ID NO: 6189	anti-NKp30 15H6	Hamster anti-NKp30 scFv of 15H6 in VH to	QIQLQESGPGLVKPSQSLSLTCSVTGFSINTGGYHWN WIRQFPGKKLEWMGYIYSSGTTRYNPSLKSRISITRDT SKNQFFLQLNSVTPEDTATYYCTRGNWHYFDYWGQ

	scFv (VH-VL)	VL orientation	GTLVAVSSGGGGSGGGGSGGGGSGGGGSSYTLTQPP SLSVAPGQKATIICSGENLSDKYVHWYQQKPGRAPV MVIYENEKRPSGIPDQFSGSNSGNIATLTISKAQPGSE ADYYCHYWESINSVVFGSGTHLTVL
SEQ ID NO: 6190	anti-NKp30 15H6 scFv (VL-VH)	Hamster anti-NKp30 scFv of 15H6 in VL to VH orientation	SYTLTQPPSLSVAPGQKATIICSGENLSDKYVHWYQQ KPGRAPVMVIYENEKRPSGIPDQFSGSNSGNIATLTIS KAQPGSEADYYCHYWESINSVVFGSGTHLTVLGGGG SGGGGSGGGGSGGGGSQIQLQESGPGLVKPSQSLSLT CSVTGFSINTGGYHWNWIRQFPGKKLEWMGYIYSSG TTRYNPSLKSRISITRDTSKNQFFLQLNSVTPEDTATY YCTRGNWHYFDYWGQGTLVAVSS
SEQ ID NO: 7310	BJM0859 lambda scFv		EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWN WVRQAPGKGLEWVGYIYSSGSTSYNPSLKSRFTISRD TSKNTFYLQMNSLRAEDTAVYYCARGDWHYFDYW GQGTMVTVSSGGGGSGGGGSGGGGSGGGGSSSETTQ PPSVSVSPGQTASITCSGEKLSDKYVHWYQQKPGQSP VMVIYENDRRPSGIPERFSGSNSGNTATLTISGTQAMD EADYFCQFWDSTNSAVFGGGTQLTVL
SEQ ID NO: 7311	BJM0860 kappa scFv		EIQLLESGGGLVQPGGSLRLSCAVSGFSITTTGYHWN WVRQAPGKGLEWVGYIYSSGSTSYNPSLKSRFTISRD TSKNTFYLQMNSLRAEDTAVYYCARGDWHYFDYW GQGTMVTVSSGGGGSGGGGSGGGGSGGGGSDSVTT QSPLSLPVTLGQPASISCSGEKLSDKYVHWYQQRPGQ SPRMLIYENDRRPSGVPDRFSGSNSGNDATLKISRVEA EDVGVYFCQFWDSTNSAVFGGGTKVEIK

In some embodiments, the NK cell engager is an antigen binding domain that binds to NKp46 (e.g., NKp46 present, e.g., expressed or displayed, on the surface of an NK cell) and comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in Table 15. In some embodiments, binding of the NK cell engager, e.g., antigen binding domain that binds to NKp46, to the NK cell activates the NK cell. An antigen binding domain that binds to NKp46 (e.g., NKp46 present, e.g., expressed or displayed, on the surface of an NK cell) may be said to target NKp46, the NK cell, or both.

In some embodiments, the NK cell engager is an antigen binding domain that binds to NKG2D (e.g., NKG2D present, e.g., expressed or displayed, on the surface of an NK cell) and comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in Table 15. In some embodiments, binding of the NK cell engager, e.g., antigen binding domain that binds to NKG2D, to the NK cell activates the

NK cell. An antigen binding domain that binds to NKG2D (e.g., NKG2D present, e.g., expressed or displayed, on the surface of an NK cell) may be said to target NKG2D, the NK cell, or both.

In some embodiments, the NK cell engager is an antigen binding domain that binds to CD16 (e.g., CD16 present, e.g., expressed or displayed, on the surface of an NK cell) and comprises any CDR amino acid sequence, framework region (FWR) amino acid sequence, or variable region amino acid sequence disclosed in Table 15. In some embodiments, binding of the NK cell engager, e.g., antigen binding domain that binds to CD16, to the NK cell activates the NK cell. An antigen binding domain that binds to CD16 (e.g., CD16 present, e.g., expressed or displayed, on the surface of an NK cell) may be said to target CD16, the NK cell, or both.

Table 15. Exemplary variable regions of NKp46, NKG2D, or CD16-targeting antigen binding domains

SEQ ID NO	Ab ID	Description	Sequence
SEQ ID NO: 6175	NKG2D_1 scFV	scFV that binds NKG2D	QVHLQESGPGLVKPSETLSLTCTVSDDSISSYYWSWIR QPPGKGLEWIGHISYSGSANYNPSLKSRVTISVDTSKN QFSLKLSSVTAADTAVYYCANWDDAFNIWGQGTMV TVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSL SPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQQYGSSPWTFGQGTKVEIK
SEQ ID NO: 6176	NKG2D_1 VH	VH that binds NKG2D	QVHLQESGPGLVKPSETLSLTCTVSDDSISSYYWSWIR QPPGKGLEWIGHISYSGSANYNPSLKSRVTISVDTSKN QFSLKLSSVTAADTAVYYCANWDDAFNIWGQGTMV TVSS
SEQ ID NO: 6177	NKG2D_1 VL	VL that binds NKG2D	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTIS RLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK
SEQ ID NO: 6178	NKG2D_2 scFV	scFV that binds NKG2D	EVQLVQSGAEVKEPGESLKISCKNSGYSFTNYWVGW VRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISAD KSINTAYLQWSSLKASDTAMYYCGRLTMFRGIIIGYF DYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEI VLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSL EPEDFAVYYCQQRSNWPWTFGQGTKVEIK
SEQ ID NO: 6179	NKG2D_2 VH	VH that binds NKG2D	EVQLVQSGAEVKEPGESLKISCKNSGYSFTNYWVGW VRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISAD KSINTAYLQWSSLKASDTAMYYCGRLTMFRGIIIGYF DYWGQGTLVTVSS
SEQ ID NO: 6180	NKG2D_2 VL	VL that binds NKG2D	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISS LEPEDFAVYYCQQRSNWPWTFGQGTKVEIK

SEQ ID NO: 6181	NKp46scFV	scFV that binds NKp46	QVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINW GKQRSGQGLEWIGEIYPGSGTNYYNEKFKAKATLTA DKSSNIAYMQLSSLTSEDSAVYFCARRGRYGLYAMD YWGQGTSVTVSSGGGGSGGGGSGGGGSGGGGSDIQ MTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKP DGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTINNL EQEDIATYFCQQGNTRPWTFGGGTKLEIK
SEQ ID NO: 6182	NKp46VH	VH that binds NKp46	QVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINW GKQRSGQGLEWIGEIYPGSGTNYYNEKFKAKATLTA DKSSNIAYMQLSSLTSEDSAVYFCARRGRYGLYAMD YWGQGTSVTVSS
SEQ ID NO: 6183	NKp46VL	VL that binds NKp46	DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQ QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTI NNLEQEDIATYFCQQGNTRPWTFGGGTKLEIK
SEQ ID NO: 6184	CD16scFV	scFV that binds CD16	EVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARG RSLLFDYWGQ GTLVTVSRGG GGSGGGGSGG GGSSELTQDP AVSVALGQTV RITCQGDSLR SYYASWYQQK PGQAPVLVIY GKNNRPSGIP DRFSGSSSGN TASLTITGAQ AEDEADYYCN SRDSSGNHVV FGGGTKLTVL
SEQ ID NO: 6185	CD16VH	VH that binds CD16	EVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARG RSLLFDYWGQ GTLVTVSR
SEQ ID NO: 6186	CD16VL	VL that binds CD16	SSELTQDP AVSVALGQTVRITCQGDSLR SYYASWYQQK PGQAPVLVIY GKNNRPSGIP DRFSGSSSGNTASLTITGAQ AEDEADYYCN SRDSSGNHVV FGGGTKLTVL

In one embodiment, the NK cell engager is a ligand of NKp30, e.g., is a B7-6, e.g., comprises the amino acid sequence of:

DLKVEMMAGGTQITPLNDNVTIFCNIFYSQPLNITSMGITWFWKSLTFDKEVKVFEFFGD HQEAFRPGAIVSPWRLKSGDASLRLPGIQLEEAGEYRCEVVVTPLKAQGTVQLEVVASP ASRLLLDQVGMKENEDKYMCESSGFYPEAINITWEKQTQKFPHPIEISEDVITGPTIKNM DGTFNVTSCLKLNSSQEDPGTVYQCVVRHASLHTPLRSNFTLTAARHSLSETEKTDNFS (SEQ ID NO: 7233), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not

more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7233.

In other embodiments, the NK cell engager is a ligand of NKp44 or NKp46, which is a viral HA. Viral hemagglutinins (HA) are glyco proteins which are on the surface of viruses. HA proteins allow viruses to bind to the membrane of cells via sialic acid sugar moieties which contributes to the fusion of viral membranes with the cell membranes (see e.g., Eur J Immunol. 2001 Sep;31(9):2680-9 "Recognition of viral hemagglutinins by NKp44 but not by NKp30"; and Nature. 2001 Feb 22;409(6823):1055-60 "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells" the contents of each of which are incorporated by reference herein).

In other embodiments, the NK cell engager is a ligand of NKG2D chosen from MICA, MICB, or ULBP1, e.g., wherein:

(i) MICA comprises the amino acid sequence:
EPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRCDRQKCRAKPQGQWAEDVLGNK
TWDRETRDLTGNGKDLRMTLAHIKDQKEGLHSLQEIRVCEIHEDNSTRSSQHFYYDGEL
FLSQNLETKEWTMPQSSRAQTLAMNVRNFLKEDAMKTKTHYHAMHADCLQELRRYLK
SGVVLRRTVPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWG
DVLPDGNGTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPSGKVLVLQSHW (SEQ ID NO: 7234), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7234;

(ii) MICB comprises the amino acid sequence:
AEPHSLRYNLMVLSQDESVQSGFLAEGHLDGQPFLRYDRQKRRAKPQGQWAEDVLGA
KTWDTETEDLTENGQDLRRTLTHIKDQKGGLHSLQEIRVCEIHEDSSTRGSRHFYYDGEL
FLSQNLETQESTVPQSSRAQTLAMNVTNFWKEDAMKTKTHYRAMQADCLQKLQRYLK
SGVAIRRTVPPMVNVTCSEVSEGNITVTCRASSFYPRNITLTWRQDGVSLSHNTQQWGD
VLPDGNGTYQTWVATRIRQGEEQRFTCYMEHSGNHGTHPVPSGKVLVLQSQRTD (SEQ ID NO: 7235), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than

five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7235; or

(iii) ULBP1 comprises the amino acid sequence:
GWVDTHCLCYDFIITPKSRPEPQWCEVQGLVDERPFLHYDCVNHKAKAFASLGKKVNV
TKTWEEQTETLRDVVDFLKGQLLDIQVENLIPIEPLTLQARMSCEHEAHGHGRGSWQFL
FNGQKFLLFDSNNRKWTALHPGAKKMTEKWEKNRDVTMFFQKISLGDCKMWLEEFL
MYWEQMLDPTKPPSLAPG (SEQ ID NO: 7236), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7236.

In other embodiments, the NK cell engager is a ligand of DNAM1 chosen from NECTIN2 or NECL5, e.g., wherein:

(i) NECTIN2 comprises the amino acid sequence:
QDVRVQVLPEVRGQLGGTVELPCHLLPPVPGLYISLVTWQRPDAPANHQNVAAFHPKM
GPSFPSPKPGSERLSFVSAKQSTGQDTEAELQDATLALHGLTVEDEGNYTCEFATFPKGS
VRGMTWLRVIAKPKNQAEAQKVTFSQDPTTVALCISKEGRPPARISWLSSLDWEAKETQ
VSGTLAGTVTVTSRFTLVPSGRADGVTVTCKVEHESFEEPALIPVTLSVRYPPEVSISGYD
DNWYLGRTDATLSCDVRSNPEPTGYDWSTTSGTFPTSAVAQGSQLVIHAVDSLFNTTFV
CTVTNAVGMGRAEQVIFVRETPNTAGAGATGG (SEQ ID NO: 7237), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7237; or

(ii) NECL5 comprises the amino acid sequence:
WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHGESGSMAV
FHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTCLFVTFPQGSRSVD
IWLRVLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNTSQVPG
FLSGTVTVTSLWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLTVYYPPEVSISGYDNN
WYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLICN

VTNALGARQAELTVQVKEGPPSEHSGISRN (SEQ ID NO: 7238), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7238.

In yet other embodiments, the NK cell engager is a ligand of DAP10, which is an adapter for NKG2D (see e.g., Proc Natl Acad Sci U S A. 2005 May 24; 102(21): 7641–7646; and Blood, 15 September 2011 Volume 118, Number 11, the full contents of each of which is incorporated by reference herein).

In other embodiments, the NK cell engager is a ligand of CD16, which is a CD16a/b ligand, e.g., a CD16a/b ligand further comprising an antibody Fc region (see e.g., Front Immunol. 2013; 4: 76 discusses how antibodies use the Fc to trigger NK cells through CD16,the full contents of which are incorporated herein).

In other embodiments, the NK cell engager is a ligand of CRTAM, which is NECL2, e.g., wherein NECL2 comprises the amino acid sequence:
QNLFTKDVTVIEGEVATISCQVNKSDDSVIQLLNPNRQTIYFRDFRPLKDSRFQLLNFSSS
ELKVSLTNVSISDEGRYFCQLYTDPPQESYTTITVLVPPRNLMIDIQKDTAVEGEEIEVNC
TAMASKPATTIRWFKGNTELKGKSEVEEWSDMYTVTSQLMLKVHKEDDGVPVICQVE
HPAVTGNLQTQRYLEVQYKPQVHIQMTYPLQGLTREGDALELTCEAIGKPQPVMVTWV
RVDDEMPQHAVLSGPNLFINNLNKTDNGTYRCEASNIVGKAHSDYMLYVYDPPTTIPPP
TTTTTTTTTTTTTILTIITDSRAGEEGSIRAVDH (SEQ ID NO: 7239), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7239.

In other embodiments, the NK cell engager is a ligand of CD27, which is CD70, e.g., wherein CD70 comprises the amino acid sequence:
QRFAQAQQQLPLESLGWDVAELQLNHTGPQQDPRLYWQGGPALGRSFLHGPELDKGQ
LRIHRDGIYMVHIQVTLAICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQR

LTPLARGDTLCTNLTGTLLPSRNTDETFFGVQWVRP (SEQ ID NO: 7240), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7240.

In other embodiments, the NK cell engager is a ligand of PSGL1, which is L-selectin (CD62L), e.g., wherein L-selectin comprises the amino acid sequence: WTYHYSEKPMNWQRARRFCRDNYTDLVAIQNKAEIEYLEKTLPFSRSYYWIGIRKIGGI WTWVGTNKSLTEEAENWGDGEPNNKKNKEDCVEIYIKRNKDAGKWNDDACHKLKAA LCYTASCQPWSCSGHGECVEIINNYTCNCDVGYYGPQCQFVIQCEPLEAPELGTMDCTH PLGNFSFSSQCAFSCSEGTNLTGIEETTCGPFGNWSSPEPTCQVIQCEPLSAPDLGIMNCSH PLASFSFTSACTFICSEGTELIGKKKTICESSGIWSNPSPICQKLDKSFSMIKEGDYN (SEQ ID NO: 7241), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7241.

In other embodiments, the NK cell engager is a ligand of CD96, which is NECL5, e.g., wherein NECL5 comprises the amino acid sequence: WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHGESGSMAV FHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTCLFVTFPQGSRSVD IWLRVLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNTSQVPG FLSGTVTVTSLWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLTVYYPPEVSISGYDNN WYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLICN VTNALGARQAELTVQVKEGPPSEHSGISRN (SEQ ID NO: 7238), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7238.

In other embodiments, the NK cell engager is a ligand of CD100 (SEMA4D), which is CD72, e.g., wherein CD72 comprises the amino acid sequence:

RYLQVSQQLQQTNRVLEVTNSSLRQQLRLKITQLGQSAEDLQGSRRELAQSQEALQVEQ
RAHQAAEGQLQACQADRQKTKETLQSEEQQRRALEQKLSNMENRLKPFFTCGSADTCC
PSGWIMHQKSCFYISLTSKNWQESQKQCETLSSKLATFSEIYPQSHSYYFLNSLLPNGGS
GNSYWTGLSSNKDWKLTDDTQRTRTYAQSSKCNKVHKTWSWWTLESESCRSSLPYICE
MTAFRFPD (SEQ ID NO: 7242), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7242.

In other embodiments, the NK cell engager is a ligand of NKp80, which is CLEC2B (AICL), e.g., wherein CLEC2B (AICL) comprises the amino acid sequence: KLTRDSQSLCPYDWIGFQNKCYYFSKEEGDWNSSKYNCSTQHADLTIIDNIEEMNFLRR
YKCSSDHWIGLKMAKNRTGQWVDGATFTKSFGMRGSEGCAYLSDDGAATARCYTER
KWICRKRIH (SEQ ID NO: 7243), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7243.

In other embodiments, the NK cell engager is a ligand of CD244, which is CD48, e.g., wherein CD48 comprises the amino acid sequence:
QGHLVHMTVVSGSNVTLNISESLPENYKQLTWFYTFDQKIVEWDSRKSKYFESKFKGR
VRLDPQSGALYISKVQKEDNSTYIMRVLKKTGNEQEWKIKLQVLDPVPKPVIKIEKIEDM
DDNCYLKLSCVIPGESVNYTWYGDKRPFPKELQNSVLETTLMPHNYSRCYTCQVSNSVS
SKNGTVCLSPPCTLARS (SEQ ID NO: 7244), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7244.

In some embodiments, the NK cell engager is a viral hemagglutinin (HA), HA is a glycoprotein found on the surface of influenza viruses. It is responsible for binding the virus to cells with sialic acid on the membranes, such as cells in the upper respiratory tract or erythrocytes. HA has at least 18 different antigens. These subtypes are named H1 through H18.

NCRs can recognize viral proteins. NKp46 has been shown to be able to interact with the HA of influenza and the HA-NA of Paramyxovirus, including Sendai virus and Newcastle disease virus. Besides NKp46, NKp44 can also functionally interact with HA of different influenza subtypes.

Death Receptor Signal Engagers

Death receptors, e.g., death receptors 4 and 5 (DR4 and DR5, also known as TRAIL-R1 and TRAIL-R2 respectively), are trimeric type I transmembrane proteins widely expressed in normal human tissues. Activation of death receptors causes intracellular signaling that induces cell death. TNF-related apoptosis-inducing ligand (TRAIL) (also known as Apo2L) is a trimeric protein that binds to Death receptors, activating their cell death-inducing signaling (Amarante-Mendes and Griffith. Pharmacol Ther. 2015 Nov;155:117-31).

The present disclosure provides, *inter alia*, multispecific (e.g., bi-, tri-, quad- specific) or multifunctional molecules, that are engineered to contain one or more death receptor signal engagers that mediate binding to death receptors and/or activation of death receptor signaling on a target cell (e.g., a tumor antigen presenting cell (e.g., cancer cell, e.g., a lymphoma cell), or a lymphocyte expressing TRBC1 or TRBC2). Accordingly, in some embodiments, the death receptor signal engager comprises one or more TRAIL polypeptides or a fragment thereof (TRAIL molecule), one or more death receptors or a fragment thereof (death receptor molecule), or one or more antigen binding domains that specifically binds to a death receptor (e.g., and activates death receptor signaling). Without wishing to be bound by theory, it is thought that a death receptor signal engager that can activate death receptor signaling on a target cell can induce the death of the target cell, e.g., a target disease cell, e.g., a target cancer cell.

Death receptor signal engagers may comprise TRAIL molecules and/or death receptor molecules from or derived from versions of TRAIL and death receptors known to those skilled in the art. In some embodiments, the death receptor signal engager comprises a human TRAIL molecule or death receptor molecule. In some embodiments, the death receptor signal engager comprises a mouse TRAIL molecule or death receptor molecule. In some embodiments, the death receptor signal engager comprises a mammalian TRAIL molecule or death receptor molecule. In some embodiments, the death receptor signal engager comprises a truncated TRAIL

molecule or death receptor molecule (e.g., relative to a wild-type TRAIL molecule or death receptor molecule).

In some embodiments, the death receptor signal engager comprises a truncated TRAIL molecule comprising at least residues corresponding to amino acids 95-281 of human TRAIL, e.g., a truncated TRAIL molecule comprising residues corresponding to amino acids 95-281 of human TRAIL. In some embodiments, the death receptor signal engager comprises a truncated TRAIL molecule comprising residues of 95-281 of human TRAIL.

In some embodiments, the death receptor signal engager comprises a truncated TRAIL molecule comprising at least residues corresponding to amino acids 122-281 of human TRAIL, e.g., a truncated TRAIL molecule comprising residues corresponding to amino acids 122-281 of human TRAIL. In some embodiments, the death receptor signal engager comprises a truncated TRAIL molecule comprising residues of 122-281 of human TRAIL.

In some embodiments, the death receptor signal engager comprises one, two, or three TRAIL molecules (e.g., the death receptor signal engager is a monomeric, dimeric, or trimeric TRAIL molecule, respectively). In some embodiments, the death receptor signal engager comprises one, two, or three death receptor molecules (e.g., the death receptor signal engager is a monomeric, dimeric, or trimeric death receptor molecule, respectively). In some embodiments, the death receptor signal engager comprises one, two, or three antigen binding domains that specifically bind to a death receptor (e.g., to one or more death receptors, e.g., the same or different death receptors)

In some embodiments, the death receptor signal engager comprises an amino acid sequence selected from Table 11 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to a sequence selected from Table 11).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6157 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6157).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6158 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6158).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6159 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6159).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6160 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6160).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6161 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6161).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6162 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6162).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6163 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6163).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6164 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6164).

In some embodiments, the death receptor signal engager comprises an amino acid sequence of SEQ ID NO: 6165 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6165).

In some embodiments, the death receptor signal engager is comprised on the same polypeptide chain as another component of a multifunctional molecule of the present disclosure, e.g., the death receptor signal engager is comprised on the same polypeptide chain as a heavy and/or light chain of a first antigen binding domain that preferentially binds to a tumor antigen on a lymphoma cell (e.g., T cell), wherein the tumor antigen is T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2), a heavy and/or light chain of a first antigen binding domain that selectively targets lymphocytes expressing T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2), an immune cell engager, a cytokine molecule, or a stromal

modified moiety, e.g., as a fusion protein. In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and light chain of a first antigen binding domain that preferentially binds to a tumor antigen on a lymphoma cell (e.g., T cell), wherein the tumor antigen is T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2). In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain that selectively targets lymphocytes expressing T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2).

In some embodiments, the fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprises an amino acid sequence of SEQ ID NO: 6170 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6170).

In some embodiments, the fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprises an amino acid sequence of SEQ ID NO: 6171 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6171).

In some embodiments, the fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprises an amino acid sequence of SEQ ID NO: 6172 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6172).

In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6170 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6170), and a heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6167 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6167). In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1

comprising an amino acid sequence of SEQ ID NO: 6170 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6170), and a heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6168 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6168).

In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6171 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6171), and a heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6167 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6167). In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6171 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6171), and a heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6168 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6168).

In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6172 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6172), and a heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6167 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6167). In some embodiments, the multifunctional molecule comprises a fusion protein comprising a death receptor signal engager and a light chain of a first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6172 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6172), and a

heavy chain of the first antigen binding domain targeting TRBC1 comprising an amino acid sequence of SEQ ID NO: 6168 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6168).

Table 11. Exemplary death receptor signal engagers

SEQ ID NO	ID Ref.	Description	Sequence
SEQ ID NO: 6157	monomeric_hTRAIL_aa122_281-hFc_Knob_Cys-Blank	Monomeric human TRAIL comprising residues 122-281	METDTLLLWVLLLWVPGSTGDYKDDDDKGGGGSGT GGAAAHITGTRGRSNTLSSPNSKNEKALGRKINSWES SRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQE EIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSC WSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLID MDHEASFFGAFAVSGSGNGTSNGTSGSSGGDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
SEQ ID NO: 6158	dimeric_hTRAIL_aa122_281-hFc_Knob_Cys-Blank	Dimeric human TRAIL comprising residues 122-281	METDTLLLWVLLLWVPGSTGDYKDDDDKGGGGSGT GGAAAHITGTRGRSNTLSSPNSKNEKALGRKINSWES SRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQE EIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSC WSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLID MDHEASFFGAFAVSGAAAHITGTRGRSNTLSSPNSKN EKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYP DPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKEND RIFVSVTNEHLIDMDHEASFFGAFAVSGSGNGTSNGT SGSSGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
SEQ ID NO: 6159	trimeric_hTRAIL_aa122_281-hFc_Knob_Cys-Blank	Trimeric human TRAIL comprising residues 122-281	METDTLLLWVLLLWVPGSTGDYKDDDDKGGGGSGT GGAAAHITGTRGRSNTLSSPNSKNEKALGRKINSWES SRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQE EIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSC WSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLID MDHEASFFGAFAVSGAAAHITGTRGRSNTLSSPNSKN EKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYP DPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKEND RIFVSVTNEHLIDMDHEASFFGAFAVSGAAAHITGTR

			GRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLH LRNGELVIHEKGFYYIYSQTYFRFQEEIKENTKNDKQ MVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLYSI YQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFA VSGSGNGTSNGTSGSSGGDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6160	monomeric_hTRAIL_95-281-hFc_Knob_Cys-Blank	Monomeric human TRAIL comprising residues 95-281	METDTLLLWVLLLWVPGSTGTSEETISTVQEKQQNIS PLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALG RKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYS QTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLM KSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSV TNEHLIDMDHEASFFGAFLVGGGGGSGGGGSDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
SEQ ID NO: 6161	dimeric_hTRAIL_95-281-hFc_Knob_Cys-Blank	Dimeric human TRAIL comprising residues 95-281	METDTLLLWVLLLWVPGSTGTSEETISTVQEKQQNIS PLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALG RKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYS QTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLM KSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSV TNEHLIDMDHEASFFGAFLVGGGGGSGGGGSGTSEET ISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLS SPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGEL VIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIY KYTSYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIF ELKENDRIFVSVTNEHLIDMDHEASFFGAFLVGGGGG SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMT KNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
SEQ ID NO: 6162	trimeric_hTRAIL_95-281-hFc_Knob_Cys-Blank	Trimeric human TRAIL comprising residues 95-281	METDTLLLWVLLLWVPGSTGTSEETISTVQEKQQNIS PLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALG RKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYS QTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLM KSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSV TNEHLIDMDHEASFFGAFLVGGGGGSGGGGSGTSEET ISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLS

			SPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGEL VIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIY KYTSYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIF ELKENDRIFVSVTNEHLIDMDHEASFFGAFLVGGGGG SGGGGSGTSEETISTVQEKQQNISPLVRERGPQRVAA HITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHS FLSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENT KNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAE YGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASF FGAFLVGGGGGSGGGGSDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6163	a_hDR5_ Tigatuzumab_scFv_VH_VL-hFc_Knob_Cys-Blank	Antigen binding domain specific to DR5, a.k.a. tigatuzumab	METDTLLLWVLLLWVPGSTGEVQLVESGGGLVQPG GSLRLSCAASGFTFSSYVMSWVRQAPGKGLEWVATI SSGGSYTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYCARRGDSMITTDYWGQGTLVTVSSGGGG SGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHT GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSY RTFGQGTKVEIKGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6164	a_hDR5_ Drozitumab_scFv_VH_VL-hFc_Knob_Cys	Antigen binding domain specific to DR5, a.k.a. drozitumab	METDTLLLWVLLLWVPGSTGEVQLVQSGGGVERPG GSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSGI NWQGGSTGYADSVKGRVTISRDNAKNSLYLQMNSL RAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVSS GGGGSGGGGSGGGGSGGGGSSELTQDPAVSVALGQT VRITCSGDSLRSYYASWYQQKPGQAPVLVIYGANNR PSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSAD SSGNHVVFGGGTKLTVLGGGGSGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K
SEQ ID NO: 6165	a_hDR5_ Conatumumab_scFv_VH_	Antigen binding domain specific to DR5, a.k.a. conatumumab	METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQ TLSLTCTVSGGSISSGDYFWSWIRQLPGKGLEWIGHIH NSGTTYYNPSLKSRVTISVDTSKKQFSLRLSSVTAADT AVYYCARDRGGDYYYGMDVWGQGTTVTVSSGGGG

	VL-hFc_Knob_Cys		SGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLS CRASQGISRSYLAWYQQKPGQAPSLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQFGSSPWT FGQGTKVEIKRGGGGSGGGGSDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 6170	a_hTRBC1_Jovi1_Hum1_VL-hCLlg_vk-a_hDR5_Tigatuzumab_scFv_VH_VL	Antigen binding domain specific to DR5, a.k.a. tigatuzumab, with anti-TRBC1 light chain	METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTPG EPASISCRSSQRLVHSNGNTYLHWYLQKPGQSPQLLI YRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGV YFCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSEV QLVESGGGLVQPGGSLRLSCAASGFTFSSYVMSWVR QAPGKGLEWVATISSGGSYTYYPDSVKGRFTISRDNA KNTLYLQMNSLRAEDTAVYYCARRGDSMITTDYWG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQS PSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKA PKLLIYWASTRHTGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYSSYRTFGQGTKVEIK
SEQ ID NO: 6171	a_hTRBC1_Jovi1_Hum1_VL-hCLlg_vk-a_hDR5_Conatumumab_scFv_VH_VL	Antigen binding domain specific to DR5, a.k.a. drozitumab, with anti-TRBC1 light chain	METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTPG EPASISCRSSQRLVHSNGNTYLHWYLQKPGQSPQLLI YRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGV YFCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSQV QLQESGPGLVKPSQTLSLTCTVSGGSISSGDYFWSWIR QLPGKGLEWIGHIHNSGTTYYNPSLKSRVTISVDTSK KQFSLRLSSVTAADTAVYYCARDRGGDYYYGMDVW GQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQ SPGTLSLSPGERATLSCRASQGISRSYLAWYQQKPGQ APSLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQFGSSPWTFGQGTKVEIKR
SEQ ID NO: 6172	a_hTRBC1_Jovi1_Hum1_VL-hCLlg_vk-a_hDR5_Drozitumab_scFv_VH_VL	Antigen binding domain specific to DR5, a.k.a. conatumumab, with anti-TRBC1 light chain	METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTPG EPASISCRSSQRLVHSNGNTYLHWYLQKPGQSPQLLI YRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDVGV YFCSQSTHVPYTFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSEV QLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVR QAPGKGLEWVSGINWQGGSTGYADSVKGRVTISRDN AKNSLYLQMNSLRAEDTAVYYCAKILGAGRGWYFD YWGKGTTVTVSSGGGGSGGGGSGGGGSGGGGSSEL

			TQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPG QAPVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCNSADSSGNHVVFGGGTKLTVL

T Cell Engagers

The present disclosure provides, *inter alia*, multispecific (e.g., bi-, tri-, quad- specific) or multifunctional molecules, that are engineered to contain one or more T cell engager that mediate binding to and/or activation of a T cell. Accordingly, in some embodiments, the T cell engager is selected from an antigen binding domain or ligand that binds to (e.g., and in some embodiments activates) one or more of CD3, TCRα, TCRβ, TCRγ, TCRζ, ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-4BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226. In other embodiments, the T cell engager is selected from an antigen binding domain or ligand that binds to and does not activate one or more of CD3, TCRα, TCRβ, TCRγ, TCRζ, ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-4BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226.

TCR beta V Antigen Binding Domains

In some embodiments, the T cell engager is an antigen binding domain (e.g., an antibody molecule or fragment thereof) that binds to (e.g., and in some embodiments activates) TCRβ. This disclosure provides, inter alia, antibody molecules and fragments thereof, that bind, *e.g.*, specifically bind, to a human TCR beta V chain (TCRβV), *e.g.*, a TCRβV gene family, *e.g.*, a TCRβV subfamily, *e.g.*, as described herein. TCR beta V families and subfamilies are known in the art, *e.g.*, as described in Yassai et al., (2009) *Immunogenetics* 61(7)pp:493-502; Wei S. and Concannon P. (1994) *Human Immunology* 41(3) pp: 201-206. The antibodies described herein can be recombinant antibodies, *e.g.*, recombinant non-murine antibodies, *e.g.*, recombinant human or humanized antibodies. Throughout this disclosure, TCRβV and TCRBV are used interchangeably.

In some embodiments, the disclosure provides T cell engagers comprising an anti-TCRβV antibody molecule that binds to human TCRβV, *e.g.*, a TCRβV family, *e.g.*, gene family. In some embodiments a TCRBV gene family comprises one or more subfamilies, *e.g.*, as described herein, *e.g.*, in FIG. 6. In some embodiments, the TCRβV gene family comprises

subfamilies comprising: a TCRβ V6 subfamily, a TCRβ V10 subfamily, a TCRβ V12 subfamily, a TCRβ V5 subfamily, a TCRβ V7 subfamily, a TCRβ V11 subfamily, a TCRβ V14 subfamily, a TCRβ V16 subfamily, a TCRβ V18 subfamily, a TCRβ V9 subfamily, a TCRβ V13 subfamily, a TCRβ V4 subfamily, a TCRβ V3 subfamily, a TCRβ V2 subfamily, a TCRβ V15 subfamily, a TCRβ V30 subfamily, a TCRβ V19 subfamily, a TCRβ V27 subfamily, a TCRβ V28 subfamily, a TCRβ V24 subfamily, a TCRβ V20 subfamily, TCRβ V25 subfamily, or a TCRβ V29 subfamily.

In some embodiments, TCRβ V6 subfamily is also known as TCRβ V13.1. In some embodiments, the TCRβ V6 subfamily comprises: TCRβ V6-4*01, TCRβ V6-4*02, TCRβ V6-9*01, TCRβ V6-8*01, TCRβ V6-5*01, TCRβ V6-6*02, TCRβ V6-6*01, TCRβ V6-2*01, TCRβ V6-3*01 or TCRβ V6-1*01. In some embodiments, TCRβ V6 comprises TCRβ V6-5*01. In some embodiments, TCRβ V6, *e.g.*, TCRβ V6-5*01, is recognized, *e.g.*, bound, by SEQ ID NO: 1 and/or SEQ ID NO: 2. In some embodiments, TCRβ V6, *e.g.*, TCRβ V6-5*01, is recognized, *e.g.*, bound, by SEQ ID NO: 9 and/or SEQ ID NO: 10. In some embodiments, TCRβ V6 is recognized, *e.g.*, bound, by SEQ ID NO: 9 and/or SEQ ID NO: 11.

In some embodiments, TCRβ V10 subfamily is also known as TCRβ V12. In some embodiments, the TCRβ V10 subfamily comprises: TCRβ V10-1*01, TCRβ V10-1*02, TCRβ V10-3*01 or TCRβ V10-2*01.

In some embodiments, TCRβ V12 subfamily is also known as TCRβ V8.1. In some embodiments, the TCRβ V12 subfamily comprises: TCRβ V12-4*01, TCRβ V12-3*01, or TCRβ V12-5*01. In some embodiments, TCRβ V12 is recognized, *e.g.*, bound, by SEQ ID NO: 15 and/or SEQ ID NO: 16. In some embodiments, TCRβ V12 is recognized, *e.g.*, bound, by any one of SEQ ID NOs 23-25, and/or any one of SEQ ID NO: 26-30:

In some embodiments, the TCRβ V5 subfamily is chosen from: TCRβ V5-5*01, TCRβ V5-6*01, TCRβ V5-4*01, TCRβ V5-8*01, TCRβ V5-1*01.

In some embodiments, the TCRβ V7 subfamily comprises TCRβ V7-7*01, TCRβ V7-6*01, TCRβ V7 -8*02, TCRβ V7 -4*01, TCRβ V7-2*02, TCRβ V7-2*03, TCRβ V7-2*01, TCRβ V7-3*01, TCRβ V7-9*03, or TCRβ V7-9*01.

In some embodiments, the TCRβ V11 subfamily comprises: TCRβ V11-1*01, TCRβ V11-2*01 or TCRβ V11-3*01.

In some embodiments, the TCRβ V14 subfamily comprises TCRβ V14*01.

In some embodiments, the TCRβ V16 subfamily comprises TCRβ V16*01.

In some embodiments, the TCRβ V18 subfamily comprises TCRβ V18*01.

In some embodiments, the TCRβ V9 subfamily comprises TCRβ V9*01 or TCRβ V9*02.

In some embodiments, the TCRβ V13 subfamily comprises TCRβ V13*01.

In some embodiments, the TCRβ V4 subfamily comprises TCRβ V4-2*01, TCRβ V4-3*01, or TCRβ V4-1*01.

In some embodiments, the TCRβ V3 subfamily comprises TCRβ V3-1*01.

In some embodiments, the TCRβ V2 subfamily comprises TCRβ V2*01.

In some embodiments, the TCRβ V15 subfamily comprises TCRβ V15*01.

In some embodiments, the TCRβ V30 subfamily comprises TCRβ V30*01, or TCRβ V30*02.

In some embodiments, the TCRβ V19 subfamily comprises TCRβ V19*01, or TCRβ V19*02.

In some embodiments, the TCRβ V27 subfamily comprises TCRβ V27*01.

In some embodiments, the TCRβ V28 subfamily comprises TCRβ V28*01.

In some embodiments, the TCRβ V24 subfamily comprises TCRβ V24-1*01.

In some embodiments, the TCRβ V20 subfamily comprises TCRβ V20-1*01, or TCRβ V20-1*02.

In some embodiments, the TCRβ V25 subfamily comprises TCRβ V25-1*01.

In some embodiments, the TCRβ V29 subfamily comprises TCRβ V29-1*01.

Table 12: List of TCRβV subfamilies and subfamily members

Reference in Fig. 6	**Subfamily**	**Subfamily members**
A	TCRβ V6 *Also referred to as:* *TCR VB 13.1*	TCRβ V6-4*01, TCRβ V6-4*02, TCRβ V6-9*01, TCRβ V6-8*01, TCRβ V6-5*01, TCRβ V6-6*02, TCRβ V6-6*01, TCRβ V6-2*01, TCRβ V6-3*01 or TCRβ V6-1*01.
B	TCRβ V10	TCRβ V10-1*01, TCRβ V10-1*02, TCRβ V10-

	Also referred to as: TCRβ V12	3*01 or TCRβ V10-2*01
C	TCRβ V12 *Also referred to as: TCRβ V8.1*	TCRβ V12-4*01, TCRβ V12-3*01, or TCRβ V12-5*01
D	TCRβ V5	TCRβ V5-5*01, TCRβ V5-6*01, TCRβ V5-4*01, TCRβ V5-8*01, TCRβ V5-1*01
E	TCRβ V7	TCRβ V7-7*01, TCRβ V7-6*01, TCRβ V7 -8*02, TCRβ V7 -4*01, TCRβ V7-2*02, TCRβ V7-2*03, TCRβ V7-2*01, TCRβ V7-3*01, TCRβ V7-9*03, or TCRβ V7-9*01
F	TCRβ V11	TCRβ V11-1*01, TCRβ V11-2*01 or TCRβ V11-3*01
G	TCRβ V14	TCRβ V14*01
H	TCRβ V16	TCRβ V16*01
I	TCRβ V18	TCRβ V18*01
J	TCRβ V9	TCRβ V9*01 or TCRβ V9*02
K	TCRβ V13	TCRβ V13*01
L	TCRβ V4	TCRβ V4-2*01, TCRβ V4-3*01, or TCRβ V4-1*01
M	TCRβ V3	TCRβ V3-1*01
N	TCRβ V2	TCRβ V2*01
O	TCRβ V15	TCRβ V15*01
P	TCRβ V30	TCRβ V30*01, or TCRβ V30*02
Q	TCRβ V19	TCRβ V19*01, or TCRβ V19*02
R	TCRβ V27	TCRβ V27*01.
S	TCRβ V28	TCRβ V28*01.
T	TCRβ V24	TCRβ V24-1*01

U	TCRβ V20	TCRβ V20-1*01, or TCRβ V20-1*02
V	TCRβ V25	TCRβ V25-1*01
W	TCRβ V29	TCRβ V29-1*01

Anti-TCRβV antibodies

In an aspect, the disclosure provides an anti-TCRβV antibody molecule that binds to human TCRβV, *e.g.*, a TCRβV gene family, *e.g.*, one or more of a TCRβV subfamily, *e.g.*, as described herein, *e.g.*, in FIG. 6. In some embodiments, the anti-TCRβV antibody molecule binds to one or more TCRβV subfamilies chosen from: a TCRβ V6 subfamily, a TCRβ V10 subfamily, a TCRβ V12 subfamily, a TCRβ V5 subfamily, a TCRβ V7 subfamily, a TCRβ V11 subfamily, a TCRβ V14 subfamily, a TCRβ V16 subfamily, a TCRβ V18 subfamily, a TCRβ V9 subfamily, a TCRβ V13 subfamily, a TCRβ V4 subfamily, a TCRβ V3 subfamily, a TCRβ V2 subfamily, a TCRβ V15 subfamily, a TCRβ V30 subfamily, a TCRβ V19 subfamily, a TCRβ V27 subfamily, a TCRβ V28 subfamily, a TCRβ V24 subfamily, a TCRβ V20 subfamily, TCRβ V25 subfamily, or a TCRβ V29 subfamily. In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβ V6 subfamily comprising: TCRβ V6-4*01, TCRβ V6-4*02, TCRβ V6-9*01, TCRβ V6-8*01, TCRβ V6-5*01, TCRβ V6-6*02, TCRβ V6-6*01, TCRβ V6-2*01, TCRβ V6-3*01 or TCRβ V6-1*01. In some embodiments the TCRβ V6 subfamily comprises TCRβ V6-5*01. In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβ V10 subfamily comprising: TCRβ V10-1*01, TCRβ V10-1*02, TCRβ V10-3*01 or TCRβ V10-2*01. In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβ V12 subfamily comprising: TCRβ V12-4*01, TCRβ V12-3*01 or TCRβ V12-5*01. In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβ V5 subfamily comprising: TCRβ V5-5*01, TCRβ V5-6*01, TCRβ V5-4*01, TCRβ V5-8*01, TCRβ V5-1*01.

In some embodiments, the anti-TCRβV antibody molecule does not bind to TCRβ V12, or binds to TCRβ V12 with an affinity and/or binding specificity that is less than (e.g., less than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the 16G8 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some embodiments, the anti-TCRβV antibody molecule binds to TCRβ V12 with an affinity and/or binding specificity that is greater than (e.g., greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the 16G8 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβV region other than TCRβ V12 (e.g., TCRβV region as described herein, e.g., TCRβ V6 subfamily (e.g., TCRβ V6-5*01) with an affinity and/or binding specificity that is greater than (e.g., greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the 16G8 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some embodiments, the anti-TCRβV antibody molecule does not bind to TCRβ V5-5*01 or TCRβ V5-1*01, or binds to TCRβ V5-5*01 or TCRβ V5-1*01 with an affinity and/or binding specificity that is less than (e.g., less than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the TM23 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some embodiments, the anti-TCRβV antibody molecule binds to TCRβ V5-5*01 or TCRβ V5-1*01with an affinity and/or binding specificity that is greater than (e.g., greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the TM23 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some embodiments, the anti-TCRβV antibody molecule binds to a TCRβV region other than TCRβ V5-5*01 or TCRβ V5-1*01 (e.g., TCRβV region as described herein, e.g., TCRβ V6 subfamily (e.g., TCRβ V6-5*01) with an affinity and/or binding specificity that is greater than (e.g., greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the TM23 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

Anti-TCRβ V6 antibodies

Accordingly, in one aspect, the disclosure provides an anti-TCRβV antibody molecule that binds to human TCRβ V6, *e.g.*, a TCRβ V6 subfamily comprising: TCRβ V6-4*01, TCRβ V6-4*02, TCRβ V6-9*01, TCRβ V6-8*01, TCRβ V6-5*01, TCRβ V6-6*02, TCRβ V6-6*01, TCRβ V6-2*01, TCRβ V6-3*01 or TCRβ V6-1*01. In some embodiments the TCRβ V6 subfamily comprises TCRβ V6-5*01.

In some embodiments, TCRβ V6-5*01 is encoded by the nucleic acid sequence of SEQ ID NO: 43, or a sequence having 85%, 90%, 95%, 99% or more identity thereof.

SEQ ID NO: 43

ATGAGCATCGGCCTCCTGTGCTGTGCAGCCTTGTCTCTCCTGTGGGCAGGTCCAGTG
AATGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGAGCAT
GACACTGCAGTGTGCCCAGGATATGAACCATGAATACATGTCCTGGTATCGACAAG
ACCCAGGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACC
AAGGAGAAGTCCCCAATGGCTACAATGTCTCCAGATCAACCACAGAGGATTTCCCG
CTCAGGCTGCTGTCGGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTT
ACTC

In some embodiments, TCRβ V6-5*01 comprises the amino acid sequence of SEQ ID NO: 44, or an amino acid sequence having 85%, 90%, 95%, 99% or more identity thereof.

SEQ ID NO: 44

MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQ
DPGMGLRLIHYSVGAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSY

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, is a non-murine antibody molecule, *e.g.*, a human or humanized antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule is a human antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule is a humanized antibody molecule.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, is isolated or recombinant.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises at least one antigen-binding region, *e.g.*, a variable region or an antigen-binding fragment thereof, from an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises at least one, two, three or four variable regions from an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises at least one or two heavy chain variable regions from an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises at least one or two light chain variable regions from an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a heavy chain constant region for an IgG4, *e.g.*, a human IgG4. In still another embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes a heavy chain constant region

for an IgG1, *e.g.*, a human IgG1. In one embodiment, the heavy chain constant region comprises an amino sequence set forth in **Table 17**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes a kappa light chain constant region, *e.g.*, a human kappa light chain constant region. In one embodiment, the light chain constant region comprises an amino sequence set forth in **Table 17**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, or three complementarity determining regions (CDRs) from a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid

sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 13**, or encoded by a nucleotide sequence shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, molecule includes all six CDRs from an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in Table 13, or closely related CDRs, *e.g.*, CDRs which are identical or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions). In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.,* at least one, two, or three CDRs according to the Kabat definition as set out in **Table 13**) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat et al. shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.,* at least one, two, or three CDRs according to the Kabat definition as set out in **Table 13**) from a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat et al. shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, three, four, five, or six CDRs according to Kabat *et al.* (*e.g.,* at least one, two, three, four, five, or six CDRs according to the Kabat definition as set out in **Table 13**) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to Kabat et al. shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes all six CDRs according to Kabat *et al.* (*e.g.,* all six CDRs according to the Kabat definition as set out in Table 13) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**; or encoded by the nucleotide sequence in **Table 13**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to Kabat et al. shown in **Table 13**. In one embodiment, the anti-TCRβV

antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, or three hypervariable loops that have the same canonical structures as the corresponding hypervariable loop of an antibody described herein, *e.g.*, an antibody chosen from chosen from BHM1709 or BHM1710 *e.g.*, the same canonical structures as at least loop 1 and/or loop 2 of the heavy and/or light chain variable domains of an antibody described herein. See, *e.g.*, Chothia et al., (1992) J. Mol. Biol. 227:799-817; Tomlinson et al., (1992) J. Mol. Biol. 227:776-798 for descriptions of hypervariable loop canonical structures. These structures can be determined by inspection of the tables described in these references.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes at least one, two, or three CDRs according to Chothia et al. (*e.g.*, at least one, two, or three CDRs according to the Chothia definition as set out in Table 13) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in Table 13, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Chothia et al. shown in Table 13.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes at least one, two, or three CDRs according to Chothia *et al.* (*e.g.,* at least one, two, or three CDRs according to the Chothia definition as set out in **Table 13**) from a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Chothia et al. shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes at least one, two, three, four, five, or six CDRs according to Chothia *et al.* (*e.g.,* at least one, two, three, four, five, or six CDRs according to the Chothia definition as set out in **Table 13**) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to Chothia et al. shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes all six CDRs according to Chothia *et al.* (*e.g.,* all six CDRs according to the Chothia definition as set out in Table 13) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**; or encoded by the nucleotide sequence in **Table 13**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to Chothia et al. shown in **Table 13**. In one embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, molecule includes a combination of CDRs or hypervariable loops defined according to Kabat et al., Chothia et al., or as described in Table 13.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, can contain any combination of CDRs or hypervariable loops according to the Kabat and Chothia definitions.

