

ANTI-CD123 BINDING MOLECULES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial Nos. 63/150,488, filed February 17, 2021; and 63/249,455, filed September 28, 2021, which are all each incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on February 17, 2022, is named 038WO1-Sequence-Listing.txt and is 84,588 bytes in size.

BACKGROUND

[0003] Acute Myeloid Leukemia (AML) is the leading cause of leukemia mortality in the United States, with >20,000 new patients per year with a 5-year survival of less than 30%, with the survival rate decreasing to 10% in patients over 60 years old (National Cancer Institute Surveillance, Epidemiology and End-Result Program (SEER) data; Oran and Weisdorf 2012, *Haematologica* 97(12) 1916). Few advances have been made in the treatment of AML patients for the past 40 years, and current treatment options primarily consist of intense chemotherapy and stem cell transplantation (Luppi *et al.* 2018, *Cancers* 10, 429). Several approaches have been taken to target cell surface molecules on AML cells to direct T cells to engage and kill AML cells. One such surface molecule is CD123 (also known as IL-3 receptor alpha chain or IL-3R α) that is expressed in >90% of AML patients on leukemic cells as well as leukemic stem cells, a cell type which is often responsible for disease relapse after therapy (Kovtun *et al.* 2018, *Blood Advances* 2(8) 848; Xie *et al.* 2017, *Blood Cancer Journal* 7, e567). In addition, CD123 is highly expressed in patients that have genetic mutations associated with a very poor prognosis, such as FLT3 (Xie *et al.* 2017, *Blood Cancer Journal* 7, e567). The amino acid sequences of two human isoforms of CD123 are presented as SEQ ID NO: 14 (isoform 1, mature protein: approximately amino acids 23 to 378 of SEQ ID NO: 14) and SEQ ID NO: 15

(isoform 2, mature protein: approximately amino acids 23 to 300 of SEQ ID NO: 15), the cynomolgus monkey CD123 amino acid sequence is presented as SEQ ID NO: 16 (about 87% identical to human isoform 1; mature protein: approximately amino acids 23 to 378 of SEQ ID NO: 16), and the mouse CD123 amino acid sequence is presented as SEQ ID NO: 17 (about 30% identical to human isoform 1; mature protein: approximately amino acids 17 to 396 of SEQ ID NO: 17).

[0004] CD123 is a clinically validated target for some hematological malignancies as evidence by the FDA approval of a recombinant IL-3 cytokine conjugated with diphtheria toxin for the treatment of blastic plasmacytoid dendritic cell neoplasms (Pemmaraju *et al* 2019, *NEJM* 380:1628). This and other CD123 targeting agents are being tested in preclinical and clinical trials. Early Phase 1 clinical studies have been conducted with CD123 x CD3 bispecific antibodies by Xencor (XmAb14045 – IgG based), Macrogenics (flotetuzumab - DART) and Jansen (JNJ-63709178 - duobody). Though early signs of clinical efficacy have been reported in some of these patients, severe cytokine release syndrome and some patient deaths have also been observed with this class of bispecific drugs (Ravandi *et al* 2018 *Blood* 132:763; Jacobs *et al* 2018, *Blood* 132:2738; Uy *et al* 2018, *Blood* 132:764). Cytokine release syndrome (or CRS) is characterized by fever, hypotension, blood coagulation abnormalities and capillary leak which can be life threatening and such findings are also associated with other T cell engaging approaches, including CAR-Ts and BiTEs (Teachley *et al* 2016, *Cancer Discovery* 6(6) 664; Hay *et al* 2017, *Blood* 130(21) 2295). These adverse safety events related to cytokine release tend to constitute dose limiting toxicities of IgG based CD3 engaging bispecific antibodies and manifest as challenges to the safe, efficient, and tolerable administration of such agents and potentially to the ability to optimize efficacy of these therapeutic agents due to the resulting limitations to dosing.

[0005] Antibodies and antibody-like molecules that can multimerize, such as IgA and IgM antibodies, have emerged as promising drug candidates in the fields of, *e.g.*, immuno-oncology and infectious diseases allowing for improved specificity, improved avidity, and the ability to bind to multiple binding targets. See, *e.g.*, U.S. Patent Nos. 9,951,134, 9,938,347, 10,570,191, 10,604,559, 10,618,978, 10,787,520, and 10,899,835 and U.S. Patent Application Publication No. US 2019-0185570, US 2019-0330374, US

2019-0338041, US 2019-0330360, and US 2019-0338040, the contents of which are incorporated herein by reference in their entireties.

[0006] There remains a need to target CD123-expressing AML cells and induce T-cell mediated killing of those cells, while minimizing CRS. We have evaluated whether targeting CD123 with our CD3 bispecific IgM technology will not only effectively target CD123 expressing AML tumor cells for T cell mediated cytotoxicity but will also produce responses with a favorable safety profile for the cytokine release syndrome that has sometimes been severe in patients treated with IgG based CD123 x CD3 bispecific antibodies. In addition, the high avidity binding of IgM antibodies may allow our CD123 x CD3 bispecific IgM to target tumor cells that express relatively lower levels of cell surface expression of CD123, as compared with IgG based bispecific antibodies.

SUMMARY

[0007] Provided herein is an antibody or antigen-binding fragment or derivative thereof comprising an antigen-binding domain that specifically binds to CD123, where the antigen-binding domain comprises a heavy chain variable region (VH) and light chain variable region (VL), where the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79, SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83. In some embodiments, the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79.

[0008] In some embodiments, the antibody or fragment or derivative thereof is an Fv fragment, a single-chain Fv fragment (scFv), or a disulfide-linked Fv fragment (sdFv).

[0009] In some embodiments, the antibody or fragment or derivative thereof is a multimeric antibody comprising five, six, or two bivalent binding units and ten, twelve, or four antigen-binding domains where at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123; where each binding unit comprises two heavy chains each comprising an IgM or IgA constant region or a multimerizing fragment or variant thereof, and where at least one two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy chain constant

regions of the multimeric antibody is associated with a copy of the VH. In some embodiments, the antibody or fragment or derivative thereof comprises a bivalent binding unit comprising two antigen-binding domains where at least one antigen-binding domain specifically binds to CD123, where the binding unit comprises two heavy chains each comprising a heavy chain constant region or fragment or variant thereof, and where at least one heavy chain constant region or fragment or variant thereof of the binding unit is fused to a copy of the VH.

[0010] In some embodiments, the antibody or fragment or derivative thereof comprises a single bivalent binding unit comprising two antigen-binding domains where at least one antigen-binding domain specifically binds to CD123, where the binding unit comprises two heavy chains each comprising a heavy chain constant region or fragment or variant thereof, and where at least one heavy chain constant region or fragment or variant thereof of the binding unit is associated with a copy of the VH. In some embodiments, the heavy chains comprise IgG heavy chain constant regions or fragments or variants thereof.

[0011] In some embodiments, the antibody or fragment or derivative thereof is a multimeric antibody comprising two, five, or six bivalent binding units and four, ten, or twelve antigen-binding domains where at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123; where each binding unit comprises two heavy chains each comprising an IgA or IgM constant region or a multimerizing fragment or variant thereof, and where at least one two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy chain constant regions of the multimeric antibody is fused to a copy of the VH. In some embodiments, the antibody or fragment or derivative thereof is a multimeric antibody comprising two, five, or six bivalent binding units and four, ten, or twelve antigen-binding domains where at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123; where each binding unit comprises two heavy chains each comprising an IgA or IgM constant region or a multimerizing fragment or variant thereof, and where at least one two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy chain constant regions of the multimeric antibody is fused to a copy of the VH. In some embodiments, each heavy chain constant region or multimerizing fragment or variant thereof is associated with a copy of the VH.