In some embodiments, a combined CDR as set out in Table 13 is a CDR that comprises a Kabat CDR and a Chothia CDR.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, molecule includes a combination of CDRs or hypervariable loops identified as combined CDRs in Table 13. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, can contain any combination of CDRs or hypervariable loops according the "combined" CDRs are described in Table 13.

In an embodiment, *e.g.*, an embodiment comprising a variable region, a CDR (*e.g.*, a combined CDR, Chothia CDR or Kabat CDR), or other sequence referred to herein, *e.g.*, in **Table 13**, the antibody molecule is a monospecific antibody molecule, a bispecific antibody molecule, a bivalent antibody molecule, a biparatopic antibody molecule, or an antibody molecule that comprises an antigen binding fragment of an antibody, *e.g.*, a half antibody or antigen binding fragment of a half antibody. In certain embodiments the antibody molecule comprise a multispecific molecule, *e.g.*, a bispecific molecule, *e.g.*, as described herein.

In an embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes:

(i) one, two or all of a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2),and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 2, SEQ ID NO: 10 or SEQ ID NO: 11, and/or

(ii) one, two or all of a heavy chain complementarity determining region 1 (HC CDR1), heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 1 or SEQ ID NO: 9.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises a LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NO: 2, and a HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NO: 1.

In some embodiments the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises a LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NO: 10, and a HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NO: 9.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises a LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NO: 11, and a HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NO: 9.

In an embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 6, a LC CDR2 amino acid sequence of SEQ ID NO: 7, or a LC CDR3 amino acid sequence of SEQ ID NO: 8; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 3, a HC CDR2 amino acid sequence of SEQ ID NO: 4, or a HC CDR3 amino acid sequence of SEQ ID NO: 5.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 6, a LC CDR2 amino acid sequence of SEQ ID NO: 7, or a LC CDR3 amino acid sequence of SEQ ID NO: 8; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 3, a HC CDR2 amino acid sequence of SEQ ID NO: 4, or a HC CDR3 amino acid sequence of SEQ ID NO: 5.

In an embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 51, a LC CDR2 amino acid sequence of SEQ ID NO: 52, or a LC CDR3 amino acid sequence of SEQ ID NO: 53; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 45, a HC CDR2 amino acid sequence of SEQ ID NO: 46, or a HC CDR3 amino acid sequence of SEQ ID NO: 47.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 51, a LC CDR2 amino acid sequence of SEQ ID NO: 52, or a LC CDR3 amino acid sequence of SEQ ID NO: 53; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 45, a HC CDR2 amino acid sequence of SEQ ID NO: 46, or a HC CDR3 amino acid sequence of SEQ ID NO: 47.

In an embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 54, a LC CDR2 amino acid sequence of SEQ ID NO: 55, or a LC CDR3 amino acid sequence of SEQ ID NO: 56; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 48, a HC CDR2 amino acid sequence of SEQ ID NO: 49, or a HC CDR3 amino acid sequence of SEQ ID NO: 50.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 54, a LC CDR2 amino acid sequence of SEQ ID NO: 55, or a LC CDR3 amino acid sequence of SEQ ID NO: 56; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 48, a HC CDR2 amino acid sequence of SEQ ID NO: 49, or a HC CDR3 amino acid sequence of SEQ ID NO: 50.

In one embodiment, the light or the heavy chain variable framework (*e.g.*, the region encompassing at least FR1, FR2, FR3, and optionally FR4) of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule can be chosen from: (a) a light or heavy chain variable framework including at least 80%, 85%, 87% 90%, 92%, 93%, 95%, 97%, 98%, or 100% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (b) a light or heavy chain variable framework including from 20% to 80%, 40% to 60%, 60% to 90%, or 70% to 95% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (c) a non-human framework (*e.g.*, a rodent framework); or (d) a non-human framework that has been modified, *e.g.*, to remove antigenic or

cytotoxic determinants, *e.g.*, deimmunized, or partially humanized. In one embodiment, the light or heavy chain variable framework region (particularly FR1, FR2 and/or FR3) includes a light or heavy chain variable framework sequence at least 70, 75, 80, 85, 87, 88, 90, 92, 94, 95, 96, 97, 98, 99% identical or identical to the frameworks of a VL or VH segment of a human germline gene.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a heavy chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence of BHM1709 or BHM1710 .*g.*, the amino acid sequence of the FR region in the entire variable region, *e.g.*, shown in **FIG. 4A**, or in SEQ ID NO: 9.

Alternatively, or in combination with the heavy chain substitutions described herein, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more amino acid changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence of BHM1709 or BHM1710 .*e.g.*, the amino acid sequence of the FR region in the entire variable region, *e.g.*, shown in **FIG. 4B,** or in SEQ ID NO: 10 or SEQ ID NO: 11.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes one, two, three, or four heavy chain framework regions shown in **FIG. 4A,** or a sequence substantially identical thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes one, two, three, or four light chain framework regions shown in **FIG. 4B,** or a sequence substantially identical thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework region 1 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework region 2 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework region 3 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework region 4 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at position 10 according to Kabat numbering. In some embodiments, the FR1 comprises a Phenylalanine at position 10, *e.g.*, a Serine to Phenyalanine substitution. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising a framework region, *e.g.*, framework region 2 (FR2), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at a position disclosed herein according to Kabat numbering. In some embodiments, FR2 comprises a Histidine at position 36, *e.g.*, a substitution at position 36 according to Kabat numbering, *e.g.*, a Tyrosine to Histidine substitution. In some embodiments, FR2 comprises an Alanine at position 46, *e.g.*, a substitution at position 46 according to Kabat numbering, *e.g.*, a Arginine to Alanine substitution. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at a position disclosed herein according to Kabat numbering. In some embodiments, FR3 comprises a Phenyalanine at position 87, *e.g.*, a substitution at position 87 according to Kabat numbering, *e.g.*, a Tyrosine to Phenyalanine substitution. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising: (a) a framework region 1 (FR1) comprising a Phenylalanine at position 10, *e.g.*, a substitution at position 10 according to Kabat numbering, *e.g.*, a Serine to Phenyalanine substitution; (b) a framework region 2 (FR2) comprising a Histidine at position 36, *e.g.*, a substitution at position 36 according to Kabat numbering, *e.g.*, a Tyrosine to Histidine substitution, and a Alanine at position 46, *e.g.*, a substitution at position 46 according to Kabat numbering, *e.g.*, a Arginine to Alanine substitution; and (c) a framework region 3 (FR3) comprising a Phenylalanine at position 87, *e.g.*, a substitution at position 87 according to Kabat numbering, *e.g.*, a Tyrosine to Phenyalanine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 10. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising: (a) a framework region 2 (FR2) comprising a Histidine at position 36, *e.g.*, a substitution at position 36 according to Kabat numbering, *e.g.*, a Tyrosine to Histidine substitution, and a Alanine at position 46, *e.g.*, a substitution at position 46 according to Kabat numbering, *e.g.*, a Arginine to Alanine substitution; and (b) a framework region 3 (FR3) comprising a Phenylalanine at position 87, *e.g.*, a substitution at position 87 according to Kabat numbering, *e.g.*, a Tyrosine to Phenyalanine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 11. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a light chain variable domain comprising: (a) a framework region 1 (FR1) comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more (*e.g.*, all) positions disclosed herein according to Kabat numbering, ; (b) a framework region 2 (FR2) comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more (*e.g.*, all) position disclosed herein according to Kabat numbering and (c) a framework region 3 (FR3) comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more (*e.g.*, all) position disclosed herein according to Kabat numbering.

In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework region 1 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework region 2 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4A**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework region 3 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4A**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework region 4 of BHM1709 or BHM1710, *e.g.*, as shown in **FIG. 4A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a heavy chain variable domain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at a position disclosed herein according to Kabat numbering. In some embodiments, FR3 comprises a Threonine at position 73, *e.g.*, a substitution at position 73 according to Kabat numbering, *e.g.*, a Glutamic Acid to Threonine substitution. In some embodiments, FR3 comprises a Glycine at position 94, *e.g.*, a substitution at position 94 according to Kabat numbering, *e.g.*, a Arginine to Glycine substitution. In some embodiments, the substitution is relative to a human germline heavy chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises a heavy chain variable domain comprising a framework region 3 (FR3) comprising a Threonine at position 73, *e.g.*, a substitution at position 73 according to Kabat numbering, *e.g.*, a Glutamic Acid to Threonine substitution, and a Glycine at position 94, *e.g.*, a substitution at position 94 according to Kabat numbering, *e.g.*, a Arginine to Glycine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 10.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework regions 1-4 of BHM1709 or BHM1710, *e.g.*, SEQ ID NO: 9, or as shown in **FIGs. 4A** and **4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework regions 1-4 of BHM1709, *e.g.*, SEQ ID NO: 10, or as shown in **FIGs. 4A** and **4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the light chain framework regions 1-4 of BHM1710, *e.g.*, SEQ ID NO: 11, or as shown in **FIGs. 4A** and **4B**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework regions 1-4 of BHM1709, *e.g.*, SEQ ID NO: 9; and the light chain framework regions 1-4 of BHM1709, *e.g.*, SEQ ID NO: 10, or as shown in **FIGs. 4A** and **4B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises the heavy chain framework regions 1-4 of BHM1710, *e.g.*, SEQ ID NO: 9; and the light chain framework regions 1-4 of BHM1710, *e.g.*, SEQ ID NO: 11, or as shown in **FIGs. 4A** and **4B.**

In some embodiments, the heavy or light chain variable domain, or both, of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, includes an amino acid sequence, which is substantially identical to an amino acid disclosed herein, *e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical to a variable region of an antibody described herein, *e.g.*, an antibody chosen from BHM1709 or BHM1710, or as described in **Table 13**, or encoded by the nucleotide sequence in **Table 13**; or which differs at least 1 or 5 residues, but less than 40, 30, 20, or 10 residues, from a variable region of an antibody described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises at least one, two, three, or four antigen-binding regions, *e.g.*, variable regions, having an amino acid sequence as set forth in **Table 13**, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 1, 2, 5, 10, or 15 amino acid residues

from the sequences shown in **Table 13**. In another embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule includes a VH and/or VL domain encoded by a nucleic acid having a nucleotide sequence as set forth in **Table 13**, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in **Table 13**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 9, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 9; and/or

a VL domain comprising the amino acid sequence of SEQ ID NO: 10, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 10.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 9, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 9; and/or

a VL domain comprising the amino acid sequence of SEQ ID NO: 11, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence of SEQ ID NO: 11, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule is a full antibody or fragment thereof (*e.g.*, a Fab, $F(ab')_2$, Fv, or a single chain Fv fragment (scFv)). In embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule is a monoclonal

antibody or an antibody with single specificity. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, can also be a humanized, chimeric, camelid, shark, or an *in vitro*-generated antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, is a humanized antibody molecule. The heavy and light chains of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, can be full-length (*e.g.*, an antibody can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or can include an antigen-binding fragment (*e.g.*, a Fab, F(ab')2, Fv, a single chain Fv fragment, a single domain antibody, a diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody).

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, is in the form of a multispecific molecule, *e.g.*, a bispecific molecule, *e.g.*, as described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, has a heavy chain constant region (Fc) chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. In some embodiments, the Fc region is chosen from the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In some embodiments, the Fc region is chosen from the heavy chain constant region of IgG1 or IgG2 (*e.g.*, human IgG1, or IgG2). In some embodiments, the heavy chain constant region is human IgG1.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule, has a light chain constant region chosen from, *e.g.*, the light chain constant regions of kappa or lambda, preferably kappa (*e.g.*, human kappa). In one embodiment, the constant region is altered, *e.g.,* mutated, to modify the properties of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). For example, the constant region is mutated at positions 296 (M to Y), 298 (S to T), 300 (T to E), 477 (H to K) and 478 (N to F) to alter Fc receptor binding (*e.g.*, the mutated positions correspond to positions

132 (M to Y), 134 (S to T), 136 (T to E), 313 (H to K) and 314 (N to F) of SEQ ID NOs: 212 or 214; or positions 135 (M to Y), 137 (S to T), 139 (T to E), 316 (H to K) and 317 (N to F) of SEQ ID NOs: 215, 216, 217 or 218), *e.g.*, relative to human IgG1.

Table 13: Amino acid and nucleotide sequences for murine, chimeric and humanized antibody molecules. The antibody molecules include murine mAb H131, and humanized mAb H131 Clones BHM1709 and BHM1710. The amino acid the heavy and light chain CDRs, and the amino acid and nucleotide sequences of the heavy and light chain variable regions, and the heavy and light chains are shown.

H131 (murine)		
SEQ ID NO: 3	HC CDR1 (Combined)	GYSFTTYYIH
SEQ ID NO: 4	HC CDR2 (Combined)	WFFPGSGNIKYNEKFKG
SEQ ID NO: 5	HC CDR3 (Combined)	SYYSYDVLDY
SEQ ID NO: 45	HC CDR1 (Kabat)	TYYIH
SEQ ID NO: 46	HC CDR2 (Kabat)	WFFPGSGNIKYNEKFKG
SEQ ID NO: 47	HC CDR3 (Kabat)	SYYSYDVLDY
SEQ ID NO:48	HC CDR1 (Chothia)	GYSFTTY
SEQ ID NO: 49	HC CDR2 (Chothia)	FPGSGN
SEQ ID NO: 50	HC CDR3 (Chothia)	SYYSYDVLDY
SEQ ID NO: 1	VH	QVQLQQSGPELVKPGTSVKISCKASGYSFTTYYI HWVKQRPGQGLEWIGWFFPGSGNIKYNEKFKG KATLTADTSSSTAYMQLSSLTSEESAVYFCAGS YYSYDVLDYWGHGTTLTVSS
SEQ ID NO: 6	LC CDR1 (Combined)	KASQNVGINVV
SEQ ID NO: 7	LC CDR2 (Combined))	SSSHRYS
SEQ ID NO: 8	LC CDR3 (Combined)	QQFKSYPLT
SEQ ID NO: 51	LC CDR1 (Kabat)	KASQNVGINVV

SEQ ID NO: 52	LC CDR2 (Kabat)	SSSHRYS
SEQ ID NO: 53	LC CDR3 (Kabat)	QQFKSYPLT
SEQ ID NO: 54	LC CDR1 (Chothia)	KASQNVGINVV
SEQ ID NO: 55	LC CDR2 (chothia)	SSSHRYS
SEQ ID NO: 56	LC CDR3 (chothia)	QQFKSYPLT
SEQ ID NO: 2	VL	DILMTQSQKFMSTSLGDRVSVSCKASQNVGINV VWHQQKPGQSPKALIYSSSHRYSGVPDRFTGSG SGTDFTLTINNVQSEDLAEYFCQQFKSYPLTFGA GTKLELK
BHM1709 (humanized)		
SEQ ID NO: 3	HC CDR1 (Combined)	GYSFTTYYIH
SEQ ID NO: 4	HC CDR2 (Combined)	WFFPGSGNIKYNEKFKG
SEQ ID NO: 5	HC CDR3 (Combined)	SYYSYDVLDY
SEQ ID NO: 9	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYSFTTY YIHWVRQAPGQGLEWMGWFFPGSGNIKYNEKF KGRVTITADTSTSTAYMELSSLRSEDTAVYYCA GSYYSYDVLDYWGQGTTVTVSS
SEQ ID NO: 12	DNA VH	CAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGT GAAGAAACCTGGCTCCTCCGTGAAGGTGTCCT GCAAGGCTTCCGGCTACTCCTTCACCACCTAC TACATCCACTGGGTCCGACAGGCCCCTGGACA AGGATTGGAATGGATGGGCTGGTTCTTCCCCG GCTCCGGCAACATCAAGTACAACGAGAAGTT CAAGGGCCGCGTGACCATCACCGCCGACACC TCTACCTCTACCGCCTACATGGAACTGTCCAG CCTGAGATCTGAGGACACCGCCGTGTACTACT GCGCCGGCTCCTACTACTCTTACGACGTGCTG GATTACTGGGGCCAGGGCACCACAGTGACAG TGTCCTCT
SEQ ID NO: 6	LC CDR1 (Combined)	KASQNVGINVV
SEQ ID NO: 7	LC CDR2 (Combined))	SSSHRYS
SEQ ID NO: 8	LC CDR3 (Combined)	QQFKSYPLT

SEQ ID NO: 10	VL	DIQMTQSPSFLSASVGDRVTITCKASQNVGINVV WHQQKPGKAPKALIYSSSHRYSGVPSRFSGSGS GTEFTLTISSLQPEDFATYFCQQFKSYPLTFGQG TKLEIK
SEQ ID NO: 13	DNA VL	GACATCCAGATGACCCAGTCTCCATCCTTCCT GTCCGCCTCTGTGGGCGACAGAGTGACCATCA CATGCAAGGCCTCTCAGAACGTGGGCATCAA CGTCGTGTGGCACCAGCAGAAGCCTGGCAAG GCTCCTAAGGCTCTGATCTACTCCTCCAGCCA CCGGTACTCTGGCGTGCCCTCTAGATTTTCCG GCTCTGGCTCTGGCACCGAGTTTACCCTGACA ATCTCCAGCCTGCAGCCTGAGGACTTCGCCAC CTACTTTTGCCAGCAGTTCAAGAGCTACCCTC TGACCTTTGGCCAGGGCACCAAGCTGGAAAT CAAG
BHM1710 (humanized)		
SEQ ID NO: 3	HC CDR1 (Combined)	GYSFTTYYIH
SEQ ID NO: 4	HC CDR2 (Combined)	WFFPGSGNIKYNEKFKG
SEQ ID NO: 5	HC CDR3 (Combined)	SYYSYDVLDY
SEQ ID NO: 9	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYSFTTY YIHWVRQAPGQGLEWMGWFFPGSGNIKYNEKF KGRVTITADTSTSTAYMELSSLRSEDTAVYYCA GSYYSYDVLDYWGQGTTVTVSS
SEQ ID NO: 12	DNA VH	CAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGT GAAGAAACCTGGCTCCTCCGTGAAGGTGTCCT GCAAGGCTTCCGGCTACTCCTTCACCACCTAC TACATCCACTGGGTCCGACAGGCCCCTGGACA AGGATTGGAATGGATGGGCTGGTTCTTCCCCG GCTCCGGCAACATCAAGTACAACGAGAAGTT CAAGGGCCGCGTGACCATCACCGCCGACACC TCTACCTCTACCGCCTACATGGAACTGTCCAG CCTGAGATCTGAGGACACCGCCGTGTACTACT GCGCCGGCTCCTACTACTCTTACGACGTGCTG GATTACTGGGGCCAGGGCACCACAGTGACAG TGTCCTCT
SEQ ID NO: 6	LC CDR1 (Combined)	KASQNVGINVV

SEQ ID NO: 7	LC CDR2 (Combined))	SSSHRYS
SEQ ID NO: 8	LC CDR3 (Combined)	QQFKSYPLT
SEQ ID NO: 11	VL	DIQMTQSPSSLSASVGDRVTITCKASQNVGINVV WHQQKPGKVPKALIYSSSHRYSGVPSRFSGSGS GTDFTLTISSLQPEDVATYFCQQFKSYPLTFGQG TKLEIK
SEQ ID NO: 14	DNA VL	GACATCCAGATGACCCAGTCTCCATCCTCTCT GTCCGCCTCTGTGGGCGACAGAGTGACCATCA CATGCAAGGCCTCTCAGAACGTGGGCATCAA CGTCGTGTGGCACCAGCAGAAACCTGGCAAG GTGCCCAAGGCTCTGATCTACTCCTCCAGCCA CAGATACTCCGGCGTGCCCTCTAGATTCTCCG GCTCTGGCTCTGGCACCGACTTTACCCTGACA ATCTCCAGCCTGCAGCCTGAGGACGTGGCCAC CTACTTTTGCCAGCAGTTCAAGAGCTACCCTC TGACCTTTGGCCAGGGCACCAAGCTGGAAAT CAAG

Anti-TCRβ V12 antibodies

Accordingly, in one aspect, the disclosure provides an anti-TCRβV antibody molecule that binds to human TCRβ V12, *e.g.*, a TCRβ V12 subfamily comprising: TCRβ V12-4*01, TCRβ V12-3*01 or TCRβ V12-5*01. In some embodiments the TCRβ V12 subfamily comprises TCRβ V12-4*01. In some embodiments the TCRβ V12 subfamily comprises TCRβ V12-3*01.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, is a non-murine antibody molecule, *e.g.*, a human or humanized antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule is a human antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule is a humanized antibody molecule.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, is isolated or recombinant.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises at least one antigen-binding region, *e.g.*, a variable region or an antigen-binding fragment thereof, from an antibody described herein, *e.g.*, an antibody described in

Table 14, or encoded by the nucleotide sequence in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises at least one, two, three or four variable regions from an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in Table 14, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises at least one or two heavy chain variable regions from an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in Table 14, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises at least one or two light chain variable regions from an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in Table 14, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises a heavy chain constant region for an IgG4, *e.g.*, a human IgG4. In still another embodiment, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes a heavy chain constant region for an IgG1, *e.g.*, a human IgG1. In one embodiment, the heavy chain constant region comprises an amino sequence set forth in **Table 17**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes a kappa light chain constant region, *e.g.*, a human kappa light chain constant region. In one embodiment, the light chain constant region comprises an amino sequence set forth in **Table 17**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14,** or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes at least one, two, or three complementarity determining regions (CDRs) from a light chain variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**. In one embodiment, one

or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in **Table 14**, or encoded by a nucleotide sequence shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, molecule includes all six CDRs from an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in Table 14, or closely related CDRs, *e.g.*, CDRs which are identical or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions). In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.,* at least one, two, or three CDRs according to the Kabat definition as set out in **Table 14**) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.,* at least one, two, or three CDRs according to the Kabat definition as set out in **Table 14**) from a light chain variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, three, four, five, or six CDRs according to Kabat *et al.* (*e.g.,*

at least one, two, three, four, five, or six CDRs according to the Kabat definition as set out in **Table 14**) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to Kabat et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes all six CDRs according to Kabat *et al.* (*e.g.,* all six CDRs according to the Kabat definition as set out in Table 14) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or encoded by the nucleotide sequence in **Table 14**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to Kabat et al. shown in **Table 14**. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three hypervariable loops that have the same canonical structures as the corresponding hypervariable loop of an antibody described herein, *e.g.*, an antibody described in Table 14, *e.g.*, the same canonical structures as at least loop 1 and/or loop 2 of the heavy and/or light chain variable domains of an antibody described herein. See, *e.g.*, Chothia et al., (1992) J. Mol. Biol. 227:799-817; Tomlinson et al., (1992) J. Mol. Biol. 227:776-798 for descriptions of hypervariable loop canonical structures. These structures can be determined by inspection of the tables described in these references.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to Chothia *et al.* (*e.g.,* at least one, two, or three CDRs according to the Chothia definition as set out in **Table 14**) from a heavy

chain variable region of an antibody described herein, *e.g.*, an antibody chosen as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Chothia et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to Chothia *et al.* (*e.g.,* at least one, two, or three CDRs according to the Chothia definition as set out in **Table 14**) from a light chain variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Chothia et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, three, four, five, or six CDRs according to Chothia *et al.* (*e.g.,* at least one, two, three, four, five, or six CDRs according to the Chothia definition as set out in **Table 14**) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to Chothia et al. shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes all six CDRs according to Chothia *et al.* (*e.g.,* all six CDRs according to the Chothia definition as set out in Table 14) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or encoded by the nucleotide sequence in **Table 14**; or a

sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to Chothia et al. shown in **Table 14**. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule may include any CDR described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to a combined CDR (*e.g.,* at least one, two, or three CDRs according to the combined CDR definition as set out in **Table 14**) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to combined CDR shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, or three CDRs according to a combined CDR (*e.g.,* at least one, two, or three CDRs according to the combined CDR definition as set out in **Table 14**) from a light chain variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to a combined CDR shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes at least one, two, three, four, five, or six CDRs according to a combined CDR. (*e.g.,* at least one, two, three, four, five, or six CDRs according to the combined CDR definition as set out in **Table 14**) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in

Table 14; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to a combined CDR shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes all six CDRs according to a combined CDR (*e.g.*, all six CDRs according to the combined CDR definition as set out in Table 14) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or encoded by the nucleotide sequence in **Table 14**; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to a combined CDR shown in **Table 14**. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule may include any CDR described herein.

In some embodiments, a combined CDR as set out in Table 13 is a CDR that comprises a Kabat CDR and a Chothia CDR.

In some embodiments, the anti-TCRβV antibody molecule, e *e.g.*, anti-TCRβ V12 antibody molecule, molecule includes a combination of CDRs or hypervariable loops identified as combined CDRs in Table 13. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, can contain any combination of CDRs or hypervariable loops according the "combined" CDRs are described in Table 13.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes a combination of CDRs or hypervariable loops defined according to the Kabat et al. and Chothia et al., or as described in Table 13

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule can contain any combination of CDRs or hypervariable loops according to the Kabat and Chothia definitions.

In an embodiment, *e.g.*, an embodiment comprising a variable region, a CDR (*e.g.,* a combined CDR, Chothia CDR or Kabat CDR), or other sequence referred to herein, *e.g.,* in **Table 14**, the antibody molecule is a monospecific antibody molecule, a bispecific antibody molecule, a bivalent antibody molecule, a biparatopic antibody molecule, or an antibody molecule that comprises an antigen binding fragment of an antibody, *e.g.,* a half antibody or antigen binding fragment of a half antibody. In certain embodiments the antibody molecule comprise a multispecific molecule, *e.g.*, a bispecific molecule, *e.g.*, as described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes:

(i) one, two or all of a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 16, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30, and/or

(ii) one, two or all of a heavy chain complementarity determining region 1 (HC CDR1), heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 15, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 20, a LC CDR2 amino acid sequence of SEQ ID NO: 21, or a LC CDR3 amino acid sequence of SEQ ID NO: 22; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 17, a HC CDR2 amino acid sequence of SEQ ID NO: 18, or a HC CDR3 amino acid sequence of SEQ ID NO: 19.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 20, a LC CDR2 amino acid sequence of SEQ ID NO: 21, and a LC CDR3 amino acid sequence of SEQ ID NO: 2; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 17, a HC CDR2 amino acid sequence of SEQ ID NO: 18, and a HC CDR3 amino acid sequence of SEQ ID NO: 19.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 63, a LC CDR2 amino acid sequence of SEQ ID NO: 64, or a LC CDR3 amino acid sequence of SEQ ID NO: 65; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 57, a HC CDR2 amino acid sequence of SEQ ID NO: 58, or a HC CDR3 amino acid sequence of SEQ ID NO: 59.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 63, a LC CDR2 amino acid sequence of SEQ ID NO: 64, or a LC CDR3 amino acid sequence of SEQ ID NO: 65; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 57, a HC CDR2 amino acid sequence of SEQ ID NO: 58, or a HC CDR3 amino acid sequence of SEQ ID NO: 59.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a LC CDR1 amino acid sequence of SEQ ID NO: 66, a LC CDR2 amino acid sequence of SEQ ID NO: 67, or a LC CDR3 amino acid sequence of SEQ ID NO: 68; and/or

(ii) a HC CDR1 amino acid sequence of SEQ ID NO: 60, a HC CDR2 amino acid sequence of SEQ ID NO: 61, or a HC CDR3 amino acid sequence of SEQ ID NO: 62.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

(i) a light chain variable region (VL) comprising a LC CDR1 amino acid sequence of SEQ ID NO: 63, a LC CDR2 amino acid sequence of SEQ ID NO: 64, or a LC CDR3 amino acid sequence of SEQ ID NO: 65; and/or

(ii) a heavy chain variable region (VH) comprising a HC CDR1 amino acid sequence of SEQ ID NO: 57, a HC CDR2 amino acid sequence of SEQ ID NO: 58, or a HC CDR3 amino acid sequence of SEQ ID NO: 59.

In one embodiment, the light or the heavy chain variable framework (*e.g.*, the region encompassing at least FR1, FR2, FR3, and optionally FR4) of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule can be chosen from: (a) a light or heavy chain variable framework including at least 80%, 85%, 87% 90%, 92%, 93%, 95%, 97%, 98%, or 100% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (b) a light or heavy chain variable framework including from 20% to 80%, 40% to 60%, 60% to 90%, or 70% to 95% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (c) a non-human framework (*e.g.*, a rodent framework); or (d) a non-human framework that has been modified, *e.g.*, to remove antigenic or cytotoxic determinants, *e.g.*, deimmunized, or partially humanized. In one embodiment, the light or heavy chain variable framework region (particularly FR1, FR2 and/or FR3) includes a light or heavy chain variable framework sequence at least 70, 75, 80, 85, 87, 88, 90, 92, 94, 95, 96, 97, 98, 99% identical or identical to the frameworks of a VL or VH segment of a human germline gene.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, comprises a heavy chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence described in Table 14 .*e.g.*, the amino acid sequence of the FR region in the entire variable region, *e.g.*, shown in **FIGs. 5A** and **5B**, or in SEQ ID NOs: 23-25.

Alternatively, or in combination with the heavy chain substitutions described herein the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more amino acid changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence of

an antibody described herein .*e.g.*, the amino acid sequence of the FR region in the entire variable region, *e.g.*, shown in **FIGs. 5A** and **5B,** or in SEQ ID NOs: 26-30.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes one, two, three, or four heavy chain framework regions shown in **FIG. 5A,** or a sequence substantially identical thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes one, two, three, or four light chain framework regions shown in **FIG. 5B,** or a sequence substantially identical thereto.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the light chain framework region 1 *e.g.*, as shown in **FIG. 5B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the light chain framework region 2 *e.g.*, as shown in **FIG. 5B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the light chain framework region 3, *e.g.*, as shown in **FIG. 5B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the light chain framework region 4, *e.g.*, as shown in **FIG. 5B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more, *e.g.*, all, position disclosed herein according to Kabat numbering. In some embodiments, FR1 comprises an Aspartic Acid at position 1, *e.g.*, a substitution at position 1 according to Kabat numbering, *e.g.*, an Alanine to Aspartic Acid substitution. In some embodiments, FR1 comprises an Asparagine at position 2, *e.g.*, a substitution at position 2 according to Kabat numbering, *e.g.*, an Isoleucine to Asparagine substitution, Serine to Asparagine substitution or Tyrosine to Asparagine substitution. In some embodiments, FR1 comprises a Leucine at position 4, *e.g.*, a substitution at position 4 according to Kabat numbering, *e.g.*, a Methionine to Leucine substitution.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a substitution at position 1 according to Kabat numbering, *e.g.*, an Alanine to

Aspartic Acid substitution, a substitution at position 2 according to Kabat numbering, *e.g.*, an Isoleucine to Asparagine substitution, Serine to Asparagine substitution or Tyrosine to Asparagine substitution, and a substitution at position 4 according to Kabat numbering, *e.g.*, a Methionine to Leucine substitution. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a substitution at position 1 according to Kabat numbering, *e.g.*, an Alanine to Aspartic Acid substitution, and a substitution at position 2 according to Kabat numbering, *e.g.*, an Isoleucine to Asparagine substitution, Serine to Asparagine substitution or Tyrosine to Asparagine substitution. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a substitution at position 1 according to Kabat numbering, *e.g.*, an Alanine to Aspartic Acid substitution, and a substitution at position 4 according to Kabat numbering, *e.g.*, a Methionine to Leucine substitution. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 1 (FR1), comprising a substitution at position 2 according to Kabat numbering, *e.g.*, an Isoleucine to Asparagine substitution, Serine to Asparagine substitution or Tyrosine to Asparagine substitution, and a substitution at position 4 according to Kabat numbering, *e.g.*, a Methionine to Leucine substitution. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more, *e.g.*, all, position disclosed herein according to Kabat numbering. In some embodiments, FR3 comprises a Glycine at position 66, *e.g.*, a substitution at position 66 according to Kabat numbering, *e.g.*, a Lysine to Glycine substitution, or a Serine to Glycine substitution. In some embodiments, FR3 comprises an Asparagine at position 69, *e.g.*, a substitution at position 69 according to Kabat numbering, *e.g.*, a Tyrosine to Asparagine substitution. In some embodiments, FR3 comprises a Tyrosine at position 71, *e.g.*, a substitution at position 71

according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, or an Alanine to Tyrosine substitution.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a substitution at position 66 according to Kabat numbering, *e.g.*, a Lysine to Glycine substitution, or a Serine to Glycine substitution, and a substitution at position 69 according to Kabat numbering, *e.g.*, a Tyrosine to Asparagine substitution. . In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a substitution at position 66 according to Kabat numbering, *e.g.*, Lysine to Glycine substitution, or a Serine to Glycine substitution, and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, or an Alanine to Tyrosine substitution. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a substitution at position 69 according to Kabat numbering, *e.g.*, a Tyrosine to Asparagine substitution and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, or an Alanine to Tyrosine substitution. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising a framework region, *e.g.*, framework region 3 (FR3), comprising a substitution at position 66 according to Kabat numbering, *e.g.*, a Lysine to Glycine substitution, or a Serine to Glycine substitution, a substitution at position 69 according to Kabat numbering, *e.g.*, a Tyrosine to Asparagine substitution and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, or an Alanine to Tyrosine substitution. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising: a framework region 1 (FR1) comprising a substitution at position 2 according to Kabat numbering, *e.g.*, a Isoleucine to Asparagine substitution; and a framework region 3 (FR3), comprising a substitution at position 69 according

to Kabat numbering, *e.g.*, a Threonine to Asparagine substitution and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 26. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising: (a) a framework region 1 (FR1) comprising a substitution at position 1 according to Kabat numbering, *e.g.*, a Alanine to Aspartic Acid substitution, and a substitution at position 2 according to Kabat numbering, *e.g.*, a Isoleucine to Asparagine substitution; and (b) a framework region 3 (FR3), comprising a substitution at position 69 according to Kabat numbering, *e.g.*, a Threonine to Asparagine substitution and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 27 In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising: (a) a framework region 1 (FR1) comprising a substitution at position 2 according to Kabat numbering, *e.g.*, a Serine to Asparagine substitution; and a substitution at position 4 according to Kabat numbering, *e.g.*, a Methionine to Leucine substitution; and (b) a framework region 3 (FR3), comprising a substitution at position 69 according to Kabat numbering, *e.g.*, a Threonine to Asparagine substitution and a substitution at position 71 according to Kabat numbering, *e.g.*, a Phenylalanine to Tyrosine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 28 In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising: (a) a framework region 1 (FR1) comprising a substitution at position 2 according to Kabat numbering, *e.g.*, a Serine to Asparagine substitution; and (b) a framework region 3 (FR3) comprising a substitution at position 66 according to Kabat numbering, *e.g.*, a Lysine to Glycine substitution; a substitution at position 69 according to Kabat numbering, *e.g.*, a Threonine to Asparagine substitution; and a substitution at position 71 according to Kabat numbering, *e.g.*, a Alanine to Tyrosine substitution, *e.g.*, as

shown in the amino acid sequence of SEQ ID NO: 29. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain comprising: (a) a framework region 1 (FR1) comprising a substitution at position 2 according to Kabat numbering, *e.g.*, a Tyrosine to Asparagine substitution; and (b) a framework region 3 (FR3) comprising a substitution at position 66 according to Kabat numbering, *e.g.*, a Serine to Glycine substitution; a substitution at position 69 according to Kabat numbering, *e.g.*, a Threonine to Asparagine substitution; and a substitution at position 71 according to Kabat numbering, *e.g.*, a Alanine to Tyrosine substitution, *e.g.*, as shown in the amino acid sequence of SEQ ID NO: 29. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises a light chain variable domain comprising: (a) a framework region 1 (FR1) comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more (*e.g.*, all) positions disclosed herein according to Kabat numbering, and (b) a framework region 3 (FR3) comprising a change, *e.g.*, a substitution (*e.g.*, a conservative substitution) at one or more (*e.g.*, all) position disclosed herein according to Kabat numbering. In some embodiments, the substitution is relative to a human germline light chain framework region sequence.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework region 1, *e.g.*, as shown in **FIG. 5A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework region 2, *e.g.*, as shown in **FIG. 5A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework region 3, *e.g.*, as shown in **FIG. 5A**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework region 4, *e.g.*, as shown in **FIG. 5A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework regions 1-4, *e.g.*, SEQ ID NOS: 20-23, or as shown in **FIG. 5A.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the light chain framework regions 1-4, *e.g.*, SEQ ID NOs: 26-30, or as shown in **FIG. 5B.**

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises the heavy chain framework regions 1-4, *e.g.*, SEQ ID NOs: 23-25; and the light chain framework regions 1-4, *e.g.*, SEQ ID NOs: 26-30, or as shown in **FIGs. 5A** and **5B.**

In some embodiments, the heavy or light chain variable domain, or both, of , the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes an amino acid sequence, which is substantially identical to an amino acid disclosed herein, *e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical to a variable region of an antibody described herein, *e.g.*, an antibody as described in **Table 14**, or encoded by the nucleotide sequence in **Table 14**; or which differs at least 1 or 5 residues, but less than 40, 30, 20, or 10 residues, from a variable region of an antibody described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises at least one, two, three, or four antigen-binding regions, *e.g.*, variable regions, having an amino acid sequence as set forth in **Table 14**, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the sequences shown in **Table 14**. In another embodiment, , the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule includes a VH and/or VL domain encoded by a nucleic acid having a nucleotide sequence as set forth in **Table 14**, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in **Table 14**.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising an amino acid sequence chosen from the amino acid sequence of SEQ ID NO: 23, SEQ ID NO:24 or SEQ ID NO:25, an amino acid sequence at least about

85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, SEQ ID NO:24 or SEQ ID NO:25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23, SEQ ID NO:24 or SEQ ID NO:25; and/or

a VL domain comprising an amino acid sequence chosen from the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 23, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 26, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 26, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 23, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 27, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ

ID NO: 27, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 23, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 28, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 28, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 28.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 23, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 29, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 29, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 29.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 23, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 23, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 23; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 30, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ

ID NO: 30, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 30.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 24, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 24, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 24; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 26, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 26, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 24, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 24, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 24; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 27, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 27, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 24, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 24, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 24; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 28, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 28, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 28.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 24, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 24, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 24; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 29, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 29, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 29.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 24, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 24, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 24; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 30, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 30, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 30.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 25, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ

ID NO: 25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 25; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 26, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 26, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 25, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 25; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 27, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 27, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 25, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 25; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 28, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 28, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 28.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 25, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ

ID NO: 25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 25; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 29, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 29, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 29.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule comprises:

a VH domain comprising the amino acid sequence of SEQ ID NO: 25, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 25, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 25; and

a VL domain comprising the amino acid sequence of SEQ ID NO: 30, an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to the amino acid sequence SEQ ID NO: 30, or an amino acid sequence which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the amino acid sequence of SEQ ID NO: 30.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule is a full antibody or fragment thereof (*e.g.*, a Fab, $F(ab')_2$, Fv, or a single chain Fv fragment (scFv)). In embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V6 (*e.g.*, anti-TCRβ V6-5*01) antibody molecule is a monoclonal antibody or an antibody with single specificity. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule, can also be a humanized, chimeric, camelid, shark, or an *in vitro*-generated antibody molecule. In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule is a humanized antibody molecule. The heavy and light chains of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule can be full-length (*e.g.*, an antibody can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or can include an antigen-binding fragment (*e.g.*, a Fab, F(ab')2, Fv, a single chain Fv fragment, a single domain antibody, a

diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody).

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule is in the form of a multispecific molecule, *e.g.*, a bispecific molecule, *e.g.*, as described herein.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule has a heavy chain constant region (Fc) chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. In some embodiments, the Fc region is chosen from the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In some embodiments, the Fc region is chosen from the heavy chain constant region of IgG1 or IgG2 (*e.g.*, human IgG1, or IgG2). In some embodiments, the heavy chain constant region is human IgG1.

In some embodiments, the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule has a light chain constant region chosen from, *e.g.*, the light chain constant regions of kappa or lambda, preferably kappa (*e.g.*, human kappa). In one embodiment, the constant region is altered, *e.g.,* mutated, to modify the properties of the anti-TCRβV antibody molecule, *e.g.*, anti-TCRβ V12 antibody molecule (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). For example, the constant region is mutated at positions 296 (M to Y), 298 (S to T), 300 (T to E), 477 (H to K) and 478 (N to F) to alter Fc receptor binding (*e.g.*, the mutated positions correspond to positions 132 (M to Y), 134 (S to T), 136 (T to E), 313 (H to K) and 314 (N to F) of SEQ ID NOs: 212 or 214; or positions 135 (M to Y), 137 (S to T), 139 (T to E), 316 (H to K) and 317 (N to F) of SEQ ID NOs: 215, 216, 217 or 218).

Table 14: Amino acid and nucleotide sequences for murine and humanized antibody molecules. The antibody molecules include murine mAb 16G8 and humanized mAb 16G8. The amino acid the heavy and light chain CDRs, and the amino acid and nucleotide sequences of the heavy and light chain variable regions, and the heavy and light chains are shown.

16G8 (murine)		
SEQ ID NO: 17	HC CDR1 (Combined)	GFTFSNFGMH

SEQ ID NO: 18	HC CDR2 (Combined)	YISSGSSTIYYADTLKG
SEQ ID NO: 19	HC CDR3 (Combined)	RGEGAMDY
SEQ ID NO: 57	HC CDR1 (Kabat)	NFGMH
SEQ ID NO: 58	HC CDR2 (Kabat)	YISSGSSTIYYADTLKG
SEQ ID NO: 59	HC CDR3 (Kabat)	RGEGAMDY
SEQ ID NO: 60	HC CDR1 (Chothia)	GFTFSNF
SEQ ID NO: 61	HC CDR2 (Chothia)	SSGSST
SEQ ID NO: 62	HC CDR3 (Chothia)	RGEGAMDY
SEQ ID NO: 15	VH	DVQLVESGGGLVQPGGSRKLSCAASGF TFSNFGMHWVRQAPDKGLEWVAYISS GSSTIYYADTLKGRFTISRDNPKNTLFL QMTSLRSEDTAMYYCARRGEGAMDY WGQGTSVTVSS
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT
SEQ ID NO: 63	LC CDR1 (Kabat)	RASSSVNYIY
SEQ ID NO: 64	LC CDR2 (Kabat)	YTSNLAP
SEQ ID NO: 65	LC CDR3 (Kabat)	QQFTSSPFT
SEQ ID NO: 66	LC CDR1 (Chothia)	RASSSVNYIY
SEQ ID NO: 67	LC CDR2 (Chothia)	YTSNLAP
SEQ ID NO: 68	LC CDR3 (Chothia)	QQFTSSPFT
SEQ ID NO: 16	VL	ENVLTQSPAIMSASLGEKVTMSCRASSS VNYIYWYQQKSDASPKLWIYYTSNLAP GVPTRFSGSGSGNSYSLTISSMEGEDAA TYYCQQFTSSPFTFGSGTKLEIK
16G8 humanized HC-1		
SEQ ID NO: 17	HC CDR1 (Combined)	GFTFSNFGMH

SEQ ID NO: 18	HC CDR2 (Combined)	YISSGSSTIYYADTLKG
SEQ ID NO: 19	HC CDR3 (Combined)	RGEGAMDY
SEQ ID NO: 23	VH	EVQLVESGGGLVQPGGSLRLSCAASGF TFSNFGMHWVRQAPGKGLEWVSYISSG SSTIYYADTLKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARRGEGAMDYW GQGTTVTVSS
SEQ ID NO: 31	DNA VH	GAGGTGCAGCTGGTTGAATCTGGCGG AGGATTGGTTCAGCCTGGCGGCTCTCT GAGACTGTCTTGTGCCGCTTCTGGCTT CACCTTCTCCAACTTCGGCATGCACTG GGTCCGACAGGCCCCTGGAAAAGGAC TGGAATGGGTGTCCTACATCTCCTCCG GCTCCTCCACCATCTACTACGCTGACA CCCTGAAGGGCAGATTCACCATCTCT CGGGACAACGCCAAGAACTCCCTGTA CCTGCAGATGAACAGCCTGAGAGCCG AGGACACCGCCGTGTACTACTGTGCT AGAAGAGGCGAGGGCGCCATGGATTA TTGGGGCCAGGGAACCACAGTGACCG TGTCTAGC
16G8 humanized HC-2		
SEQ ID NO: 17	HC CDR1 (Combined)	GFTFSNFGMH
SEQ ID NO: 18	HC CDR2 (Combined)	YISSGSSTIYYADTLKG
SEQ ID NO: 19	HC CDR3 (Combined)	RGEGAMDY
SEQ ID NO: 24	VH	EVQLVESGGGLVQPGGSLRLSCAASGF TFSNFGMHWVRQAPGKGLEWVSYISSG SSTIYYADTLKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARRGEGAMDYW GQGTTVTVSS
SEQ ID NO: 32	DNA VH	GAGGTGCAGCTGGTTGAATCTGGCGG AGGATTGGTTCAGCCTGGCGGCTCTCT GAGACTGTCTTGTGCCGCTTCTGGCTT CACCTTCTCCAACTTCGGCATGCACTG GGTCCGACAGGCCCCTGGAAAAGGAC TGGAATGGGTGTCCTACATCTCCTCCG GCTCCTCCACCATCTACTACGCTGACA

		CCCTGAAGGGCAGATTCACCATCAGC CGGGACAACTCCAAGAACACCCTGTA CCTGCAGATGAACTCCCTGAGAGCCG AGGACACCGCCGTGTACTACTGTGCT AGAAGAGGCGAGGGCGCCATGGATTA TTGGGGCCAGGGAACCACAGTGACCG TGTCTAGC
16G8 humanized HC-3		
SEQ ID NO: 17	HC CDR1 (Combined)	GFTFSNFGMH
SEQ ID NO: 18	HC CDR2 (Combined)	YISSGSSTIYYADTLKG
SEQ ID NO: 19	HC CDR3 (Combined)	RGEGAMDY
SEQ ID NO: 25	VH	QVQLVESGGGVVQPGRSLRLSCAASGF TFSNFGMHWVRQAPGKGLEWVAYISS GSSTIYYADTLKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCARRGEGAMDY WGQGTTVTVSS
SEQ ID NO: 33	DNA VH	CAGGTGCAGCTGGTGGAATCTGGTGG CGGAGTTGTGCAGCCTGGCAGATCCC TGAGACTGTCTTGTGCCGCCTCTGGCT TCACCTTCTCCAACTTCGGCATGCACT GGGTCCGACAGGCCCCTGGAAAAGGA TTGGAGTGGGTCGCCTACATCTCCTCC GGCTCCTCCACCATCTACTACGCTGAC ACCCTGAAGGGCAGATTCACCATCAG CCGGGACAACTCCAAGAACACCCTGT ACCTGCAGATGAACTCCCTGAGAGCC GAGGACACCGCCGTGTACTACTGTGC TAGAAGAGGCGAGGGCGCCATGGATT ATTGGGGCCAGGGAACCACAGTGACC GTGTCTAGC
16G8 humanized LC-1		
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT

SEQ ID NO: 26	VL	DNQLTQSPSFLSASVGDRVTITCRASSS VNYIYWYQQKPGKAPKLLIYYTSNLAP GVPSRFSGSGSGNEYTLTISSLQPEDFAT YYCQQFTSSPFTFGQGTKLEIK
SEQ ID NO: 34	DNA VL	GATAACCAGCTGACCCAGTCTCCTAG CTTCCTGTCTGCCTCTGTGGGCGACAG AGTGACAATTACCTGCCGGGCCTCCT CCTCCGTGAACTACATCTACTGGTATC AGCAGAAGCCCGGCAAGGCCCCTAAG CTGCTGATCTACTACACCTCCAATCTG GCCCCTGGCGTGCCCTCTAGATTTTCC GGATCTGGCTCCGGCAACGAGTATAC CCTGACAATCTCCAGCCTGCAGCCTG AGGACTTCGCCACCTACTACTGCCAG CAGTTCACCTCCTCTCCATTCACCTTT GGCCAGGGCACCAAGCTGGAAATCAA A
16G8 humanized LC-2		
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT
SEQ ID NO: 27	VL	DNQLTQSPSSLSASVGDRVTITCRASSS VNYIYWYQQKPGKAPKLLIYYTSNLAP GVPSRFSGSGSGNDYTLTISSLQPEDFAT YYCQQFTSSPFTFGQGTKLEIK
SEQ ID NO: 35	DNA VL	ATAACCAGCTGACCCAGTCTCCTTCCA GCCTGTCTGCTTCTGTGGGCGACAGA GTGACAATTACCTGCCGGGCCTCCTCC TCCGTGAACTACATCTACTGGTATCAG CAGAAGCCCGGCAAGGCCCCTAAGCT GCTGATCTACTACACCTCCAATCTGGC CCCTGGCGTGCCCTCTAGATTTTCCGG ATCTGGCTCCGGCAACGACTATACCC TGACAATCTCCAGCCTGCAGCCTGAG GACTTCGCCACCTACTACTGCCAGCA GTTCACCTCCTCTCCATTCACCTTTGG CCAGGGCACCAAGCTGGAAATCAAA

16G8 humanized LC-3		
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT
SEQ ID NO: 28	VL	ENVLTQSPATLSVSPGERATLSCRASSS VNYIYWYQQKPGQAPRLLIYYTSNLAP GIPARFSGSGSGNEYTLTISSLQSEDFAV YYCQQFTSSPFTFGQGTKLEIK
SEQ ID NO: 36	DNA VL	GAGAATGTGCTGACCCAGTCTCCTGC CACACTGTCTGTTAGCCCTGGCGAGA GAGCTACCCTGAGCTGCAGAGCCTCT TCCTCCGTGAACTACATCTACTGGTAT CAGCAGAAGCCCGGCCAGGCTCCTAG ACTGCTGATCTACTACACCTCCAATCT GGCCCCTGGCATCCCTGCCAGATTTTC CGGATCTGGCTCCGGCAACGAGTATA CCCTGACCATCTCCAGCCTGCAGTCCG AGGACTTTGCTGTGTACTATTGCCAGC AGTTCACAAGCAGCCCTTTCACCTTTG GCCAGGGCACCAAGCTGGAAATCAAA
16G8 humanized LC-4		
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT
SEQ ID NO: 29	VL	QNVLTQPPSASGTPGQRVTISCRASSSV NYIYWYQQLPGTAPKLLIYYTSNLAPG VPDRFSGSGSGNSYSLAISGLRSEDEAD YYCQQFTSSPFTFGTGTKVTVL
SEQ ID NO: 37	DNA VL	CAGAATGTGCTGACCCAACCTCCTTCC GCCTCTGGCACACCTGGACAGAGAGT GACAATCTCCTGCCGGGCCTCCTCCTC CGTGAACTACATCTACTGGTATCAGC AGCTGCCCGGCACCGCTCCTAAACTG CTGATCTACTACACCTCCAATCTGGCC CCTGGCGTGCCCGATAGATTTTCCGG

		ATCTGGCTCCGGCAACTCCTACAGCCT GGCTATCTCTGGCCTGAGATCTGAGG ACGAGGCCGACTACTACTGCCAGCAG TTCACCTCCTCTCCATTCACCTTTGGC ACCGGCACCAAAGTGACAGTTCTT
16G8 humanized LC-5		
SEQ ID NO: 20	LC CDR1 (Combined)	RASSSVNYIY
SEQ ID NO: 21	LC CDR2 (Combined))	YTSNLAP
SEQ ID NO: 22	LC CDR3(Combined)	QQFTSSPFT
SEQ ID NO: 30	VL	SNELTQPPSVSVSPGQTARITCRASSSVN YIYWYQQKSGQAPVLVIYYTSNLAPGIP ERFSGSGSGNMYTLTISGAQVEDEADY YCQQFTSSPFTFGTGTKVTVL
SEQ ID NO: 38	DNA VL	TCTAATGAGCTGACCCAGCCTCCTTCC GTGTCCGTGTCTCCTGGACAGACCGC CAGAATTACCTGCCGGGCCTCCTCCTC CGTGAACTACATCTACTGGTATCAGC AGAAGTCCGGCCAGGCTCCTGTGCTC GTGATCTACTACACCTCCAATCTGGCC CCTGGCATCCCTGAGAGATTCTCCGG ATCTGGCTCCGGCAACATGTACACCC TGACCATCTCTGGCGCCCAGGTGGAA GATGAGGCCGACTACTACTGCCAGCA GTTCACCTCCTCTCCATTCACCTTTGG CACCGGCACCAAAGTGACAGTTCTT

Table 17. Constant region amino acid sequences of human IgG heavy chains and human kappa light chain

Human kappa constant region SEQ ID NO: 39	LC	RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
IgG4 (S228P) mutant constant region (EU	HC	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV

Numbering) SEQ ID NO: 40		SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLG
IgG1 wild type SEQ ID NO: 41	HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
IgG1 (N297A) mutant constant region (EU Numbering) SEQ ID NO: 42	HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK

B Cell, Macrophage & Dendritic Cell Engagers

Broadly, B cells, also known as B lymphocytes, are a type of white blood cell of the lymphocyte subtype. They function in the humoral immunity component of the adaptive immune system by secreting antibodies. Additionally, B cells present antigen (they are also classified as professional antigen-presenting cells (APCs)) and secrete cytokines. Macrophages are a type of white blood cell that engulfs and digests cellular debris, foreign substances, microbes, cancer cells via phagocytosis. Besides phagocytosis, they play important roles in nonspecific defense (innate immunity) and also help initiate specific defense mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes. For example, they are important as antigen presenters to T cells. Beyond increasing inflammation and stimulating the immune system, macrophages also play an important anti-inflammatory role and can decrease immune reactions through the release of cytokines. Dendritic cells (DCs) are antigen-presenting cells that function

in processing antigen material and present it on the cell surface to the T cells of the immune system.

The present disclosure provides, *inter alia*, multispecific (e.g., bi-, tri-, quad- specific) or multifunctional molecules, that include, e.g., are engineered to contain, one or more B cell, macrophage, and/or dendritic cell engager that mediate binding to and/ or activation of a B cell, macrophage, and/or dendritic cell.

Accordingly, in some embodiments, the immune cell engager comprises a B cell, macrophage, and/or dendritic cell engager chosen from one or more of CD40 ligand (CD40L) or a CD70 ligand; an antibody molecule that binds to CD40 or CD70; an antibody molecule to OX40; an OX40 ligand (OX40L); an agonist of a Toll-like receptor (e.g., as described herein, e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4), or a TLR9 agonists); a 41BB; a CD2; a CD47; or a STING agonist, or a combination thereof.

In some embodiments, the B cell engager is a CD40L, an OX40L, or a CD70 ligand, or an antibody molecule that binds to OX40, CD40 or CD70.

In some embodiments, the macrophage engager is a CD2 agonist. In some embodiments, the macrophage engager is an antigen binding domain that binds to: CD40L or antigen binding domain or ligand that binds CD40, a Toll like receptor (TLR) agonist (e.g., as described herein), e.g., a TLR9 or TLR4 (e.g., caTLR4 (constitutively active TLR4), CD47, or a STING agonist. In some embodiments, the STING agonist is a cyclic dinucleotide, e.g., cyclic di-GMP (cdGMP) or cyclic di-AMP (cdAMP). In some embodiments, the STING agonist is biotinylated.

In some embodiments, the dendritic cell engager is a CD2 agonist. In some embodiments, the dendritic cell engager is a ligand, a receptor agonist, or an antibody molecule that binds to one or more of: OX40L, 41BB, a TLR agonist (e.g., as described herein) (e.g., TLR9 agonist, TLR4 (e.g., caTLR4 (constitutively active TLR4)), CD47, or and a STING agonist. In some embodiments, the STING agonist is a cyclic dinucleotide, e.g., cyclic di-GMP (cdGMP) or cyclic di-AMP (cdAMP). In some embodiments, the STING agonist is biotinylated.

In other embodiments, the immune cell engager mediates binding to, or activation of, one or more of a B cell, a macrophage, and/or a dendritic cell. Exemplary B cell, macrophage, and/or dendritic cell engagers can be chosen from one or more of CD40 ligand (CD40L) or a

CD70 ligand; an antibody molecule that binds to CD40 or CD70; an antibody molecule to OX40; an OX40 ligand (OX40L); a Toll-like receptor agonist (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); a 41BB agonist; a CD2; a CD47; or a STING agonist, or a combination thereof.

In some embodiments, the B cell engager is chosen from one or more of a CD40L, an OX40L, or a CD70 ligand, or an antibody molecule that binds to OX40, CD40 or CD70.

In other embodiments, the macrophage cell engager is chosen from one or more of a CD2 agonist; a CD40L; an OX40L; an antibody molecule that binds to OX40, CD40 or CD70; a Toll-like receptor agonist or a fragment thereof (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4)); a CD47 agonist; or a STING agonist.

In other embodiments, the dendritic cell engager is chosen from one or more of a CD2 agonist, an OX40 antibody, an OX40L, 41BB agonist, a Toll-like receptor agonist or a fragment thereof (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4)), CD47 agonist, or a STING agonist.

In one embodiment, the OX40L comprises the amino acid sequence: QVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCDGFYLISLKGYFSQ EVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDNTSLDDFHVNGGE LILIHQNPGEFCVL (SEQ ID NO: 7245), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7245.

In another embodiment, the CD40L comprises the amino acid sequence: MQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTVKRQGLY YIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFE LQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ ID NO: 7246), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7246.

In yet other embodiments, the STING agonist comprises a cyclic dinucleotide, e.g., a cyclic di-GMP (cdGMP), a cyclic di-AMP (cdAMP), or a combination thereof, optionally with 2',5' or 3',5' phosphate linkages.

In one embodiment, the immune cell engager includes 41BB ligand, e.g., comprising the amino acid sequence:
ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLS
WYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALH
LQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARH
AWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 7247), a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7247.

Toll-Like Receptors

Toll-Like Receptors (TLRs) are evolutionarily conserved receptors are homologues of the Drosophila Toll protein, and recognize highly conserved structural motifs known as pathogen-associated microbial patterns (PAMPs), which are exclusively expressed by microbial pathogens, or danger-associated molecular patterns (DAMPs) that are endogenous molecules released from necrotic or dying cells. PAMPs include various bacterial cell wall components such as lipopolysaccharide (LPS), peptidoglycan (PGN) and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. DAMPs include intracellular proteins such as heat shock proteins as well as protein fragments from the extracellular matrix. Stimulation of TLRs by the corresponding PAMPs or DAMPs initiates signaling cascades leading to the activation of transcription factors, such as AP-1, NF-κB and interferon regulatory factors (IRFs). Signaling by TLRs results in a variety of cellular responses, including the production of interferons (IFNs), pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response. TLRs are implicated in a number of inflammatory and immune disorders and play a role in cancer (Rakoff-Nahoum S. & Medzhitov R., 2009. Toll-like receptors and cancer. Nat Revs Cancer 9:57- 63.)

TLRs are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats (LRRs) and a cytoplasmic tail that contains a conserved region called the Toll/IL-1 receptor (TIR) domain. Ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. TLR2 is essential for the recognition of a variety of PAMPs from Gram-positive bacteria, including bacterial lipoproteins, lipomannans and lipoteichoic acids. TLR3 is implicated in virus-derived double-stranded RNA. TLR4 is predominantly activated by lipopolysaccharide. TLR5 detects bacterial flagellin and TLR9 is required for response to unmethylated CpG DNA. Finally, TLR7 and TLR8 recognize small synthetic antiviral molecules, and single-stranded RNA was reported to be their natural ligand. TLR11 has been reported to recognize uropathogenic E.coli and a profilin-like protein from Toxoplasma gondii. The repertoire of specificities of the TLRs is apparently extended by the ability of TLRs to heterodimerize with one another. For example, dimers of TLR2 and TLR6 are required for responses to diacylated lipoproteins while TLR2 and TLR1 interact to recognize triacylated lipoproteins. Specificities of the TLRs are also influenced by various adapter and accessory molecules, such as MD-2 and CD14 that form a complex with TLR4 in response to LPS.

TLR signaling consists of at least two distinct pathways: a MyD88-dependent pathway that leads to the production of inflammatory cytokines, and a MyD88-independent pathway associated with the stimulation of IFN-β and the maturation of dendritic cells. The MyD88-dependent pathway is common to all TLRs, except TLR3 (Adachi O. et al., 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity. 9(1):143-50). Upon activation by PAMPs or DAMPs, TLRs hetero- or homodimerize inducing the recruitment of adaptor proteins via the cytoplasmic TIR domain. Individual TLRs induce different signaling responses by usage of the different adaptor molecules. TLR4 and TLR2 signaling requires the adaptor TIRAP/Mal, which is involved in the MyD88-dependent pathway. TLR3 triggers the production of IFN-β in response to double-stranded RNA, in a MyD88-independent manner, through the adaptor TRIF/TICAM-1. TRAM/TICAM-2 is another adaptor molecule involved in the MyD88-independent pathway which function is restricted to the TLR4 pathway.

TLR3, TLR7, TLR8 and TLR9 recognize viral nucleic acids and induce type I IFNs. The signaling mechanisms leading to the induction of type I IFNs differ depending on the TLR activated. They involve the interferon regulatory factors, IRFs, a family of transcription factors known to play a critical role in antiviral defense, cell growth and immune regulation. Three IRFs (IRF3, IRF5 and IRF7) function as direct transducers of virus-mediated TLR signaling. TLR3 and TLR4 activate IRF3 and IRF7, while TLR7 and TLR8 activate IRF5 and IRF7 (Doyle S. et al., 2002. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity. 17(3):251-63). Furthermore, type I IFN production stimulated by TLR9 ligand CpG-A has been shown to be mediated by PI(3)K and mTOR (Costa-Mattioli M. & Sonenberg N. 2008. RAPping production of type I interferon in pDCs through mTOR. Nature Immunol. 9: 1097-1099).

TLR-9

TLR9 recognizes unmethylated CpG sequences in DNA molecules. CpG sites are relatively rare (~1%) on vertebrate genomes in comparison to bacterial genomes or viral DNA. TLR9 is expressed by numerous cells of the immune system such as B lymphocytes, monocytes, natural killer (NK) cells, and plasmacytoid dendritic cells. TLR9 is expressed intracellularly, within the endosomal compartments and functions to alert the immune system of viral and bacterial infections by binding to DNA rich in CpG motifs. TLR9 signals leads to activation of the cells initiating pro-inflammatory reactions that result in the production of cytokines such as type-I interferon and IL-12.

TLR Agonists

A TLR agonist can agonize one or more TLR, e.g., one or more of human TLR- 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, an adjunctive agent described herein is a TLR agonist. In some embodiments, the TLR agonist specifically agonizes human TLR-9. In some embodiments, the TLR-9 agonist is a CpG moiety. As used herein, a CpG moiety, is a linear dinucleotide having the sequence: 5'—C—phosphate—G—3', that is, cytosine and guanine separated by only one phosphate.

In some embodiments, the CpG moiety comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more CpG

dinucleotides. In some embodiments, the CpG moiety consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 CpG dinucleotides. In some embodiments, the CpG moiety has 1-5, 1-10, 1-20, 1-30, 1-40, 1-50, 5-10, 5-20, 5-30, 10-20, 10-30, 10-40, or 10-50 CpG dinucleotides.

In some embodiments, the TLR-9 agonist is a synthetic ODN (oligodeoxynucleotides). CpG ODNs are short synthetic single-stranded DNA molecules containing unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs). CpG ODNs possess a partially or completely phosphorothioated (PS) backbone, as opposed to the natural phosphodiester (PO) backbone found in genomic bacterial DNA. There are three major classes of CpG ODNs: classes A, B and C, which differ in their immunostimulatory activities. CpG-A ODNs are characterized by a PO central CpG-containing palindromic motif and a PS-modified 3' poly-G string. They induce high IFN-α production from pDCs but are weak stimulators of TLR9-dependent NF-κB signaling and pro-inflammatory cytokine (e.g. IL-6) production. CpG-B ODNs contain a full PS backbone with one or more CpG dinucleotides. They strongly activate B cells and TLR9-dependent NF-κB signaling but weakly stimulate IFN-α secretion. CpG-C ODNs combine features of both classes A and B. They contain a complete PS backbone and a CpG-containing palindromic motif. C-Class CpG ODNs induce strong IFN-α production from pDC as well as B cell stimulation.

Stromal Modifying Moieties

Solid tumors have a distinct structure that mimics that of normal tissues and comprises two distinct but interdependent compartments: the parenchyma (neoplastic cells) and the stroma that the neoplastic cells induce and in which they are dispersed. All tumors have stroma and require stroma for nutritional support and for the removal of waste products. In the case of tumors which grow as cell suspensions (e.g., leukemias, ascites tumors), the blood plasma serves as stroma (Connolly JL et al. Tumor Structure and Tumor Stroma Generation. In: Kufe DW et al., editors. Holland-Frei *Cancer Medicine*. 6th edition. Hamilton: BC Decker; 2003). The stroma includes a variety of cell types, including fibroblasts/myofibroblasts, glial, epithelial, fat, vascular, smooth muscle, and immune cells along with extracellular matrix (ECM) and

extracellular molecules (Li Hanchen et al. Tumor Microenvironment: The Role of the Tumor Stroma in Cancer. *J of Cellular Biochemistry* 101: 805-815 (2007)).

Stromal modifying moieties described herein include moieties (e.g., proteins, e.g., enzymes) capable of degrading a component of the stroma, e.g., an ECM component, e.g., a glycosaminoglycan, e.g., hyaluronan (also known as hyaluronic acid or HA), chondroitin sulfate, chondroitin, dermatan sulfate, heparin sulfate, heparin, entactin, tenascin, aggrecan and keratin sulfate; or an extracellular protein, e.g., collagen, laminin, elastin, fibrinogen, fibronectin, and vitronectin.

Stromal Modifying Enzymes

In some embodiments, the stromal modifying moiety is an enzyme. For example, the stromal modifying moiety can include, but is not limited to a hyaluronidase, a collagenase, a chondroitinase, a matrix metalloproteinase (e.g., macrophage metalloelastase).

Hyaluronidases

Hyaluronidases are a group of neutral- and acid-active enzymes found throughout the animal kingdom. Hyaluronidases vary with respect to substrate specificity, and mechanism of action. There are three general classes of hyaluronidases: (1) Mammalian-type hyaluronidases, (EC 3.2.1.35) which are endo-beta-N-acetylhexosaminidases with tetrasaccharides and hexasaccharides as the major end products. They have both hydrolytic and transglycosidase activities, and can degrade hyaluronan and chondroitin sulfates; (2) Bacterial hyaluronidases (EC 4.2.99.1) degrade hyaluronan and, and to various extents, chondroitin sulfate and dermatan sulfate. They are endo-beta-N-acetylhexosaminidases that operate by a beta elimination reaction that yields primarily disaccharide end products; (3) Hyaluronidases (EC 3.2.1.36) from leeches, other parasites, and crustaceans are endo-beta-glucuronidases that generate tetrasaccharide and hexasaccharide end products through hydrolysis of the beta 1-3 linkage.

Mammalian hyaluronidases can be further divided into two groups: (1) neutral active and (2) acid active enzymes. There are six hyaluronidase-like genes in the human genome, HYAL1, HYAL2, HYAL3 HYAL4 HYALP1 and PH20/SPAM1. HYALP1 is a pseudogene, and HYAL3 has not been shown to possess enzyme activity toward any known substrates. HYAL4 is a

chondroitinase and lacks activity towards hyaluronan. HYAL1 is the prototypical acid-active enzyme and PH20 is the prototypical neutral-active enzyme. Acid active hyaluronidases, such as HYAL1 and HYAL2 lack catalytic activity at neutral pH. For example, HYAL1 has no catalytic activity in vitro over pH 4.5 (Frost and Stern, "A Microtiter-Based Assay for Hyaluronidase Activity Not Requiring Specialized Reagents", Analytical Biochemistry, vol. 251, pp. 263-269 (1997). HYAL2 is an acid active enzyme with a very low specific activity in vitro.

In some embodiments the hyaluronidase is a mammalian hyaluronidase. In some embodiments the hyaluronidase is a recombinant human hyaluronidase. In some embodiments, the hyaluronidase is a neutral active hyaluronidase. In some embodiments, the hyaluronidase is a neutral active soluble hyaluronidase. In some embodiments, the hyaluronidase is a recombinant PH20 neutral-active enzyme. In some embodiments, the hyaluronidase is a recombinant PH20 neutral-active soluble enzyme. In some embodiments the hyaluronidase is glycosylated. In some embodiments, the hyaluronidase possesses at least one N-linked glycan. A recombinant hyaluronidase can be produced using conventional methods known to those of skill in the art, e.g., US7767429, the entire contents of which are incorporated by reference herein.

In some embodiments the hyaluronidase is rHuPH20 (also referred to as Hylenex®; presently manufactured by Halozyme; approved by the FDA in 2005 (*see e.g.,* Scodeller P (2014) Hyaluronidase and other Extracellular Matrix Degrading Enzymes for Cancer Therapy: New Uses and Nano- Formulations. *J Carcinog Mutage* 5:178; US7767429; US8202517; US7431380; US8450470; US8772246; US8580252, the entire contents of each of which is incorporated by reference herein). rHuPH20 is produced by genetically engineered CHO cells containing a DNA plasmid encoding for a soluble fragment of human hyaluronidase PH20. In some embodiments the hyaluronidase is glycosylated. In some embodiments, the hyaluronidase possesses at least one N-linked glycan. A recombinant hyaluronidase can be produced using conventional methods known to those of skill in the art, e.g., US7767429, the entire contents of which are incorporated by reference herein. In some embodiments, rHuPH20 has a sequence at least 95% (e.g., at least 96%, 97%, 98%, 99%, 100%) identical to the amino acid sequence of LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRL GYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTW ARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRP

NHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQS
PVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVA
LGASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRK
NWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADV
KDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTLS (SEQ ID NO: 7248).

In any of the methods provided herein, the anti-hyaluronan agent can be an agent that degrades hyaluronan or can be an agent that inhibits the synthesis of hyaluronan. For example, the anti-hyaluronan agent can be a hyaluronan degrading enzyme. In another example, the anti-hyaluronan agent or is an agent that inhibits hyaluronan synthesis. For example, the anti-hyaluronan agent is an agent that inhibits hyaluronan synthesis such as a sense or antisense nucleic acid molecule against an HA synthase or is a small molecule drug. For example, an anti-hyaluronan agent is 4- methylumbelliferone (MU) or a derivative thereof, or leflunomide or a derivative thereof. Such derivatives include, for example, a derivative of 4-methylumbelliferone (MU) that is 6,7-dihydroxy-4-methyl coumarin or 5,7-dihydroxy-4-methyl coumarin.

In further examples of the methods provided herein, the hyaluronan degrading enzyme is a hyaluronidase. In some examples, the hyaluronan-degrading enzyme is a PH20 hyaluronidase or truncated form thereof to lacking a C-terminal glycosylphosphatidylinositol (GPI) attachment site or a portion of the GPI attachment site. In specific examples, the hyaluronidase is a PH20 selected from a human, monkey, bovine, ovine, rat, mouse or guinea pig PH20. For example, the hyaluronan- degrading enzyme is a human PH20 hyaluronidase that is neutral active and N-glycosylated and is selected from among (a) a hyaluronidase polypeptide that is a full- length PH20 or is a C-terminal truncated form of the PH20, wherein the truncated form includes at least amino acid residues 36-464 of SEQ ID NO: 7248, such as 36-481 , 36-482, 36-483, where the full-length PH20 has the sequence of amino acids set forth in SEQ ID NO: 7248; or (b) a hyaluronidase polypeptide comprising a sequence of amino acids having at least 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with the polypeptide or truncated form of sequence of amino acids set forth in SEQ ID NO: 7248; or (c) a hyaluronidase polypeptide of (a) or (b) comprising amino acid substitutions, whereby the hyaluronidase polypeptide has a sequence of amino acids having at least 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %,

99 % or more sequence identity with the polypeptide set forth in SEQ ID NO: 7248 or the with the corresponding truncated forms thereof. In exemplary examples, the hyaluronan- degrading enzyme is a PH20 that comprises a composition designated rHuPH20.

In other examples, the anti-hyaluronan agent is a hyaluronan degrading enzyme that is modified by conjugation to a polymer. The polymer can be a PEG and the anti-hyaluronan agent a PEGylated hyaluronan degrading enzyme. Hence, in some examples of the methods provided herein the hyaluronan-degrading enzyme is modified by conjugation to a polymer. For example, the hyaluronan-degrading enzyme is conjugated to a PEG, thus the hyaluronan degrading enzyme is PEGylated. In an exemplary example, the hyaluronan-degrading enzyme is a PEGylated PH20 enzyme (PEGPH20). In the methods provided herein, the corticosteroid can be a glucocorticoid that is selected from among cortisones, dexamethasones, hydrocortisones, methylprednisolones, prednisolones and prednisones.

Chondroitinases

Chondroitinases are enzymes found throughout the animal kingdom which degrade glycosaminoglycans, specifically chondroitins and chondroitin sulfates, through an endoglycosidase reaction. In some embodiments the chondroitinase is a mammalian chondroitinase. In some embodiments the chondroitinase is a recombinant human chondroitinase. In some embodiments the chondroitinase is HYAL4. Other exemplary chondroitinases include chondroitinase ABC (derived from Proteus vulgaris; Japanese Patent Application Laid-open No 6-153947, T. Yamagata et al. J. Biol. Chem., 243, 1523 (1968), S. Suzuki et al, J. Biol. Chem., 243, 1543 (1968)), chondroitinase AC (derived from Flavobacterium heparinum; T. Yamagata et al., J. Biol. Chem., 243, 1523 (1968)), chondroitinase AC II (derived from Arthrobacter aurescens; K. Hiyama, and S. Okada, J. Biol. Chem., 250, 1824 (1975), K. Hiyama and S. Okada, J. Biochem. (Tokyo), 80, 1201 (1976)), Hyaluronidase ACIII (derived from Flavobacterium sp. Hp102; Hirofumi Miyazono et al., Seikagaku, 61, 1023 (1989)), chondroitinase B (derived from Flavobacterium heparinum; Y. M. Michelacci and C. P. Dietrich, Biochem. Biophys. Res. Commun., 56, 973 (1974), Y. M. Michelacci and C. P. Dietrich, Biochem. J., 151, 121 (1975), Kenichi Maeyama et al,

Seikagaku, 57, 1189 (1985)), chondroitinase C (derived from Flavobacterium sp. Hp102; Hirofumi Miyazono et al, Seikagaku, 61, 1023 (1939)), and the like.

Matrix Metalloproteinases

Matrix metalloproteases (MMPs) are zinc-dependent endopeptidases that are the major proteases involved in extracellular matrix (ECM) degradation. MMPs are capable of degrading a wide range of extracellular molecules and a number of bioactive molecules. Twenty-four MMP genes have been identified in humans, which can be organized into six groups based on domain organization and substrate preference: Collagenases (MMP-1, -8 and -13), Gelatinases (MMP-2 and MMP-9), Stromelysins (MMP-3, -10 and -11), Matrilysin (MMP-7 and MMP-26), Membrane-type (MT)-MMPs (MMP-14, -15, -16, -17, -24 and -25) and others (MMP-12, -19, -20, -21, -23, -27 and -28). In some embodiments, the stromal modifying moiety is a human recombinant MMP (e.g., MMP -1, -2, -3, -4, -5, -6, -7, -8, -9, 10, -11, -12, -13, -14, 15, -15, -17, -18, -19, 20, -21, -22, -23, or -24).

Collagenases

The three mammalian collagenases (MMP-1, -8, and -13) are the principal secreted endopeptidases capable of cleaving collagenous extracellular matrix. In addition to fibrillar collagens, collagenases can cleave several other matrix and non-matrix proteins including growth factors. Collagenases are synthesized as inactive pro-forms, and once activated, their activity is inhibited by specific tissue inhibitors of metalloproteinases, TIMPs, as well as by non-specific proteinase inhibitors (Ala-aho R et al. *Biochimie*. Collagenases in cancer. 2005 Mar-Apr;87(3-4):273-86). In some embodiments, the stromal modifying moiety is a collagenase. In some embodiments, the collagenase is a human recombinant collagenase. In some embodiments, the collagenase is MMP-1. In some embodiments, the collagenase is MMP-8. In some embodiments, the collagenase is MMP-13.

Macrophage metalloelastase

Macrophage metalloelastase (MME), also known as MMP-12, is a member of the stromelysin subgroup of MMPs and catalyzes the hydrolysis of soluble and insoluble elastin and

a broad selection of matrix and nonmatrix substrates including type IV collagen, fibronectin, laminin, vitronectin, entactin, heparan, and chondroitin sulfates (Erja Kerkelä et al. Journal of Investigative Dermatology (2000) 114, 1113–1119; doi:10.1046/j.1523-1747.2000.00993). In some embodiments, the stromal modifying moiety is a MME. In some embodiments, the MME is a human recombinant MME. In some embodiments, the MME is MMP-12.

Additional stromal modifying moieties

In some embodiments, the stromal modifying moiety causes one or more of: decreases the level or production of a stromal or extracellular matrix (ECM) component; decreases tumor fibrosis; increases interstitial tumor transport; improves tumor perfusion; expands the tumor microvasculature; decreases interstitial fluid pressure (IFP) in a tumor; or decreases or enhances penetration or diffusion of an agent, e.g., a cancer therapeutic or a cellular therapy, into a tumor or tumor vasculature.

In some embodiments, the stromal or ECM component decreased is chosen from a glycosaminoglycan or an extracellular protein, or a combination thereof. In some embodiments, the glycosaminoglycan is chosen from hyaluronan (also known as hyaluronic acid or HA), chondroitin sulfate, chondroitin, dermatan sulfate, heparin, heparin sulfate, entactin, tenascin, aggrecan and keratin sulfate. In some embodiments, the extracellular protein is chosen from collagen, laminin, elastin, fibrinogen, fibronectin, or vitronectin. In some embodiments, the stromal modifying moiety includes an enzyme molecule that degrades a tumor stroma or extracellular matrix (ECM). In some embodiments, the enzyme molecule is chosen from a hyaluronidase molecule, a collagenase molecule, a chondroitinase molecule, a matrix metalloproteinase molecule (e.g., macrophage metalloelastase), or a variant (e.g., a fragment) of any of the aforesaid. The term "enzyme molecule" includes a full length, a fragment or a variant of the enzyme, e.g., an enzyme variant that retains at least one functional property of the naturally-occurring enzyme.

In some embodiments, the stromal modifying moiety decreases the level or production of hyaluronic acid. In other embodiments, the stromal modifying moiety comprises a hyaluronan degrading enzyme, an agent that inhibits hyaluronan synthesis, or an antibody molecule against hyaluronic acid.

In some embodiments, the hyaluronan degrading enzyme is a hyaluronidase molecule, e.g., a full length or a variant (e.g., fragment thereof) thereof. In some embodiments, the hyaluronan degrading enzyme is active in neutral or acidic pH, e.g., pH of about 4-5. In some embodiments, the hyaluronidase molecule is a mammalian hyaluronidase molecule, e.g., a recombinant human hyaluronidase molecule, e.g., a full length or a variant (e.g., fragment thereof, e.g., a truncated form) thereof. In some embodiments, the hyaluronidase molecule is chosen from HYAL1, HYAL2, or PH-20/SPAM1, or a variant thereof (e.g., a truncated form thereof). In some embodiments, the truncated form lacks a C-terminal glycosylphosphatidylinositol (GPI) attachment site or a portion of the GPI attachment site. In some embodiments, the hyaluronidase molecule is glycosylated, e.g., comprises at least one N-linked glycan.

In some embodiments, the hyaluronidase molecule comprises the amino acid sequence: LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTW ARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQS PVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVA LGASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRK NWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADV KDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTLS (SEQ ID NO: 7256), or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7256.

In some embodiments, the hyaluronidase molecule comprises:

(i) the amino acid sequence of 36-464 of SEQ ID NO: 7256;

(ii) the amino acid sequence of 36-481, 36-482, or 36-483 of PH20, wherein PH20 has the sequence of amino acids set forth in SEQ ID NO: 7256; or

(iii) an amino acid sequence having at least 95% to 100 % sequence identity to the polypeptide or truncated form of sequence of amino acids set forth in SEQ ID NO: 7256; or

(iv) an amino acid sequence having 30, 20, 10, 5 or fewer amino acid substitutions to the amino acid sequence set forth in SEQ ID NO: 7256. In some embodiments, the hyaluronidase molecule comprises an amino acid sequence at least 95% (e.g., at least 95%, 96%, 97%, 98%, 99%, 100%) identical to the amino acid sequence of SEQ ID NO: 7256. In some embodiments, the hyaluronidase molecule is encoded by a nucleotide sequence at least 95% (e.g., at least 96%, 97%, 98%, 99%, 100%) identical to the nucleotide sequence of SEQ ID NO: 7256.

In some embodiments, the hyaluronidase molecule is PH20, e.g., rHuPH20. In some embodiments, the hyaluronidase molecule is HYAL1 and comprises the amino acid sequence: FRGPLLPNRPFTTVWNANTQWCLERHGVDVDVSVFDVVANPGQTFRGPDMTIFYSSQG TYPYYTPTGEPVFGGLPQNASLIAHLARTFQDILAAIPAPDFSGLAVIDWEAWRPRWAFN WDTKDIYRQRSRALVQAQHPDWPAPQVEAVAQDQFQGAARAWMAGTLQLGRALRPR GLWGFYGFPDCYNYDFLSPNYTGQCPSGIRAQNDQLGWLWGQSRALYPSIYMPAVLEG TGKSQMYVQHRVAEAFRVAVAAGDPNLPVLPYVQIFYDTTNHFLPLDELEHSLGESAA QGAAGVVLWVSWENTRTKESCQAIKEYMDTTLGPFILNVTSGALLCSQALCSGHGRCV RRTSHPKALLLLNPASFSIQLTPGGGPLSLRGALSLEDQAQMAVEFKCRCYPGWQAPWC ERKSMW (SEQ ID NO: 7253), or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7253.

In some embodiments, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule, further comprises a polymer, e.g., is conjugated to a polymer, e.g., PEG. In some embodiments, the hyaluronan-degrading enzyme is a PEGylated PH20 enzyme (PEGPH20). In some embodiments, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule, further comprises an immunoglobulin chain constant region (e.g., Fc region) chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4. In some embodiments, the immunoglobulin constant region (e.g., the Fc region) is linked, e.g., covalently linked to, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule. In some embodiments, the immunoglobulin chain constant region (e.g., Fc region) is altered, e.g., mutated, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine

residues, effector cell function, or complement function. In some embodiments, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule forms a dimer.

In some embodiments, the stromal modifying moiety comprises an inhibitor of the synthesis of hyaluronan, e.g., an HA synthase. In some embodiments, the inhibitor comprises a sense or an antisense nucleic acid molecule against an HA synthase or is a small molecule drug. In some embodiments, the inhibitor is 4- methylumbelliferone (MU) or a derivative thereof (e.g., 6,7-dihydroxy-4-methyl coumarin or 5,7-dihydroxy-4-methyl coumarin), or leflunomide or a derivative thereof.

In some embodiments, the stromal modifying moiety comprises antibody molecule against hyaluronic acid.

In some embodiments, the stromal modifying moiety comprises a collagenase molecule, e.g., a mammalian collagenase molecule, or a variant (e.g., fragment) thereof. In some embodiments, the collagenase molecule is collagenase molecule IV, e.g., comprising the amino acid sequence of:

YNFFPRKPKWDKNQITYRIIGYTPDLDPETVDDAFARAFQVWSDVTPLRFSRIHDGEADI
MINFGRWEHGDGYPFDGKDGLLAHAFAPGTGVGGDSHFDDDELWTLGEGQVVRVKY
GNADGEYCKFPFLFNGKEYNSCTDTGRSDGFLWCSTTYNFEKDGKYGFCPHEALFTMG
GNAEGQPCKFPFRFQGTSYDSCTTEGRTDGYRWCGTTEDYDRDKKYGFCPETAMSTVG
GNSEGAPCVFPFTFLGNKYESCTSAGRSDGKMWCATTANYDDDRKWGFCPDQGYSLF
LVAAHEFGHAMGLEHSQDPGALMAPIYTYTKNFRLSQDDIKGIQELYGASPDIDLGTGP
TPTLGPVTPEICKQDIVFDGIAQIRGEIFFFKDRFIWRTVTPRDKPMGPLLVATFWPELPEK
IDAVYEAPQEEKAVFFAGNEYWIYSASTLERGYPKPLTSLGLPPDVQRVDAAFNWSKNK
KTYIFAGDKFWRYNEVKKKMDPGFPKLIADAWNAIPDNLDAVVDLQGGGHSYFFKGA
YYLKLENQSLKSVKFGSIKSDWLGC (SEQ ID NO: 7254), or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 7254.

Linkers

The multispecific or multifunctional molecule disclosed herein can further include a linker, e.g., a linker between one or more of: the antigen binding domain and the cytokine molecule, the antigen binding domain and the immune cell engager, the antigen binding domain and the stromal modifying moiety, the cytokine molecule and the immune cell engager, the cytokine molecule and the stromal modifying moiety, the immune cell engager and the stromal modifying moiety, the antigen binding domain and the immunoglobulin chain constant region, the cytokine molecule and the immunoglobulin chain constant region, the immune cell engager and the immunoglobulin chain constant region, or the stromal modifying moiety and the immunoglobulin chain constant region. In embodiments, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker, or a combination thereof.

In one embodiment, the multispecific molecule can include one, two, three or four linkers, e.g., a peptide linker. In one embodiment, the peptide linker includes Gly and Ser. In some embodiments, the peptide linker is selected from GGGGS (SEQ ID NO: 7249); GGGGSGGGGS (SEQ ID NO: 7250); GGGGSGGGGSGGGGS (SEQ ID NO: 7251); and DVPSGPGGGGGSGGGGS (SEQ ID NO: 7252). In some embodiments, the peptide linker is a A(EAAAK)nA (SEQ ID NO: 7255) family of linkers (e.g., as described in Protein Eng. (2001) 14 (8): 529-532). These are stiff helical linkers with n ranging from 2 – 5. In some embodiments, the peptide linker is selected from AEAAAKEAAAKAAA (SEQ ID NO: 75); AEAAAKEAAAKEAAAKAAA (SEQ ID NO: 76); AEAAAKEAAAKEAAAKEAAAKAAA (SEQ ID NO: 77); and AEAAAKEAAAKEAAAKEAAAKEAAAKAAA(SEQ ID NO: 78).

Nucleic Acids

Nucleic acids encoding the aforementioned multispecific or multifunctional molecules are also disclosed.

In certain embodiments, the invention features nucleic acids comprising nucleotide sequences that encode heavy and light chain variable regions and CDRs or hypervariable loops of the antibody molecules, as described herein. For example, the invention features a first and second nucleic acid encoding heavy and light chain variable regions, respectively, of an antibody

molecule chosen from one or more of the antibody molecules disclosed herein. The nucleic acid can comprise a nucleotide sequence as set forth in the tables herein, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in the tables herein.

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In other embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In yet another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions).

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having the nucleotide sequence as set forth in the tables herein, a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In yet another embodiment, the

nucleic acid can comprise a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein).

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding a cytokine molecule, an immune cell engager, or a stromal modifying moiety disclosed herein.

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail hereinbelow.

Vectors

Further provided herein are vectors comprising the nucleotide sequences encoding a multispecific or multifunctional molecule described herein. In one embodiment, the vectors comprise nucleotides encoding a multispecific or multifunctional molecule described herein. In one embodiment, the vectors comprise the nucleotide sequences described herein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototropy to an auxotrophic host, biocide resistance (*e.g.,* antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for

optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors may be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid based transfection or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity.

Methods and conditions for culturing the resulting transfected cells and for recovering the antibody molecule produced are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

Cells

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell. The host cell can be a eukaryotic cell, *e.g.*, a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, *e.g.*, *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (*e.g.*, NSO), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, *e.g.*, mammary epithelial cell.

The invention also provides host cells comprising a nucleic acid encoding an antibody molecule as described herein.

In one embodiment, the host cells are genetically engineered to comprise nucleic acids encoding the antibody molecule.

In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for

example, an inducible promoter.

The invention also provides host cells comprising the vectors described herein.

The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

Uses and Combination Therapies

Methods described herein include treating a cancer in a subject by using a multispecific molecule described herein, e.g., using a pharmaceutical composition described herein. Also provided are methods for reducing or ameliorating a symptom of a cancer in a subject, as well as methods for inhibiting the growth of a cancer and/or killing one or more cancer cells. In embodiments, the methods described herein decrease the size of a tumor and/or decrease the number of cancer cells in a subject administered with a described herein or a pharmaceutical composition described herein.

In embodiments, the cancer is a hematological cancer. In embodiments, the hematological cancer is a leukemia or a lymphoma. As used herein, a "hematologic cancer" refers to a tumor of the hematopoietic or lymphoid tissues, *e.g.,* a tumor that affects blood, bone marrow, or lymph nodes. Exemplary hematologic malignancies include, but are not limited to, leukemia (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, acute monocytic leukemia (AMoL), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), or large granular lymphocytic leukemia), lymphoma (*e.g.,* AIDS-related lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma (*e.g.,* classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma), mycosis fungoides, non-Hodgkin lymphoma (*e.g.,* B-cell non-Hodgkin lymphoma (*e.g.,* Burkitt lymphoma, small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, or mantle cell lymphoma) or T-cell non-Hodgkin lymphoma (mycosis fungoides, anaplastic large cell lymphoma, or precursor T-lymphoblastic lymphoma)), primary central nervous system

lymphoma, Sézary syndrome, Waldenström macroglobulinemia), chronic myeloproliferative neoplasm, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, or myelodysplastic/myeloproliferative neoplasm.

In embodiments, the cancer is a solid cancer. Exemplary solid cancers include, but are not limited to, ovarian cancer, rectal cancer, stomach cancer, testicular cancer, cancer of the anal region, uterine cancer, colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, Kaposi's sarcoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, brain stem glioma, pituitary adenoma, epidermoid cancer, carcinoma of the cervix squamous cell cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, sarcoma of soft tissue, cancer of the urethra, carcinoma of the vulva, cancer of the penis, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, spinal axis tumor, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, metastatic lesions of said cancers, or combinations thereof.

In certain embodiments, the cancer is an epithelial, mesenchymal or hematologic malignancy. In certain embodiments, the cancer treated is a solid tumor (e.g., carcinoid, carcinoma or sarcoma), a soft tissue tumor (e.g., a heme malignancy), and a metastatic lesion, e.g., a metastatic lesion of any of the cancers disclosed herein. In one embodiment, the cancer treated is a fibrotic or desmoplastic solid tumor, e.g., a tumor having one or more of: limited tumor perfusion, compressed blood vessels, fibrotic tumor interstitium, or increased interstitial fluid pressure. In one embodiment, the solid tumor is chosen from one or more of pancreatic (e.g., pancreatic adenocarcinoma or pancreatic ductal adenocarcinoma), breast, colon, colorectal, lung (e.g., small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC)), skin, ovarian, liver cancer, esophageal cancer, endometrial cancer, gastric cancer, head and neck cancer, kidney, or prostate cancer.

Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers are noted below and include: squamous cell cancer (e.g. epithelial squamous cell cancer), lung

cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer. The term "cancer" includes primary malignant cells or tumors (e.g., those whose cells have not migrated to sites in the subject's body other than the site of the original malignancy or tumor) and secondary malignant cells or tumors (e.g., those arising from metastasis, the migration of malignant cells or tumor cells to secondary sites that are different from the site of the original tumor).