In some embodiments, each heavy chain constant region or fragment or variant thereof is fused to a copy of the VH.

[0012] In some embodiments, each binding unit further comprises two light chains each comprising a light chain constant region or fragment or variant thereof, and where at least one, two, three, four, five, six, seven eight, nine, ten, eleven, or twelve light chain constant regions or fragments or variants thereof is/are associated with a copy of the VL. In some embodiments, each binding unit further comprises two light chains each comprising a light chain constant region or fragment or variant thereof, and where at least one, two, three, four, five, six, seven eight, nine, ten, eleven, or twelve light chain constant regions or fragments or variants thereof is/are fused to a copy of the VL. In some embodiments, each light chain constant region or fragment or variant thereof is associated with a copy of the VL. In some embodiments, each light chain constant region or fragment or variant thereof is/are fused to a copy of the VL.

[0013] In some embodiments, the antibody or fragment or derivative thereof comprises a complete antibody, an Fab fragment, an Fab' fragment, or an F(ab')₂ fragment.

[0014] In some embodiments, the antibody or fragment or derivative thereof is dimeric and comprises two bivalent IgA binding units and a J chain or fragment or variant thereof, where each binding unit comprises a C α 3 domain and an α -tailpiece (α tp) domain. In some embodiments, the IgA heavy chain constant regions or fragments or variants thereof each further comprise a C α 1 domain, a C α 2 domain, an IgA hinge region, or any combination thereof.

[0015] In some embodiments, the antibody or fragment or derivative thereof is hexameric or pentameric and comprises five or six bivalent IgM binding units, where each binding unit comprises a C μ 4 domain and a μ -tail piece (μ tp) domain or fragment or variant thereof. In some embodiments, the IgM heavy chain constant regions or multimerizing fragments or variants thereof each further comprise a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof. In some embodiments, the IgA heavy chain constant regions or fragments or variants thereof are IgA1 heavy chain constant regions or fragments or variants thereof. In some embodiments, the IgA heavy chain constant regions or fragments or variants thereof comprise SEQ ID NO: 3. In some embodiments, the IgA heavy chain constant regions or fragments or variants thereof are

IgA2 heavy chain or fragments or variants thereof. In some embodiments, the IgA heavy chain constant regions or fragments or variants thereof comprise SEQ ID NO: 4.

- 5 [0016] In some embodiments, each IgM heavy chain constant region is a human IgM constant region or multimerizing variant or fragment thereof, comprising the amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, or a variant or fragment thereof. In some embodiments, the antibody or fragment or derivative thereof comprises a variant human IgM constant region, where the antibody or fragment or derivative thereof has reduced CDC activity relative to an antibody or antigen-binding fragment or derivative thereof comprising IgM heavy chain constant regions comprising the amino acid sequence SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, each variant human IgM constant region comprises an amino acid substitution corresponding to position P311 of SEQ ID NO: 1 or SEQ ID NO: 2, an amino acid substitution corresponding to position P313 of SEQ ID NO: 1 or SEQ ID NO: 2, or amino acid substitutions corresponding to positions P311 and P313 of SEQ ID NO: 1 or SEQ ID NO: 2.
- 10 [0017] In some embodiments, each IgM heavy chain constant region or multimerizing variant or fragment thereof is a variant human IgM constant region with one or more half-life altering single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more half-life altering single amino acid substitutions, deletions, or insertions; where the antibody or fragment or derivative thereof exhibits increased serum half-life upon administration to a subject animal relative to an antibody or antigen-binding fragment or derivative thereof comprising the reference IgM heavy chain constant regions, which is administered in the same way to the same animal species. In some embodiments, the variant IgM heavy chain constant regions comprise amino acid half-life altering substitutions at one or more amino acid positions corresponding to amino acid E345A, S401A, E402A, or E403A of the wild-type human IgM constant region SEQ ID NO: 1 or SEQ ID NO: 2.
- 15 [0018] In some embodiments, the IgM heavy chain constant regions or multimerizing variant or fragment thereof each comprise one or more glycosylation substitutions corresponding to N46, N209, N272, or N440 of SEQ ID NO: 1 or SEQ ID NO: 2, where the one or more glycosylation substitutions prevent asparagine (N)-linked glycosylation.
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[0019] In some embodiments, the antibody or fragment or derivative thereof is pentameric, and further comprises a J chain, or fragment thereof, or variant thereof.

5 [0020] In some embodiments, the J-chain or fragment or variant thereof is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 7 or a fragment thereof, or a variant thereof. In some embodiments, the antibody or fragment or derivative thereof comprises a variant J-chain or fragment thereof, where the variant J-chain comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type mature human J-chain of SEQ ID NO: 7, and where an IgM antibody comprising the variant J-chain exhibits an increased serum half-life upon administration to an animal relative to a reference IgM antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the J-chain, and is administered in the same way to the same animal species. In some embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 7 is substituted with alanine (A). In some embodiments, the J-chain comprises the amino acid sequence SEQ ID NO: 8.

15 [0021] In some embodiments, the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, where the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof. In some embodiments, the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof. In some embodiments, the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids. In some embodiments, the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, to the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof, where the heterologous polypeptides fused to both the N-terminus and C-terminus can be the same or different. In some embodiments, the polypeptide fused to the J-chain or fragment or variant thereof is an antibody antigen-binding domain, or a subunit thereof. In some embodiments, the antibody antigen-binding domain comprises a scFv fragment.

25 [0022] In some embodiments, the heterologous polypeptide binds to CD3. In some embodiments, the antibody or fragment or derivative thereof can bind CD3. In some embodiments, the antibody or fragment or derivative thereof comprises a CD3-binding VH and VL sequence disclosed herein. In some embodiments, the antibody antigen-

binding domain binds to CD3 and comprises a heavy chain variable region (VH) and a light chain variable region (VL), where the VH comprises VH complementarity-determining regions VHCDR1, VHCDR2, and VHCDR3 and the VL comprises VL complementarity-determining regions VLCDR1, VLCDR2, and VLCDR3, where the

5 VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise, respectively, the amino acid sequences SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25; SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, and

10 SEQ ID NO: 41; SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49; SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57; SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 64, and SEQ ID NO: 65; or SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73. In some embodiments, the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise, respectively, the amino acid sequences SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33. In some embodiments, the antibody antigen-binding domain comprises VH and VL amino acid sequences at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 18 and SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 38, SEQ ID NO: 42 and SEQ ID NO: 46, SEQ ID NO: 50 and SEQ ID NO: 54, SEQ ID NO: 58 and SEQ ID NO: 62, or SEQ ID NO: 66 and SEQ ID NO: 70, respectively. In some embodiments, the antibody antigen-binding domain comprises the VH and VL amino acid sequences SEQ ID NO: 18 and SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 38, SEQ ID NO: 42 and SEQ ID NO: 46, SEQ ID NO: 50 and SEQ ID NO: 54, SEQ ID NO: 58 and SEQ ID NO: 62, or SEQ ID NO: 66 and SEQ ID NO: 70, respectively. In some embodiments, the antibody antigen-binding domain comprises the VH and VL amino acid sequences SEQ ID NO: 26 and SEQ ID NO: 30, respectively.

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30 **[0023]** In some embodiments, the antibody or fragment or derivative thereof further comprises a secretory component, or fragment or variant thereof.

[0024] In some embodiments, the antibody or fragment or derivative thereof is multispecific. In some embodiments, the antibody or fragment or derivative thereof is bispecific.

5 [0025] In some embodiments, the antibody or fragment or derivative thereof can specifically bind to human CD123. In some embodiments, the antibody or fragment or derivative thereof specifically binds to human CD123 with an affinity characterized by a dissociation constant K_D no greater than 500 nM, 100 nM, 50.0 nM, 40.0 nM, 30.0 nM, 20.0 nM, 10.0 nM, 9.0 nM, 8.0 nM, 7.0 nM, 6.0 nM, 5.0 nM, 4.0 nM, 3.0 nM, 2.0 nM, 1.0 nM, 0.50 nM, 0.10 nM, 0.050 nM, 0.01 nM, 0.005 nM, or 0.001 nM.

10 [0026] Also provided herein is a composition comprising an antibody or fragment or derivative thereof disclosed herein. Also provided herein is a polynucleotide comprising a nucleic acid sequence that encodes the antibody or fragment or derivative thereof disclosed herein or a subunit thereof. Further provided herein is a vector comprising a polynucleotide disclosed herein. Also provided herein is a host cell comprising a vector
15 disclosed herein.

[0027] Further provided herein is a method of producing an antibody or fragment or derivative thereof disclosed herein, comprising culturing a host cell disclosed herein, and recovering the antibody or fragment or derivative thereof.

20 [0028] Also provided herein is a method of treating cancer comprising administering to a subject in need of treatment an effective amount of an antibody or fragment or derivative thereof disclosed herein. In some embodiments, the subject is human. In some embodiments, the cancer is a hematological cancer. In some embodiments, the hematological cancer is acute myeloid leukemia (AML).

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

25 [0029] **FIGS. 1A-1D** show a comparison of parental and humanized anti-CD123 VH (**FIG. 1A, 1C**) and VL (**FIG. 1B, 1D**) sequences based on the 4D9Q (**FIG. 1A, 1B**) or 4NWT (**FIG. 1C, 1D**) frameworks. 32716-VH: SEQ ID NO: 74, h32716-VL: SEQ ID NO: 75, h32716-VH1: SEQ ID NO: 76, h32716-VH2: SEQ ID NO: 77, h32716-VH3: SEQ ID NO: 78, h32716-VL1: SEQ ID NO: 79, h32716-VH4: SEQ ID NO: 80, h32716-

VH5: SEQ ID NO: 81, h32716-VH6: SEQ ID NO: 82, and h32716-VL2: SEQ ID NO: 83.

[0030] FIGS. 2A-2G show Octet measurements of association and dissociation of 32716 IgG (FIG. 2A), h32716-1-1 IgG (FIG. 2B), h32716-2-1 IgG (FIG. 2C), h32716-3-1 IgG (FIG. 2D), h32716-4-2 IgG (FIG. 2E), h32716-5-2 IgG (FIG. 2F), and h32716-6-2 IgG (FIG. 2G). The vertical line denotes the beginning of the dissociation phase of the procedure.

[0031] FIG. 3 shows binding of anti-CD123 IgG antibodies to human CD123 on MV411 cells at different concentrations measured by flow cytometry.

10 [0032] FIGS. 4A-4C show Octet measurements of association and dissociation of 32716 IgM (FIG. 4A), h32716-1-1 IgM (FIG. 4B), and h32716-4-2 IgM (FIG. 4C). The vertical line denotes the beginning of the dissociation phase of the procedure.

[0033] FIGS. 5A-5B show binding of anti-CD123xCD3 IgM antibodies to human CD123 (FIG. 5A) or human CD3ε (FIG. 5B) at different concentrations measured by ELISA.

[0034] FIG. 6 shows binding of anti-CD123xCD3 IgM antibodies to human CD123 on MV411 cells at different concentrations measured by flow cytometry.

[0035] FIGS. 7A-7B show tumor viability after treatment with anti-CD123xCD3 IgM antibodies in a pan- T-cell dependent cellular cytotoxicity (TDCC) assay on KG1a (FIG. 7A) and MV411 cells (FIG. 7B) after 72 hours.

[0036] FIG. 8 shows the percentage of high molecular weight aggregates (%HMW) in solutions of h32716-1-1 IgM, h32716-4-2 IgM, and 32716 IgM antibody under various conditions.

[0037] FIG. 9A shows the average tumor volumes over time through day 75 of mice treated with vehicle, anti-CD123XCD3 IgG #1 treatment, 5 mg/kg h32716-1-1 IgM antibody, and 15 mg/kg h32716-1-1 IgM antibody. FIG. 9B shows individual tumor volumes on day 75 of mice treated with vehicle, anti-CD123XCD3 IgG #1 treatment, 5 mg/kg h32716-1-1 IgM antibody, and 15 mg/kg h32716-1-1 IgM antibody.

[0038] FIGS. 10A-D show individual tumor volumes over time through day 75 for vehicle (FIG. 10A), anti-CD123XCD3 IgG #1 (FIG. 10B), 5 mg/kg h32716-1-1 IgM antibody (FIG. 10C), and 15 mg/kg h32716-1-1 IgM antibody (FIG. 10D).

- [0039] FIG. 11 shows *in vitro* colony formation of AML cells from four different donors, following treatment with h32716-1-1 IgM antibody. The calculation was normalized to solvent control.
- 5 [0040] FIG. 12 shows the serum concentrations of antibody after one dose of 5 mg/kg of h32716, h32716-4-2, or h32716-1-1 over time in a mouse model.
- [0041] FIG. 13A shows the number of live tumor cells following 48 hours of TDCC with either CD123xCD3 IgG #1 or h32716-1-1. FIGS. 13B-13D show that amount of IFN γ (FIG. 13B), IL-6 (FIG. 13C), and IL-10 (FIG. 13D) was detected in the media after following 48 hours of TDCC with either CD123xCD3 IgG #1 or h32716-1-1.
- 10 [0042] FIG. 14A shows the number of live tumor cells following 72 hours of TDCC with either CD123xCD3 IgG #1 or h32716-1-1. FIGS. 14B-14D show that amount of IFN γ (FIG. 14B), IL-6 (FIG. 14C), and IL-10 (FIG. 14D) was detected in the media after following 72 hours of TDCC with either CD123xCD3 IgG #1 or h32716-1-1.

DETAILED DESCRIPTION

15 Definitions

- [0043] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.
- 20 [0044] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following
- 25 embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).
- [0045] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular
- 30 Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and

Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary of Biochemistry and Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

5 [0046] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various embodiments or embodiments of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more
10 fully defined by reference to the specification in its entirety.

[0047] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids and
15 does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression
20 modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology but is not necessarily translated from a designated nucleic acid sequence. It
25 can be generated in any manner, including by chemical synthesis.

[0048] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such
30 structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations and are referred to as

unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine.

5 [0049] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially
10 purified by any suitable technique.

[0050] As used herein, the term "a non-naturally occurring polypeptide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polypeptide that are, or might be, determined or interpreted by a judge or an administrative or judicial body, to be "naturally-occurring."