Other examples of cancers or malignancies include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia,

Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal

Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In other embodiments, the multispecific molecule, as described above and herein, is used to treat a hyperproliferative disorder, e.g., a hyperproliferative connective tissue disorder (e.g., a hyperproliferative fibrotic disease). In one embodiment, the hyperproliferative fibrotic disease is multisystemic or organ-specific. Exemplary hyperproliferative fibrotic diseases include, but are not limited to, multisystemic (e.g., systemic sclerosis, multifocal fibrosclerosis, sclerodermatous graft-versus-host disease in bone marrow transplant recipients, nephrogenic systemic fibrosis, scleroderma), and organ-specific disorders (e.g., fibrosis of the eye, lung, liver, heart, kidney, pancreas, skin and other organs). In other embodiments, the disorder is chosen from liver cirrhosis or tuberculosis. In other embodiments, the disorder is leprosy.

In embodiments, the multispecific molecules (or pharmaceutical composition) are administered in a manner appropriate to the disease to be treated or prevented. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Appropriate dosages may be determined by clinical trials. For example, when "an effective amount" or "a therapeutic amount" is indicated, the precise amount of the pharmaceutical composition (or multispecific molecules) to be administered can be determined by a physician with consideration of individual differences in tumor size, extent of infection or metastasis, age, weight, and condition of the subject. In embodiments, the pharmaceutical composition described herein can be administered at a dosage of 10^4 to 10^9 cells/kg body weight, e.g., 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. In embodiments, the pharmaceutical composition described herein can be administered multiple times at these dosages. In embodiments, the pharmaceutical composition described herein can be administered using infusion techniques described in immunotherapy (see, *e.g.*, Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

In embodiments, the cancer is a myeloproliferative neoplasm, e.g., primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), or chronic myelogenous leukemia (CML). In embodiments, the cancer is myelofibrosis. In embodiments, the subject has myelofibrosis. In embodiments, the subject has a calreticulin mutation, e.g., a

calreticulin mutation disclosed herein. In embodiments, the subject does not have the JAK2-V617F mutation. In embodiments, the subject has the JAK2-V617F mutation. In embodiments, the subject has a MPL mutation. In embodiments, the subject does not have a MPL mutation.

In embodiments, the cancer is a solid cancer. Exemplary solid cancers include, but are not limited to, ovarian cancer, rectal cancer, stomach cancer, testicular cancer, cancer of the anal region, uterine cancer, colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, Kaposi's sarcoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, brain stem glioma, pituitary adenoma, epidermoid cancer, carcinoma of the cervix squamous cell cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, sarcoma of soft tissue, cancer of the urethra, carcinoma of the vulva, cancer of the penis, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, spinal axis tumor, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, metastatic lesions of said cancers, or combinations thereof.

In embodiments, the multispecific molecules or pharmaceutical composition is administered to the subject parenterally. In embodiments, the cells are administered to the subject intravenously, subcutaneously, intratumorally, intranodally, intramuscularly, intradermally, or intraperitoneally. In embodiments, the cells are administered, e.g., injected, directly into a tumor or lymph node. In embodiments, the cells are administered as an infusion (e.g., as described in Rosenberg et al., New Eng. J. of Med. 319:1676, 1988) or an intravenous push. In embodiments, the cells are administered as an injectable depot formulation.
In embodiments, the subject is a mammal. In embodiments, the subject is a human, monkey, pig, dog, cat, cow, sheep, goat, rabbit, rat, or mouse. In embodimnets, the subject is a human. In embodiments, the subject is a pediatric subject, *e.g.*, less than 18 years of age, e.g., less than 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or less years of age. In embodiments, the subject is an adult, *e.g.*, at least 18 years of age, e.g., at least 19, 20, 21, 22, 23, 24, 25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, or 80-90 years of age.

Combination Therapies

The multispecific or multifunctional molecules disclosed herein can be used in combination with a second therapeutic agent or procedure.

In embodiments, the multispecific or multifunctional molecule and the second therapeutic agent or procedure are administered/performed after a subject has been diagnosed with a cancer, e.g., before the cancer has been eliminated from the subject. In embodiments, the multispecific or multifunctional molecule and the second therapeutic agent or procedure are administered/performed simultaneously or concurrently. For example, the delivery of one treatment is still occurring when the delivery of the second commences, e.g., there is an overlap in administration of the treatments. In other embodiments, the multispecific or multifunctional molecule and the second therapeutic agent or procedure are administered/performed sequentially. For example, the delivery of one treatment ceases before the delivery of the other treatment begins.

In embodiments, combination therapy can lead to more effective treatment than monotherapy with either agent alone. In embodiments, the combination of the first and second treatment is more effective (e.g., leads to a greater reduction in symptoms and/or cancer cells) than the first or second treatment alone. In embodiments, the combination therapy permits use of a lower dose of the first or the second treatment compared to the dose of the first or second treatment normally required to achieve similar effects when administered as a monotherapy. In embodiments, the combination therapy has a partially additive effect, wholly additive effect, or greater than additive effect.

In one embodiment, the multispecific or multifunctional molecule is administered in combination with a therapy, *e.g.*, a cancer therapy (*e.g.*, one or more of anti-cancer agents, immunotherapy, photodynamic therapy (PDT), surgery and/or radiation). The terms "chemotherapeutic," "chemotherapeutic agent," and "anti-cancer agent" are used interchangeably herein. The administration of the multispecific or multifunctional molecule and the therapy, *e.g.*, the cancer therapy, can be sequential (with or without overlap) or simultaneous. Administration of the multispecific or multifunctional molecule can be continuous or intermittent during the course of therapy (*e.g.*, cancer therapy). Certain therapies described herein can be used to treat cancers and non-cancerous diseases. For example, PDT efficacy can be enhanced in

cancerous and non-cancerous conditions (*e.g.*, tuberculosis) using the methods and compositions described herein (reviewed in, *e.g.*, Agostinis, P. *et al.* (2011) *CA Cancer J. Clin.* 61:250-281).

Anti-cancer therapies

In other embodiments, the multispecific or multifunctional molecule is administered in combination with a low or small molecular weight chemotherapeutic agent. Exemplary low or small molecular weight chemotherapeutic agents include, but not limited to, 13-cis-retinoic acid (isotretinoin, ACCUTANE®), 2-CdA (2-chlorodeoxyadenosine, cladribine, LEUSTATIN™), 5-azacitidine (azacitidine, VIDAZA®), 5-fluorouracil (5-FU, fluorouracil, ADRUCIL®), 6-mercaptopurine (6-MP, mercaptopurine, PURINETHOL®), 6-TG (6-thioguanine, thioguanine, THIOGUANINE TABLOID®), abraxane (paclitaxel protein-bound), actinomycin-D (dactinomycin, COSMEGEN®), alitretinoin (PANRETIN®), all-transretinoic acid (ATRA, tretinoin, VESANOID®), altretamine (hexamethylmelamine, HMM, HEXALEN®), amethopterin (methotrexate, methotrexate sodium, MTX, TREXALL™, RHEUMATREX®), amifostine (ETHYOL®), arabinosylcytosine (Ara-C, cytarabine, CYTOSAR-U®), arsenic trioxide (TRISENOX®), asparaginase (Erwinia L-asparaginase, L-asparaginase, ELSPAR®, KIDROLASE®), BCNU (carmustine, BiCNU®), bendamustine (TREANDA®), bexarotene (TARGRETIN®), bleomycin (BLENOXANE®), busulfan (BUSULFEX®, MYLERAN®), calcium leucovorin (Citrovorum Factor, folinic acid, leucovorin), camptothecin-11 (CPT-11, irinotecan, CAMPTOSAR®), capecitabine (XELODA®), carboplatin (PARAPLATIN®), carmustine wafer (prolifeprospan 20 with carmustine implant, GLIADEL® wafer), CCI-779 (temsirolimus, TORISEL®), CCNU (lomustine, CeeNU), CDDP (cisplatin, PLATINOL®, PLATINOL-AQ®), chlorambucil (leukeran), cyclophosphamide (CYTOXAN®, NEOSAR®), dacarbazine (DIC, DTIC, imidazole carboxamide, DTIC-DOME®), daunomycin (daunorubicin, daunorubicin hydrochloride, rubidomycin hydrochloride, CERUBIDINE®), decitabine (DACOGEN®), dexrazoxane (ZINECARD®), DHAD (mitoxantrone, NOVANTRONE®), docetaxel (TAXOTERE®), doxorubicin (ADRIAMYCIN®, RUBEX®), epirubicin (ELLENCE™), estramustine (EMCYT®), etoposide (VP-16, etoposide phosphate, TOPOSAR®, VEPESID®, ETOPOPHOS®), floxuridine (FUDR®), fludarabine (FLUDARA®), fluorouracil (cream) (CARAC™, EFUDEX®, FLUOROPLEX®), gemcitabine

(GEMZAR®), hydroxyurea (HYDREA®, DROXIA™, MYLOCEL™), idarubicin (IDAMYCIN®), ifosfamide (IFEX®), ixabepilone (IXEMPRA™), LCR (leurocristine, vincristine, VCR, ONCOVIN®, VINCASAR PFS®), L-PAM (L-sarcolysin, melphalan, phenylalanine mustard, ALKERAN®), mechlorethamine (mechlorethamine hydrochloride, mustine, nitrogen mustard, MUSTARGEN®), mesna (MESNEX™), mitomycin (mitomycin-C, MTC, MUTAMYCIN®), nelarabine (ARRANON®), oxaliplatin (ELOXATIN™), paclitaxel (TAXOL®, ONXAL™), pegaspargase (PEG-L-asparaginase, ONCOSPAR®), PEMETREXED (ALIMTA®), pentostatin (NIPENT®), procarbazine (MATULANE®), streptozocin (ZANOSAR®), temozolomide (TEMODAR®), teniposide (VM-26, VUMON®), TESPA (thiophosphoamide, thiotepa, TSPA, THIOPLEX®), topotecan (HYCAMTIN®), vinblastine (vinblastine sulfate, vincaleukoblastine, VLB, ALKABAN-AQ®, VELBAN®), vinorelbine (vinorelbine tartrate, NAVELBINE®), and vorinostat (ZOLINZA®).

In another embodiment, the multispecific or multifunctional molecule is administered in conjunction with a biologic. Biologics useful in the treatment of cancers are known in the art and a binding molecule of the invention may be administered, for example, in conjunction with such known biologics. For example, the FDA has approved the following biologics for the treatment of breast cancer: HERCEPTIN® (trastuzumab, Genentech Inc., South San Francisco, Calif.; a humanized monoclonal antibody that has anti-tumor activity in HER2-positive breast cancer); FASLODEX® (fulvestrant, AstraZeneca Pharmaceuticals, LP, Wilmington, Del.; an estrogen-receptor antagonist used to treat breast cancer); ARIMIDEX® (anastrozole, AstraZeneca Pharmaceuticals, LP; a nonsteroidal aromatase inhibitor which blocks aromatase, an enzyme needed to make estrogen); Aromasin® (exemestane, Pfizer Inc., New York, N.Y.; an irreversible, steroidal aromatase inactivator used in the treatment of breast cancer); FEMARA® (letrozole, Novartis Pharmaceuticals, East Hanover, N.J.; a nonsteroidal aromatase inhibitor approved by the FDA to treat breast cancer); and NOLVADEX® (tamoxifen, AstraZeneca Pharmaceuticals, LP; a nonsteroidal antiestrogen approved by the FDA to treat breast cancer). Other biologics with which the binding molecules of the invention may be combined include: AVASTIN® (bevacizumab, Genentech Inc.; the first FDA-approved therapy designed to inhibit angiogenesis); and ZEVALIN® (ibritumomab tiuxetan, Biogen Idec, Cambridge, Mass.; a radiolabeled monoclonal antibody currently approved for the treatment of B-cell lymphomas).

In addition, the FDA has approved the following biologics for the treatment of colorectal cancer: AVASTIN®; ERBITUX® (cetuximab, ImClone Systems Inc., New York, N.Y., and Bristol-Myers Squibb, New York, N.Y.; is a monoclonal antibody directed against the epidermal growth factor receptor (EGFR)); GLEEVEC® (imatinib mesylate; a protein kinase inhibitor); and ERGAMISOL® (levamisole hydrochloride, Janssen Pharmaceutica Products, LP, Titusville, N.J.; an immunomodulator approved by the FDA in 1990 as an adjuvant treatment in combination with 5-fluorouracil after surgical resection in patients with Dukes' Stage C colon cancer).

For the treatment of lung cancer, exemplary biologics include TARCEVA® (erlotinib HCL, OSI Pharmaceuticals Inc., Melville, N.Y.; a small molecule designed to target the human epidermal growth factor receptor 1 (HER1) pathway).

For the treatment of multiple myeloma, exemplary biologics include VELCADE® Velcade (bortezomib, Millennium Pharmaceuticals, Cambridge Mass.; a proteasome inhibitor). Additional biologics include THALIDOMID® (thalidomide, Clegene Corporation, Warren, N.J.; an immunomodulatory agent and appears to have multiple actions, including the ability to inhibit the growth and survival of myeloma cells and anti-angiogenesis).

Additional exemplary cancer therapeutic antibodies include, but are not limited to, 3F8, abagovomab, adecatumumab, afutuzumab, alacizumab pegol, alemtuzumab (CAMPATH®, MABCAMPATH®), altumomab pentetate (HYBRI-CEAKER®), anatumomab mafenatox, anrukinzumab (IMA-638), apolizumab, arcitumomab (CEA-SCAN®), bavituximab, bectumomab (LYMPHOSCAN®), belimumab (BENLYSTA®, LYMPHOSTAT-B®), besilesomab (SCINTIMUN®), bevacizumab (AVASTIN®), bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide (PROSTASCINT®), catumaxomab (REMOVAB®), CC49, cetuximab (C225, ERBITUX®), citatuzumab bogatox, cixutumumab, clivatuzumab tetraxetan, conatumumab, dacetuzumab, denosumab (PROLIA®), detumomab, ecromeximab, edrecolomab (PANOREX®), elotuzumab, epitumomab cituxetan, epratuzumab, ertumaxomab (REXOMUN®), etaracizumab, farletuzumab, figitumumab, fresolimumab, galiximab, gemtuzumab ozogamicin (MYLOTARG®), girentuximab, glembatumumab vedotin, ibritumomab (ibritumomab tiuxetan, ZEVALIN®), igovomab (INDIMACIS-125®), intetumumab, inotuzumab ozogamicin,

ipilimumab, iratumumab, labetuzumab (CEA-CIDE®), lexatumumab, lintuzumab, lucatumumab, lumiliximab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, nacolomab tafenatox, naptumomab estafenatox, necitumumab, nimotuzumab (THERACIM®, THERALOC®), nofetumomab merpentan (VERLUMA®), ofatumumab (ARZERRA®), olaratumab, oportuzumab monatox, oregovomab (OVAREX®), panitumumab (VECTIBIX®), pemtumomab (THERAGYN®), pertuzumab (OMNITARG®), pintumomab, pritumumab, ramucirumab, ranibizumab (LUCENTIS®), rilotumumab, rituximab (MABTHERA®, RITUXAN®), robatumumab, satumomab pendetide, sibrotuzumab, siltuximab, sontuzumab, tacatuzumab tetraxetan (AFP-CIDE®), taplitumomab paptox, tenatumomab, TGN1412, ticilimumab (tremelimumab), tigatuzumab, TNX-650, tositumomab (BEXXAR®), trastuzumab (HERCEPTIN®), tremelimumab, tucotuzumab celmoleukin, veltuzumab, volociximab, votumumab (HUMASPECT®), zalutumumab (HUMAX-EGFR®), and zanolimumab (HUMAX-CD4®).

In other embodiments, the multispecific or multifunctional molecule is administered in combination with a viral cancer therapeutic agent. Exemplary viral cancer therapeutic agents include, but not limited to, vaccinia virus (vvDD-CDSR), carcinoembryonic antigen-expressing measles virus, recombinant vaccinia virus (TK-deletion plus GM-CSF), Seneca Valley virus-001, Newcastle virus, coxsackie virus A21, GL-ONC1, EBNA1 C-terminal/LMP2 chimeric protein-expressing recombinant modified vaccinia Ankara vaccine, carcinoembryonic antigen-expressing measles virus, G207 oncolytic virus, modified vaccinia virus Ankara vaccine expressing p53, OncoVEX GM-CSF modified herpes-simplex 1 virus, fowlpox virus vaccine vector, recombinant vaccinia prostate-specific antigen vaccine, human papillomavirus 16/18 L1 virus-like particle/AS04 vaccine, MVA-EBNA1/LMP2 Inj. vaccine, quadrivalent HPV vaccine, quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine (GARDASIL®), recombinant fowlpox-CEA(6D)/TRICOM vaccine; recombinant vaccinia-CEA(6D)-TRICOM vaccine, recombinant modified vaccinia Ankara-5T4 vaccine, recombinant fowlpox-TRICOM vaccine, oncolytic herpes virus NV1020, HPV L1 VLP vaccine V504, human papillomavirus bivalent (types 16 and 18) vaccine (CERVARIX®), herpes simplex virus HF10, Ad5CMV-p53 gene, recombinant vaccinia DF3/MUC1 vaccine, recombinant vaccinia-MUC-1 vaccine, recombinant vaccinia-TRICOM vaccine, ALVAC MART-1 vaccine, replication-defective herpes

simplex virus type I (HSV-1) vector expressing human Preproenkephalin (NP2), wild-type reovirus, reovirus type 3 Dearing (REOLYSIN®), oncolytic virus HSV1716, recombinant modified vaccinia Ankara (MVA)-based vaccine encoding Epstein-Barr virus target antigens, recombinant fowlpox-prostate specific antigen vaccine, recombinant vaccinia prostate-specific antigen vaccine, recombinant vaccinia-B7.1 vaccine, rAd-p53 gene, Ad5-delta24RGD, HPV vaccine 580299, JX-594 (thymidine kinase-deleted vaccinia virus plus GM-CSF), HPV-16/18 L1/AS04, fowlpox virus vaccine vector, vaccinia-tyrosinase vaccine, MEDI-517 HPV-16/18 VLP AS04 vaccine, adenoviral vector containing the thymidine kinase of herpes simplex virus TK99UN, HspE7, FP253/Fludarabine, ALVAC(2) melanoma multi-antigen therapeutic vaccine, ALVAC-hB7.1, canarypox-hIL-12 melanoma vaccine, Ad-REIC/Dkk-3, rAd-IFN SCH 721015, TIL-Ad-INFg, Ad-ISF35, and coxsackievirus A21 (CVA21, CAVATAK®).

In other embodiments, the multispecific or multifunctional molecule is administered in combination with a nanopharmaceutical. Exemplary cancer nanopharmaceuticals include, but not limited to, ABRAXANE® (paclitaxel bound albumin nanoparticles), CRLX101 (CPT conjugated to a linear cyclodextrin-based polymer), CRLX288 (conjugating docetaxel to the biodegradable polymer poly (lactic-co-glycolic acid)), cytarabine liposomal (liposomal Ara-C, DEPOCYT™), daunorubicin liposomal (DAUNOXOME®), doxorubicin liposomal (DOXIL®, CAELYX®), encapsulated-daunorubicin citrate liposome (DAUNOXOME®), and PEG anti-VEGF aptamer (MACUGEN®).

In some embodiments, the multispecific or multifunctional molecule is administered in combination with paclitaxel or a paclitaxel formulation, e.g., TAXOL®, protein-bound paclitaxel (e.g., ABRAXANE®). Exemplary paclitaxel formulations include, but are not limited to, nanoparticle albumin-bound paclitaxel (ABRAXANE®, marketed by Abraxis Bioscience), docosahexaenoic acid bound-paclitaxel (DHA-paclitaxel, Taxoprexin, marketed by Protarga), polyglutamate bound-paclitaxel (PG-paclitaxel, paclitaxel poliglumex, CT-2103, XYOTAX, marketed by Cell Therapeutic), the tumor-activated prodrug (TAP), ANG105 (Angiopep-2 bound to three molecules of paclitaxel, marketed by ImmunoGen), paclitaxel-EC-1 (paclitaxel bound to the erbB2-recognizing peptide EC-1; see Li *et al., Biopolymers* (2007) 87:225-230), and glucose-conjugated paclitaxel (*e.g.,* 2'-paclitaxel methyl 2-glucopyranosyl succinate, see Liu *et al., Bioorganic & Medicinal Chemistry Letters* (2007) 17:617-620).

Exemplary RNAi and antisense RNA agents for treating cancer include, but not limited to, CALAA-01, siG12D LODER (Local Drug EluteR), and ALN-VSP02.

Other cancer therapeutic agents include, but not limited to, cytokines (e.g., aldesleukin (IL-2, Interleukin-2, PROLEUKIN®), alpha Interferon (IFN-alpha, Interferon alfa, INTRON® A (Interferon alfa-2b), ROFERON-A® (Interferon alfa-2a)), Epoetin alfa (PROCRIT®), filgrastim (G-CSF, Granulocyte - Colony Stimulating Factor, NEUPOGEN®), GM-CSF (Granulocyte Macrophage Colony Stimulating Factor, sargramostim, LEUKINE™), IL-11 (Interleukin-11, oprelvekin, NEUMEGA®), Interferon alfa-2b (PEG conjugate) (PEG interferon, PEG-INTRON™), and pegfilgrastim (NEULASTA™)), hormone therapy agents (e.g., aminoglutethimide (CYTADREN®), anastrozole (ARIMIDEX®), bicalutamide (CASODEX®), exemestane (AROMASIN®), fluoxymesterone (HALOTESTIN®), flutamide (EULEXIN®), fulvestrant (FASLODEX®), goserelin (ZOLADEX®), letrozole (FEMARA®), leuprolide (ELIGARD™, LUPRON®, LUPRON DEPOT®, VIADUR™), megestrol (megestrol acetate, MEGACE®), nilutamide (ANANDRON®, NILANDRON®), octreotide (octreotide acetate, SANDOSTATIN®, SANDOSTATIN LAR®), raloxifene (EVISTA®), romiplostim (NPLATE®), tamoxifen (NOVALDEX®), and toremifene (FARESTON®)), phospholipase A2 inhibitors (e.g., anagrelide (AGRYLIN®)), biologic response modifiers (e.g., BCG (THERACYS®, TICE®), and Darbepoetin alfa (ARANESP®)), target therapy agents (e.g., bortezomib (VELCADE®), dasatinib (SPRYCEL™), denileukin diftitox (ONTAK®), erlotinib (TARCEVA®), everolimus (AFINITOR®), gefitinib (IRESSA®), imatinib mesylate (STI-571, GLEEVEC™), lapatinib (TYKERB®), sorafenib (NEXAVAR®), and SU11248 (sunitinib, SUTENT®)), immunomodulatory and antiangiogenic agents (e.g., CC-5013 (lenalidomide, REVLIMID®), and thalidomide (THALOMID®)), glucocorticosteroids (e.g., cortisone (hydrocortisone, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, ALA-CORT®, HYDROCORT ACETATE®, hydrocortone phosphate LANACORT®, SOLU-CORTEF®), decadron (dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, DEXASONE®, DIODEX®, HEXADROL®, MAXIDEX®), methylprednisolone (6-methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, DURALONE®, MEDRALONE®, MEDROL®, M-PREDNISOL®, SOLU-MEDROL®), prednisolone (DELTA-CORTEF®, ORAPRED®, PEDIAPRED®, PRELONE®), and

prednisone (DELTASONE®, LIQUID PRED®, METICORTEN®, ORASONE®)), and bisphosphonates (e.g., pamidronate (AREDIA®), and zoledronic acid (ZOMETA®))

In some embodiments, the multispecific or multifunctional molecule is used in combination with a tyrosine kinase inhibitor (*e.g.*, a receptor tyrosine kinase (RTK) inhibitor). Exemplary tyrosine kinase inhibitor include, but are not limited to, an epidermal growth factor (EGF) pathway inhibitor (*e.g.*, an epidermal growth factor receptor (EGFR) inhibitor), a vascular endothelial growth factor (VEGF) pathway inhibitor (*e.g.*, an antibody against VEGF, a VEGF trap, a vascular endothelial growth factor receptor (VEGFR) inhibitor (*e.g.*, a VEGFR-1 inhibitor, a VEGFR-2 inhibitor, a VEGFR-3 inhibitor)), a platelet derived growth factor (PDGF) pathway inhibitor (*e.g.*, a platelet derived growth factor receptor (PDGFR) inhibitor (*e.g.*, a PDGFR-ß inhibitor)), a RAF-1 inhibitor, a KIT inhibitor and a RET inhibitor. In some embodiments, the anti-cancer agent used in combination with the AHCM agent is selected from the group consisting of: axitinib (AG013736), bosutinib (SKI-606), cediranib (RECENTIN™, AZD2171), dasatinib (SPRYCEL®, BMS-354825), erlotinib (TARCEVA®), gefitinib (IRESSA®), imatinib (Gleevec®, CGP57148B, STI-571), lapatinib (TYKERB®, TYVERB®), lestaurtinib (CEP-701), neratinib (HKI-272), nilotinib (TASIGNA®), semaxanib (semaxinib, SU5416), sunitinib (SUTENT®, SU11248), toceranib (PALLADIA®), vandetanib (ZACTIMA®, ZD6474), vatalanib (PTK787, PTK/ZK), trastuzumab (HERCEPTIN®), bevacizumab (AVASTIN®), rituximab (RITUXAN®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), ranibizumab (Lucentis®), nilotinib (TASIGNA®), sorafenib (NEXAVAR®), alemtuzumab (CAMPATH®), gemtuzumab ozogamicin (MYLOTARG®), ENMD-2076, PCI-32765, AC220, dovitinib lactate (TKI258, CHIR-258), BIBW 2992 (TOVOK™), SGX523, PF-04217903, PF-02341066, PF-299804, BMS-777607, ABT-869, MP470, BIBF 1120 (VARGATEF®), AP24534, JNJ-26483327, MGCD265, DCC-2036, BMS-690154, CEP-11981, tivozanib (AV-951), OSI-930, MM-121, XL-184, XL-647, XL228, AEE788, AG-490, AST-6, BMS-599626, CUDC-101, PD153035, pelitinib (EKB-569), vandetanib (zactima), WZ3146, WZ4002, WZ8040, ABT-869 (linifanib), AEE788, AP24534 (ponatinib), AV-951(tivozanib), axitinib, BAY 73-4506 (regorafenib), brivanib alaninate (BMS-582664), brivanib (BMS-540215), cediranib (AZD2171), CHIR-258 (dovitinib), CP 673451, CYC116, E7080, Ki8751, masitinib (AB1010), MGCD-265, motesanib diphosphate (AMG-706), MP-470, OSI-930,

Pazopanib Hydrochloride, PD173074, Sorafenib Tosylate (Bay 43-9006), SU 5402, TSU-68(SU6668), vatalanib, XL880 (GSK1363089, EXEL-2880). Selected tyrosine kinase inhibitors are chosen from sunitinib, erlotinib, gefitinib, or sorafenib. In one embodiment, the tyrosine kinase inhibitor is sunitinib.

In one embodiment, the multispecific or multifunctional molecule is administered in combination with one of more of: an anti-angiogenic agent, or a vascular targeting agent or a vascular disrupting agent. Exemplary anti-angiogenic agents include, but are not limited to, VEGF inhibitors (*e.g.*, anti-VEGF antibodies (*e.g.*, bevacizumab); VEGF receptor inhibitors (*e.g.*, itraconazole); inhibitors of cell proliferatin and/or migration of endothelial cells (*e.g.*, carboxyamidotriazole, TNP-470); inhibitors of angiogenesis stimulators (*e.g.*, suramin), among others. A vascular-targeting agent (VTA) or vascular disrupting agent (VDA) is designed to damage the vasculature (blood vessels) of cancer tumors causing central necrosis (reviewed in, *e.g.*, Thorpe, P.E. (2004) *Clin. Cancer Res.* Vol. 10:415-427). VTAs can be small-molecule. Exemplary small-molecule VTAs include, but are not limited to, microtubule destabilizing drugs (e.g., combretastatin A-4 disodium phosphate (CA4P), ZD6126, AVE8062, Oxi 4503); and vadimezan (ASA404).

Immune checkpoint inhibitors

In other embodiments, methods described herein comprise use of an immune checkpoint inhibitor in combination with the multispecific or multifunctional molecule. The methods can be used in a therapeutic protocol *in vivo*.

In embodiments, an immune checkpoint inhibitor inhibits a checkpoint molecule. Exemplary checkpoint molecules include but are not limited to CTLA4, PD1, PD-L1, PD-L2, TIM3, LAG3, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1, and A2aR. See, e.g., Pardoll. Nat. Rev. Cancer 12.4(2012):252-64, incorporated herein by reference.

In embodiments, the immune checkpoint inhibitor is a PD-1 inhibitor, e.g., an anti-PD-1 antibody such as Nivolumab, Pembrolizumab or Pidilizumab. Nivolumab (also called MDX-1106, MDX-1106-04, ONO-4538, or BMS-936558) is a fully human IgG4 monoclonal antibody

that specifically inhibits PD1. See, e.g., US 8,008,449 and WO2006/121168. Pembrolizumab (also called Lambrolizumab, MK-3475, MK03475, SCH-900475 or KEYTRUDA®; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. See, e.g., Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44, US 8,354,509 and WO2009/114335. Pidilizumab (also called CT-011 or Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD1. See, e.g., WO2009/101611. In one embodiment, the inhibitor of PD-1 is an antibody molecule having a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence of Nivolumab, Pembrolizumab or Pidilizumab. Additional anti-PD1 antibodies, e.g., AMP 514 (Amplimmune), are described, e.g., in US 8,609,089, US 2010028330, and/or US 20120114649.

In some embodiments, the PD-1 inhibitor is an immunoadhesin, e.g., an immunoadhesin comprising an extracellular/PD-1 binding portion of a PD-1 ligand (e.g., PD-L1 or PD-L2) that is fused to a constant region (*e.g.*, an Fc region of an immunoglobulin). In embodiments, the PD-1 inhibitor is AMP-224 (B7-DCIg, *e.g.*, described in WO2011/066342and WO2010/027827), a PD-L2 Fc fusion soluble receptor that blocks the interaction between B7-H1 and PD-1.

In embodiments, the immune checkpoint inhibitor is a PD-L1 inhibitor, e.g., an antibody molecule. In some embodiments, the PD-L1 inhibitor is YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105. In some embodiments, the anti-PD-L1 antibody is MSB0010718C (also called A09-246-2; Merck Serono), which is a monoclonal antibody that binds to PD-L1. Exemplary humanized anti-PD-L1 antibodies are described, e.g., in WO2013/079174. In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody, e.g., YW243.55.S70. The YW243.55.S70 antibody is described, e.g., in WO 2010/077634. In one embodiment, the PD-L1 inhibitor is MDX-1105 (also called BMS-936559), which is described, e.g., in WO2007/005874. In one embodiment, the PD-L1 inhibitor is MDPL3280A (Genentech / Roche), which is a human Fc-optimized IgG1 monoclonal antibody against PD-L1. See, e.g., U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906. In one embodiment, the inhibitor of PD-L1 is an antibody molecule having a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence of YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105.

In embodiments, the immune checkpoint inhibitor is a PD-L2 inhibitor, e.g., AMP-224 (which is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD1 and B7-H1. See, e.g., WO2010/027827 and WO2011/066342.

In one embodiment, the immune checkpoint inhibitor is a LAG-3 inhibitor, e.g., an anti LAG-3 antibody molecule. In embodiments, the anti-LAG-3 antibody is BMS-986016 (also called BMS986016; Bristol-Myers Squibb). BMS-986016 and other humanized anti-LAG-3 antibodies are described, e.g., in US 2011/0150892, WO2010/019570, and WO2014/008218.

In embodiments, the immune checkpoint inhibitor is a TIM-3 inhibitor, e.g., anti-TIM3 antibody molecule, e.g., described in U.S. Patent No.: 8,552,156, WO 2011/155607, EP 2581113 and U.S Publication No.: 2014/044728.

In embodiments, the immune checkpoint inhibitor is a CTLA-4 inhibitor, e.g., anti-CTLA-4 antibody molecule. Exemplary anti-CTLA4 antibodies include Tremelimumab (IgG2 monoclonal antibody from Pfizer, formerly known as ticilimumab, CP-675,206); and Ipilimumab (also called MDX-010, CAS No. 477202-00-9). Other exemplary anti-CTLA-4 antibodies are described, *e.g.,* in U.S. Pat. No. 5,811,097.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

EXAMPLES

Example 1: Immunization of Armenian hamster to generate anti-NKp30 antibodies

Briefly, armenian hamster were immunized with the extracellular domain of human NKp30 protein in complete Freund's adjuvant and boosted twice on day 14 and day 28 with NKp30 in incomplete Freund's adjuvant (IFA). On day 56 one more boost in IFA was given and the animals harvested three days later. Spleens were collected and fused with P3X63Ag8.653 murine myeloma cell line. 0.9 x 10^5 cells/well in 125 ul were seated in 96 well plate and feed with 125 µl of I-20 + 2ME + HAT (IMDM (4g/L glucose) supplemented with 20% fetal bovine serum, 4 mM L-glutamine, 1 mM sodium pyruvate, 50 U penicillin, 50 µg streptomycin and 50

μM 2-ME in the absence or presence of HAT or HT for selection, and Hybridoma Cloning Factor (1% final) on days 7, 11 and thereafter as needed. At approximately 2 weeks after fusion (cells are about 50% confluent) supernatant was collected and assayed for binding.

Example 2: Hybridoma screen for NKp30 mAbs

Expi293 cells were transfected with BG160 (hNKp30 cell antigen) 18 hours prior to screening. The day of screening, transfected cells were diluted to 0.05 x$10^{\wedge 6}$/mL and anti-Armenian hamster Fc Alexa Fluor 488 added to a final concentration of 0.4 ug/mL. 50 uL (2,500 cells) of this mixture was added to each well of a 384 well plate. The same density of untransfected 293 cells with secondary were used as a negative control. 5 uL of hybridoma supernatant was added to the cell mixture and the plate incubated for 1 hour at 37°C. The plates were then imaged on Mirrorball. Positive clones were identified and subcloned by serial dilution to obtain clonal selected hybridoma. After reconfirmation using the same protocols the hybridoma cells were harvested and the corresponding heavy and light chain sequences recovered. The DNA was subcloned into pcDNA3.4 for subsequent expression of the corresponding antibodies and further validation.

Example 3: Binding of NKp30 antibodies to NK92 cells

NK-92 cells were washed with PBS containing 0.5% BSA and 0.1% sodium azide (staining buffer) and added to 96-well V-bottom plates with 200,000 cells/well. Hamster NKp30 antibodies were added to the cells in 2.0-fold serial dilutions and incubated for 1 hour at room temperature. The plates were washed twice with staining buffer. The secondary antibody against hamster Fc conjugated to AF647 (Jackson, 127-605-160) was added at 1:100 dilution (1.4mg/ml stock) and incubated with the cells for 30 minutes at 4°C followed by washing with staining buffer. Cells were subsequently were fixed for 10 minutes with 4% paraformaldehyde at room temperature. The plates were read on CytoFLEX LS (Beckman Coulter). Data was calculated as the percent-AF747 positive population (FIG. 9).

Example 4: Bioassay to measure activity of NKp30 antibodies using NK92 cell line

NKp30 antibodies were three-fold serially diluted in PBS and incubated at 2-8 C° overnight in flat bottom 96 well plates. Plates were washed twice in PBS and 40,000 NK-92 cells were added in growth medium containing IL-2. Plates were incubated at 37 C°, 5% CO2, humidified incubator for 16-24 hours before supernatants were collected. IFNγ levels in supernatants was measured following MSD assay instructions (FIG. 10). Supernatant collected from cells incubated with hamster isotype IgG was used as negative control and supernatants from cells incubated with NKp30 monoclonal antibody (R&D, clone 210847) was utilized as a positive control. Data were generated using hamster anti-NKp30 mABs.

Example 5: ELISA to measure binding of humanized JOVI.1 variant to human TRBC1

An ELISA assay was performed to assay binding of a humanized JOVI.1 variant to human TRBC1. Microplates were coated with 1 ug/mL of each JOVI.1 variant separately in 100 uL and blocked with 2% BSA. Serial dilutions of hTRBC1, BIM0444 (7 points, 5-fold dilutions, 100 nM to 6.4 pM) were transferred to the coated and blocked plates at 100 uL/well and incubated for 1 hr at room temperature. Plates were washed three times and incubated for 30 mins with anti-his tag Fc horseradish peroxidase conjugate followed by addition of TMB, a substrate of HRP. The plates were developed for 5 mins, stopped with 1M HCL and read at a wavelength of 450 nm. The ELISA data show direct binding of anti-TRBC1 mAbs (bivalent) to human TRBC1 (FIG. 7).

Example 6: Assay to measure binding of humanized JOVI.1 variant to human TRBC1

An Octet assay was performed to check binding of JOVI.1 humanized variants. Protein A biosensors were equilibrated in PBS at 25°C. The sensors were loaded with hTRBC1, BIM0444 at 20 ug/mL in PBS to a response of 1.5 nM followed by serial dilutions of JOVI1.1 fabs, BIM0446 and BIM0460 (7 points, 2-fold dilutions, 50 nM to 0.78 nM).

Further Octet parameters include:

Baseline: 30 sec in PBS

Load: 20 sec to a response of 1.5 nm

Baseline: 60 sec

Association: 60 sec

Dissociation: 60 sec in PBS

Octet data showed binding of anti-TRBC1 Fabs to hTRBC1 (FIG. 8). hTRBC1 was captured on the sensor tip and dipped in solution containing different concentrations of monovalent Fabs.

Example 7: Generation and characterization of humanized anti-NKp30 antibodies

A series of hamster anti-NKp30 antibodies were selected. These antibodies were shown to bind to human NKp30 and cynomolgus NKp30 and induce IFNγ production from NK-90 cells (data not shown). The VH and VL sequences of exemplary hamster anti-NKp30 antibodies 15E1, 9G1, 15H6, 9D9, 3A12, and 12D10 are disclosed in Table 9. The VH and VL sequences of exemplary humanized anti-NKp30 antibodies based on 15E1, 9G1, and 15H6 are also disclosed in Table 9. The Kabat CDRs of these antibodies are disclosed in Table 18 and Table 8.

Two humanized constructs based on 15E1 were selected. The first construct BJM0407 is a Fab comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7302 and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 7305. Its corresponding scFv construct BJM0859 comprises the amino acid sequence of SEQ ID NO: 7310. The second construct BJM0411 is a Fab comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7302 and a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 7309. Its corresponding scFv construct BJM0860 comprises the amino acid sequence of SEQ ID NO: 7311. BJM0407 and BJM0411 showed comparable biophysical characteristics, e.g., binding affinity to NKp30 and thermal stability. The scFv constructs BJM0859 and BJM0860 also showed comparable biophysical properties.

Example 8: Generation and characterization of humanized anti-TRBC1 antibodies

The murine anti-TRBC1 antibody JOVI.1 was humanized, leading to a number of humanized variants. The VH and VL sequences of exemplary humanized variants are disclosed in Table 4. One humanized variant BIM0460 was selected, which comprises a VH comprising the amino acid sequence of SEQ ID NO: 253 and a VL comprising the amino acid sequence of SEQ ID NO: 258. BIM0460 was further modified by germlining, leading to a number of

germlined variants. The VH and VL sequences of exemplary germlined variants are also disclosed in Table 4. One germlined variant BJM0578 was selected, which comprises a VH comprising the amino acid sequence of SEQ ID NO: 7351 and a VL comprising the amino acid sequence of SEQ ID NO: 258. The Kabat CDRs of these humanized and germlined variants are disclosed in Table 6 and Table 3. BIM0460 was shown to bind to human TRBC1 with an affinity of 17 nM. BJM0578 was shown to bind to human TRBC1 with an affinity of 110 nM.

Example 9: Cytokine secretion and T cell activation profiling.

This example explores whether ADCC-disabled formats would be preferable for antibodies that bind to TRBC1 and NKp30. JOVI.1 engagement upon plate coating or in solution upon Fc engagement induced T cell proliferation and activation (data not shown). This could be a liability for treating patients with T cell lymphoma, e.g., patients with peripheral T-cell lymphoma (PTCL).

Five constructs were generated as shown in FIGs. 11A-11E. BJM1052 is a bispecific antibody comprising an anti-TRBC1 Fab (based on BIM0460) and an anti-NKp30 scFv (based on BJM0407) (FIG. 11A). BJM1052 comprises the amino acid sequences of SEQ ID NO: 7379 (anti-TRBC1 HC), SEQ ID NO: 7380 (anti-TRBC1 LC), and SEQ ID NO: 7383 (anti-NKp30 scFv-Fc). BJM1052 comprises an N297A mutation in its Fc region. BJM1042 is a bispecific antibody comprising an anti-TRBC1 Fab (based on BJM0578) and an anti-NKp30 scFv (based on BJM0407) (FIG. 11B). BJM1042 comprises the amino acid sequences of SEQ ID NO: 7382 (anti-TRBC1 HC), SEQ ID NO: 7380 (anti-TRBC1 LC), and SEQ ID NO: 7383 (anti-NKp30 scFv-Fc). BJM1042 comprises an N297A mutation in its Fc region. BJM0889 is a single arm antibody comprising an anti-TRBC1 Fab (based on BIM0460) (FIG. 11C). BJM1083 is a single arm antibody comprising an anti-TRBC1 Fab (based on BJM0578) (FIG. 11D). Both BJM0889 and BJM1083 comprise an N297A mutation in the Fc region. BJM1053 is similar to BJM1052, except that BJM1053 has an ADCC enabled Fc region.

As shown in FIGs. 12A and 12B, Fc enabled antibodies BJM1053 and hIgG1 bound to THP1 cells which express Fcγ receptors, whereas N297A mutated antibodies (BJM1052, BJM1042, and BJM0889) did not show significant binding.

To test if antibodies with N297A mutation (Fc disabled) are safer, anti-TRBC1/NKp30 antibodies and control molecules were added to PBMCs in solution at 100, 10 or 1nM and T cell

proliferation was measured on Days 1 and Day 5. Fc disabled antibodies BJM1052 and BJM1042 showed less lymphocyte clustering than the Fc enabled antibody BJM1053 (data not shown). T cell activation was significantly reduced in PBMCs treated with BJM1052 and BJM1042 on Day 5, as demonstrated by the percentage of proliferating T cells (FIGs. 13A and 13B) as well as the percentage of CD69-CD25+ T cells (FIGs. 13C and 13D).

Example 10: In vitro binding to TRBC1 and NKp30

Various constructs were generated as shown in FIGs. 14A-14D. Shown in FIG. 14A is a bispecific antibody comprising an anti-TRBC1 Fab (based on BIM0460 or BJM0578) and an anti-NKp30 scFv (based on BJM0407 or BJM0411). The bispecific antibodies may or may not have an N297A mutation in their Fc regions. The molecules listed in FIG. 14B have the configuration shown in FIG. 14A.

FIG. 14C shows a bispecific antibody comprising an anti-TRBC1 Fab (based on BIM0460 or BJM0578) and an anti-NKp30 Fab (based on BJM0407 or BJM0411). The bispecific antibodies may or may not have an N297A mutation in their Fc regions. The molecules listed in FIG. 14D have the configuration shown in FIG. 14C.

All the anti-TRBC1/NKp30 antibodies tested exhibited binding to NK cell line KHYG-1 (FIGs. 15A and 15D) as well as TRBC1+ Jurkat cells (FIGs. 15B and 15D).

Example 11: In vitro cytolysis of TRBC1+ cell lines

In this example, anti-TRBC1/NKp30 antibodies were tested for their ability to induce killing of TRBC1-expressing cells in the presence of NK cells. The antibodies tested in this Example are shown in FIGs. 11A-11E.

In a first study, NK-92 effector cells were cultured in 5:1 ratio with CFSE labeled target cells for 4h. Target cell lysis was measured using flow cytometry and gating on dead target cells. Anti-TRBC1/NKp30 bispecific antibodies BJM1052 and BJM1042 induced killing of TRBC1+ Jurkat cells (FIG. 16A) and H9 cells (FIG. 16B), but not TRBC2+ HPB-ALL cells (FIG. 16C), in the presence of NK-92 effector cells.

In a second study, primary NK cells were cultured in 5:1 ratio with CFSE labeled target cells for 4h. For H9 cells, 10:1 E:T ratio was used. Target cell lysis was measured using flow cytometry. Anti-TRBC1/NKp30 bispecific antibodies BJM1052 and BJM1042 induced killing

of TRBC1+ Jurkat cells (FIG. 17A) and H9 cells (FIG. 17B), but not TRBC2+ HPB-ALL cells (FIG. 17C), in the presence of primary NK cells.

In a third study, NK cells and target cells were co-cultured for 4 hours in the presence of anti-TRBC1/NKp30 antibodies BJM1052 and BJM1042, supernatants were collected, and cytokine levels were measured using MSD. Target cell lysis correlated with NK cell activation, as demonstrated by the percentage of CD69+CD107a+ NK cells (FIG. 18A), IFNγ secretion (FIG. 18B), and TNFα secretion (FIG. 18C).

The next study examines whether anti-TRBC1/NKp30 antibodies BJM1052 and BJM1042 activates NK cells in the absence of target cells. Primary NK cells were incubated with 50nM of antibodies for 4h in the absence of target cells, and then supernatants were collected to measure IFNγ and TNFα levels. As shown in FIGs. 19A and 19B, NK cell activation mediated by anti-TRBC1/NKp30 antibodies required the presence of both NK cells and target cells.

Finally, anti-TRBC1/NKp30 antibodies BJM1052 and BJM1042 did not induce NK cell death in the presence of target cells (FIG. 20).

Example 12: Selective in vitro cytolysis of patient-derived TRBC1+ PDX

Common subtypes of T-cell lymphoma include: Peripheral T-Cell Lymphoma, Not Otherwise Specified (PTCL - NOS); Anaplastic Large Cell Lymphoma (ALCL); Angioimmunoblastic T-Cell Lymphoma (AITL); and Cutaneous T-Cell Lymphoma (CTCL). Uncommon subtypes of T-cell lymphoma include: Adult T-Cell Leukemia/Lymphoma (ATLL); T-Cell Lymphoblastic Lymphoma; Hepatosplenic Gamma-Delta T-Cell Lymphoma; Enteropathy-Type T-Cell Lymphoma; Nasal NK/T-Cell Lymphomas; Treatment-Related T-Cell Lymphomas. Similar frequency and expression of TRBC1 was observed in PBMCs isolated from healthy donors and PBMCs isolated from PTCL patients (data not shown).

Two Patient-Derived Xenograft (PDX) samples were tested to be TRBC1 positive: PDX3 was derived from a patient with Acute Lymphoblastic Leukemia (T-ALL), and PDX6 was derived from a patient with Primary cutaneous CD30+ T-Cell Lymphoproliferative Disorder (CTCL).