15 [0051] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides which retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of
20 polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, *e.g.*, a polypeptide include fragments as described above, and polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain embodiments, variants can be non-naturally occurring. Non-naturally occurring variants
25 can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions, or additions. Derivatives are polypeptides that have been altered to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a
30 "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty

standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

5 **[0052]** A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, 10 cysteine), nonpolar side chains (*e.g.*, glycine, 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). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the 15 polypeptides and antibodies of the present disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem. 32*: 1180-1 187 (1993); Kobayashi *et al.*, *Protein Eng. 12*(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA 94*:412-417 (1997)). 20

[0053] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can 25 comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or "nucleic acid sequence" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

[0054] By an "isolated" nucleic acid or polynucleotide is intended any form of the 30 nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be "isolated." Also, a

polynucleotide segment, *e.g.*, a PCR product, which has been engineered to have restriction sites for cloning is considered to be “isolated.” Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0055] As used herein, the term “a non-naturally occurring polynucleotide” or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the nucleic acid or polynucleotide that are, or might be, determined or interpreted by a judge, or an administrative or judicial body, to be “naturally-occurring.”

[0056] As used herein, a “coding region” is a portion of nucleic acid which consists of codons translated into amino acids. Although a “stop codon” (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0057] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably

associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

[0058] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0059] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0060] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

[0061] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase.

[0062] As used herein, the term "binding molecule" refers in its broadest sense to a molecule that specifically binds to a binding target, *e.g.*, an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one of more "antigen-binding domains" described herein. A non-limiting example of a binding molecule is an antibody or antibody-like molecule that retains antigen-specific binding or an antibody-like molecule or fragment thereof as described in detail herein that retains antigen-specific binding. In certain embodiments a "binding molecule" comprises an antibody or antibody-like molecule as described in detail herein.

[0063] As used herein, the terms "binding domain" or "antigen-binding domain" (can be used interchangeably) refer to a region or fragment of a binding molecule *e.g.*, an antibody or antibody-like molecule, that is necessary and sufficient to specifically bind to a binding target, *e.g.*, an epitope. For example, an "Fv," *e.g.*, a heavy chain variable region and a light chain variable region of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a "binding domain." Other antigen-binding domains include, without limitation, the heavy chain variable region (VHH) of an antibody derived from a camelid species, or six immunoglobulin

complementarity determining regions (CDRs) expressed in a scaffold, *e.g.*, a fibronectin scaffold. A “binding molecule,” *e.g.*, an antibody or antibody-like molecule as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or even more “antigen-binding domains.” As used herein, a “binding unit-associated antigen-binding domain” refers to an antigen binding domain that is part of an antibody heavy chain and/or an antibody light chain. The term “J-chain-associated antigen-binding domain” refers to an antigen binding domain that is associated with a modified J-chain as described herein, for example, a scFv fused to a wild type human J-chain, or functional fragment or variant thereof.

10 [0064] The terms “antibody” and “immunoglobulin” can be used interchangeably herein. An antibody as provided in this disclosure must specifically bind to an antigen, *i.e.*, it includes at least the variable domain of a heavy chain (for camelid species) or at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. See, *e.g.*, Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless
15 otherwise stated, the term “antibody” encompasses anything ranging from a small antigen-binding fragment of an antibody to a full sized antibody, *e.g.*, an IgG antibody that includes two IgG heavy chains or fragments thereof and two complete light chains, an IgA antibody that includes two, four, or eight heavy chains or multimerizing
20 fragments thereof and two, four, or eight light chains and optionally includes a J-chain and/or a secretory component, or an IgM antibody that includes ten or twelve complete heavy chains and ten or twelve complete light chains and optionally includes a J-chain or functional fragment or variant thereof.

[0065] The term “immunoglobulin” comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as, *e.g.*, gamma, mu, alpha, delta, or epsilon, (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, $\gamma 1$ - $\gamma 4$ or $\alpha 1$ - $\alpha 2$). It is the nature of this chain that determines the “isotype” of the antibody as IgG, IgM, IgA, IgD, or IgE, respectively. The immunoglobulin subclasses (subtypes) *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, etc.
25 are well characterized and are known to confer functional specialization. Modified versions of each of these immunoglobulins are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

[0066] Light chains are classified as either kappa or lambda (κ , λ). Each heavy chain class can be associated with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other via disulfide bonds, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are expressed, *e.g.*, by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, *e.g.*, IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a "Y" structure, also referred to herein as an "H2L2" structure, or a "binding unit."

[0067] The term "binding unit" is used herein to refer to the portion of a binding molecule, *e.g.*, an antibody or antibody-like molecule that corresponds to a standard "H2L2" immunoglobulin structure, *e.g.*, two heavy chains or fragments thereof and two light chains or fragments thereof. In certain embodiments a binding unit can correspond to two heavy chains, *e.g.*, in a camelid antibody. In certain embodiments, *e.g.*, where the binding molecule is a bivalent IgG antibody or antibody-like molecule, the terms "binding molecule" and "binding unit" are equivalent. In other embodiments, *e.g.*, where the binding molecule is multimeric, *e.g.*, a dimeric or tetrameric IgA antibody or IgA-like antibody, a pentameric IgM antibody or IgM-like antibody, or a hexameric IgM antibody or IgM-like antibody, the binding molecule comprises two or more "binding units." Two of four in the case of an IgA dimer or tetramer, or five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and light chains, but will typically be bivalent, *i.e.*, will include two "antigen-binding domains," as defined above. As used herein, certain binding molecules provided in this disclosure are "dimeric" or "tetrameric," and include two or four bivalent binding units that include IgA heavy chain constant regions or multimerizing fragments thereof. Certain binding molecules provided in this disclosure are "pentameric" or "hexameric," and include five or six bivalent binding units that include IgM heavy chain constant regions or multimerizing fragments thereof. A binding molecule, *e.g.*, an antibody or antibody-like molecule, comprising two or more, *e.g.*, two, four, five, or six binding units, is referred to herein as "multimeric."

[0068] The term “J-chain” as used herein refers to the J-chain associated with pentameric IgM or dimeric or tetrameric IgA antibodies of any animal species, any functional fragment thereof, derivative thereof, and/or variant thereof, including the mature human J-chain, the amino acid sequence of which is presented as SEQ ID NO: 7.

5 Various J-chain variants and modified J-chain derivatives are disclosed herein. As persons of ordinary skill in the art will recognize, “a functional fragment” or a “functional variant” includes those fragments and variants that can associate with IgM heavy chain constant regions to form a pentameric IgM antibody (or alternatively can associate with IgA heavy chain constant regions to form a dimeric or tetrameric IgA
10 antibody).

[0069] The term “modified J-chain” is used herein to refer to a derivative of a native sequence J-chain polypeptide comprising a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain introduced into the native sequence. The introduction can be achieved by any means, including direct or indirect fusion of the
15 heterologous polypeptide or other moiety or by attachment through a peptide or chemical linker. The term “modified human J-chain” encompasses, without limitation, a human J-chain comprising the amino acid sequence of SEQ ID NO: 7 or functional fragment thereof, or functional variant thereof, modified by the introduction of a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain. In certain
20 embodiments the heterologous moiety does not interfere with efficient polymerization of IgM into a pentamer or IgA into a dimer or tetramer, and binding of such polymers to a target. Exemplary modified J-chains can be found, *e.g.*, in U.S. Patent Nos. 9,951,134, 10,975,147, 10,400,038, and 10,618,978, and in U.S. Patent Application Publication No. US-2019-0185570, each of which is incorporated herein by reference in its entirety.