The antibodies shown in FIGs. 21A and 21B were used in a functional killing assay. BJM0145 is a single arm anti-TRBC1 antibody. BJM0773 is a bispecific antibody comprising

an anti-TRBC1 Fab and an anti-NKp30 scFv. PDX samples were labeled with CFSE, cultured with primary NK cells or KHYG1 cells at 5:1 ratio of E:T for 5 hours in the presence of BJM0145 or BJM0773 (0.01 - 10nM). Specific killing was meausred using the following calculation:

$$\frac{\%\ \text{dead treated (PDX+NK)} - \%\ \text{dead PDX}}{100\%\ \text{(Max killing)} - \%\ \text{dead PDX}}$$

As shown in FIGs. 22A-22D, anti-TRBC1/NKp30 antibody BJM0773 efficiently killed TRBC1 positive PDX3 and PDX6. The single arm anti-TRBC1 antibody BJM0145 exhibited weak killing in the presence of primary NK cells due to ADCC (FIGs. 22A and 22C), but not in the presence of KHYG1 cells, which are CD16 deficient NK cells (FIGs. 22B and 22D). The single arm anti-TRBC1 antibody or the bispecific anti-TRBC1/NKp30 antibody did not kill TRBC1 negative PDX (data not shown).

Example 13: In vitro cytolysis of TRBC1+ Jurkat cells using NK cells from PTCL patients

This example examines whether anti-TRBC1/NKp30 antibodies can mediate killing of TRBC1+ target cells in the presence of NK cells isolated from PTCL patients.

NK cells and NKp30+ NK cells are present in normal proportions in PTCL patient PBMCs (data not shown). NK cells were enriched from PTCL patients and healthy donor PBMCs by negative selection and then incubated overnight with 200U/ml IL-2. On the following day, NK cells were co-cultured with Jurkat cells for 4h in the presence of 10nM antibodies.

As shown in FIG. 23, PTCL patient derived NK cells killed TRBC1+ Jurkat cells in the presence of the anti-TRBC1/NKp30 antibody BJM1042. NK cells were activated during the killing assay, as demonstrated by the percentage of CD69+CD107+ NK cells (FIG. 24). The bispecific anti-TRBC1/NKp30 antibodies BJM1052 and BJM1042 induced higher levels of IFNγ (FIG. 25A) and TNFα (FIG. 25B) than the single arm anti-TRBC1 antibody FJM0889 did.

Example 14: Competition with B7-H6, a natural ligand for NKp30

The natural ligands of NKp30 includes B7-H6, pp65, BAT3, and BAG6. B7-H6 is found on many cancer cell lines and primary cancer cells (e.g., T- and B-cell lymphoma, leukemia, and melanoma). Membrane-bound B7-H6 can mediate activation of primary human NK cells and killing of target cells. Soluble B7-H6, on the other hand, is found in serum or tumor

microenvironment and can inhibit binding of anti-NKp30 mAbs, down-modulate NKp30 expression, and dampen NKp30-mediate activation and target cell killing.

This example examines whether the bispecific anti-TRBC1/NKp30 antibodies compete with B7-H6 for binding to NKp30.

As shown in FIGs. 26A and 26B, the bispecific anti-TRBC1/NKp30 antibody BJM1042 bound more strongly to NKp30 than B7-H6. In a competition assay, B7H6 (4μg/ml, ~143nM) and varying concentration of antibodies (BJM1042, anti-NKp30 or anti-NKp46) were added simultaneously to NKp30 coated ELISA plate. As shown in FIG. 26C, B7H6 binding signal was diminished with increasing concentrations of competing antibodies. BJM1042 competed with B7-H6 for binding with NKp30, to a similar level as a positive control anti-NKp30 antibody (FIG. 26C). A negative control anti-NKp46 antibody did not interfere with B7-H6 binding to NKp30, suggesting that the interference observed in this ELISA was specific (FIG. 26C).

Example 15: In-vivo killing of TRBC1 cell line derived model

This example examines the anti-tumor activity of the anti-TRBC1/NKp30 antibody BJM1042 in an in vivo model.

On day 0, NOG-IL-15 mice were implanted subcutaneously with H9 tumor cells. 16 days post tumor implant, mice were engrafted with in vitro expanded primary NK cells. Two weeks following NK implant (31 days post tumor implant), mice were randomized by tumor volume and dosed with 1mg/kg BJM1042 or associated controls. Tumor volume and body weight was measured daily following exposure to test articles.

The anti-TRBC1/NKp30 antibody BJM1042 induced regression of subcutaneous H9 tumors in NOG IL-15 mice engrafted with primary NK cells (FIGs. 27B and 27C). BJM1042 also inhibited tumor growth in the absence of NK cells, but to a lesser extent compared to treatment in the presence of NK cells (FIGs. 27B and 27C). Similar results were observed with the anti-TRBC1 control antibody BJM1083 (FIGs. 27B and 27C).

Example 16: In-vivo specificity for TRBC1

In this example, the specificity of BJM1042 was evaluated using TRBC2-expressing HPB-ALL xenografts in primary NK cell engrafted NOG-IL-15 mice.

On day 0, NOG-IL-15 mice were implanted subcutaneously with 5e6 TRBC2+ HPB-ALL cells. 12 days post tumor implant, mice were engrafted with 2e6 in vitro expanded primary

NK cells. 2 days following NK implant (14 days post tumor implant), mice were randomized by tumor volume and dosed with 0.5mg/kg BJM1042 or associated controls. Mice were treated with therapeutics twice a week. Tumor volume was quantified by calipers twice a week. Body weight was measured twice a week.

The anti-TRBC1/NKp30 antibody BJM1042, which induced regression of TRBC1-expressing H9 and Jurkat tumors, did not affect the growth of TRBC2-expressing HPB-ALL tumors (FIG. 28B). The molecules were well tolerated at the doses used and did not result in body weight loss or any other obvious adverse effects (data not shown).

Example 17: Biophysical analysis of anti-TRBC1/NKp30 antibodies

The anti-TRBC1/NKp 30 antibodies BJM1042 and BJM1052 were analyzed for biophysical properties. BJM1042 and BJM1052 exhibited high stability and low aggregation propensity. BJM1042 and BJM1052 showed retained binding to FcRn and reduced or negligible binding to Fcγ receptors.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A multifunctional molecule, comprising:

(i) a first antigen binding domain that binds to T cell receptor beta chain constant domain 1 (TRBC1) or T cell receptor beta chain constant domain 2 (TRBC2), and

(ii) a second antigen binding domain that binds to NKp30.

2. The multifunctional molecule of claim 1, wherein the first antigen binding domain binds to TRBC1.

3. The multifunctional molecule of claim 2, wherein the first antigen binding domain comprises one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

4. The multifunctional molecule of claim 2 or 3, wherein the first antigen binding domain comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3, wherein the VHCDR1, VHCDR2, and VHCDR3 comprise the amino acid sequences of:

SEQ ID NOs: 7346, 7355, and 202, respectively;

SEQ ID NOs: 7346, 201, and 202, respectively;

SEQ ID NOs: 7354, 201, and 202, respectively; or

SEQ ID NOs: 7354, 7355, and 202, respectively.

5. The multifunctional molecule of claim 4, wherein the VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of:

SEQ ID NOs: 223, 224, and 225, respectively;

SEQ ID NOs: 7367, 224, and 225, respectively;

SEQ ID NOs: 223, 7368, and 225, respectively;

SEQ ID NOs: 223, 224, and 7369, respectively; or

SEQ ID NOs: 7367, 7368, and 7369, respectively.

6. The multifunctional molecule of claim 4 or 5, wherein the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise the amino acid sequences of:

SEQ ID NOs: 7346, 7355, 202, 223, 224, and 225, respectively;
SEQ ID NOs: 7346, 201, 202, 223, 224, and 225, respectively;
SEQ ID NOs: 7346, 7355, 202, 7367, 224, and 225, respectively;
SEQ ID NOs: 7346, 7355, 202, 223, 7368, and 225, respectively;
SEQ ID NOs: 7346, 7355, 202, 223, 224, and 7369, respectively;
SEQ ID NOs: 7346, 7355, 202, 7367, 7368, and 7369, respectively;
SEQ ID NOs: 7346, 201, 202, 7367, 224, and 225, respectively;
SEQ ID NOs: 7346, 201, 202, 223, 7368, and 225, respectively;
SEQ ID NOs: 7346, 201, 202, 223, 224, and 7369, respectively;
SEQ ID NOs: 7346, 201, 202, 7367, 7368, and 7369, respectively;
SEQ ID NOs: 7354, 201, 202, 223, 224, and 225, respectively;
SEQ ID NOs: 7354, 201, 202, 7367, 224, and 225, respectively;
SEQ ID NOs: 7354, 201, 202, 223, 7368, and 225, respectively;
SEQ ID NOs: 7354, 201, 202, 223, 224, and 7369, respectively;
SEQ ID NOs: 7354, 201, 202, 7367, 7368, and 7369, respectively;
SEQ ID NOs: 7354, 7355, 202, 223, 224, and 225, respectively;
SEQ ID NOs: 7354, 7355, 202, 7367, 224, and 225, respectively;
SEQ ID NOs: 7354, 7355, 202, 223, 7368, and 225, respectively;
SEQ ID NOs: 7354, 7355, 202, 223, 224, and 7369, respectively; or
SEQ ID NOs: 7354, 7355, 202, 7367, 7368, and 7369, respectively.

7. The multifunctional molecule of any of claims 4-6, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7351, 253, 250-252, 254, 7343, 7344, 7350, and 7352 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL comprises an amino acid sequence selected from the group consisting of

SEQ ID NOs: 258, 255-257, 259, 260, and 7357-7360 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

8. The multifunctional molecule of any of claims 4-7, wherein the VH and VL comprise the amino acid sequences of:

SEQ ID NOs: 7351 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

SEQ ID NOs: 253 and 258, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

9. The multifunctional molecule of any of claims 2-8, wherein:

(i) the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors not comprising TRBC1, optionally wherein the KD for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the KD for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC1;

(ii) the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC1 than for T cell receptors comprising TRBC2, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC1 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC2;

(iii) binding of the first antigen binding domain to TRBC1 on a lymphoma cell or lymphocyte, e.g., T cell, does not appreciably activate the lymphoma cell or lymphocyte, e.g., T cell, (e.g., as measured by T cell proliferation, expression of a T cell activation marker (e.g., CD69 or CD25), and/or expression of a cytokine (e.g., TNFα and IFNγ).

10. The multifunctional molecule of claim 1, wherein the first antigen binding domain binds to TRBC2.

11. The multifunctional molecule of claim 10, wherein:

(i) the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than for T cell receptors not comprising TRBC2, optionally wherein the KD for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the KD for the binding between the first antigen binding domain and a T cell receptor not comprising TRBC2;

(ii) the first antigen binding domain has a higher affinity for a T cell receptor comprising TRBC2 than for T cell receptors comprising TRBC1, optionally wherein the K_D for the binding between the first antigen binding domain and TRBC2 is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and a T cell receptor comprising TRBC1; or

(iii) binding of the first antigen binding domain to TRBC2 on a lymphoma cell or lymphocyte, e.g., T cell, does not appreciably activate the lymphoma cell or lymphocyte, e.g., T cell, (e.g., as measured by T cell proliferation, expression of a T cell activation marker (e.g., CD69 or CD25), and/or expression of a cytokine (e.g., TNFα and IFNγ).

12. The multifunctional molecule of any of claims 1-11, wherein the second antigen binding domain comprises one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 7-10, 18, and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

13. The multifunctional molecule of any of claims 1-12, wherein the second antigen binding domain comprises a VH comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3, and a VL comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3, wherein the VHCDR1, VHCDR2, and VHCDR3 of the second antigen binding domain comprise the amino acid sequences of:

SEQ ID NOs: 7313, 6001, and 7315, respectively;

SEQ ID NOs: 7313, 6001, and 6002, respectively;

SEQ ID NOs: 7313, 6008, and 6009, respectively;

SEQ ID NOs: 7313, 7385, and 7315, respectively; or

SEQ ID NOs: 7313, 7318, and 6009, respectively.

14. The multifunctional molecule of claim 13, wherein the VLCDR1, VLCDR2, and VLCDR3 of the second antigen binding domain comprise the amino acid sequences of:

SEQ ID NOs: 7326, 7327, and 7329, respectively;

SEQ ID NOs: 6063, 6064, and 7293, respectively;

SEQ ID NOs: 6070, 6071, and 6072, respectively; or

SEQ ID NOs: 6070, 6064, and 7321, respectively.

15. The multifunctional molecule of claim 13 or 14, wherein the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 of the second antigen binding domain comprise the amino acid sequences of:

SEQ ID NOs: 7313, 6001, 7315, 7326, 7327, and 7329, respectively;

SEQ ID NOs: 7313, 6001, 6002, 6063, 6064, and 7293, respectively;

SEQ ID NOs: 7313, 6008, 6009, 6070, 6071, and 6072, respectively;

SEQ ID NOs: 7313, 7385, 7315, 6070, 6064, and 7321, respectively; or

SEQ ID NOs: 7313, 7318, 6009, 6070, 6064, and 7321, respectively.

16. The multifunctional molecule of any of claims 13-15, wherein:

(i) the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7302, 7298, 7300, 7301, 7303, and 7304 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7309, 7305, 7299, 7306-7308 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

(ii) the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6121 or 6123-6128 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7294 or 6137-6141 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

(iii) the VH of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6122 or 6129-6134 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto) and/or the VL of the second antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6136 or 6142-6147 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

17. The multifunctional molecule of any of claims 13-16, wherein the VH and VL of the second antigen binding domain comprise the amino acid sequences of:

SEQ ID NOs: 7302 and 7309, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

SEQ ID NOs: 7302 and 7305, respectively (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

18. The multifunctional molecule of any of claims 13-17, wherein the second antigen binding domain comprise the amino acid sequences of:

SEQ ID NO: 7311 or 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

SEQ ID NO: 6187 or 6188 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

SEQ ID NO: 6189 or 6190 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

19. The multifunctional molecule of any of claims 1-18, wherein the multifunctional molecule binds to TRBC1 or TRBC2 monovalently.

20. The multifunctional molecule of any of claims 1-19, wherein the multifunctional molecule comprises a configuration shown in any of FIGs. 29A-29D, optionally wherein:

(i) the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29A;

(ii) the multifunctional antibody molecule comprises an anti-TRBC1 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 29B;

(iii) the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC1 scFv, e.g., comprises a configuration shown in FIG. 29C; or

(iv) the multifunctional antibody molecule comprises an anti-TRBC1 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 29D.

21. The multifunctional molecule of any of claims 1-19, wherein the multifunctional molecule comprises a configuration shown in any of FIGs. 30A-30D, optionally wherein:

(i) the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30A;

(ii) the multifunctional antibody molecule comprises an anti-TRBC2 Fab and an anti-NKp30 Fab, e.g., comprises a configuration shown in FIG. 30B;

(iii) the multifunctional antibody molecule comprises an anti-NKp30 Fab and an anti-TRBC2 scFv, e.g., comprises a configuration shown in FIG. 30C; or

(iv) the multifunctional antibody molecule comprises an anti-TRBC2 scFv and an anti-NKp30 scFv, e.g., comprises a configuration shown in FIG. 30D.

22. The multifunctional molecule of any of claims 1-21, further comprising a dimerization module comprising one or more immunoglobulin chain constant regions (e.g., Fc regions) comprising one or more of: a paired cavity-protuberance ("knob-in-a hole"), an electrostatic interaction, or a strand-exchange.

23. The multifunctional molecule of any of claims 1-22, comprising an anti-TRBC1 amino acid sequence disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto, and/or an anti-NKp30 amino acid sequence disclosed in any of Tables 7-10, 18, and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

24. The multifunctional molecule of any of claims 1-23, comprising:

(i) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

(ii) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

(iii) SEQ ID NOs: 7382, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

25. The multifunctional molecule of any of claims 1-23, comprising:

(i) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7309 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

(ii) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7311 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

(iii) SEQ ID NOs: 7379, 7380, and 7383 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

26. The multifunctional molecule of any of claims 1-23, comprising:

(i) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a

sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

(ii) an anti-TRBC1 VH of SEQ ID NO: 7351 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

(iii) SEQ ID NOs: 7382, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

27. The multifunctional molecule of any of claims 1-23, comprising:

(i) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-NKp30 VH of SEQ ID NO: 7302 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 VL of SEQ ID NO: 7305 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto);

(ii) an anti-TRBC1 VH of SEQ ID NO: 253 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), an anti-TRBC1 VL of SEQ ID NO: 258 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto), and an anti-NKp30 scFv of SEQ ID NO: 7310 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto); or

(iii) SEQ ID NOs: 7379, 7380, and 7384 (or a sequence having at least 85%, 90%, 95%, or 99% identity thereto).

28. An antibody molecule that binds to TRBC1, comprising one or more CDRs, framework regions, variable regions, or antigen binding domains disclosed in any of Tables 2-6 and 19, or a sequence having at least 85%, 90%, 95%, or 99% identity thereto.

29. The multifunctional molecule of any of claims 1-27 or the antibody molecule of claim 28, further comprising a heavy chain constant region variant, e.g., an Fc region variant, that comprises one or more mutations that result in reduced or ablated affinity for at least one Fc receptor, optionally wherein the one or more mutations result in reduced or ablated antibody

dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP), or complement dependent cytotoxicity (CDC).

30. The multifunctional molecule or antibody molecule of claim 29, wherein the Fc region variant comprises one or more mutations disclosed in Table 20, optionally wherein the Fc region variant comprises an N297A mutation.

31. A nucleic acid molecule encoding the multifunctional molecule of any of claims 1-27, 29, or 30 or the antibody molecule of any of claims 28-30.

32. A vector, e.g., an expression vector, comprising the nucleic acid molecules of claim 31.

33. A cell comprising the nucleic acid molecule of claim 31 or the vector of claim 32.

34. A method of making, e.g., producing, the multifunctional molecule of any of claims 1-27, 29, or 30 or the antibody molecule of any of claims 28-30, comprising culturing the cell of claim 33, under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

35. A pharmaceutical composition comprising the multifunctional molecule of any of claims 1-27, 29, or 30 or the antibody molecule of any of claims 28-30 and a pharmaceutically acceptable carrier, excipient, or stabilizer.

36. A method of treating a cancer, comprising administering to a subject in need thereof the multifunctional molecule of any of claims 1-27, 29, or 30, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

37. The method of claim 36, further comprising identifying, evaluating, or selecting a subject in need of treatment, wherein identifying, evaluating, or selecting comprises determining

(e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2.

38. The method of claim 37, further comprising, responsive to determining that a subject has cancer cells that express a T cell receptor comprising TRBC1:

optionally, selecting the subject for treatment with a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1, and

administering a multifunctional molecule comprising an antigen binding domain that binds to a T cell receptor comprising TRBC1.

39. A method of treating a cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, comprising:

responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC1, administering to the subject the multifunctional molecule of any one of claims 1-27, 29, or 30, wherein the first antigen binding domain binds to TRBC1, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

40. A method of treating a cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, comprising:

responsive to a determination that a subject has cancer cells that express a T cell receptor comprising TRBC2, administering to the subject the multifunctional molecule of any one of claims 1-27, 29, or 30, wherein the first antigen binding domain binds to TRBC2, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

41. A method of identifying a subject in need of treatment for cancer, e.g., a lymphoma or leukemia, e.g., a T cell lymphoma or leukemia, using the multifunctional molecule of any of claims 1-27, 29, or 30, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has cancer cells that express a T cell receptor comprising TRBC1 or TRBC2, wherein:

responsive to a determination that the subject has cancer cells that express a T cell receptor comprising TRBC1, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, or

responsive to a determination that the subject has cancer cells that express a T cell receptor comprising TRBC2, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, and optionally not as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1.

42. The method of claim 41, further comprising:

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC1, or

responsive to identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to TRBC2, treating the subject with (e.g., administering to the subject) a multifunctional molecule comprising an antigen binding domain that binds to TRBC2.

43. The method of any of claims 36-42, wherein the cancer is leukemia or lymphoma.

44. The method of any of claims 36-43, wherein the cancer is selected from Acquired immune deficiency syndrome (AIDS)-associated lymphoma, Angioimmunoblastic T-cell lymphoma, Adult T-cell leukemia/lymphoma, Burkitt lymphoma, Central nervous system (CNS) lymphoma, Diffuse large B-cell lymphoma (DLBCL), Lymphoblastic lymphoma, Mantle cell lymphoma (MCL), Peripheral T-cell lymphoma (PTCL) (e.g., Hepatosplenic T-cell lymphoma (HSGDTCL), Subcutaneous paniculitis-like T-cell lymphoma, or Enteropathy-associated T-cell lymphoma), Transformed follicular and transformed mucosa-associated lymphoid tissue

(MALT) lymphomas, Cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome), Follicular lymphoma, Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, Marginal zone B-cell lymphoma, Gastric mucosa-associated lymphoid tissue (MALT) lymphoma, Chronic lymphocytic leukemia/small-cell lymphocytic lymphoma (CLL/SLL), Extranodal T-/NK-cell lymphoma (nasal type), and Anaplastic large-cell lymphoma (e.g., primary cutaneous anaplastic large-cell lymphoma or systemic anaplastic large-cell lymphoma).

45. The method of any of claims 36-43, wherein the cancer is lymphoma is Peripheral T-cell lymphoma (PTCL).

FIG. 1A

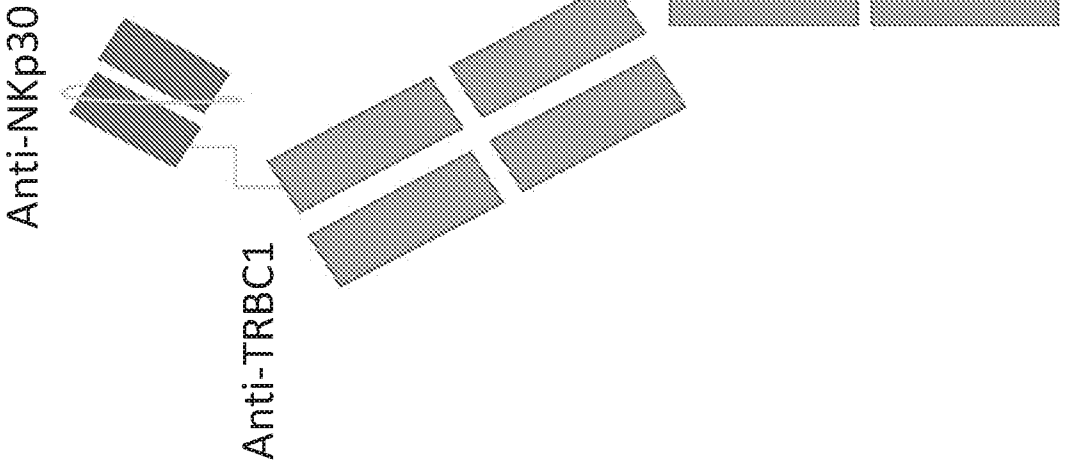
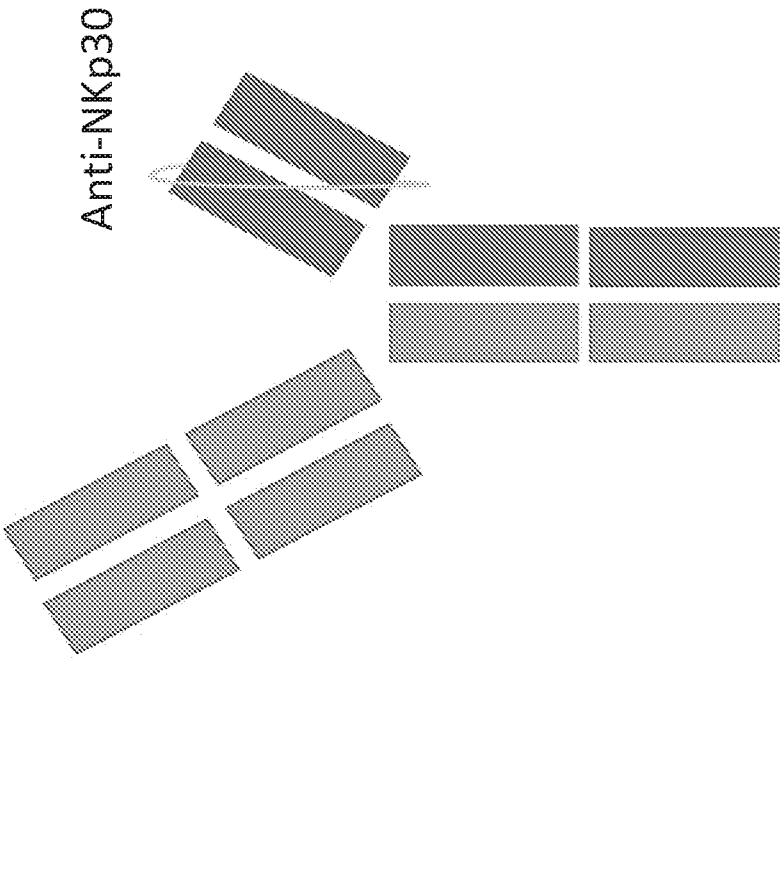


FIG. 1B



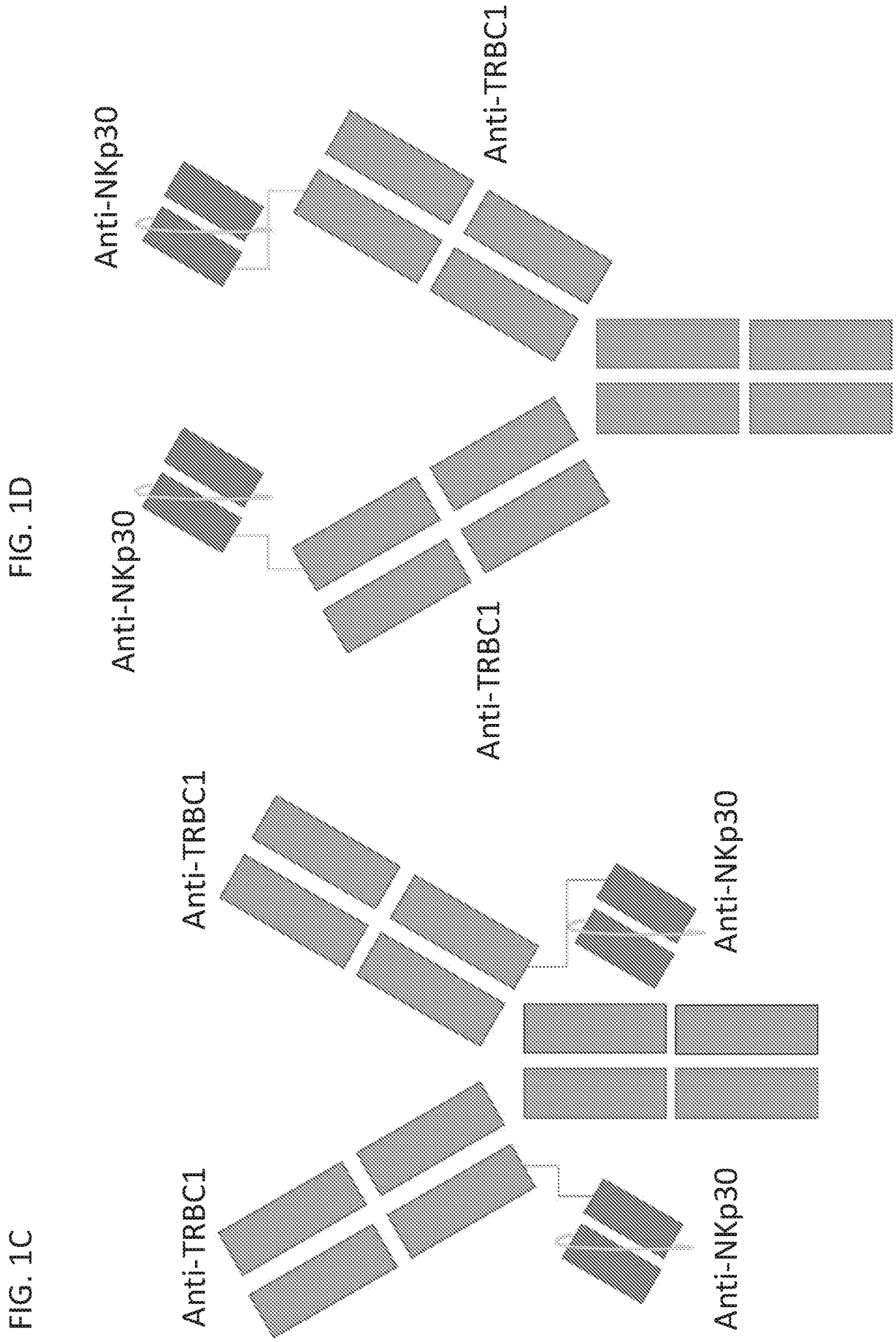

Anti-NKp30
Anti-TRBC1
Anti-NKp30
Anti-TRBC1
Anti-TRBC1
Anti-NKp30
Anti-TRBC1
Anti-NKp30

FIG. 1D

FIG. 1C

FIG. 2A

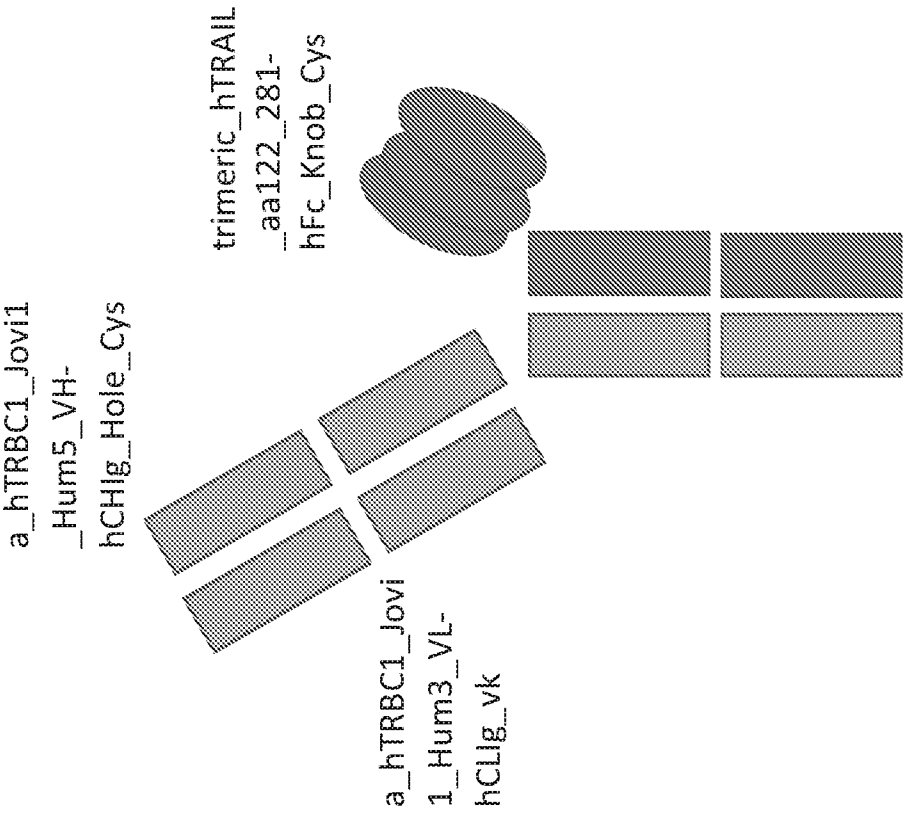

a_hTRBC1_Jovi1_Hum5_VH-hCHIg_Hole_Cys
trimeric_hTRAIL_aa122_281-hFc_Knob_Cys
a_hTRBC1_Jovi1_Hum3_VL-hCLIg_vk

FIG. 2B

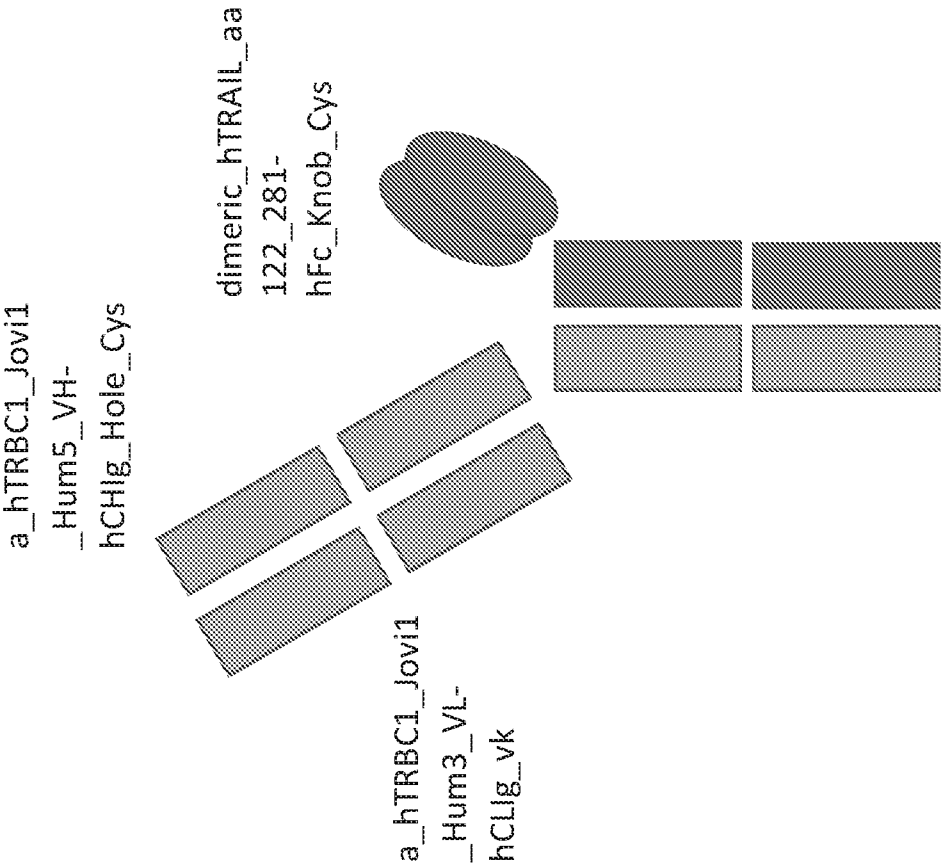

a_hTRBC1_Jovi1_Hum5_VH-hCHIg_Hole_Cys
dimeric_hTRAIL_aa122_281-hFc_Knob_Cys
a_hTRBC1_Jovi1_Hum3_VL-hCLIg_vk

FIG. 2C

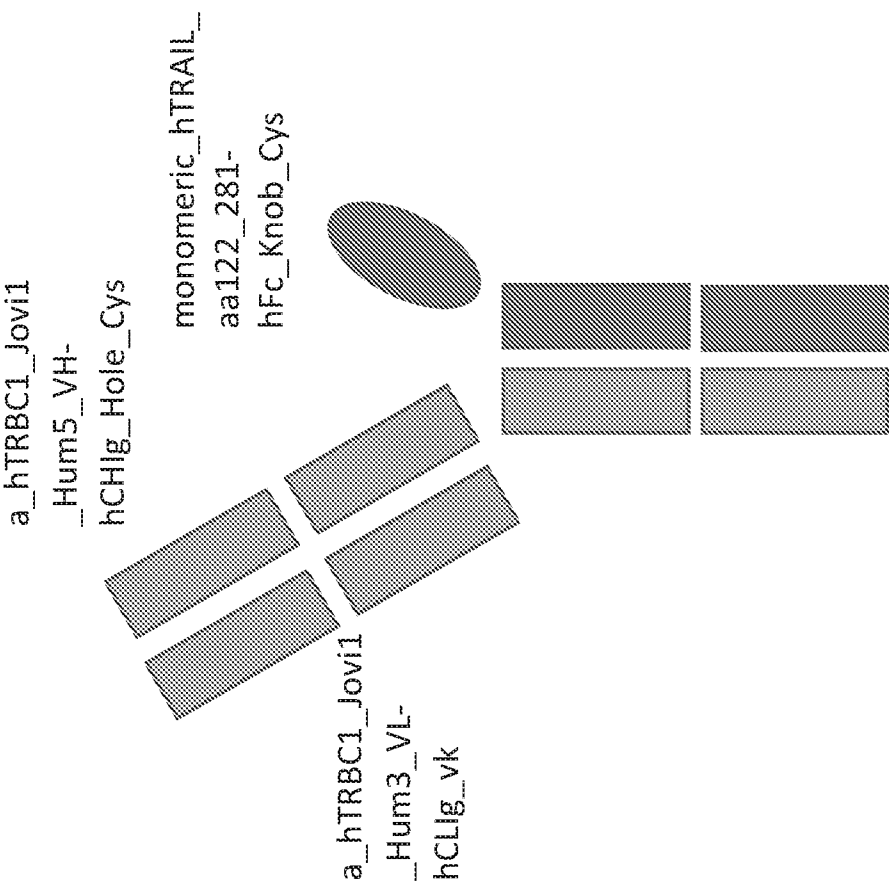

a_hTRBC1_Jovi1
_Hum5_VH-
hCHIg_Hole_Cys
monomeric_hTRAIL_
aa122_281-
hFc_Knob_Cys
a_hTRBC1_Jovi1
_Hum3_VL-
hCLIg_vk

FIG. 2D

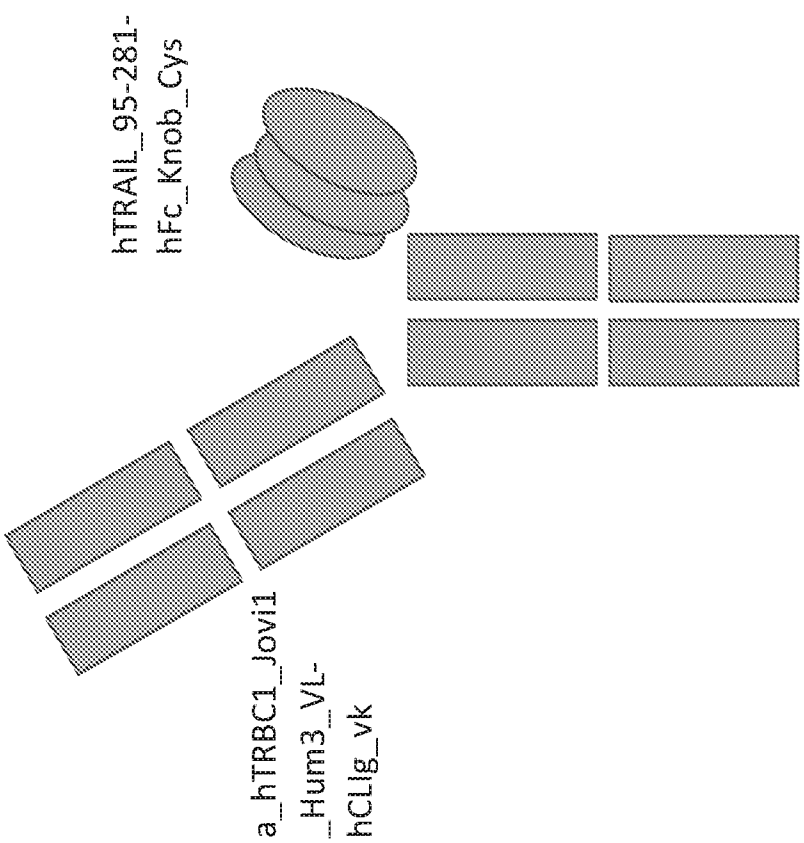

a_hTRBC1_Jovi1
_Hum5_VH-
hCHIg_Hole_Cys
hTRAIL_95-281-
hFc_Knob_Cys
a_hTRBC1_Jovi1
_Hum3_VL-
hCLIg_vk

FIG. 2E

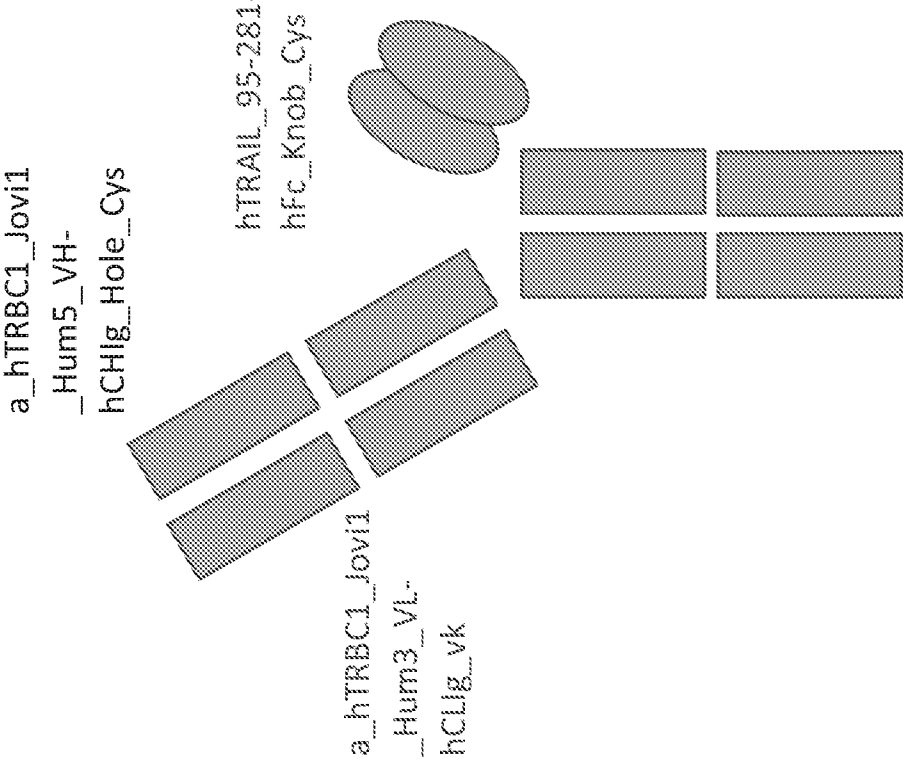

a_hTRBC1_Jovi1
_Hum5_VH-
hCHIg_Hole_Cys
hTRAIL_95-281-
hFc_Knob_Cys
a_hTRBC1_Jovi1
_Hum3_VL-
hCLIg_vk

FIG. 2F

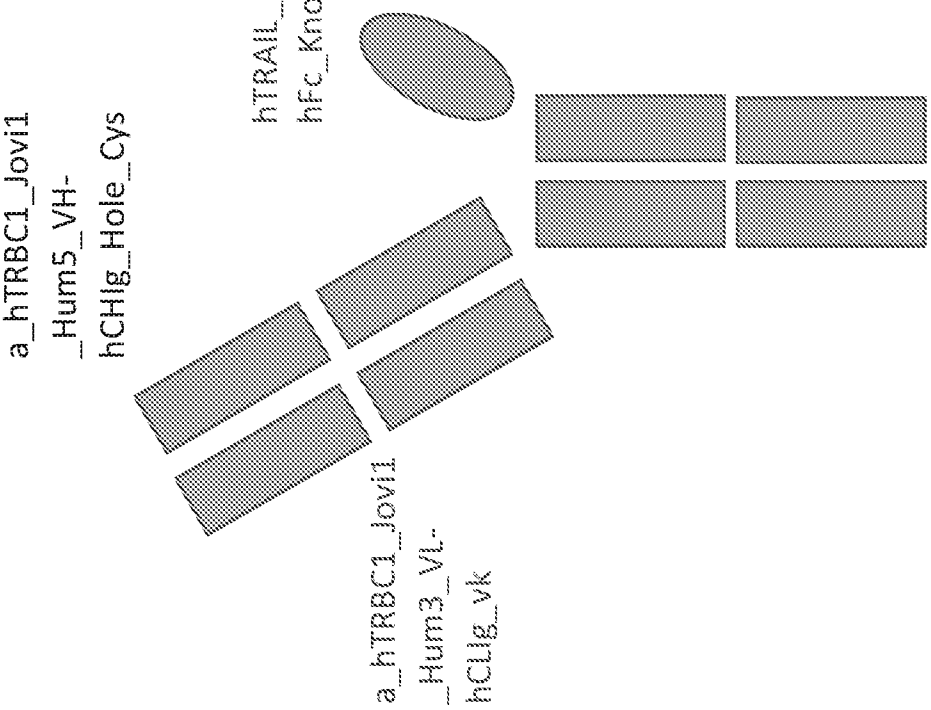

a_hTRBC1_Jovi1
_Hum5_VH-
hCHIg_Hole_Cys
hTRAIL_95-281-
hFc_Knob_Cys
a_hTRBC1_Jovi1
_Hum3_VL-
hCLIg_vk

FIG. 3A

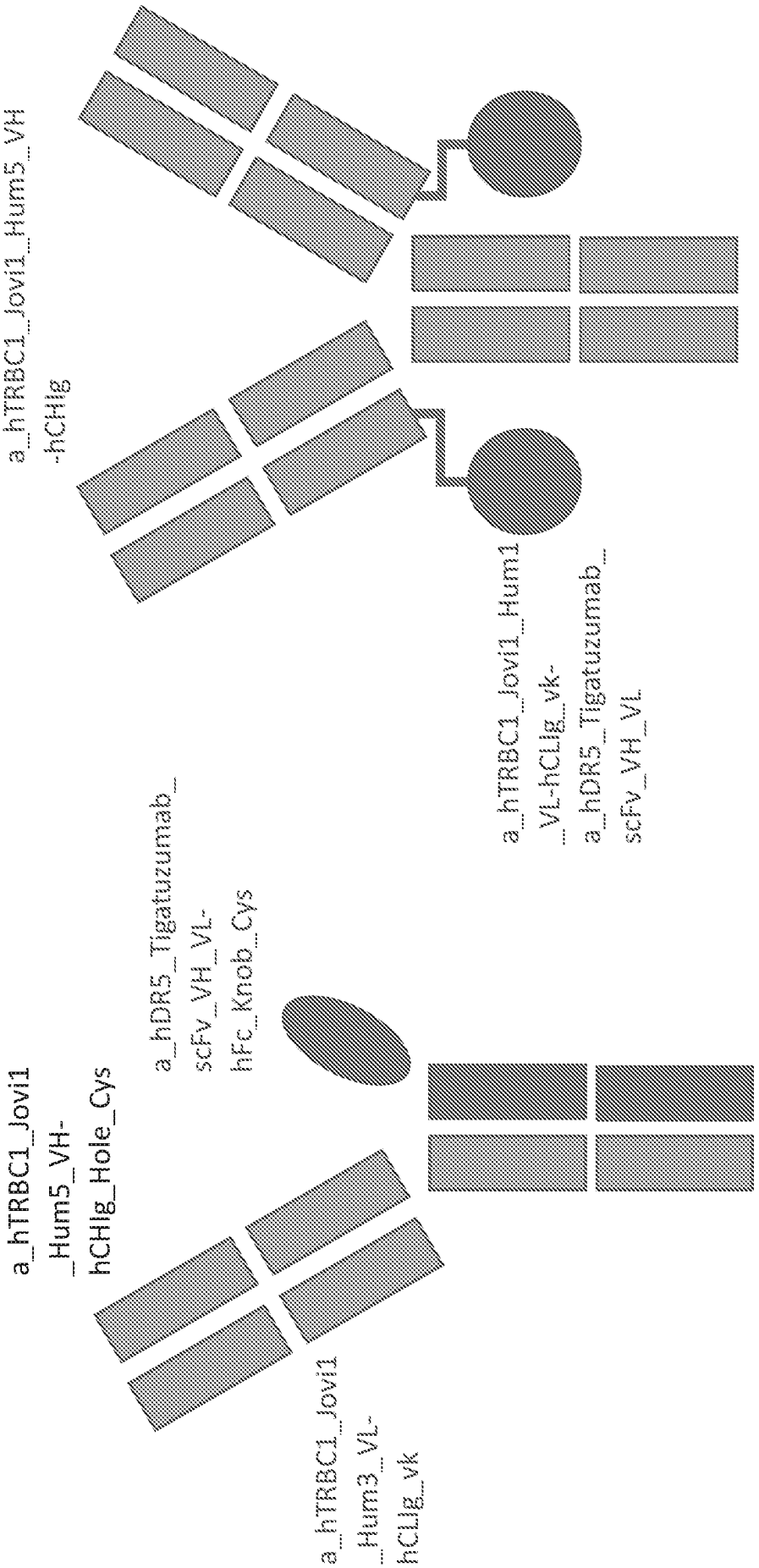

a_hTRBC1_Jovi1_Hum5_VH
-hCHIg
a_hTRBC1_Jovi1
_Hum5_VH-
hCHIg_Hole_Cys
a_hDR5_Tigatuzumab_
scFv_VH_VL-
hFc_Knob_Cys
a_hTRBC1_Jovi1_Hum1
_VL-hCLIg_vk-
a_hDR5_Tigatuzumab_
scFv_VH_VL
a_hTRBC1_Jovi1
_Hum3_VL-
hCLIg_vk