25 [0070] As used herein, the terms “IgM-derived binding molecule,” “IgM-like antibody,” “IgM-like binding unit,” or “IgM-like heavy chain constant region” refer to a variant antibody-derived binding molecule, antibody, binding unit, or heavy chain constant region that still retains the structural portions of an IgM heavy chain necessary to confer the ability to bind to antigen and to form multimers, *i.e.*, hexamers, or in association with
30 J-chain, form pentamers. An IgM-like antibody or IgM-derived binding molecule typically includes at least the C μ 4 and μ tailpiece (μ tp) domains of the IgM constant region and an antigen binding domain or subunit thereof but can include heavy chain

constant region domains from other antibody isotypes, *e.g.*, IgG, from the same species or from a different species. An IgM-like antibody or IgM-derived binding molecule can likewise be an antibody fragment in which one or more constant regions are deleted, as long as the IgM-like antibody is capable of binding antigen and of forming hexamers and/or pentamers. Thus, an IgM-like antibody or IgM-derived binding molecule can be, *e.g.*, a hybrid IgM/IgG antibody or can be a “multimerizing fragment” of an IgM antibody.

[0071] As used herein, the terms “IgA-derived binding molecule,” “IgA-like antibody,” “IgA-like binding unit,” or “IgA-like heavy chain constant region” refer to a variant antibody-derived binding molecule, antibody, binding unit, or heavy chain constant region that still retains the structural portions of an IgA heavy chain necessary to bind antigen and to confer the ability to form multimers, *i.e.*, dimers or tetramers, in association with J-chain. An IgA-like antibody or IgA-derived binding molecule typically includes at least the C α 3 and α tailpiece (α tp) domains of the IgA constant region and an antigen binding domain or subunit thereof but can include heavy chain constant region domains from other antibody isotypes, *e.g.*, IgG, from the same species or from a different species. An IgA-like antibody or IgA-derived binding molecule can likewise be an antibody fragment in which one or more constant regions are deleted, as long as the IgA-like antibody is capable of binding antigen and forming dimers in association with a J-chain. Thus, an IgA-like antibody or IgA-derived binding molecule can be, *e.g.*, a hybrid IgA/IgG antibody or can be a “multimerizing fragment” of an IgA antibody.

[0072] The terms “valency,” “monovalent,” “bivalent,” “multivalent” and grammatical equivalents, refer to the number of antigen-binding domains in given binding molecule, *e.g.*, an antibody or antibody-like molecule, or in a given binding unit. As such, the terms “bivalent,” “tetravalent,” and “hexavalent” in reference to a given binding molecule, *e.g.*, an IgM antibody, IgM-like antibody or multimerizing fragment thereof, denote the presence of two antigen-binding domains, four antigen-binding domains, and six antigen-binding domains, respectively. A typical IgM antibody or IgM-like antibody or IgM-derived binding molecule where each binding unit is bivalent, can have 10 or 12 valencies. A bivalent or multivalent binding molecule, *e.g.*, antibody or antibody-like molecule, can be monospecific, *i.e.*, all of the antigen-binding domains are the same, or

can be bispecific or multispecific, *e.g.*, where two or more antigen-binding domains are different, *e.g.*, bind to different epitopes on the same antigen, or bind to entirely different antigens.

5 [0073] The term “epitope” includes any molecular determinant capable of specific binding to an antigen-binding domain of an antibody or antibody-like molecule. In certain embodiments, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, can have three-dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antigen-binding domain of an antibody.

[0074] The term “target” is used in the broadest sense to include substances that can be bound by a binding molecule, *e.g.*, an antibody or antibody-like molecule. A target can be, *e.g.*, a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule. Moreover, a “target” can, for example, be a cell, an organ, or an organism that comprises an epitope that can be bound by a binding molecule, *e.g.*, an antibody or antibody-like molecule.

[0075] Both the light and heavy chains of an antibody or antibody-like molecule are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally, but refer to particular structures of the molecule. The variable regions of both the light (VL) and heavy (VH) chains determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (*e.g.*, CH1, CH2, CH3, or CH4) confer biological properties such as the ability to multimerize, secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding regions or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4 in the case of IgM) and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

30 [0076] A “full length IgM antibody heavy chain” is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (VH), an antibody heavy chain constant domain 1 (CH1 or C μ 1), an antibody heavy chain

constant domain 2 (CM2 or C μ 2), an antibody heavy chain constant domain 3 (CM3 or C μ 3), and an antibody heavy chain constant domain 4 (CM4 or C μ 4) that can include a tailpiece.

[0077] As indicated above, variable region(s) form the antigen-binding domain of the antibody or antibody-like molecule, allowing it to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or an antigen-binding subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody or antibody-like molecule combine to form the antigen-binding domain. More specifically, an antigen-binding domain can be defined by three CDRs on each of the VH and VL chains. Certain antibodies or antibody-like molecules form larger structures. For example, IgA heavy chains can form a molecule that includes two or four H2L2 binding units and a J-chain covalently connected via disulfide bonds, which can be further associated with a secretory component, and IgM heavy chains can form a pentameric or hexameric molecule that includes five or six H2L2 binding units and optionally a J-chain covalently connected via disulfide bonds.

[0078] The six "complementarity determining regions" or "CDRs" present in an antibody antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three-dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the target antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and

Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987), which are incorporated herein by reference in their entireties).

[0079] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact amino acid numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1 CDR Definitions*

	Kabat	Chothia
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

*Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0080] Antibody variable domains can also be analyzed, *e.g.*, using the IMGT information system (imgt_dot_cines_dot_fr/) (IMGT®/V-Quest) to identify variable

region segments, including CDRs. (See, *e.g.*, Brochet *et al.*, Nucl. Acids Res., 36:W503-508, 2008).

[0081] Kabat *et al.* also defined a numbering system for variable region and constant region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering system is explicitly noted, however, consecutive numbering is used for all amino acid sequences in this disclosure.

[0082] The Kabat numbering system for the human IgM constant domain can be found in Kabat, *et al.* "Tabulation and Analysis of Amino acid and nucleic acid Sequences of Precursors, V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen, T-Cell Surface Antigens, β -2 Microglobulins, Major Histocompatibility Antigens, Thy-1, Complement, C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin, α -2 Macroglobulins, and Other Related Proteins," U.S. Dept. of Health and Human Services (1991). IgM constant regions can be numbered sequentially (*i.e.*, amino acid #1 starting with the first amino acid of the constant region, or by using the Kabat numbering scheme. A comparison of the numbering of two alleles of the human IgM constant region sequentially (presented herein as SEQ ID NO: 1 (alleleIGHM*03) and SEQ ID NO: 2 (alleleIGHM*04)) and by the Kabat system is set out below. The underlined amino acid residues are not accounted for in the Kabat system ("X," double underlined below, can be serine (S) (SEQ ID NO: 1) or glycine (G) (SEQ ID NO: 2)):

Sequential (SEQ ID NO: 1 or SEQ ID NO: 2)/KABAT numbering key for IgM heavy chain

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1/127 GSASAPTLFP LVSCENSPSD TSSVAVGCLA QDFLPDSITF SWKYKNNSDI
51/176 SSTRGFPSVL RGGKYAATSQ VLLPSKDVMQ GTDEHVCKV QHPNGNKEKN
101/226 VPLPVIAELP PKVSVFVPPR DGFFGNPRKS KLICQATGFS PRQIQVSWLR
151/274 EGKQVGSGVT TDQVQAEAKE SGPTTYKVTS TLTIKESDWL XQSMFTCRVD
201/324 HRGLTFQQNA SSMCVPDQDT AIRVFAIPPS FASIFLTKST KLTCLVTDLT
251/374 TYDSVTISWT RQNGEAVKTH TNISESHPNA TFSAVGEASI CEDDWNNGER
301/424 FTCTVTHTDL PSPLKQTISR PKGVALHRPD VYLLPPAREQ LNLRESATIT
351/474 CLVTGFSPAD VEVQWMQRGQ PLSPEKYVTS APMPEPQAPG RYFAHSILTV

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401/524 SEEEWNTGET YTCVVAHEAL PNRVTERTVD KSTGKPTLYN VSLVMSDTAG
 451/574 TCY

- [0083]** Binding molecules, *e.g.*, antibodies, antibody-like molecules, antigen-binding fragments, variants, or derivatives thereof, and/or multimerizing fragments thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are described, *e.g.*, in US patent 5,892,019.
- [0084]** By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or antibody-like molecule binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, a binding molecule, *e.g.*, an antibody or antibody-like molecule, is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."
- [0085]** A binding molecule, *e.g.*, an antibody or antibody-like molecule disclosed herein can be said to bind a target antigen with an off rate (k_{off}) of less than or equal to $5 \times 10^{-2} \text{ sec}^{-1}$, 10^{-2} sec^{-1} , $5 \times 10^{-3} \text{ sec}^{-1}$, 10^{-3} sec^{-1} , $5 \times 10^{-4} \text{ sec}^{-1}$, 10^{-4} sec^{-1} , $5 \times 10^{-5} \text{ sec}^{-1}$, 10^{-5} sec^{-1} , $5 \times 10^{-6} \text{ sec}^{-1}$, 10^{-6} sec^{-1} , $5 \times 10^{-7} \text{ sec}^{-1}$, or 10^{-7} sec^{-1} .
- [0086]** A binding molecule, *e.g.*, an antibody or antibody-like molecule disclosed herein can be said to bind a target antigen with an on rate (k_{on}) of greater than or equal to $10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $10^4 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$, $10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $10^6 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$ or $10^7 \text{ M}^{-1} \text{ sec}^{-1}$.
- [0087]** A binding molecule, *e.g.*, an antibody or antibody-like molecule is said to competitively inhibit binding of a reference antibody or antibody-like molecule to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen-binding fragment to the epitope.

Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays or OCTET assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antibody-like molecule to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

5 **[0088]** As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen-binding domains, *e.g.*, of an antibody or antibody-like molecule. See, *e.g.*, Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a
10 population of antigen-binding domains and an antigen. See, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual antigen-binding domains in the population with specific epitopes, and the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of
15 high avidity. Likewise, the interaction between a multimeric antibody with four, eight, ten, or twelve valencies and a population of specific epitopes would be one of high avidity. An interaction between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

[0089] Binding molecules, *e.g.*, antibodies or fragments, variants, or derivatives thereof
20 as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than
25 the one that induced its formation. The cross-reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0090] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For
30 example, a binding molecule can bind to an antigen with a dissociation constant or KD no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5

$\times 10^{-10}$ M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0091] Antigen-binding fragments of a binding molecule or antibody as provided herein including single-chain antibodies or other antigen-binding domains that can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J-chain, or secretory component. Also included are antigen-binding fragments that can include any combination of variable region(s) sufficient to bind antigen with one or more of a hinge region, CH1, CH2, CH3, or CH4 domains, a J-chain, or a secretory component. Binding molecules, *e.g.*, antibodies or antibody-like molecules can be from any animal origin including birds and mammals. The antibodies can be, *e.g.*, human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be chondrichthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.* According to embodiments of the present disclosure, an IgM or IgM-like antibody or IgM-derived binding molecule as provided herein can include an antigen-binding fragment of an antibody, *e.g.*, a scFv, so long as the IgM or IgM-like antibody is able to form a multimer, *e.g.*, a hexamer or a pentamer.

[0092] As used herein, the term "heavy chain subunit" includes amino acid sequences derived from an immunoglobulin heavy chain. A binding molecule, *e.g.*, an antibody or antibody-like molecule comprising a heavy chain subunit can include a VH domain and one or more of a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, a μ tail-piece (μ tp), or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody, antibody-like molecule, or fragment, variant, or derivative thereof can include without limitation, in addition to a VH domain, any combination of a CH1 domain, a hinge, a CH2 domain; a CH3 domain; a CH4 domain; or a μ tailpiece (μ tp) of one or more antibody isotypes and/or species. In certain embodiments, a binding molecule, *e.g.*, an antibody, antibody-like molecule, or fragment, variant, or derivative thereof can include, in addition to a VH

domain, one or more of a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, a μ -tailpiece (μ tp) domain and a J-chain (in the case of IgM), or one or more of a CH1 domain, a hinge region, a CH2 domain, a CH3 domain, an α -tailpiece (α tp) domain, and a J-chain (in the case of IgA). Further, a binding molecule, *e.g.*, antibody or antibody-like molecule, provided in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH1 domain, a hinge, a CH2 domain, or a CH3 domain. These domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule. According to embodiments of the present disclosure, an IgM or IgM-like antibody as provided herein includes sufficient portions of an IgM heavy chain constant region to allow the IgM or IgM-like antibody to form a multimer, *e.g.*, a hexamer or a pentamer, *e.g.*, the IgM heavy chain constant region includes a "multimerizing fragment" of an IgM heavy chain constant region.

[0093] As used herein, the term "light chain subunit" includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least a VL, and can further include a CL (*e.g.*, C κ or C λ) domain.

[0094] Binding molecules, *e.g.*, antibodies, antibody-like molecules, antigen-binding fragments, variants, or derivatives thereof, or multimerizing fragments thereof can be described or specified in terms of the epitope(s) or portion(s) of an antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen-binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or two or more epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

[0095] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain in IgG, IgA, and IgD heavy chains. This hinge region comprises approximately 25 amino acids and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently.

[0096] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group.

[0097] As used herein, the term "chimeric antibody" refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant

region (which can be intact, partial, or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.e.g.*, mouse or primate) and the constant region is human.

[0098] The terms “multispecific antibody” or “bispecific antibody” refer to an antibody or antibody-like molecule that has antigen-binding domains for two or more different epitopes within a single antibody molecule. Other binding molecules in addition to the canonical antibody structure can be constructed with two binding specificities.

[0099] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more amino acids in either the CDR or framework regions. In certain embodiments, entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain embodiments, not all the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen-binding capacity of the donor can still be transferred to the recipient variable domains. Exemplary methods of humanization are described in U.S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370.

[0100] As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.e.g.*, by recombinant techniques, in vitro peptide synthesis, by enzymatic or chemical coupling of peptides, nucleic acids, or glycans, or some combination of these techniques).

[0101] As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original ORFs. Thus, a

recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide. The term "associated" and grammatical equivalents refers to the interaction of two or more elements function together and that can be linked or fused, but can also be in proximity, *e.g.*, interacting in trans without being connected in any particular way.

[0102] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A portion of a polypeptide that is "amino-terminal" or "N-terminal" to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly, a portion of a polypeptide that is "carboxy-terminal" or "C-terminal" to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example, in a typical antibody, the variable domain is "N-terminal" to the constant region, and the constant region is "C-terminal" to the variable domain.