FIG. 3B

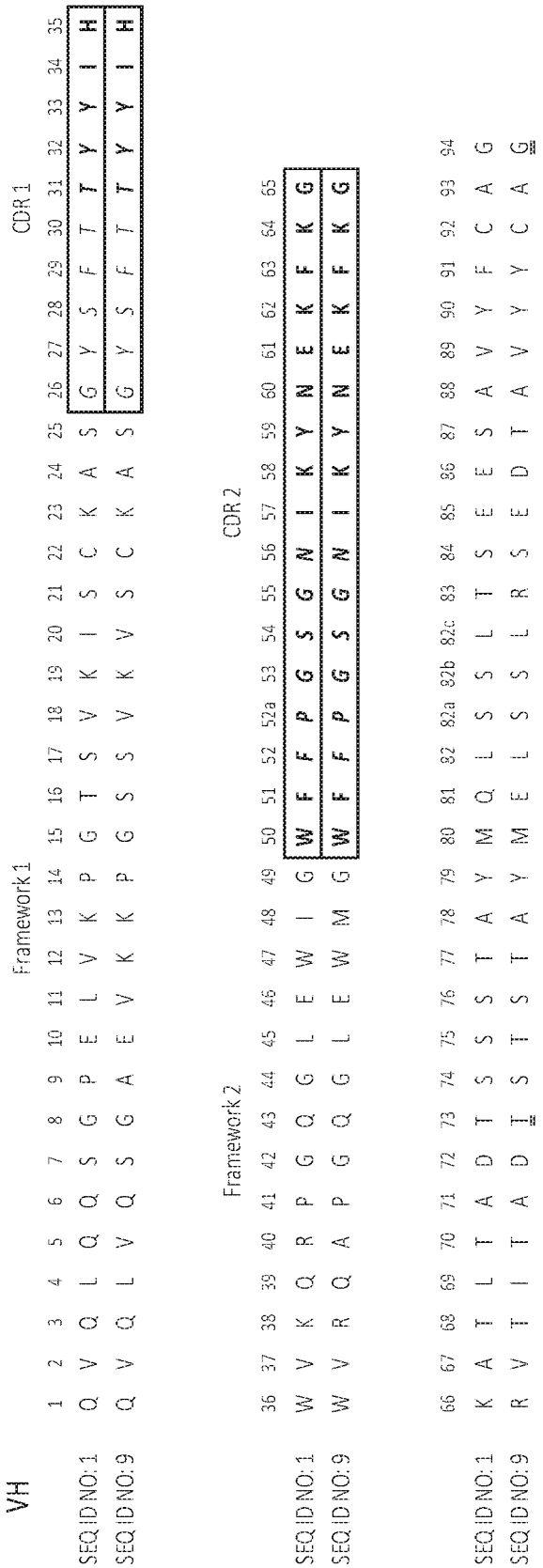

VH
Framework 1
CDR 1
Framework 2
CDR 2
CDR 3
Framework 4
SEQ ID NO: 1
SEQ ID NO: 9

FIG. 4A

VL

| | Framework 1 | | |

VH

	Framework 1																							CDR 1									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
SEQ ID NO: 15	D	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	R	K	L	S	C	A	A	S	G	F	T	F	S	N	F	G	M	H
SEQ ID NO: 25	Q	V	Q	L	V	E	S	G	G	G	V	V	Q	P	G	R	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	F	G	M	H
SEQ ID NO: 23	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	F	G	M	H
SEQ ID NO: 24	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	F	G	M	H

	Framework 2														CDR 2																
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65
SEQ ID NO: 15	W	V	R	Q	A	P	D	K	G	L	E	W	V	A	Y	I	S	S	G	S	S	T	I	Y	Y	A	D	T	L	K	G
SEQ ID NO: 25	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	Y	I	S	S	G	S	S	T	I	Y	Y	A	D	T	L	K	G
SEQ ID NO: 23	W	V	R	Q	A	P	G	K	G	L	E	W	V	S	Y	I	S	S	G	S	S	T	I	Y	Y	A	D	T	L	K	G
SEQ ID NO: 24	W	V	R	Q	A	P	G	K	G	L	E	W	V	S	Y	I	S	S	G	S	S	T	I	Y	Y	A	D	T	L	K	G

	Framework 3																														
	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94
SEQ ID NO: 15	R	F	T	I	S	R	D	N	P	K	N	T	L	F	L	Q	M	T	S	L	R	S	E	D	T	A	M	Y	Y	C	A	R
SEQ ID NO: 25	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R
SEQ ID NO: 23	R	F	T	I	S	R	D	N	A	K	N	S	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R
SEQ ID NO: 24	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R

	CDR 3								Framework 4										
	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113
SEQ ID NO: 15	R	G	E	G	A	M	D	Y	W	G	Q	G	T	S	V	T	V	S	S
SEQ ID NO: 25	R	G	E	G	A	M	D	Y	W	G	Q	G	T	T	V	T	V	S	S
SEQ ID NO: 23	R	G	E	G	A	M	D	Y	W	G	Q	G	T	T	V	T	V	S	S
SEQ ID NO: 24	R	G	E	G	A	M	D	Y	W	G	Q	G	T	T	V	T	V	S	S

FIG. 5A

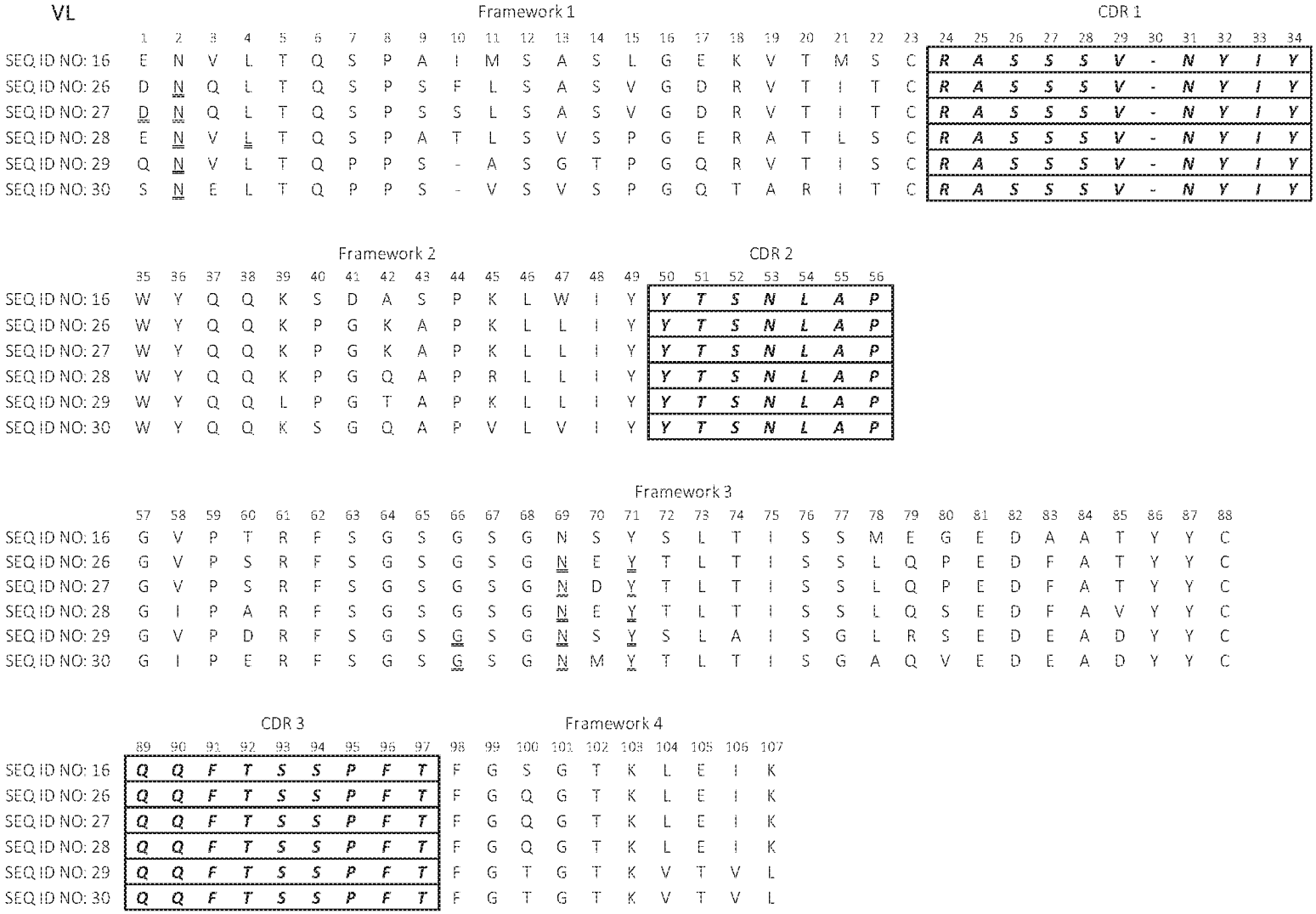

VL
Framework 1
CDR 1
Framework 2
CDR 2
Framework 3
CDR 3
Framework 4
SEQ ID NO: 16
SEQ ID NO: 26
SEQ ID NO: 27
SEQ ID NO: 28
SEQ ID NO: 29
SEQ ID NO: 30

FIG. 5B

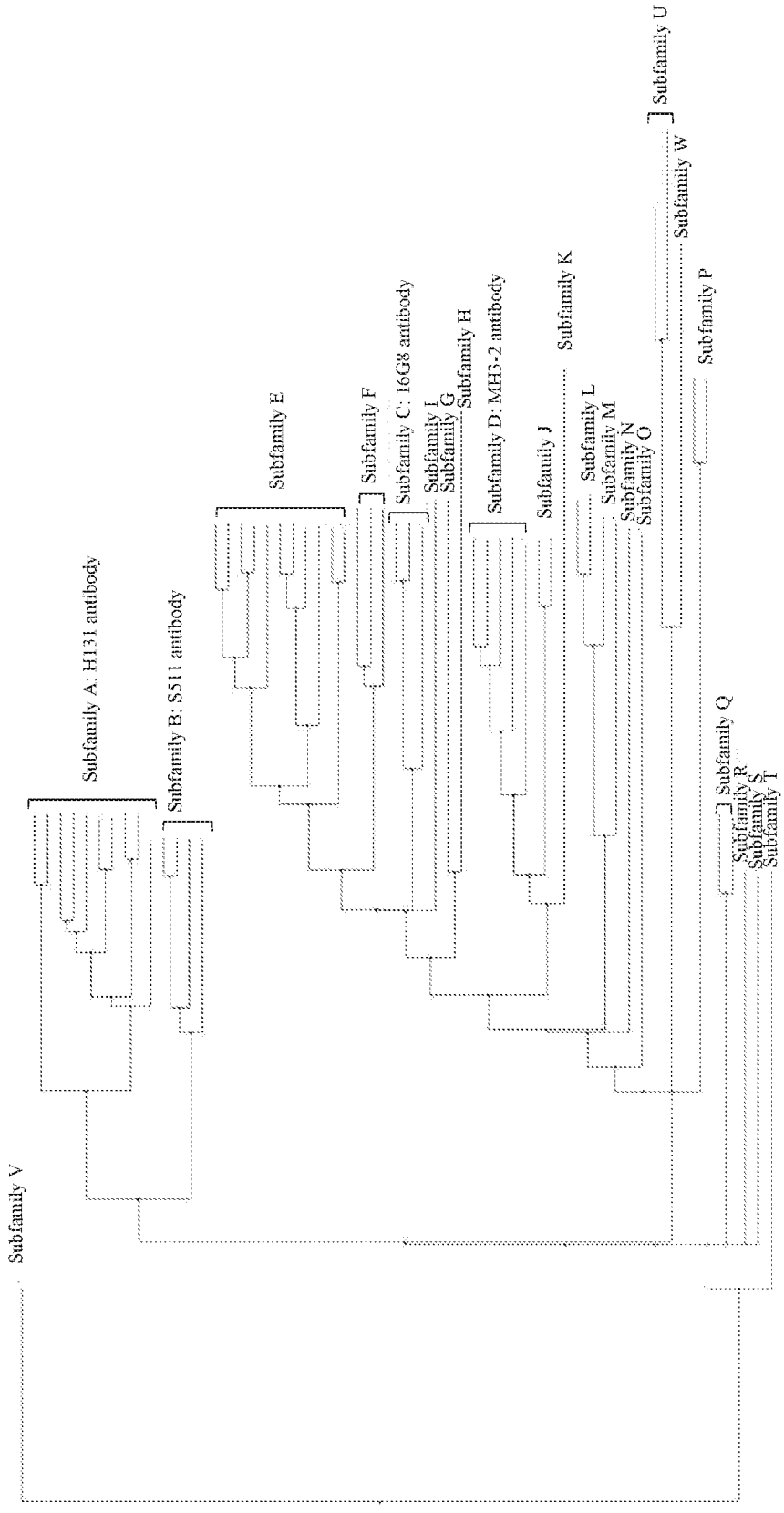
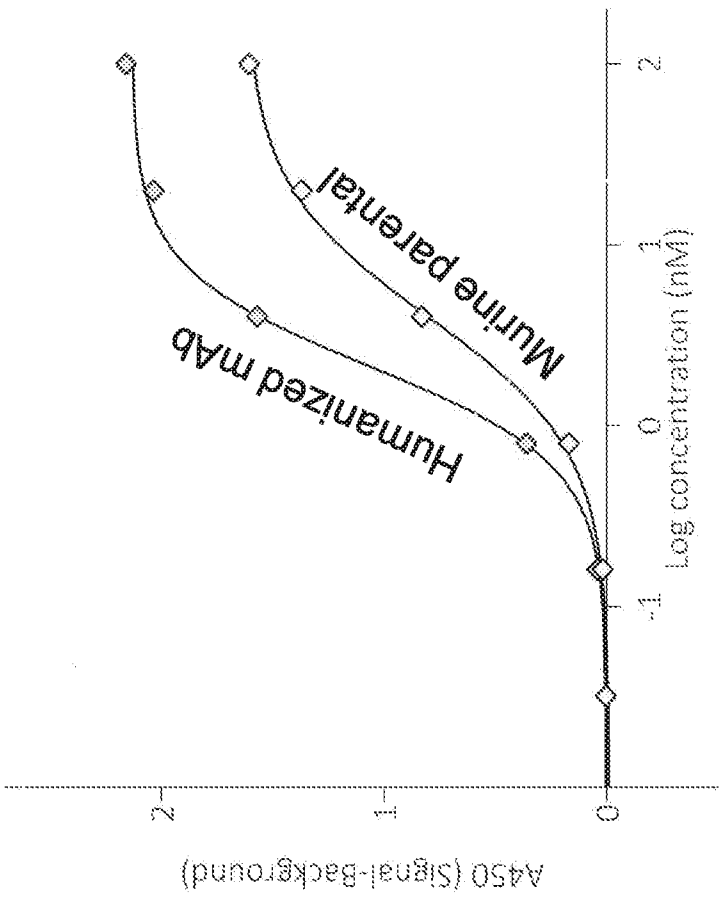
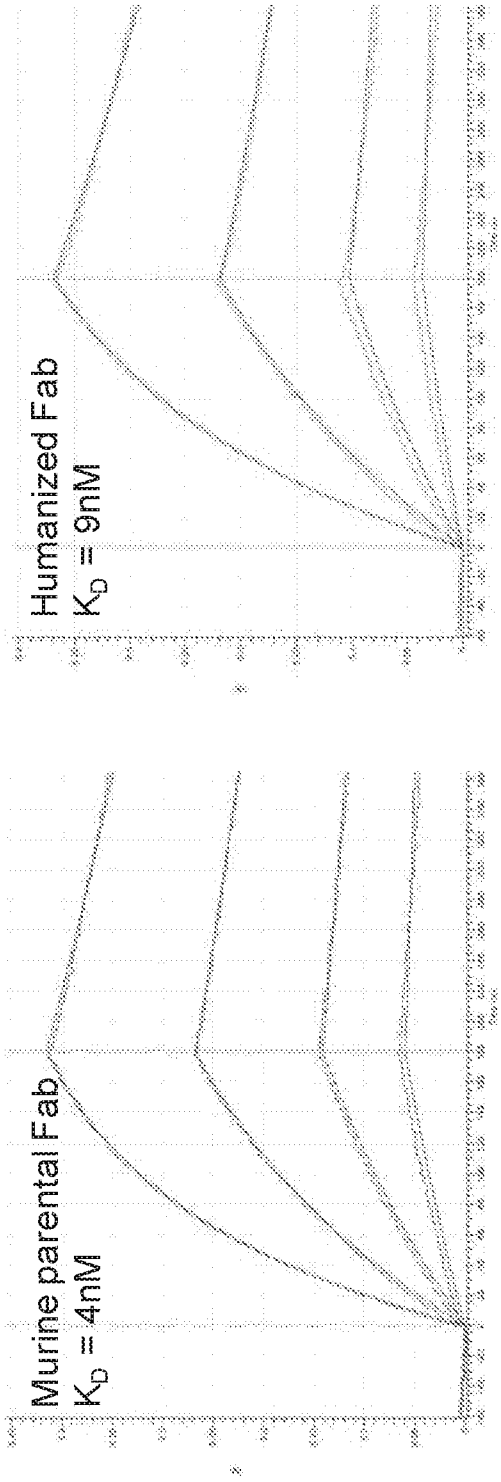

Subfamily V
Subfamily A: H131 antibody
Subfamily B: S511 antibody
Subfamily E
Subfamily F
Subfamily C: 16G8 antibody
Subfamily I
Subfamily G
Subfamily H
Subfamily D: MH3-2 antibody
Subfamily J
Subfamily K
Subfamily L
Subfamily M
Subfamily N
Subfamily O
Subfamily U
Subfamily W
Subfamily P
Subfamily Q
Subfamily R
Subfamily S
Subfamily T

FIG. 6



ELISA data showing direct binding of anti-TRBC1 mAbs (bivalent) to hTRBC1 coated on the plate

FIG. 7



Octet data showing binding of anti-TRBC1 Fabs to hTRBC1. hTRBC1 was captured on the sensor tip and dipped in solution containing different concentrations of monovalent Fabs

FIG. 8

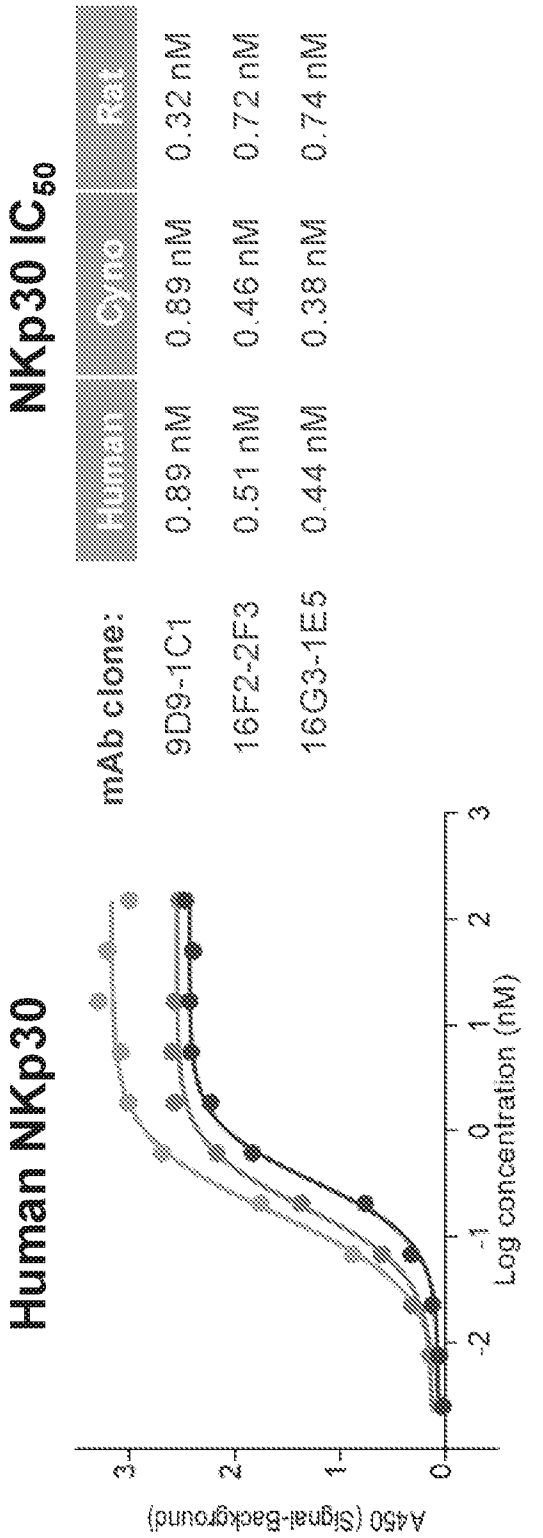

Human NKp30
A450 (Signal-Background)
Log concentration (nM)
mAb clone:
9D9-1C1
16F2-2F3
16G3-1E5
NKp30 IC50
Human
Cyno
Rat
0.89 nM
0.89 nM
0.32 nM
0.51 nM
0.46 nM
0.72 nM
0.44 nM
0.38 nM
0.74 nM

FIG. 9

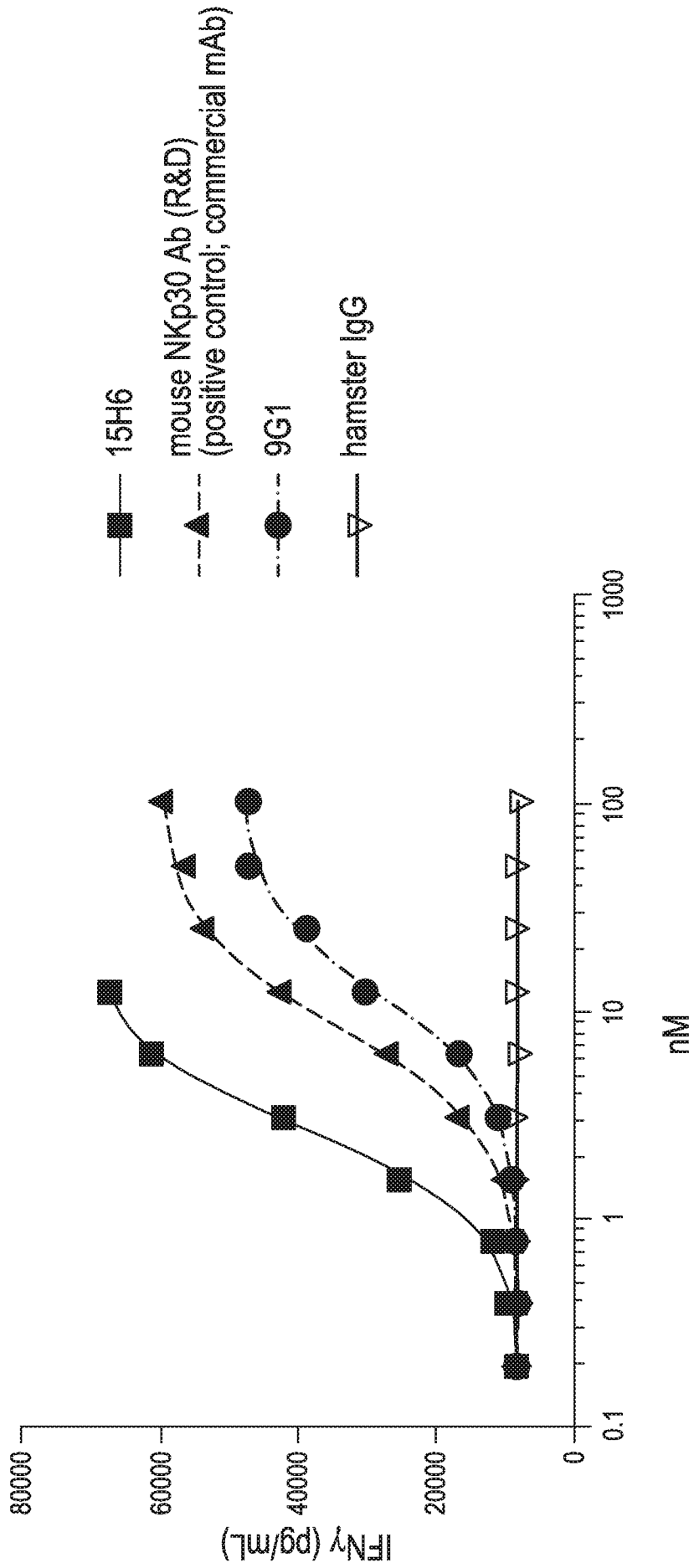

Activation of NK92 cells by NK p30 Ab
15H6
mouse NKp30 Ab (R&D)
(positive control; commercial mAb)
9G1
hamster IgG
IFNγ (pg/mL)
80000
60000
40000
20000
0
0.1
1
10
100
1000
nM

FIG. 10

FIG. 11A

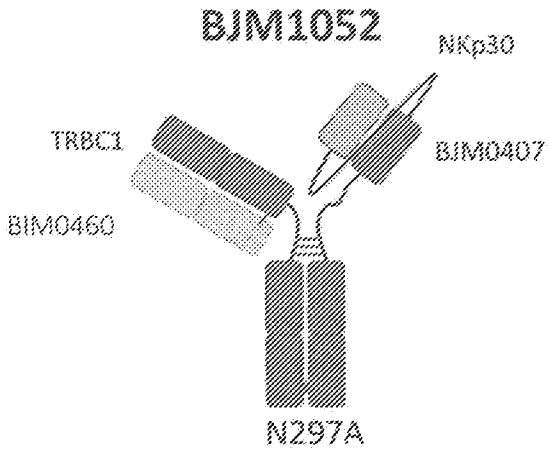


FIG. 11B

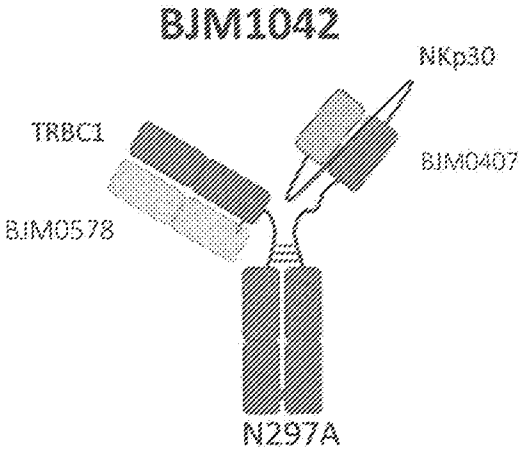


FIG. 11C

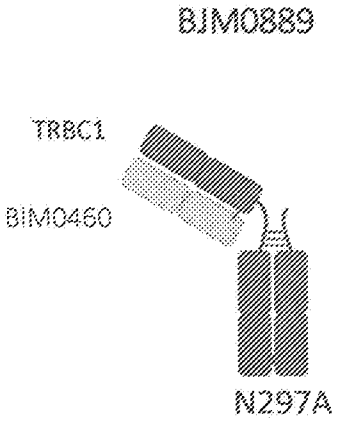


FIG. 11D

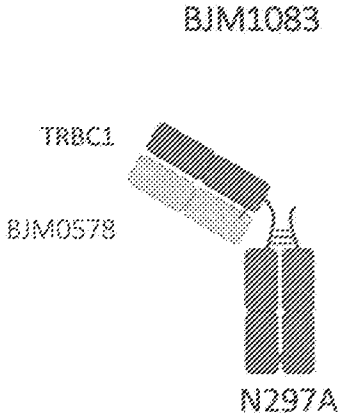
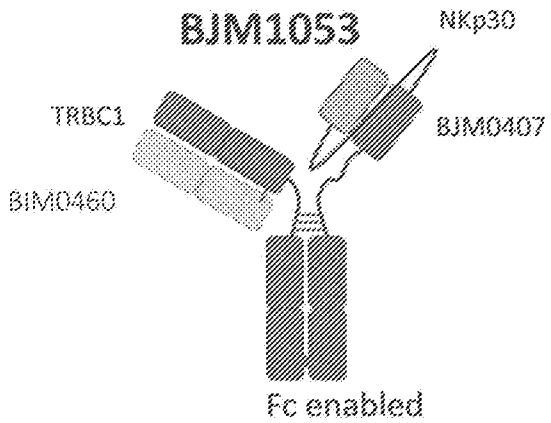


FIG. 11E



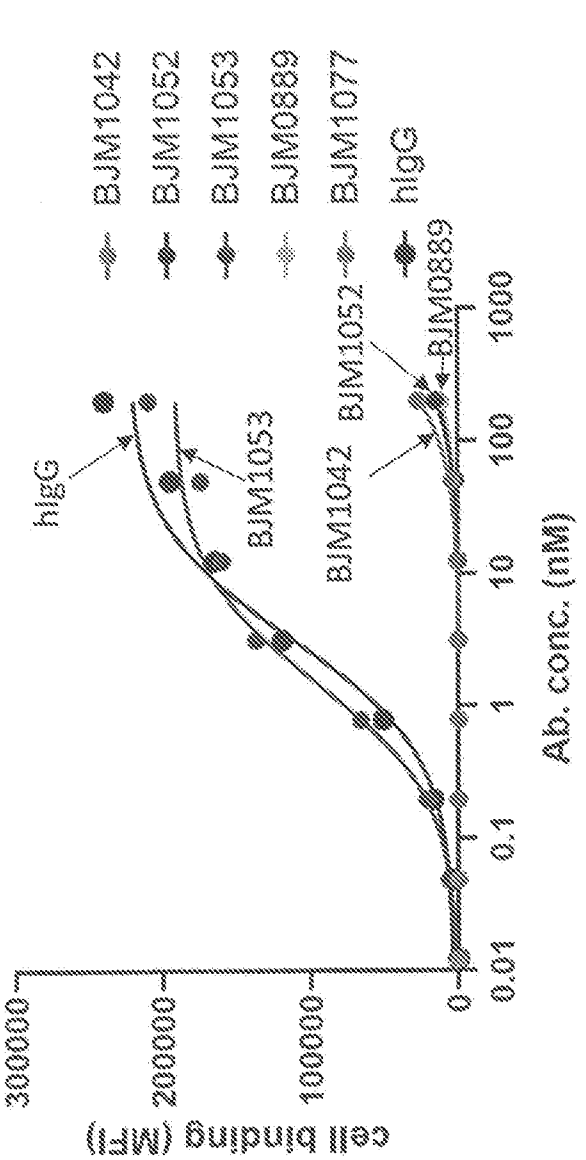


FIG. 12A

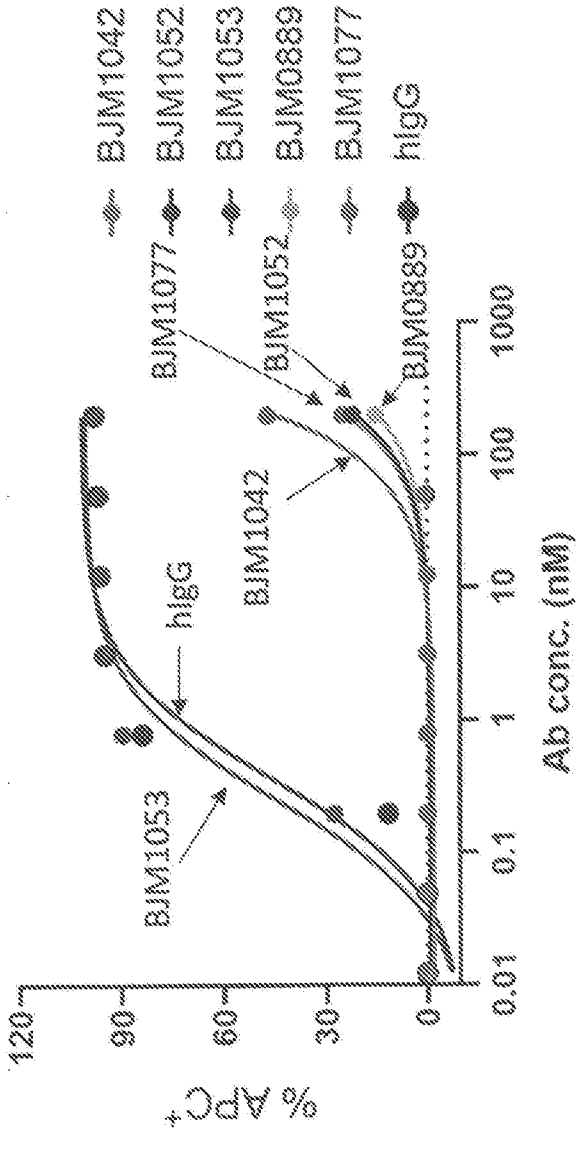


FIG. 12B

FIG. 13A

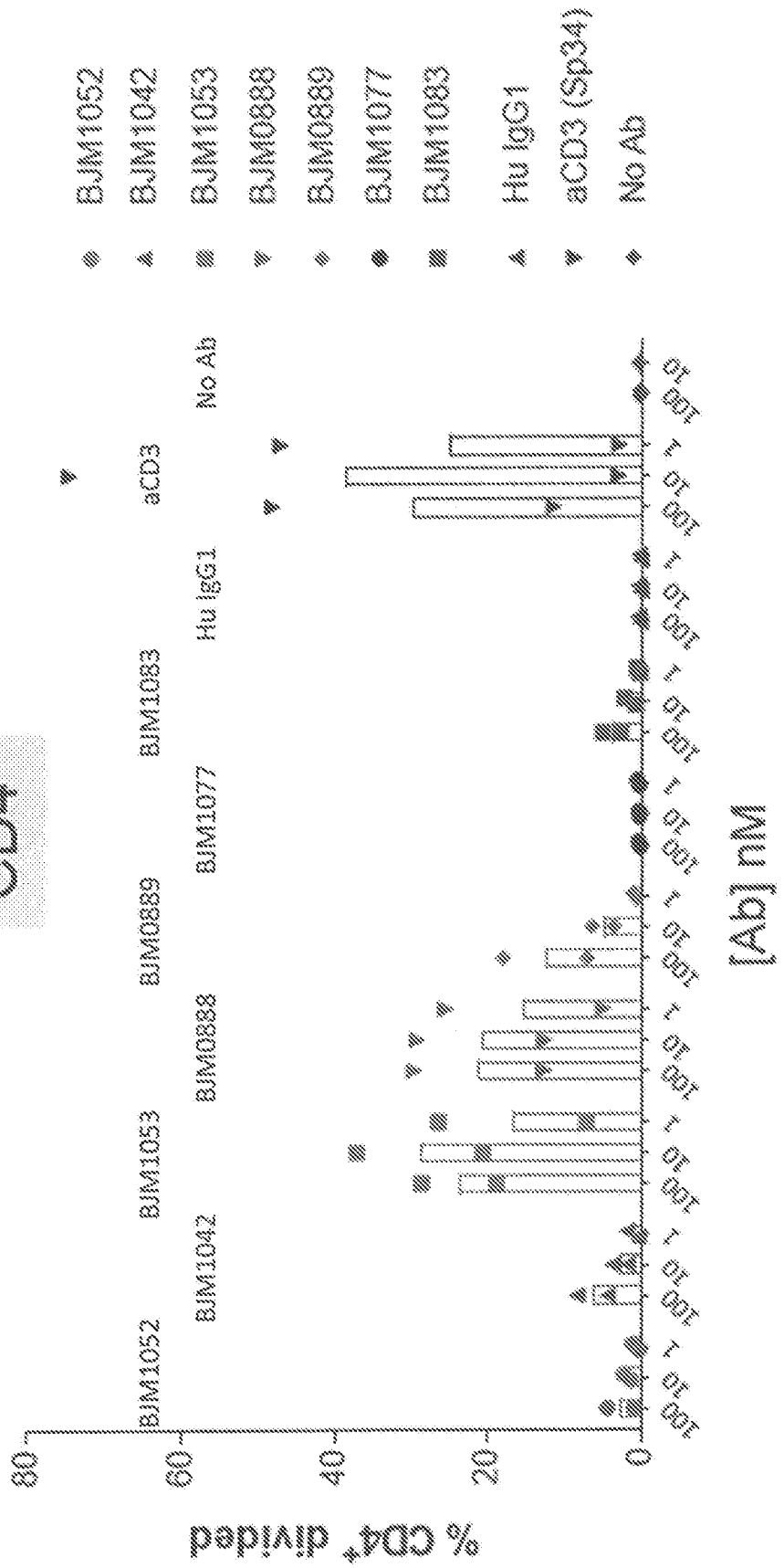

CD4
% CD4+ divided
80
60
40
20
0
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3
Mo Ab
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3 (Sp34)
No Ab
[Ab] nM

FIG. 13B

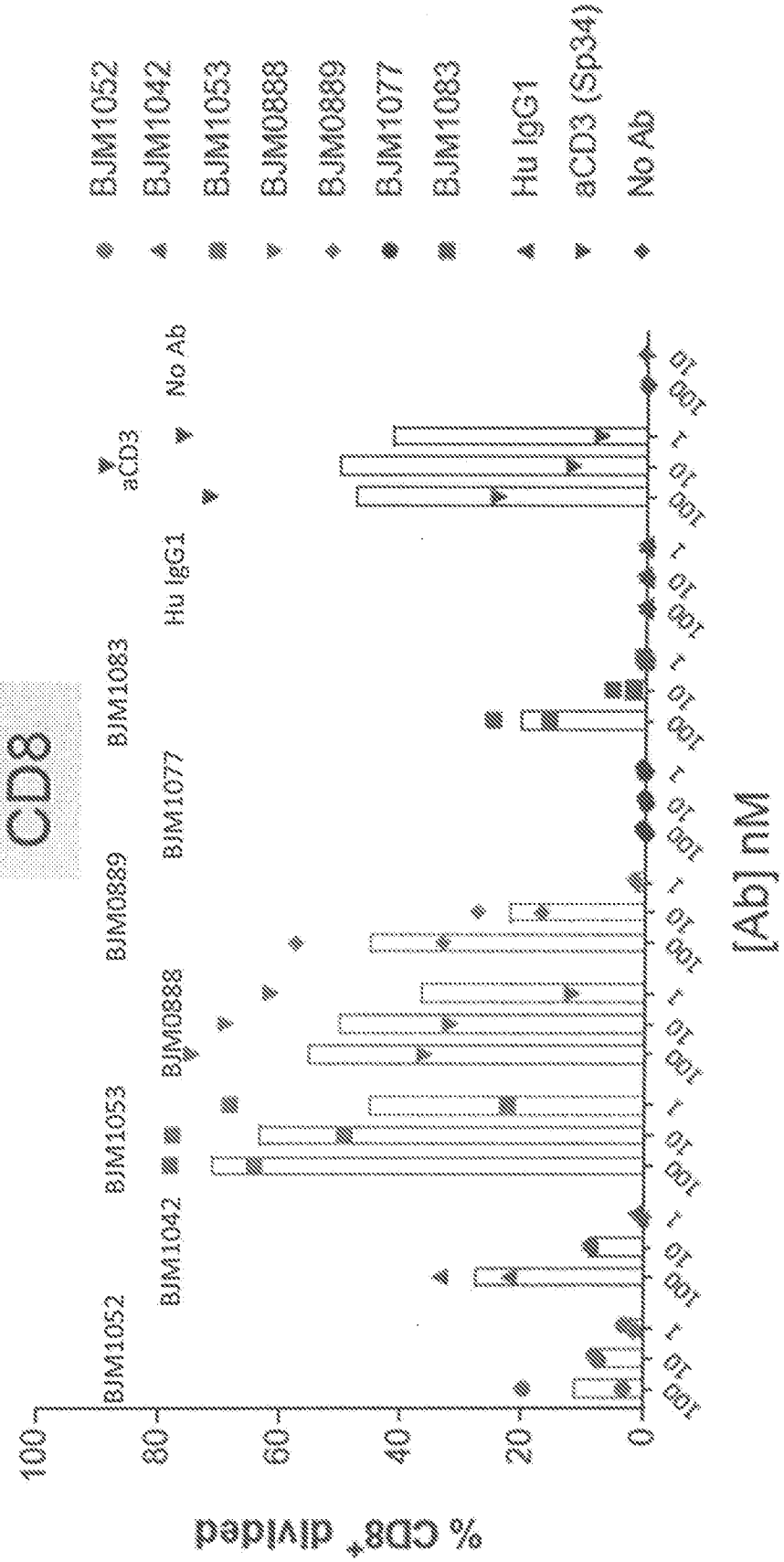

CD8
% CD8+ divided
100
80
60
40
20
0
[Ab] nM
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3
No Ab
aCD3 (Sp34)

FIG. 13C

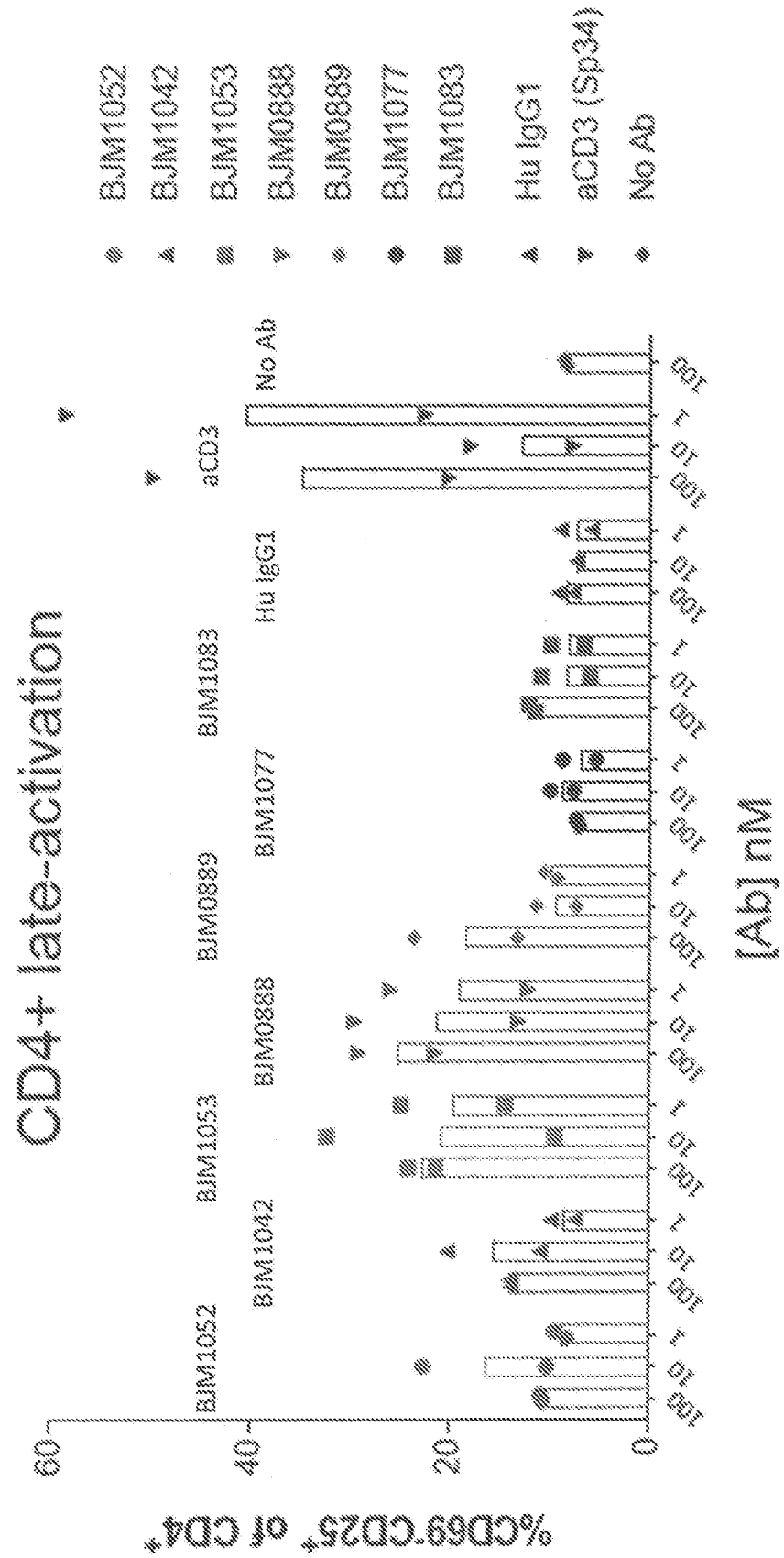

CD4+ late-activation
%CD69.CD25+ of CD4+
60
40
20
0
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3
No Ab
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3 (Sp34)
No Ab
[Ab] nM