[0103] The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into RNA, *e.g.*, messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*,

polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0104] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to
5 alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed disease, pathologic condition, or disorder. Terms such as "prevent," "prevention," "avoid," "deterrence" and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted disease, pathologic condition, or disorder. Thus, "a subject need of treatment"
10 can include those already with the disease, pathologic condition. The term "a subject in need of prevention" those subjects prone to have the disease, pathologic condition, or disorder; and those in whom the disease, pathologic condition, or disorder is to be prevented.

[0105] As used herein the terms "serum half-life" or "plasma half-life" refer to the time
15 it takes (*e.g.*, in minutes, hours, or days) following administration for the serum or plasma concentration of a protein or a drug, *e.g.*, a binding molecule such as an antibody or antibody-like molecule as described herein, to be reduced by 50%. Two half-lives can be described: the alpha half-life, α half-life, or $t_{1/2\alpha}$, which is the rate of decline in plasma concentrations due to the process of drug redistribution from the central compartment,
20 *e.g.*, the blood in the case of intravenous delivery, to a peripheral compartment (*e.g.*, a tissue or organ), and the beta half-life, β half-life, or $t_{1/2\beta}$ which is the rate of decline due to the processes of excretion or metabolism.

[0106] As used herein the term "area under the plasma drug concentration-time curve" or "AUC" reflects the actual body exposure to drug after administration of a dose of the
25 drug and is expressed in mg*h/L. This area under the curve is measured from time 0 (t_0) to infinity (∞) and is dependent on the rate of elimination of the drug from the body and the dose administered.

[0107] As used herein, the term "mean residence time" or "MRT" refers to the average length of time the drug remains in the body.

30 [0108] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian

subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

Anti-CD123 Antigen-Binding Domains

[0109] Provided herein is an antibody or antigen-binding fragment or derivative thereof comprising an antigen-binding domain that specifically binds to CD123, where the antigen-binding domain comprises a heavy chain variable region (VH) and light chain variable region (VL), where the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79, SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83.

[0110] In certain embodiments, the antigen-binding domain as provided above is an Fv fragment, *e.g.*, a single-chain Fv fragment (scFv), or a disulfide-linked Fv fragment (sdFv). In certain embodiments, the antigen-binding domain as provided above is an scFv.

[0111] In certain embodiments, the antigen-binding domain as provided above is included in an antibody antibody-like molecule as described elsewhere herein. In some embodiments, the antigen-binding domain as provided above is included in an antibody or antibody-like molecule, where the antibody or antibody-like molecule is multispecific, *e.g.*, bispecific, trispecific, or tetraspecific. In some embodiments, the multispecific antibody or antibody-like molecule as provided herein specifically binds to CD123 and to a target on an effector cell, *e.g.*, CD16 or CD3.

[0112] In certain embodiments, the antibody or antibody-like molecule comprises a bivalent binding unit comprising two antigen-binding domains, where at least one antigen-binding domain specifically binds to CD123. According to this embodiments, the binding unit comprises two heavy chains each comprising a heavy chain constant region or fragment or variant thereof, and where at least one heavy chain constant region or fragment or variant thereof of the binding unit is associated with, *e.g.*, fused to a copy of the provided VH of the antigen-binding domain. In certain embodiments, both heavy chain constant regions or fragments or variants thereof of the binding unit are associated with, *e.g.*, fused to a copy of the provided VH of the antigen-binding domain. In certain embodiments, the heavy chains comprise IgG heavy chain constant regions or fragments

or variants thereof. Various IgG heavy chain constant regions and fragments or variants thereof are known, such as those described in Kang, *et al.*, 2019, *Experimental & Molecular Medicine*, 51:1-9; Brezski, *et al.*, 2016, *Current Opinion in Immunology*, 40: 62-69; Okazaki, *et al.*, 2004, *Journal of Molecular Biology*, 336(5): 1239-1249; Kang, *et al.*, 2019, *Front. Immunol.*, 10(562):1-11, and Saxena, *et al.*, 2016, *Front. Immunol.*, 7(580):1-11.. In certain embodiments the bivalent binding unit further comprises two light chains each comprising a light chain constant region or fragment or variant thereof. In certain embodiments at least one light chain constant region is fused to a copy of the provided VL of the antigen-binding domain. In certain embodiments, the bivalent binding unit comprises a complete antibody, *e.g.*, a complete IgG antibody or an F(ab')₂ fragment. In certain embodiments both light chain constant regions or fragments or variants thereof of the binding unit are fused to a copy of the provided VL of the antigen-binding domain. In certain embodiments, the bivalent binding unit comprises a complete antibody, *e.g.*, a complete IgG antibody or an F(ab')₂ fragment. In certain embodiments, the bivalent binding unit comprises a complete antibody, *e.g.*, a complete IgG heavy chain constant regions. In certain embodiments, the bivalent binding unit is a human IgG antibody, fragment, or derivative thereof.

[0113] In certain embodiments, the provided antigen-binding domain is included in a multimeric antibody or antibody-like molecule comprising two, five, or six bivalent binding units, where the antibody comprises four, eight, ten, or twelve antigen-binding domains. In certain embodiments at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the antigen-binding domains specifically binds to CD123. As provided herein, at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the antigen-binding domains comprise the VH and VL amino acid sequences as provided herein. According to these embodiments, each binding unit comprises two heavy chains each comprising an IgA or IgM constant region or a multimerizing fragment or variant thereof, and at least one of the heavy chain constant regions of the binding unit is associated with, *e.g.*, fused to a copy of the provided VH of the provided antigen-binding domain. In certain embodiments the multimeric antibody or antibody-like molecule is a human antibody.

[0114] In certain embodiments, the provided multimeric antibody or antibody-like molecule is dimeric or tetrameric and comprises two bivalent IgA binding units and a J

chain or functional fragment or variant thereof, where each binding unit comprises two IgA heavy chain constant regions, *e.g.*, IgA1 or IgA2 heavy chain constant regions, or multimerizing fragments or variants thereof. In certain embodiments the dimeric or tetrameric antibody or antibody-like molecule can further comprise a secretory component, or fragment or variant thereof. In certain embodiments, the IgA heavy chain constant regions or multimerizing fragments or variants thereof each comprise a C α 3 domain and an α -tailpiece (α tp) domain, and can further comprise a C α 1 domain, a C α 2 domain, an IgA hinge region, or any combination thereof.

[0115] In certain embodiments, the provided multimeric antibody or antibody-like molecule is hexameric or pentameric and comprises five or six bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof. In certain embodiments the IgM heavy chain constant regions or multimerizing fragments or variants thereof each comprise a C μ 4 domain and a μ -tailpiece (μ tp) domain or fragment or variant thereof, and can further comprise a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof. In certain embodiments the multimeric antibody or antibody-like molecule is pentameric, and further comprises a J chain, or functional fragment thereof, or functional variant thereof. In certain embodiments, each binding unit further comprises two light chains each comprising a light chain constant region or fragment or variant thereof, and wherein at least one, two, three, four, five, six, seven eight, nine, ten, eleven, or twelve light chain constant regions are fused to a copy of the provided VL of the antigen-binding domain. In certain embodiments the multimeric antibody or antibody-like molecule is a human antibody.

[0116] The antibody or antibody-like molecule as provided herein can, in certain embodiments, be multispecific.