FIG. 13D

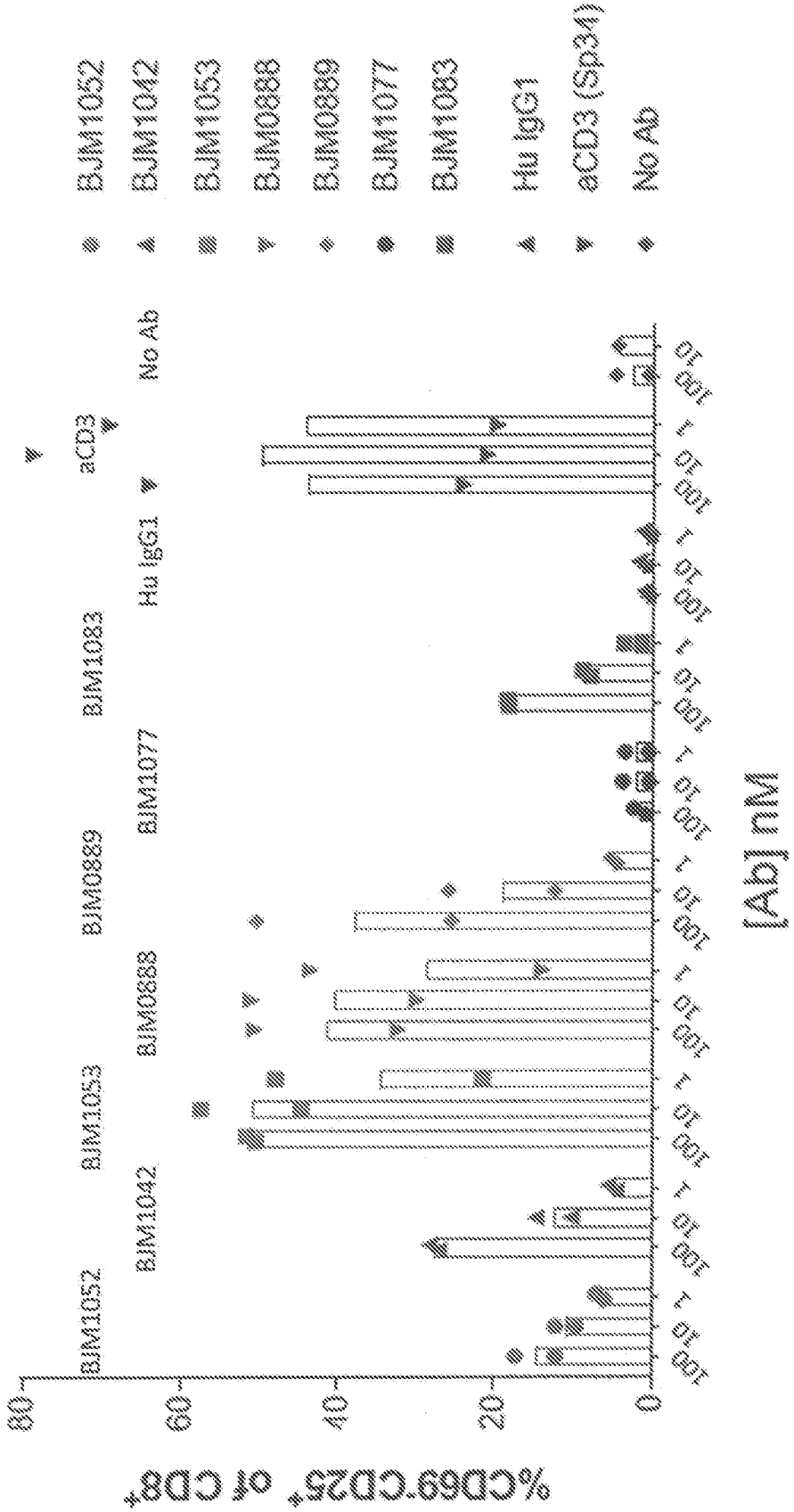

CD8+ late-activation
%CD69.CD25+ of CD8+
[Ab] nM
BJM1052
BJM1042
BJM1053
BJM0888
BJM0889
BJM1077
BJM1083
Hu IgG1
aCD3
No Ab
aCD3 (Sp34)

FIG. 14A

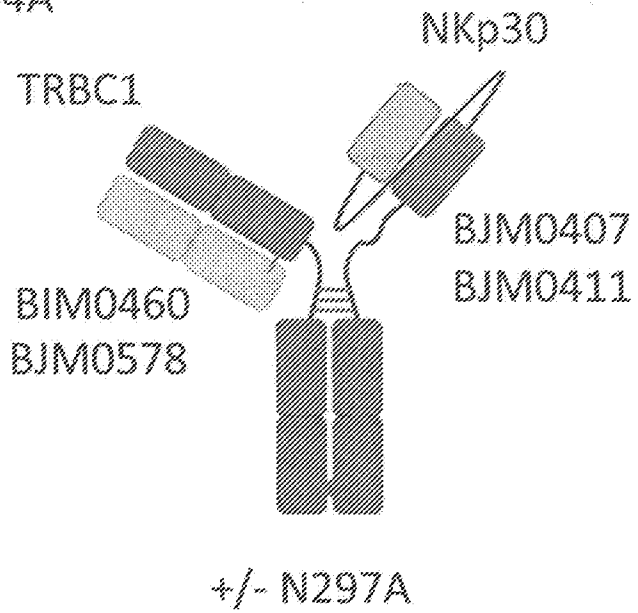


FIG. 14B

BJM1048 460/407 N297A	BJM1032 578/407
BJM1051 460/407	BJM1034 578/411
BJM1052 460/411 N297A	BJM1038 578/407 N297A
BJM1053 460/411	BJM1042 578/411 N297A

FIG. 14C

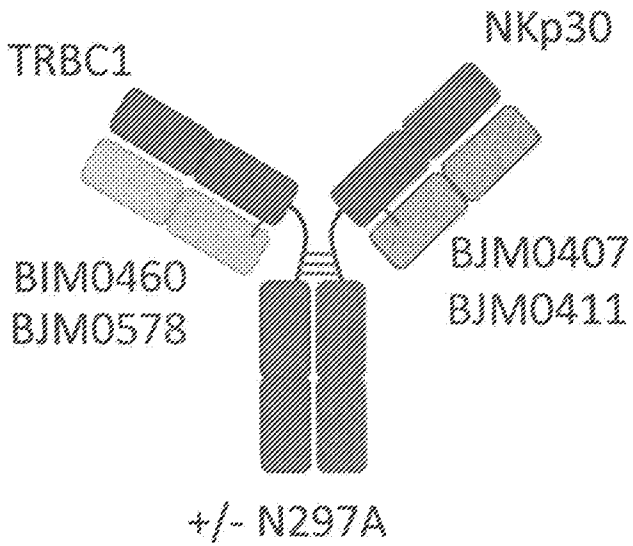
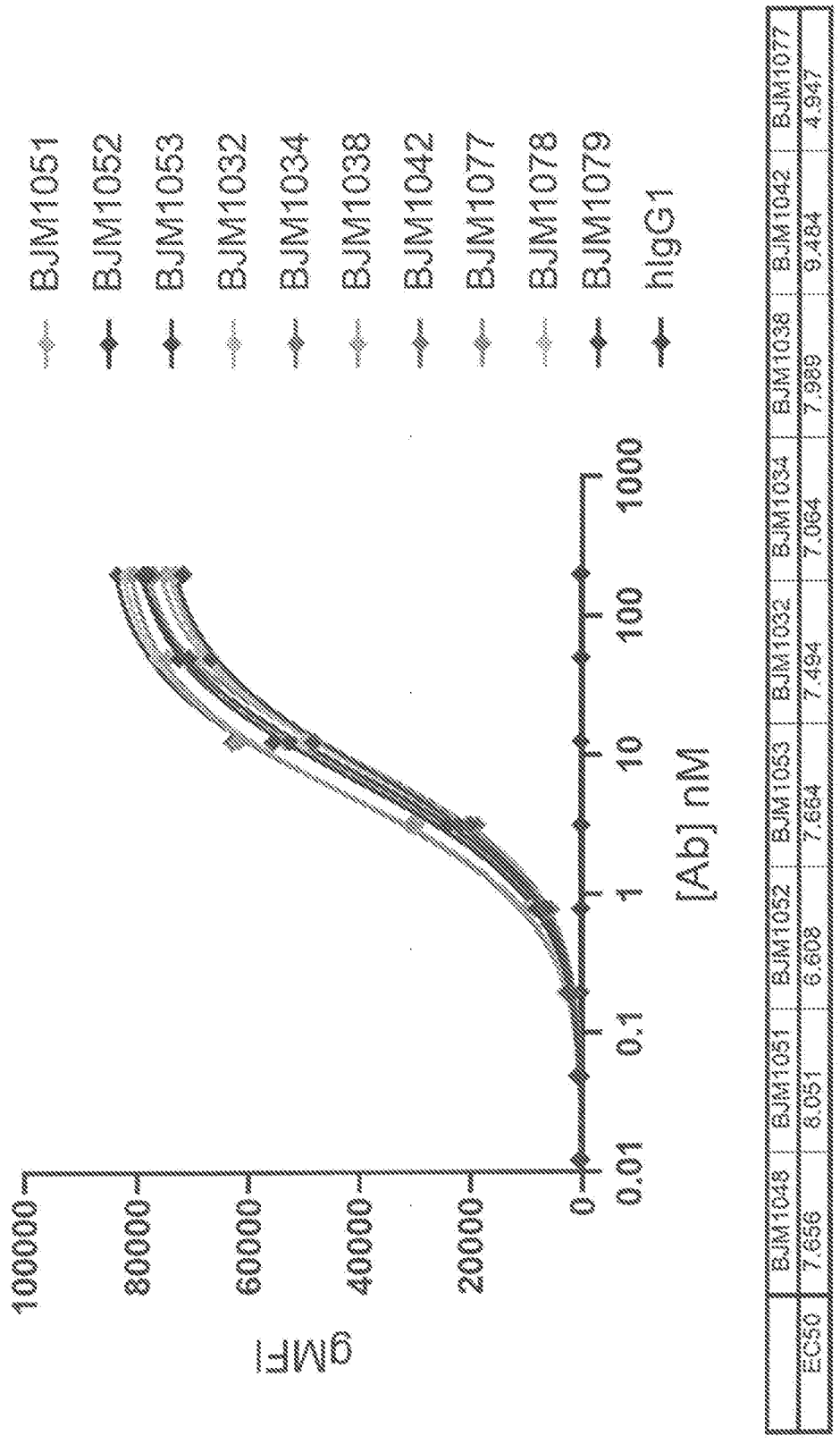


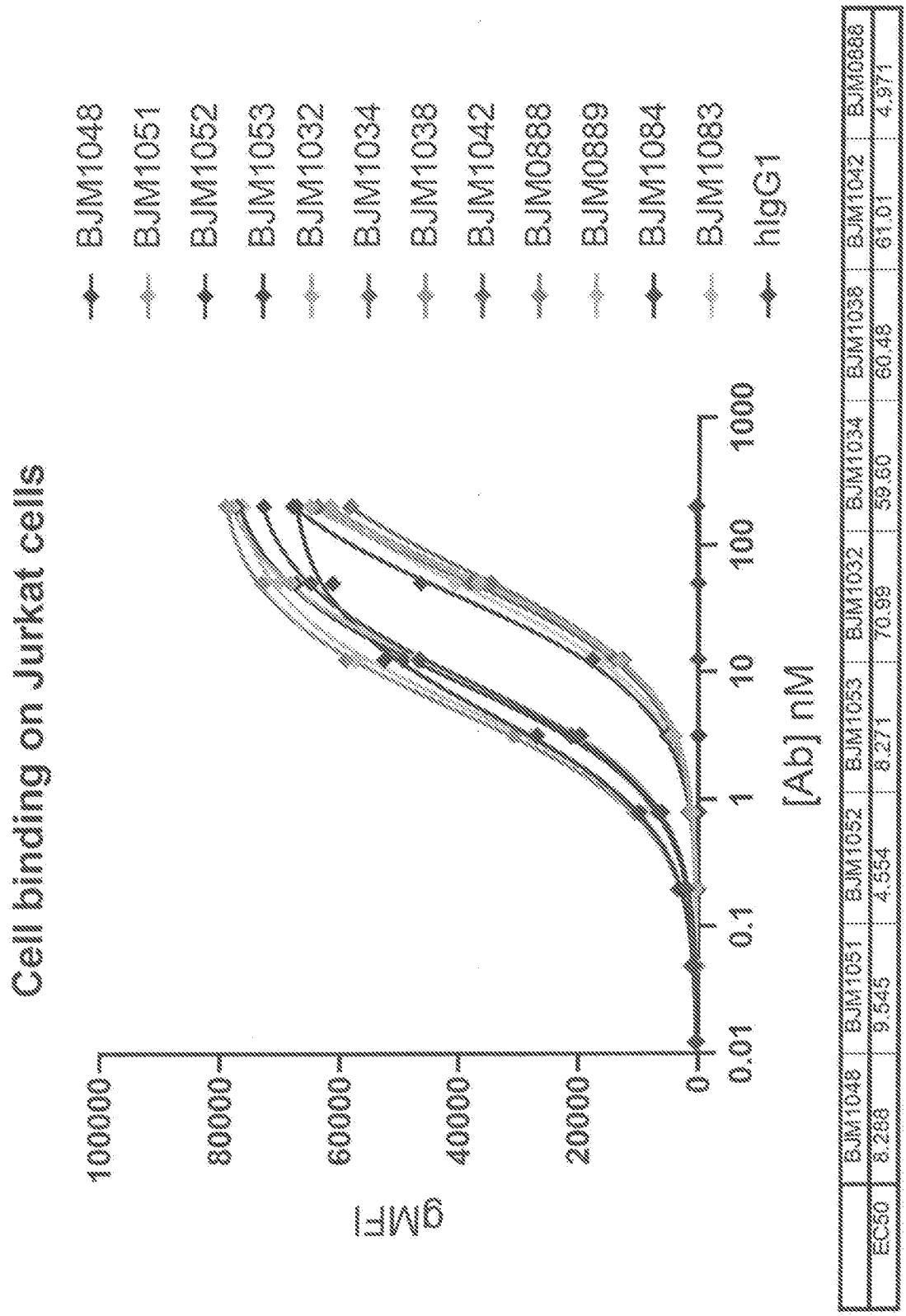
FIG. 14D

BJM0862 460/407
BJM0863 460/411
BJM1028 578/407 N297A
BJM1046 578/411 N297A

FIG. 15A



	BJM1048	BJM1051	BJM1052	BJM1053	BJM1032	BJM1034	BJM1038	BJM1042	BJM1077
EC50	7.656	8.051	6.808	7.664	7.494	7.064	7.989	9.484	4.947



	BJM1048	BJM1051	BJM1052	BJM1053	BJM1032	BJM1034	BJM1038	BJM1042	BJM0888
EC50	8.288	9.545	4.554	8.271	70.99	39.60	60.48	61.01	4.971

FIG. 15B

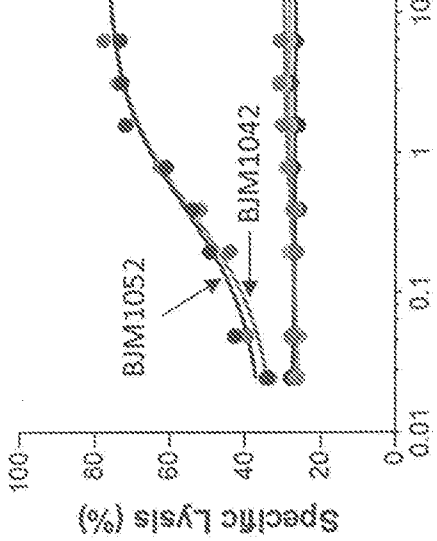
FIG. 15C

BJM1048	Nkp30/TRBC1 bispecific 407/460_N297A
BJM1051	Nkp30/TRBC1 bispecific 407/460_WT
BJM1052	Nkp30/TRBC1 bispecific 411/460_N297A
BJM1053	Nkp30/TRBC1 bispecific 411/460_WT
BJM1032	Nkp30/TRBC1 bispecific 407/578_WT
BJM1034	Nkp30/TRBC1 bispecific 411/578_WT
BJM1038	Nkp30/TRBC1 bispecific 407/578_N297A
BJM1042	Nkp30/TRBC1 bispecific 411/578_N297A
BJM1077	Nkp30 single arm control 411 N297A
BJM1078	Nkp30 single arm control 407 WT
BJM1079	Nkp30 single arm control 411 WT
BJM0888	TRBC1 single arm WT 460
BJM0889	TRBC1 single arm N297A 460
BJM1084	TRBC1 578 Single arm control WT
BJM1083	TRBC1 578 Single arm control N297A knob

FIG. 15D

	gMFI	
Sample	EC50 KHYG-1 (NKp30) [nM]	EC50 Jurkat (TRBC1) [nM]
BJM1048	7.656	8.288
BJM1051	8.051	9.545
BJM1052	6.608	4.554
BJM1053	7.664	8.271
BJM1032	7.494	70.99
BJM1034	7.064	59.6
BJM1038	7.989	60.48
BJM1042	9.484	61.01
BJM1077	4.947	
BJM1078	5.2	
BJM1079	5.357	
BJM0888		4.971
BJM0889		5.261
BJM1084		40.33
BJM1083		44.16

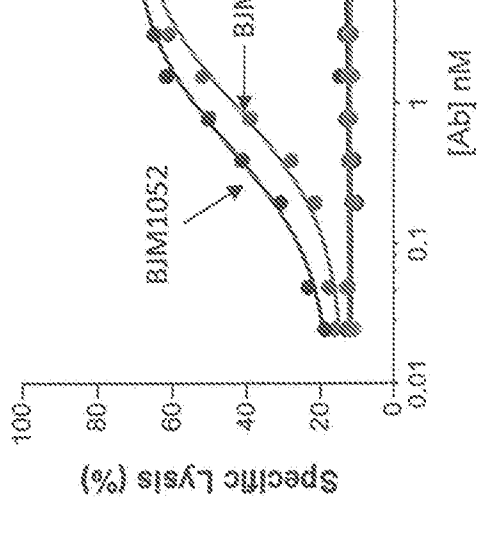
FIG. 16A



Jurkat	BJM1052	BJM1042
EC_{50}	0.3976	0.3838

BJM1052: NKp30xTRBC1(460)
BJM1042: NKp30xTRBC1(578)
BJM0889: TRBC1 (460, N297A)
BJM1077: NKp30 (N297A)
BJM1083:TRBC1 (578, N297A)

FIG. 16B



H9	BJM1052	BJM1042
EC_{50}	0.4775	0.9082

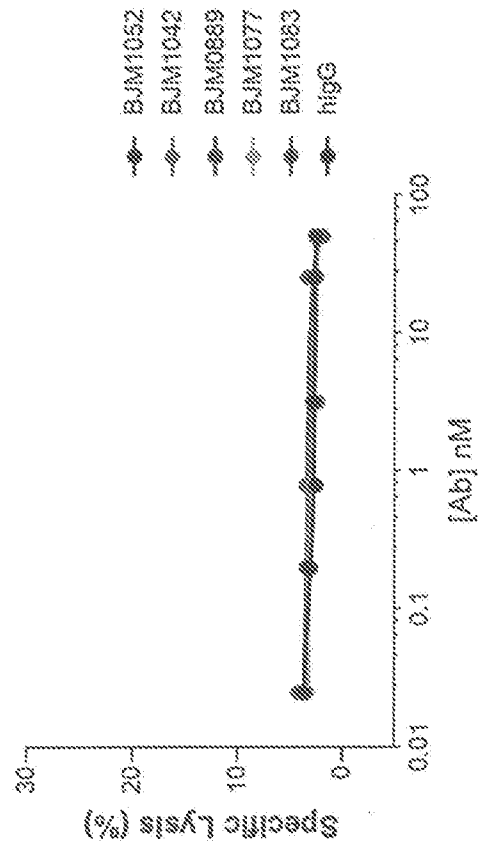
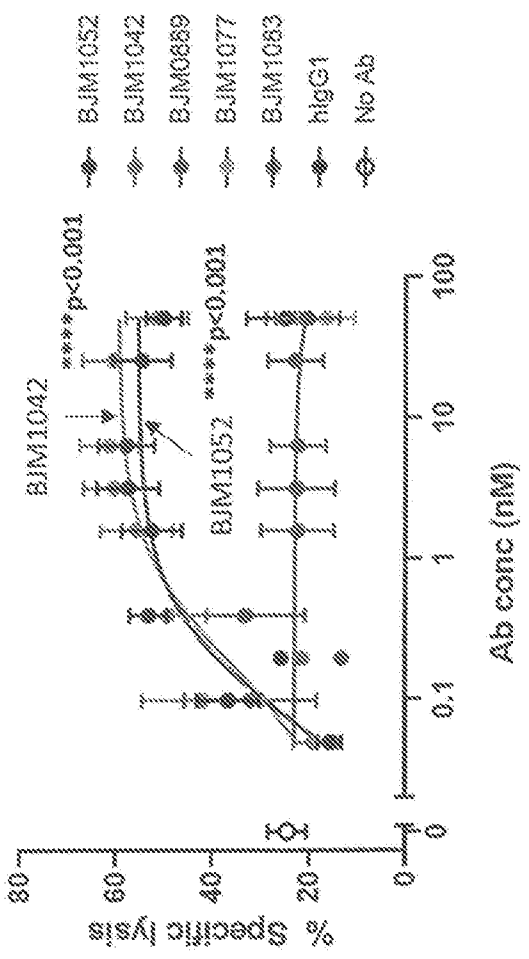

HPB-ALL cell lysis
Specific Lysis (%)
30
20
10
0
0.01
0.1
1
10
100
[Ab] nM
BJM1052
BJM1042
BJM0889
BJM1077
BJM1083
hIgG

FIG. 16C

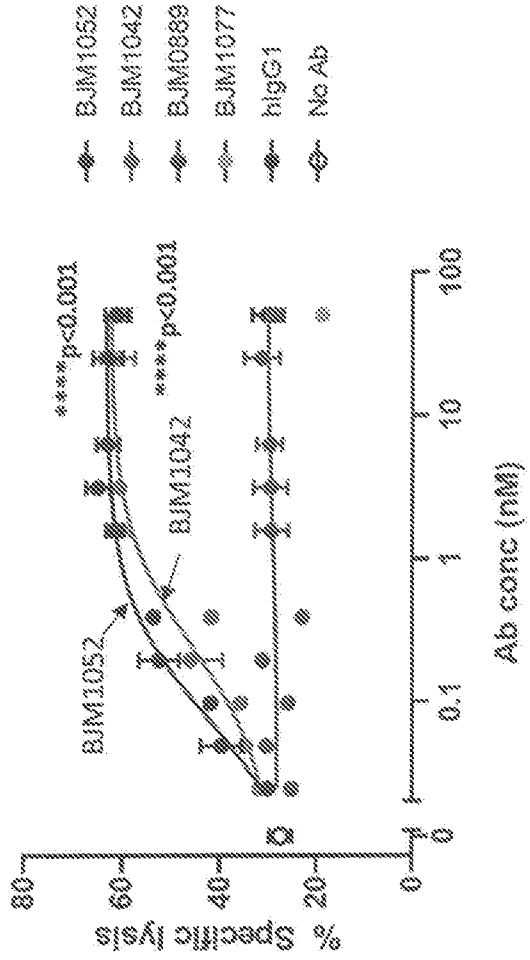
FIG. 17A



	BJM1052	BJM1042
EC50	0.064	0.1778

BJM1052: NKp30xTRBC1(460)
BJM1042: NKp30xTRBC1(578)
BJM0889: TRBC1 (460, N297A)
BJM1077: NKp30 (N297A)
BJM1083:TRBC1 (578, N297A)

FIG. 17B



	BJM1052	BJM1042
EC50	0.075	0.238

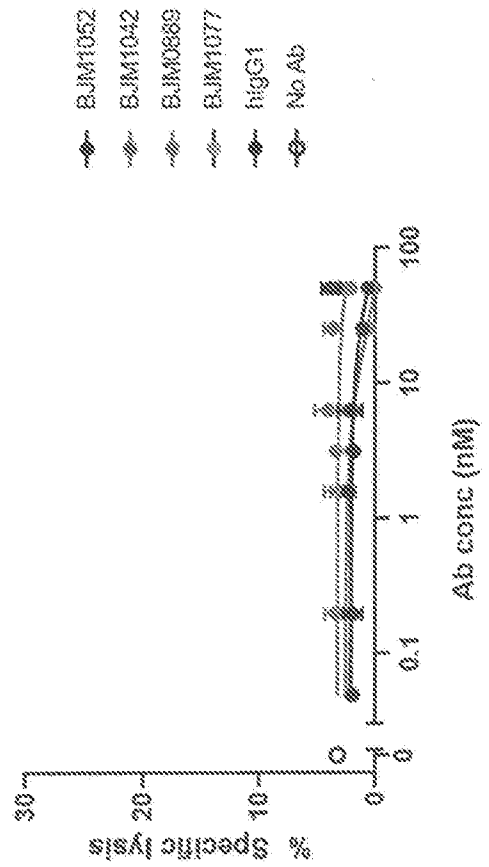

HPB-ALL cell lysis
% Specific lysis
0
10
20
30
Ab conc (nM)
0.1
1
10
100
BJM1052
BJM1042
BJM0869
BJM1077
hIgG1
No Ab

FIG. 17C

FIG. 18A

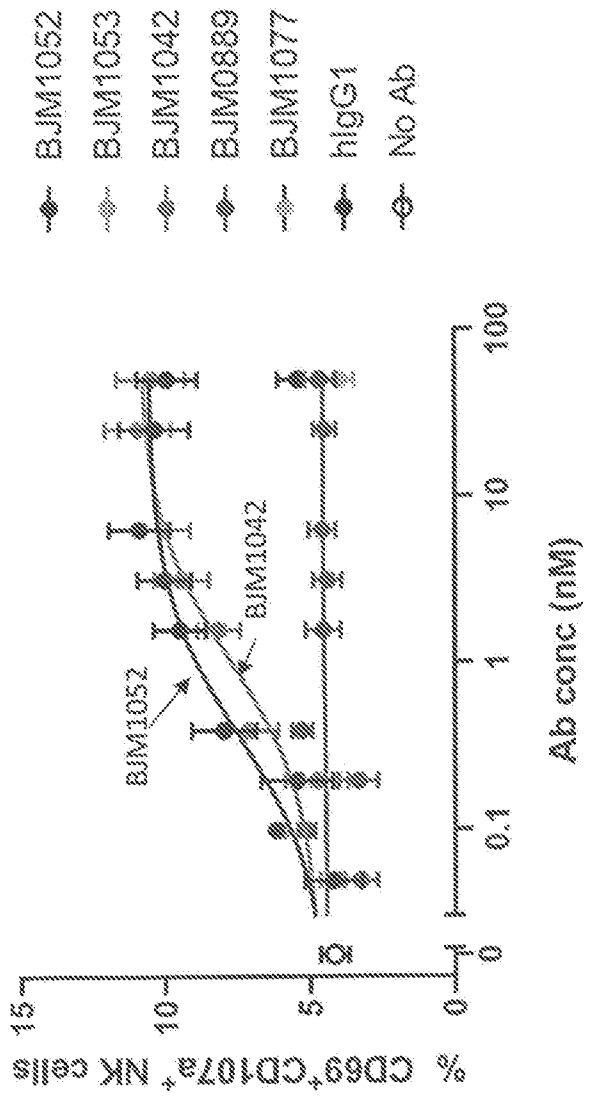
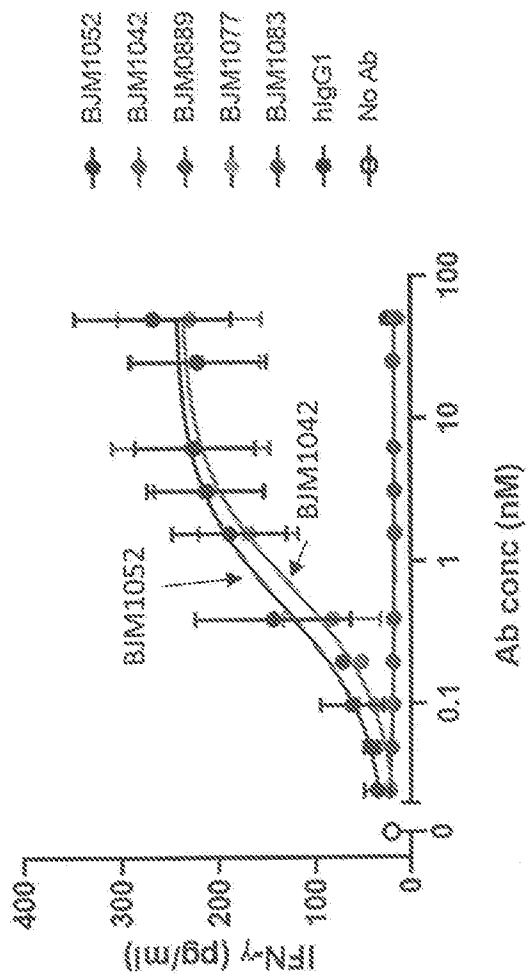


FIG. 18B



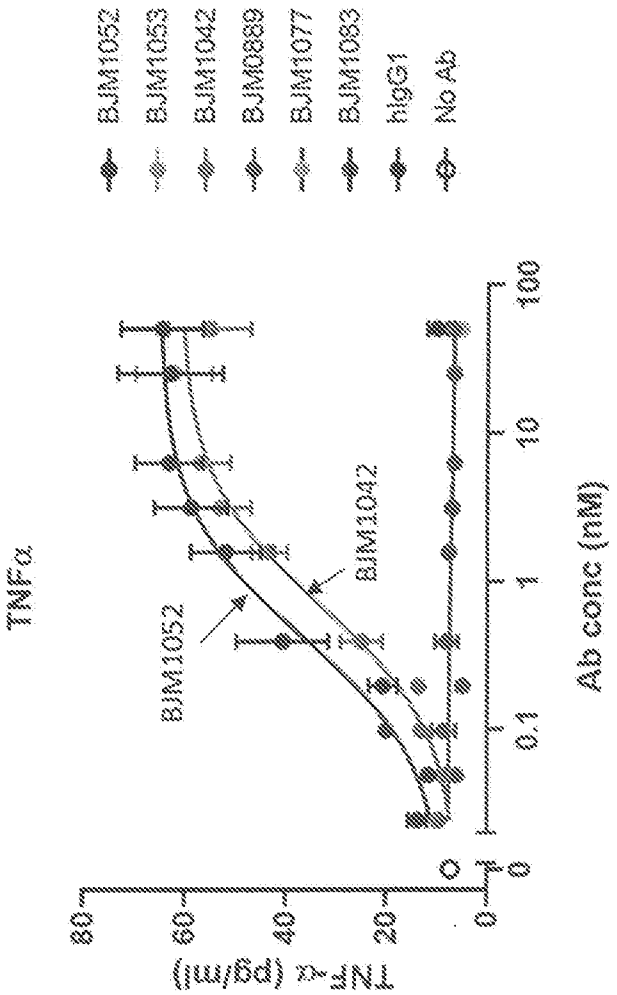

TNFα
BJM1052
BJM1053
BJM1042
BJM0889
BJM1077
BJM1083
hIgG1
No Ab
BJM1052
BJM1042
TNF-α (pg/ml)
Ab conc (nM)
0
20
40
60
80
0
0.1
1
10
100

FIG. 18C

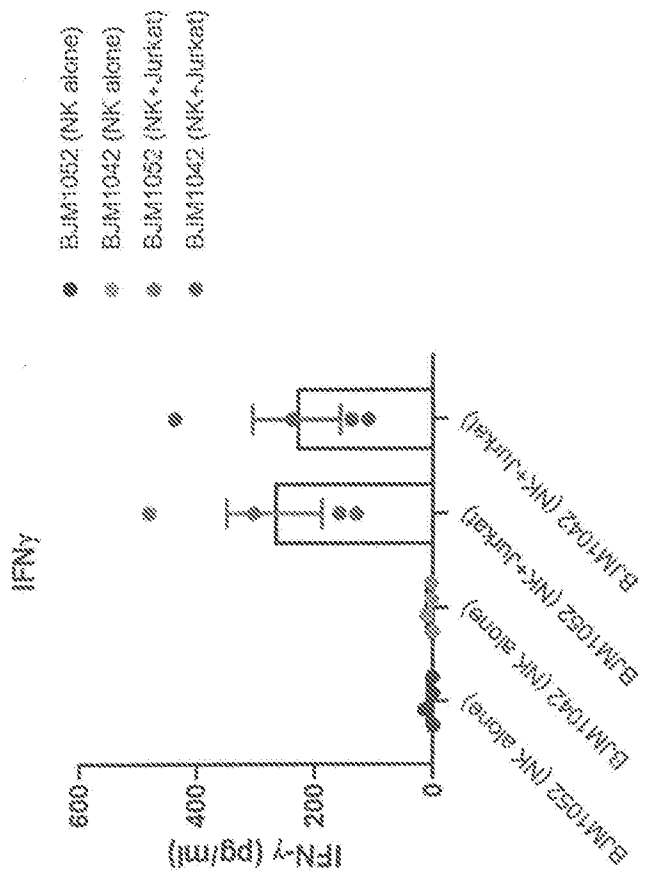


FIG. 19A

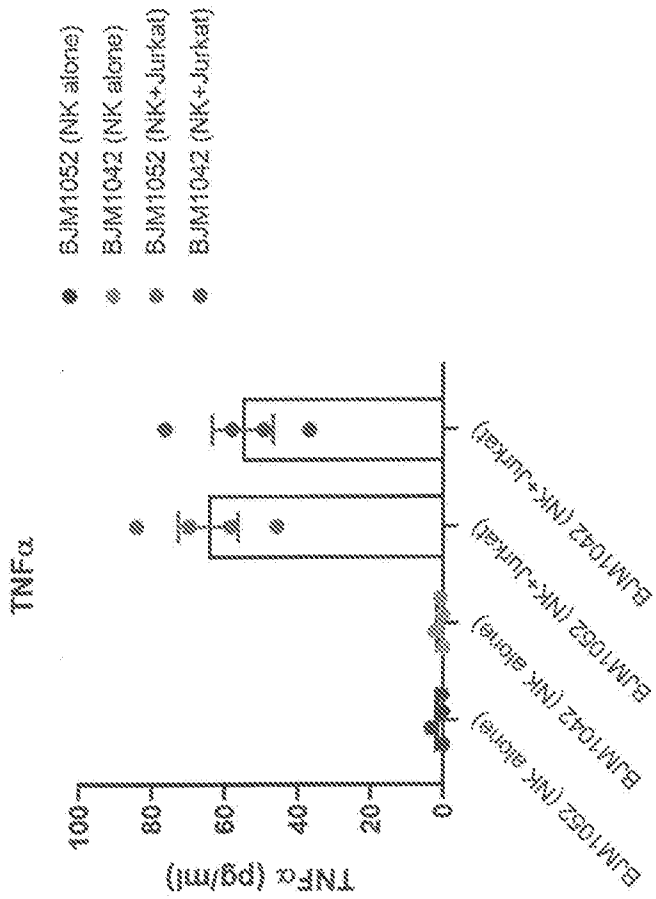


FIG. 19B

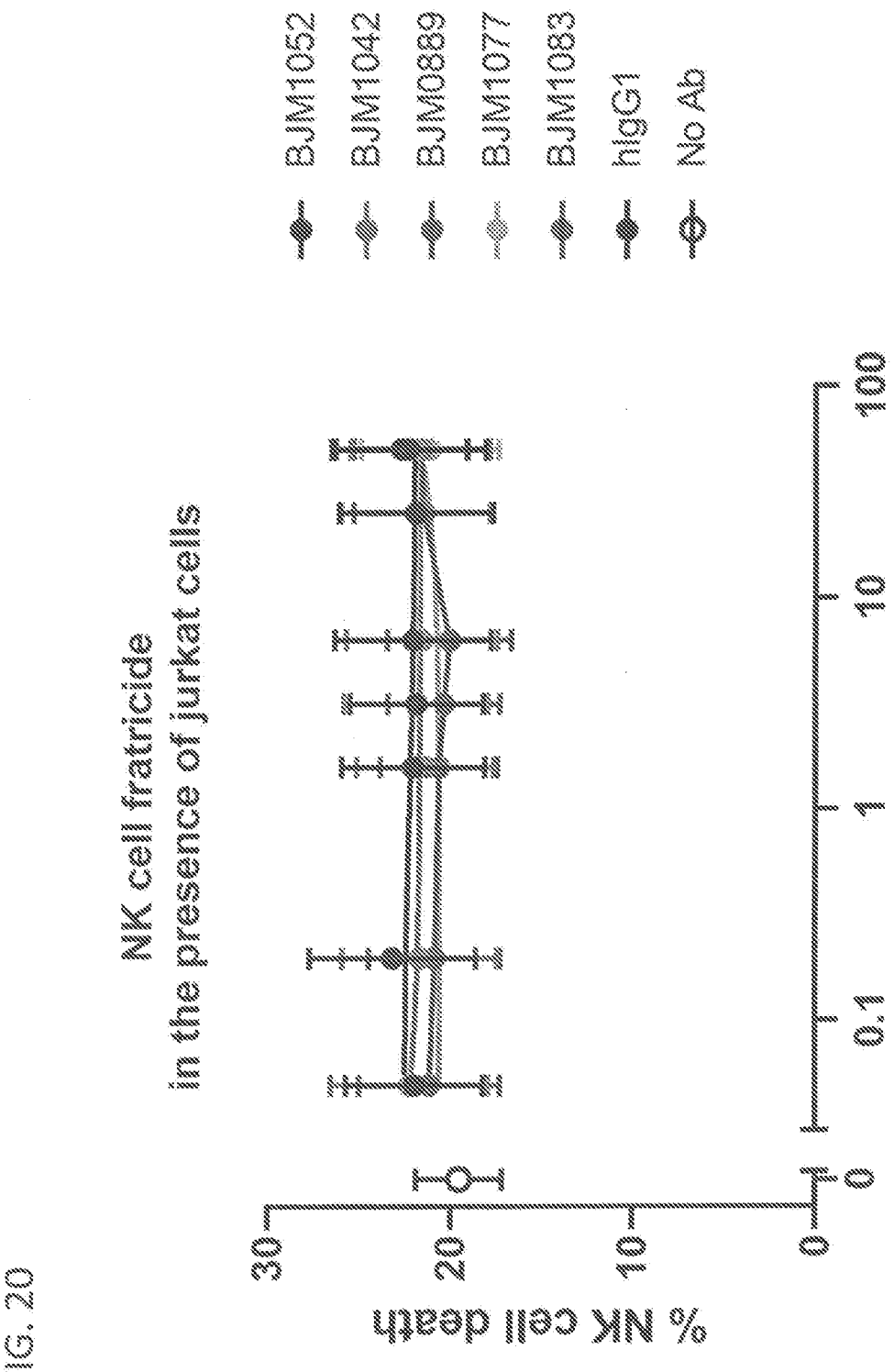

NK cell fratricide
in the presence of jurkat cells
% NK cell death
0
10
20
30
0
0.1
1
10
100
BJM1052
BJM1042
BJM0889
BJM1077
BJM1083
hIgG1
No Ab

FIG. 20

FIG. 21B

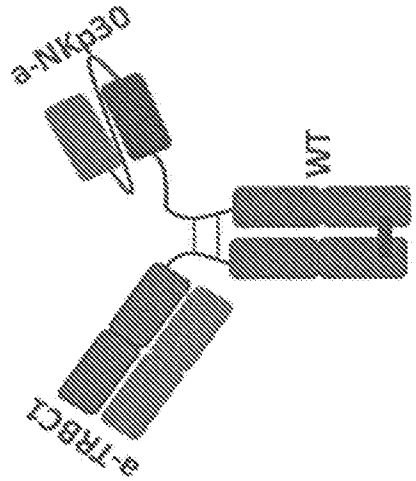


FIG. 21A

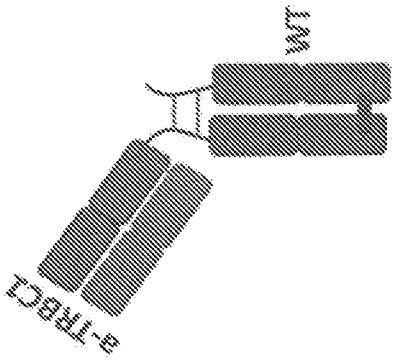


FIG. 22A

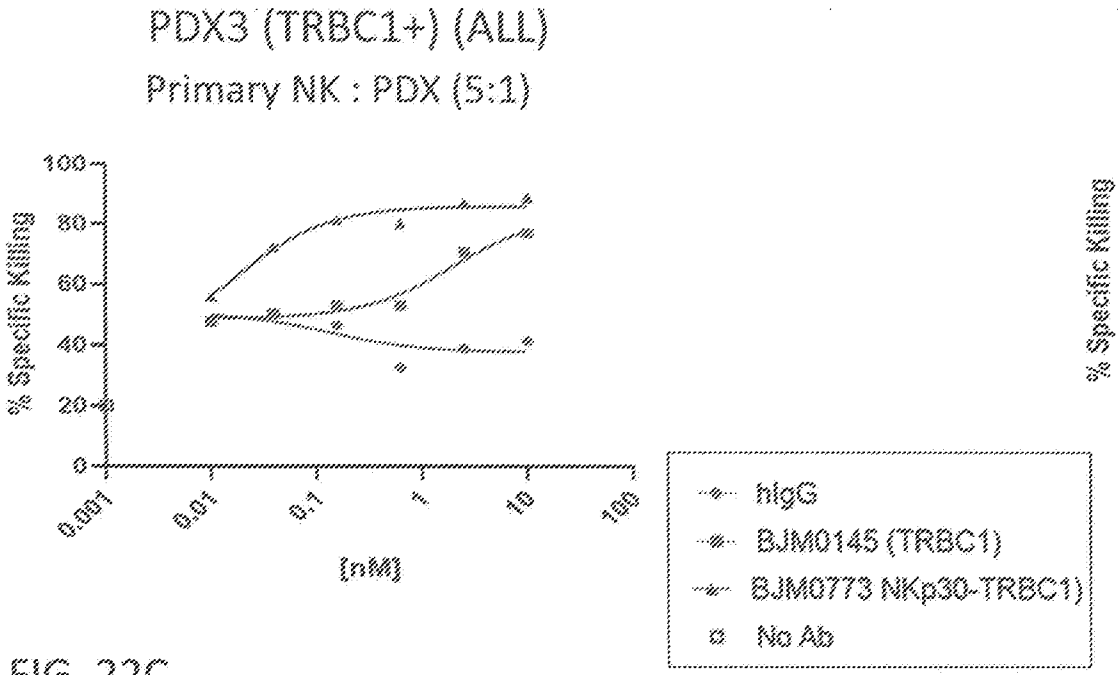


FIG. 22B

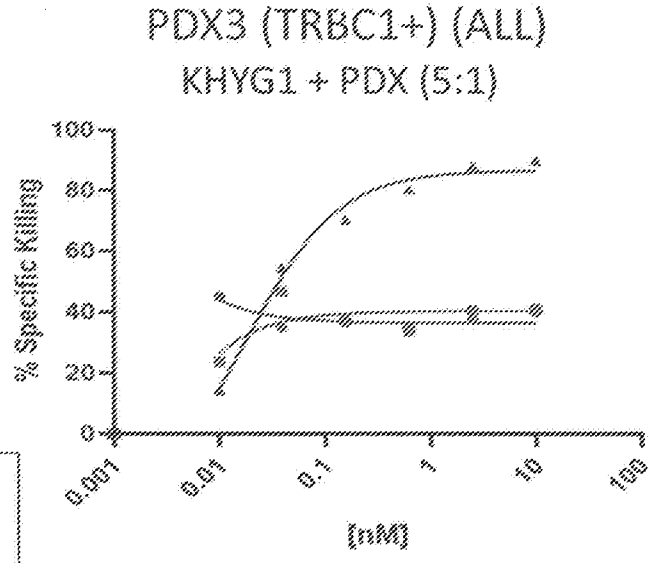


FIG. 22C

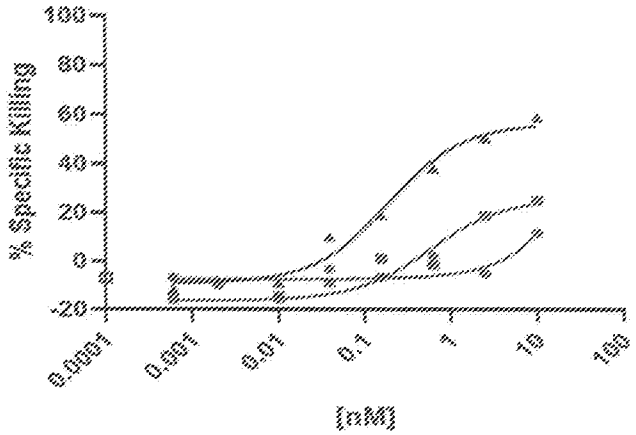


FIG. 22D

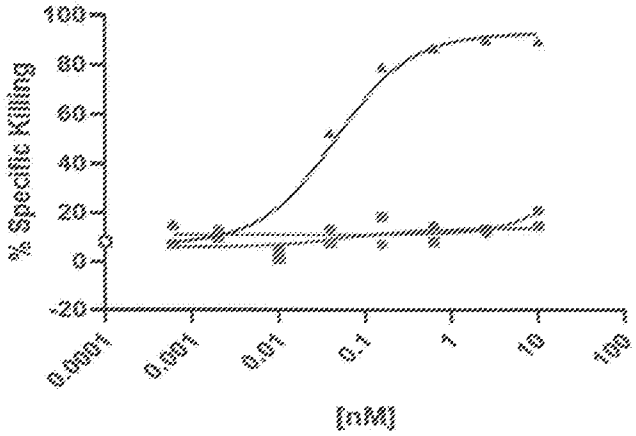
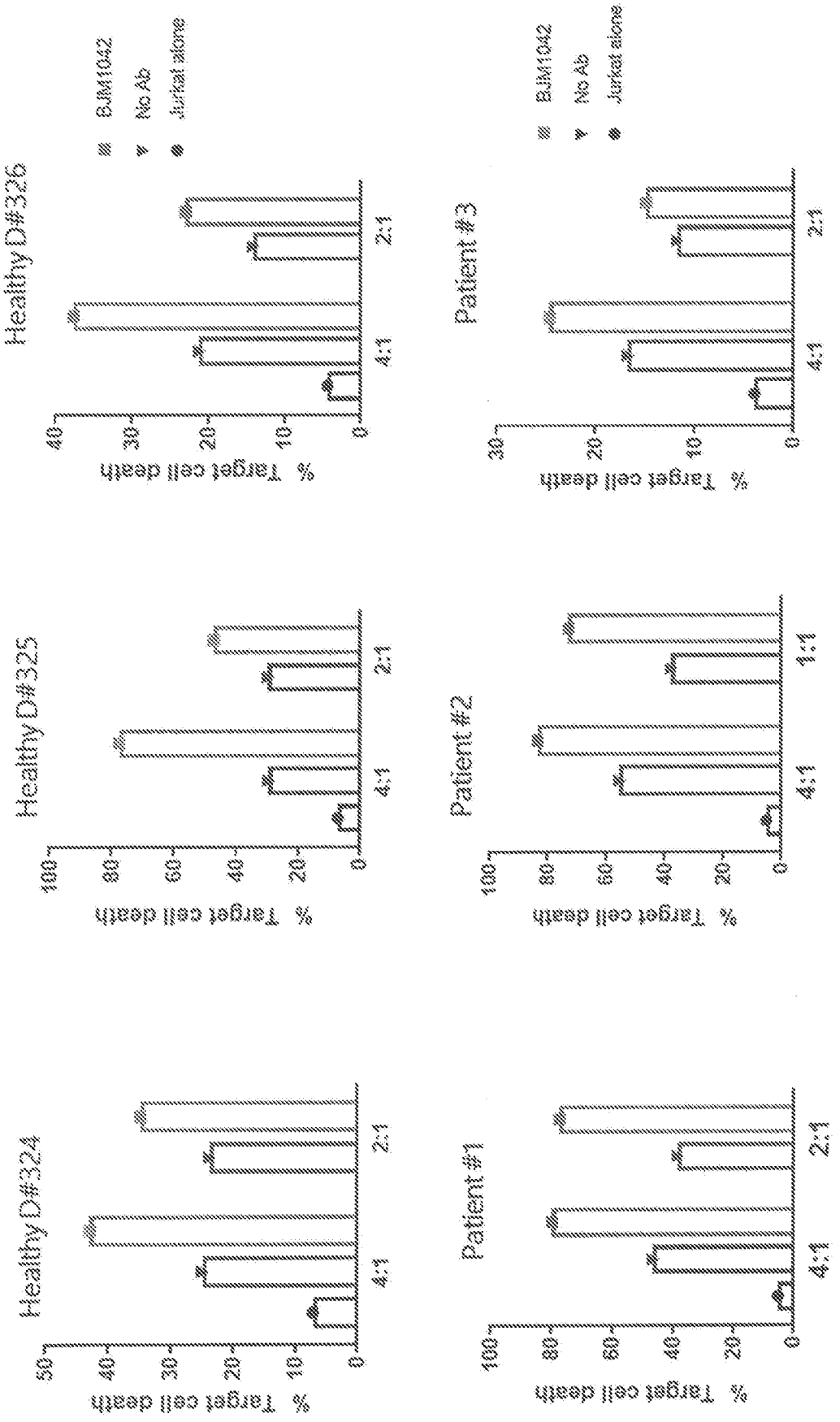


FIG. 23


Healthy D#324
Healthy D#325
Healthy D#326
Patient #1
Patient #2
Patient #3
% Target cell death
4:1
2:1
1:1
BJM1042
No Ab
Jurkat alone

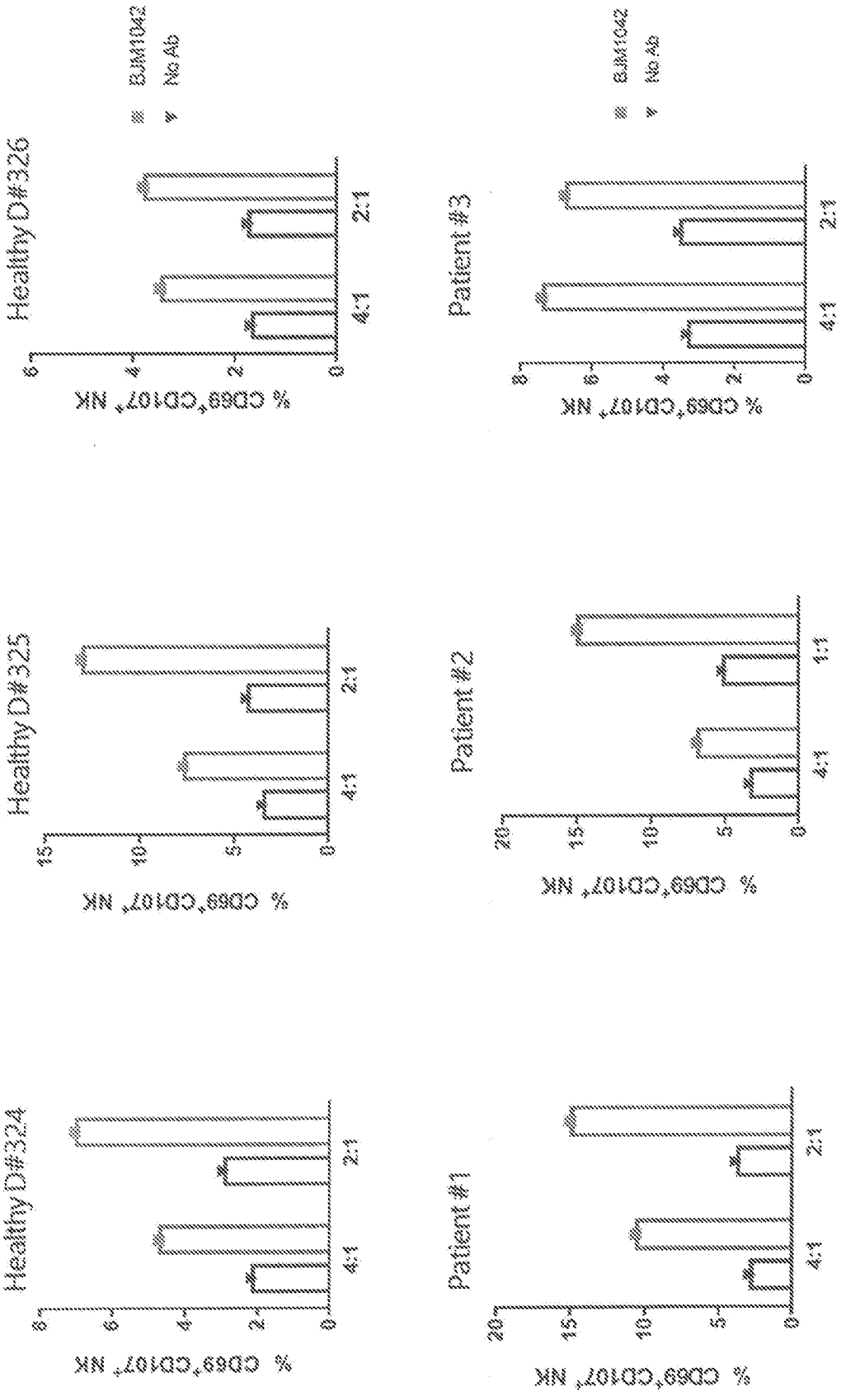

Healthy D#326
Healthy D#325
Healthy D#324
Patient #3
Patient #2
Patient #1
BJM1042
No Ab
% CD69+CD107+ NK
4:1
2:1
1:1

FIG. 24

FIG. 25A

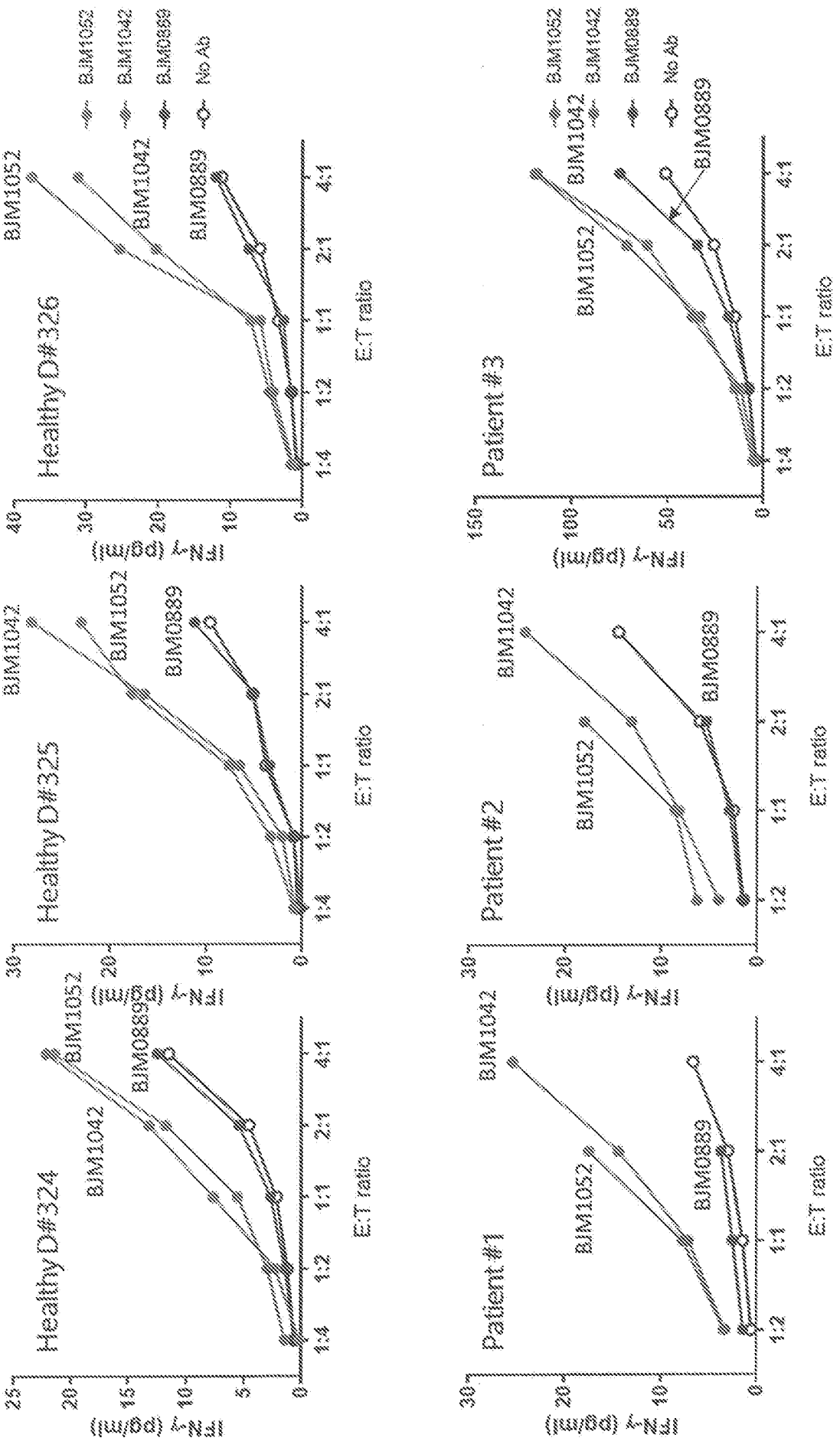

Healthy D#324
Healthy D#325
Healthy D#326
Patient #1
Patient #2
Patient #3
BJM1052
BJM1042
BJM0889
No Ab
IFN-γ (pg/ml)
E:T ratio
1:4
1:2
1:1
2:1
4:1

FIG. 25B

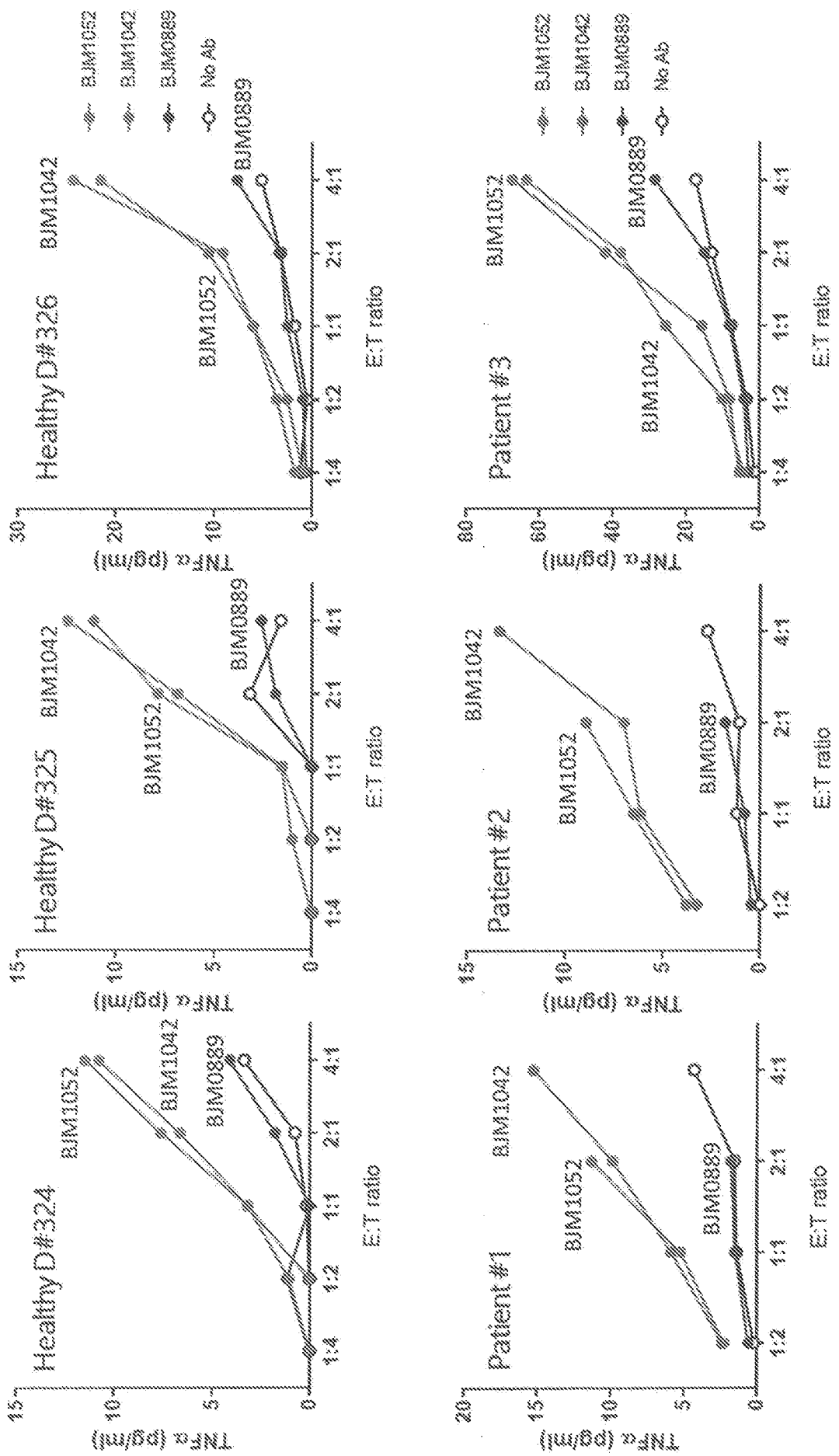

Healthy D#324
Healthy D#325
Healthy D#326
Patient #1
Patient #2
Patient #3
BJM1052
BJM1042
BJM0889
No Ab
TNFα (pg/ml)
E:T ratio
1:4
1:2
1:1
2:1
4:1

FIG. 26A

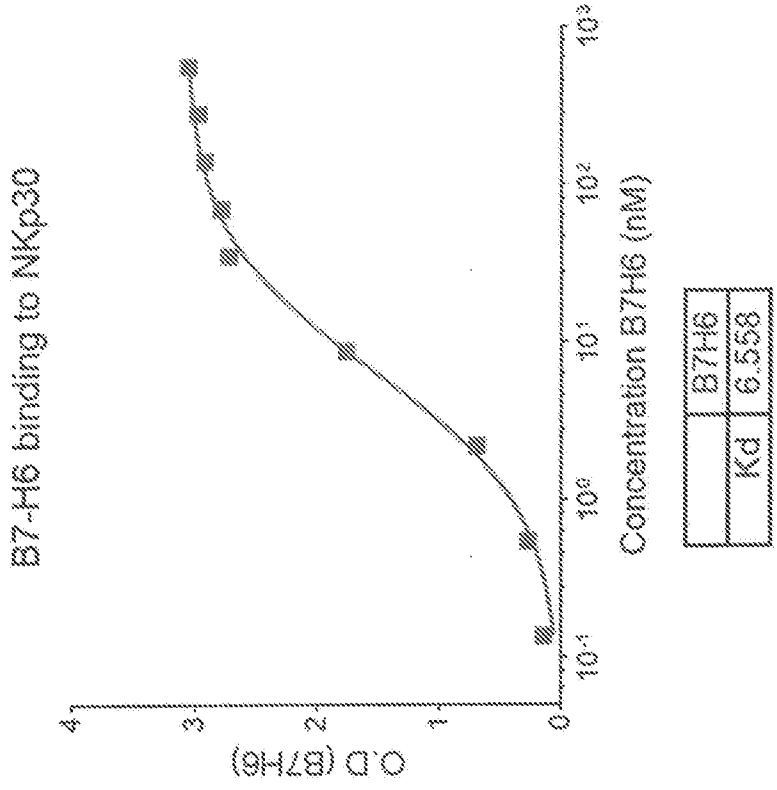
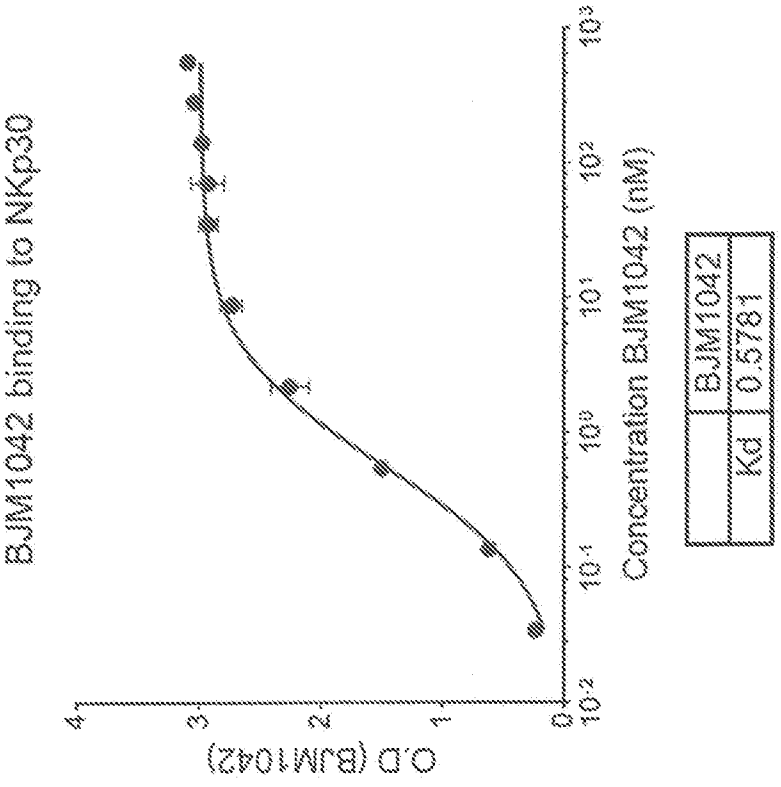
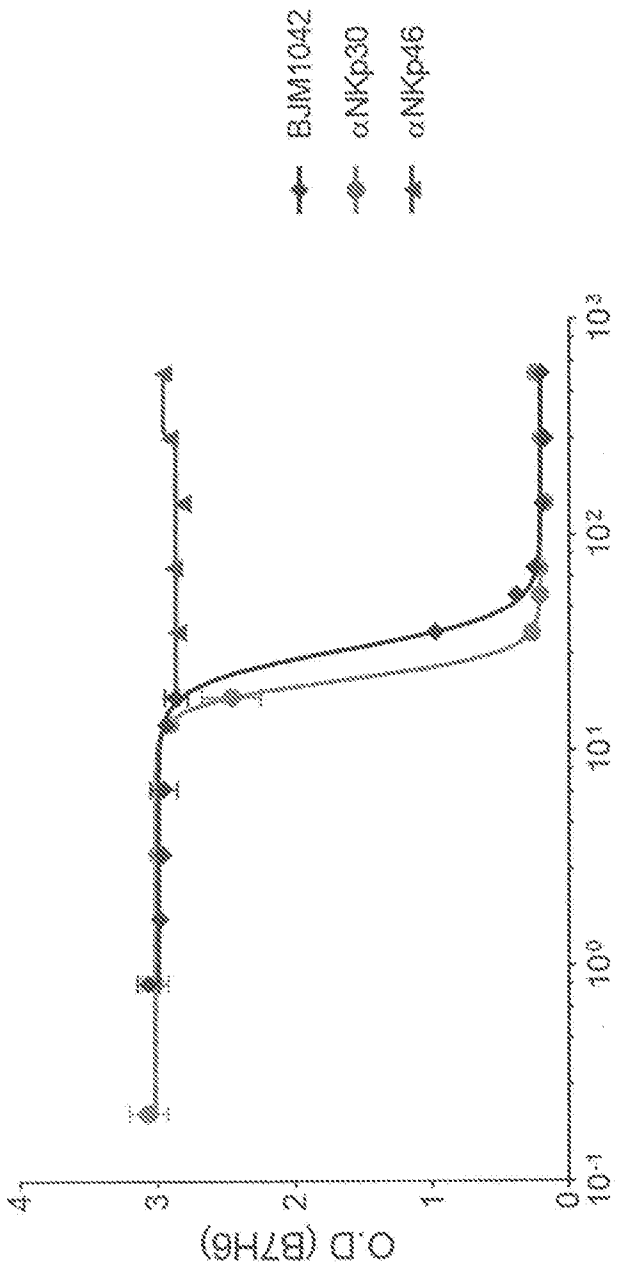


FIG. 26B

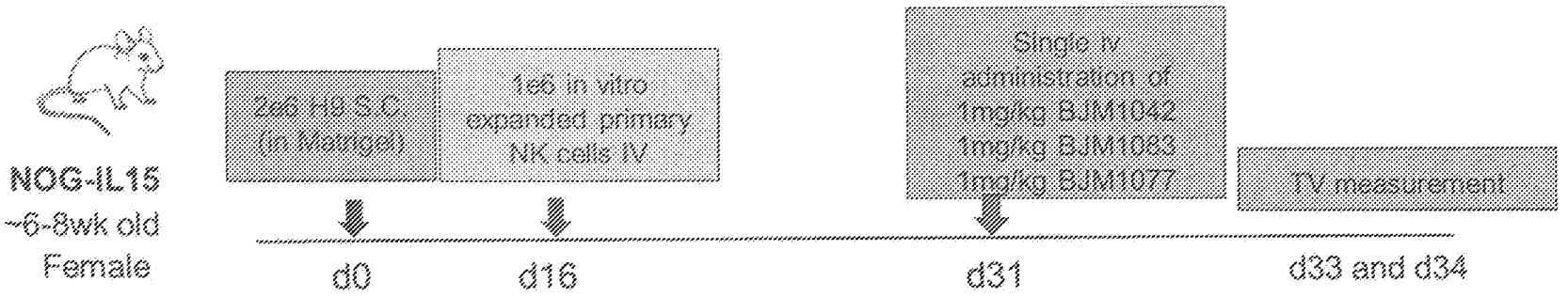




	αNKp30	αNKp46	BJM1042
IC50	21.24	~ 278.7	29.41

FIG. 26C

FIG. 27A



SN	Molecule	N	Dose (mg/kg)	Schedule	1e6 primary NK cells
1	Vehicle	5	NA	qd	-
2	**BJM1042** (NKp30/TRBC1 411/578_N297A)	5	1	qd	-
3	Vehicle	5	NA	qd	+
4	**BJM1042** (NKp30/TRBC1 411/578_N297A)	5	1	qd	+
5	**BJM1083** (TRBC1 single arm 578 N297A)	5	1	qd	+
6	**BJM1077** (NKp30 single arm 411 N297A)	5	1	qd	+

FIG. 27B

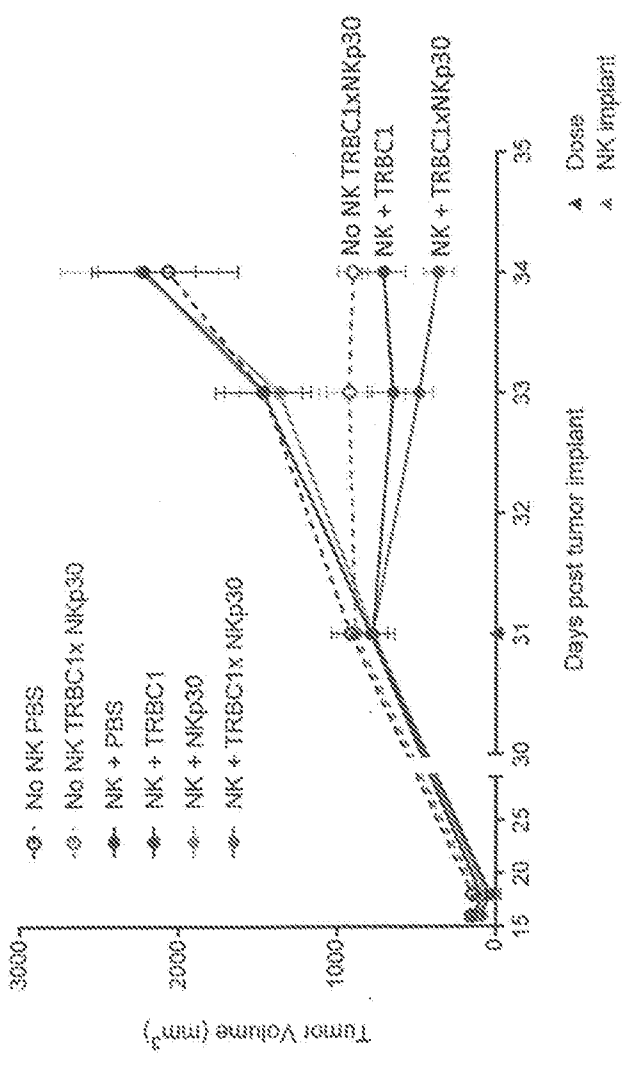


FIG. 27C

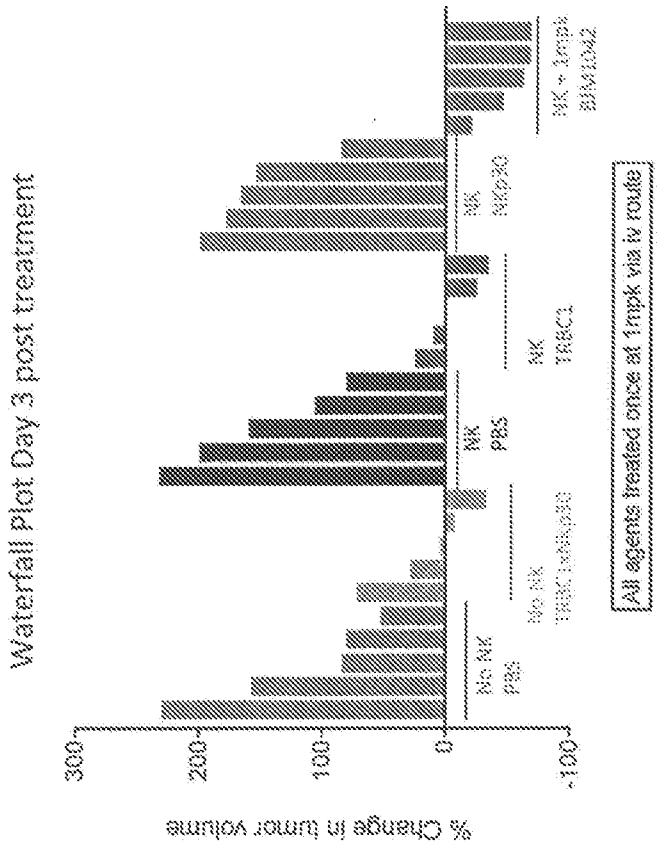


FIG. 28A

NOG-IL15
~6-8wk old
Female

Bi-weekly tumor and body weight measurements

D0
Implant HPB-ALL SC

D12
Implant 2e6 NK cells

D14
Randomize
Dose* 1

D17
Dose* 2

SN	Cell line	NK cells	n	Strain	Treatment	Schedule	Dose
1	5e6 HPB-ALL SC	2e6 IV	4	NOG-IL-15	PBS	Q3D x 2	NA
2	5e6 HPB-ALL SC	2e6 IV	5	NOG-IL-15	BJM1042	Q3D x 2	0.5mg/kg
3	5e6 HPB-ALL SC	2e6 IV	4	NOG-IL-15	BJM1083	Q3D x 2	0.5mg/kg
4	5e6 HPB-ALL SC	2e6 IV	4	NOG-IL-15	BJM1077	Q3D x 2	0.5mg/kg

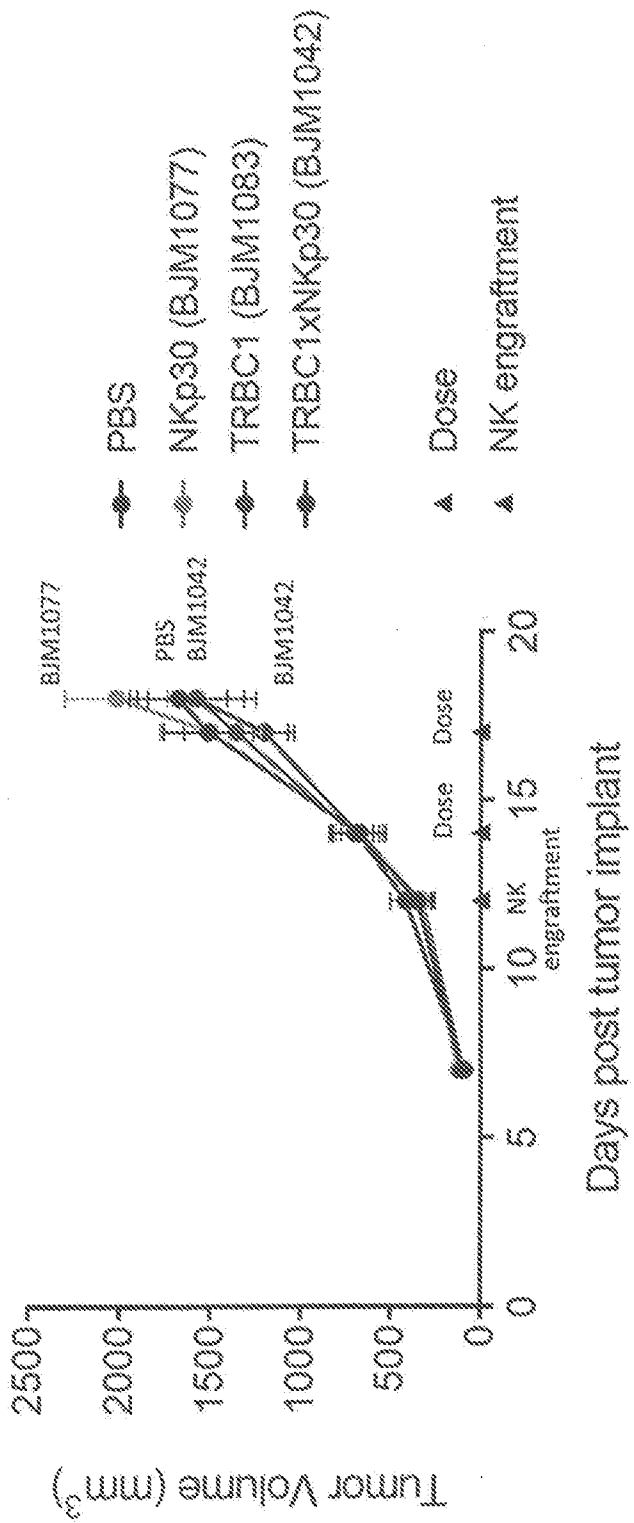

Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
5
10
15
20
Days post tumor implant
NK engraftment
Dose
Dose
Dose
BJM1077
PBS
BJM1042
BJM1042
PBS
NKp30 (BJM1077)
TRBC1 (BJM1083)
TRBC1xNKp30 (BJM1042)
Dose
NK engraftment

FIG. 28B

FIG. 29A

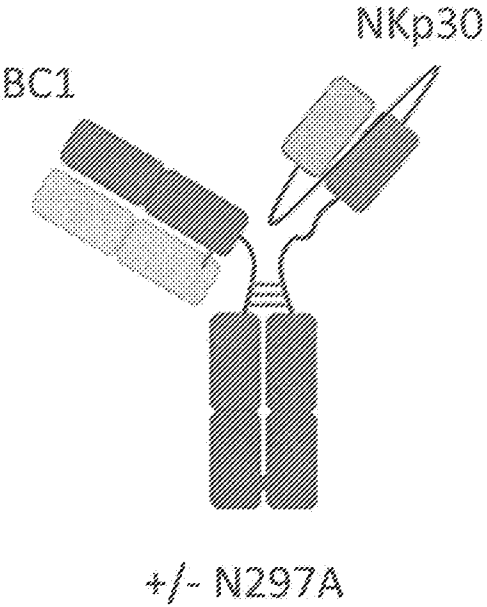


FIG. 29B

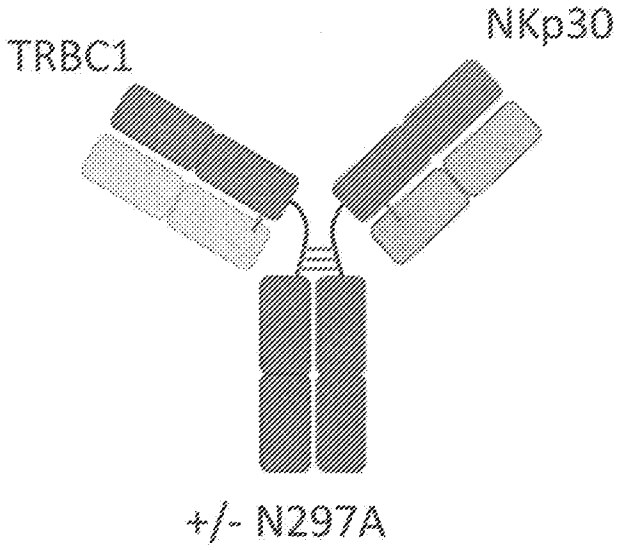


FIG. 29C

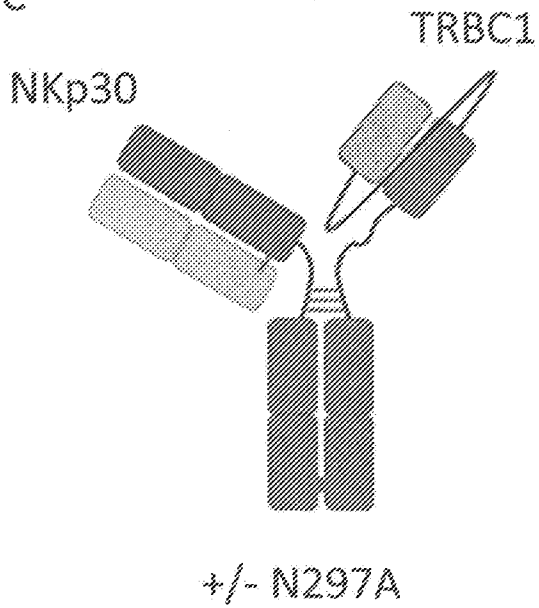


FIG. 29D

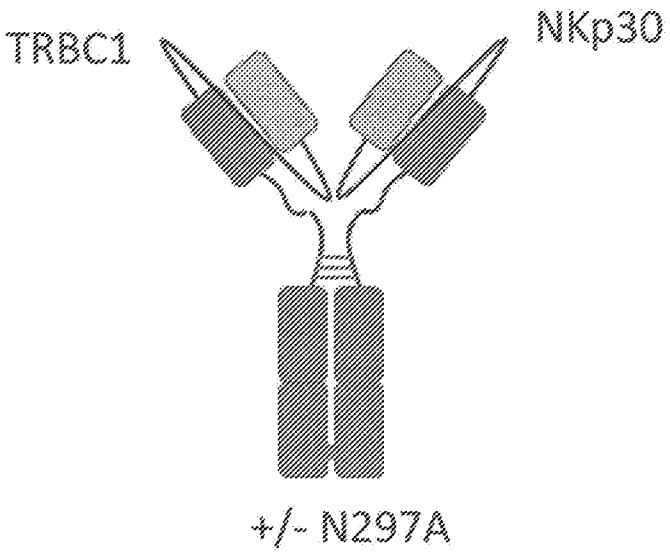


FIG. 30A

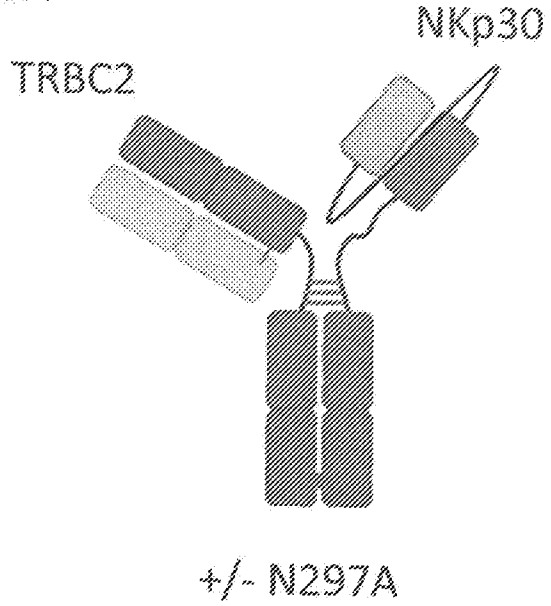


FIG. 30B

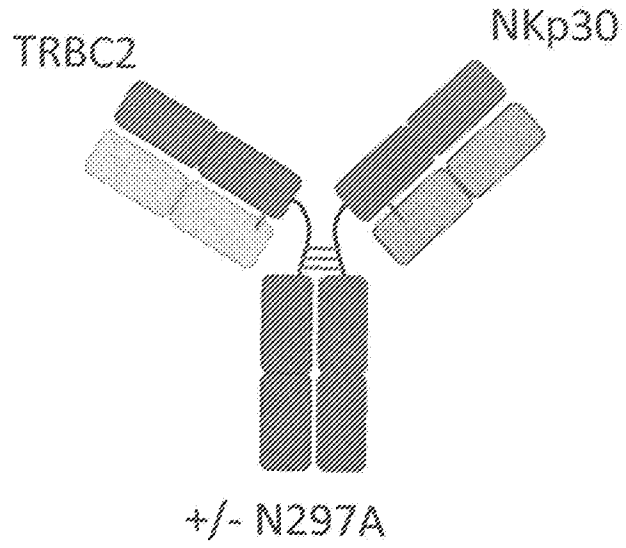


FIG. 30C

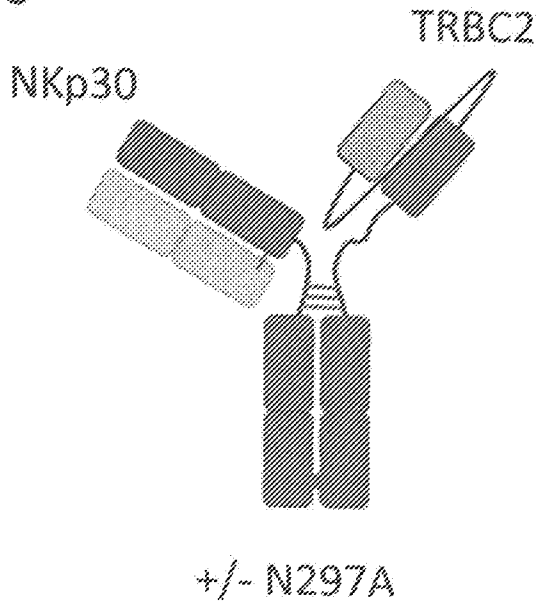
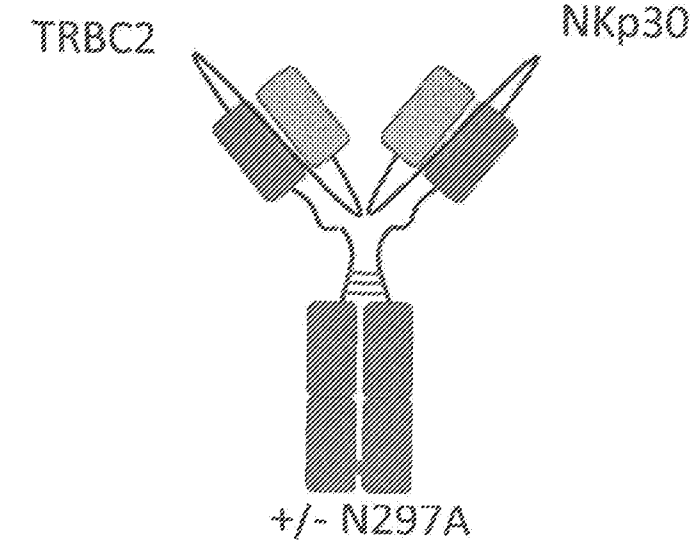


FIG. 30D



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019291

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/056873 A1 (MEDINNOVA GES MED INNOVATIONEN [DE]; JENSEN MARKUS [DE] ET AL.) 8 July 2004 (2004-07-08) page 5, line 24 - page 6, line 33 page 7, line 23 - page 9, line 13 page 10, line 19 - page 12, line 18 claims 1-3,7,10-13,15,22,23; examples 1,2 ----- -/--	1-45

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 June 2020	15/06/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Annette

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MING-RU WU ET AL: "B7H6-Specific Bispecific T Cell Engagers Lead to Tumor Elimination and Host Antitumor Immunity", THE JOURNAL OF IMMUNOLOGY, vol. 194, no. 11, 24 April 2015 (2015-04-24), pages 5305-5311, XP055640111, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.1402517 the whole document, in particular discussion -----	1-45
Y	PAUL M MACIOCIA ET AL: "Targeting the T cell receptor [beta]-chain constant region for immunotherapy of T cell malignancies", NATURE MEDICINE, vol. 23, no. 12, 13 November 2017 (2017-11-13), pages 1416-1423, XP055700312, New York ISSN: 1078-8956, DOI: 10.1038/nm.4444 the whole document, in particular abstract, discussion, page 1418, right-hand column, last paragraph - page 1420, right-hand column, second paragraph -& PAUL M MACIOCIA ET AL: "Targeting the T cell receptor [beta]-chain constant region for immunotherapy of T cell malignancies: Supplemental Figures", NATURE MEDICINE, vol. 23, no. 12, 13 November 2017 (2017-11-13), pages 1416-1423, XP055556125, New York ISSN: 1078-8956, DOI: 10.1038/nm.4444 -----	1-45
X	WO 2015/132598 A1 (UCL BUSINESS PLC [GB]) 11 September 2015 (2015-09-11) page 3, line 1 - line 33; figures 4,5 page 4, line 11 - line 31; figures 9,11,13-15 page 7, line 10 - page 10, line 34 page 13, line 31 - page 24, line 4 page 27, line 34 - page 29, line 2 page 36, line 21 - page 37, line 13 claims 1-19; examples 6,8-10,12,13 -----	28-35
X	WO 2018/224844 A1 (AUTOLUS LTD [GB]) 13 December 2018 (2018-12-13) the whole document, in particular page 8, line 10-page 13, line 26; page 15, lines 34-page 35, line 3; examples 1-6 -----	28-35

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	"33rd Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2018)", JOURNAL FOR IMMUNOTHERAPY OF CANCER, BIOMED CENTRAL LTD, LONDON, UK, vol. 6, no. 1, 6 November 2018 (2018-11-06), pages 1-192, XP021262327, DOI: 10.1186/S40425-018-0423-X P530	1-45
A	T. ZHANG ET AL: "Cancer Immunotherapy Using a Bispecific NK Receptor Fusion Protein that Engages both T Cells and Tumor Cells", CANCER RESEARCH, vol. 71, no. 6, 15 March 2011 (2011-03-15) , pages 2066-2076, XP055214113, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-10-3200 the whole document, in particular abstract and discussion	1-45
A	WO 2017/165464 A1 (ELSTAR THERAPEUTICS INC [US]) 28 September 2017 (2017-09-28) page 1, line 14 - line 30 page 14, line 3 - line 30 page 20, line 9 - page 21, line 24 page 25, line 24 - line 25 page 59, line 15 - page 60, line 25 page 78, line 16 - page 92, line 25 page 118, line 9 - page 125, line 31 page 137, line 25 - page 138, line 15; claims 1-6,18,19,54-59,145,146; examples 2,3,7-12,14-17,20-22	1-45
A	WO 2019/005641 A1 (SYSTIMMUNE INC [US]; SICHUAN BAILI PHARMACEUTICAL CO LTD [CN]) 3 January 2019 (2019-01-03) the whole document, in particular page 3, line 1-page 4, line 30; claims 1-22; figures 1-3	1-45
A	MAULIK VYAS ET AL: "Natural ligands and antibody-based fusion proteins: harnessing the immune system against cancer", TRENDS IN MOLECULAR MEDICINE, vol. 20, no. 2, 1 February 2014 (2014-02-01), pages 72-82, XP055501127, GB ISSN: 1471-4914, DOI: 10.1016/j.molmed.2013.10.006 the whole document	1-45

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/019291

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004056873 A1	08-07-2004	AU 2003296553 A1	14-07-2004
		DE 10261223 A1	08-07-2004
		EP 1590370 A1	02-11-2005
		WO 2004056873 A1	08-07-2004
WO 2015132598 A1	11-09-2015	AU 2015225944 A1	01-09-2016
		AU 2019204921 A1	01-08-2019
		AU 2019204925 A1	01-08-2019
		BR 112016020304 A2	17-10-2017
		CA 2940564 A1	11-09-2015
		CL 2016002195 A1	19-05-2017
		CL 2018003096 A1	11-01-2019
		CL 2019001567 A1	16-08-2019
		CL 2019001568 A1	16-08-2019
		CN 106068276 A	02-11-2016
		DK 3125934 T3	03-02-2020
		EP 3125934 A1	08-02-2017
		EP 3241561 A1	08-11-2017
		ES 2758173 T3	04-05-2020
		HU E047641 T2	28-05-2020
		JP 2017143838 A	24-08-2017
		JP 2017510257 A	13-04-2017
		KR 20160127130 A	02-11-2016
		KR 20200027576 A	12-03-2020
		KR 20200027577 A	12-03-2020
		PE 20170125 A1	16-03-2017
		PH 12016501646 A1	06-02-2017
		PL 3125934 T3	30-04-2020
		PT 3125934 T	16-12-2019
		RU 2016138421 A	05-04-2018
		RU 2018140694 A	10-12-2018
		SG 10201906460P A	27-09-2019
		SG 10201906461S A	27-09-2019
		SG 11201606583V A	29-09-2016
		US 2017066827 A1	09-03-2017
		US 2017334998 A1	23-11-2017
		WO 2015132598 A1	11-09-2015
WO 2018224844 A1	13-12-2018	AU 2018282038 A1	12-12-2019
		CA 3065006 A1	13-12-2018
		CL 2019003536 A1	08-05-2020
		CN 110741018 A	31-01-2020
		CO 2019013614 A2	01-04-2020
		EP 3635006 A1	15-04-2020
		KR 20200015713 A	12-02-2020
		PE 20191717 A1	06-12-2019
		US 2020140549 A1	07-05-2020
		WO 2018224844 A1	13-12-2018
WO 2017165464 A1	28-09-2017	AU 2017238172 A1	13-09-2018
		CA 3016563 A1	28-09-2017
		CN 109153728 A	04-01-2019
		EP 3433281 A1	30-01-2019
		JP 2019512272 A	16-05-2019
		US 2017368169 A1	28-12-2017
		WO 2017165464 A1	28-09-2017
WO 2019005641 A1	03-01-2019	CN 110891650 A	17-03-2020

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/019291

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		TW 201904588 A	01-02-2019
		TW 201904997 A	01-02-2019
		WO 2019005641 A1	03-01-2019
		WO 2019005642 A1	03-01-2019