[0117] In certain embodiments the provided antigen-binding domain, or an antibody or fragment or derivative thereof, antibody-like molecule comprising the antigen binding domain can specifically bind to human CD123. In certain embodiments the provided antigen-binding domain, or an antibody or fragment or derivative comprising the antigen binding domain binds to CD123 with an affinity characterized by a dissociation constant KD no greater than 500 nM, 100 nM, 50.0 nM, 40.0 nM, 30.0 nM, 20.0 nM, 10.0 nM, 9.0 nM, 8.0 nM, 7.0 nM, 6.0 nM, 5.0 nM, 4.0 nM, 3.0 nM, 2.0 nM, 1.0 nM, 0.50 nM,

0.10 nM, 0.050 nM, 0.01 nM, 0.005 nM, or 0.001 nM; and wherein the CD123 is human CD123.

IgM antibodies, IgM-like antibodies, and IgM-derived binding molecules

[0118] IgM is the first immunoglobulin produced by B cells in response to stimulation
5 by antigen and is naturally present at around 1.5 mg/ml in serum with a half-life of about 5 days. IgM is a pentameric or hexameric molecule and thus includes five or six binding units. An IgM binding unit typically includes two light and two heavy chains. While an IgG heavy chain constant region contains three heavy chain constant domains (CH1, CH2 and CH3), the heavy (μ) constant region of IgM additionally contains a fourth
10 constant domain (CH4) and includes a C-terminal μ "tailpiece" (μ tp). While several human alleles exist, the human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 1 (IMGT alleleIGHM*03, identical to, *e.g.*, GenBank Accession No. pir||S37768) or SEQ ID NO: 2 (IMGT alleleIGHM*04, identical to, *e.g.*, GenBank Accession No. sp|P01871.4). The human C μ 1 region ranges from about amino acid 5 to
15 about amino acid 102 of SEQ ID NO: 1 or SEQ ID NO: 2; the human C μ 2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 1 or SEQ ID NO: 2, the human C μ 3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 1 or SEQ ID NO: 2, the C μ 4 region ranges from about amino acid 329 to about amino acid 430 of SEQ ID NO: 1 or SEQ ID NO: 2, and the tailpiece ranges from
20 about amino acid 431 to about amino acid 453 of SEQ ID NO: 1 or SEQ ID NO: 2.

[0119] Other forms of the human IgM constant region with minor sequence variations exist, including, without limitation, GenBank Accession Nos. CAB37838.1 and pir||MHHU. The amino acid substitutions, insertions, and/or deletions at positions
25 corresponding to SEQ ID NO: 1 or SEQ ID NO: 2 described and claimed elsewhere in this disclosure can likewise be incorporated into alternate human IgM sequences, as well as into IgM constant region amino acid sequences of other species.

[0120] Each IgM heavy chain constant region is associated with an antigen-binding domain, *e.g.*, a scFv, or a subunit of an antigen-binding domain, *e.g.*, a VH region.

[0121] Five IgM binding units can form a complex with an additional small polypeptide
30 chain (the J-chain), or a functional fragment, variant, or derivative thereof, to form a pentameric IgM antibody or IgM-like antibody. The precursor form of the human J-chain

is presented as SEQ ID NO:1. The signal peptide (underlined) extends from amino acid 1 to about amino acid 22 of SEQ ID NO: 1, and the mature human J-chain extends from about amino acid 23 to amino acid 159 of SEQ ID NO: 1. The mature human J-chain has the amino acid sequence SEQ ID NO: 2

5 **[0122]** Exemplary variant and modified J-chains are provided elsewhere herein. Without the J-chain, an IgM antibody or IgM-like antibody typically assembles into a hexamer, comprising six binding units and up to twelve binding unit-associated antigen-binding domains. With a J-chain, an IgM antibody or IgM-like antibody typically assembles into a pentamer, comprising five binding units and up to ten binding unit-associated antigen-binding domains, or more, if the J-chain is a modified J-chain comprising one or more heterologous polypeptides that can be, *e.g.*, additional J-chain-associated antigen-binding domain(s). The assembly of five or six IgM binding units into a pentameric or hexameric IgM antibody or IgM-like antibody is thought to involve interactions between the C μ 4 and μ tailpiece domains. *See, e.g., Braathen, R., et al., J. Biol. Chem. 277:42755-42762*
10 (2002). Accordingly, the constant regions of a pentameric or hexameric IgM antibody or antibody-like molecule provided in this disclosure typically includes at least the C μ 4 and/or μ tailpiece domains. A “multimerizing fragment” of an IgM heavy chain constant region thus includes at least the C μ 4 domain and a μ tp domain. An IgM heavy chain constant region can additionally include a C μ 3 domain or a fragment thereof, a C μ 2
15 domain or a fragment thereof, and/or a C μ 1 domain or a fragment thereof. In certain embodiments, a binding molecule, *e.g.*, an IgM antibody or IgM-like antibody as provided herein can include a complete IgM heavy (μ) chain constant domain, *e.g.*, SEQ ID NO: 1 or SEQ ID NO: 2, or a multimerizing variant, derivative, or analog thereof, *e.g.*, as provided herein.

20 **[0123]** In certain embodiments, the disclosure provides a pentameric IgM or IgM-like antibody comprising five bivalent binding units, where each binding unit includes two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each associated with an antigen-binding domain or a subunit of an antigen-binding domain. In certain embodiments, the two IgM heavy chain constant regions are human heavy chain
25 constant regions.

30 **[0124]** Where the IgM or IgM-like antibody provided herein is pentameric, the IgM or IgM-like antibody typically further includes a J-chain, or functional fragment or variant

thereof. In some embodiments, the J-chain is a modified J-chain comprising a heterologous moiety, *e.g.*, a J-chain-associated antigen binding domain. In certain embodiments the J-chain-associated antigen binding domain specifically binds to an immune effector cell, *e.g.*, a CD8⁺ cytotoxic T cell or an NK cell. In certain
5 embodiments the modified J-chain includes one or more heterologous moieties attached thereto, *e.g.*, an immune stimulatory agent. In certain embodiments the J-chain can be mutated to affect, *e.g.*, enhance, the serum half-life of the IgM or IgM-like antibody provided herein, as discussed elsewhere in this disclosure. In certain embodiments the J-chain can be mutated to affect glycosylation, as discussed elsewhere in this disclosure.

10 **[0125]** In some embodiments, the IgM or IgM-like antibody provided herein is hexameric and comprises six bivalent binding units. In some embodiments, each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof.

15 **[0126]** An IgM heavy chain constant region can include one or more of a C μ 1 domain or fragment or variant thereof, a C μ 2 domain or fragment or variant thereof, a C μ 3 domain or fragment or variant thereof, a C μ 4 domain or fragment or variant thereof, and/or a μ tail piece (μ tp) or fragment or variant thereof, provided that the constant region can serve a desired function in the IgM or IgM-like antibody, *e.g.*, associate with second IgM
20 constant region to form a binding unit with one, two, or more antigen-binding domain(s), and/or associate with other binding units (and in the case of a pentamer, a J-chain) to form a hexamer or a pentamer. In certain embodiments the two IgM heavy chain constant regions or fragments or variants thereof within an individual binding unit each comprise a C μ 4 domain or fragment or variant thereof, a μ tailpiece (μ tp) or fragment or
25 variant thereof, or a combination of a C μ 4 domain and a μ tp or fragment or variant thereof. In certain embodiments the two IgM heavy chain constant regions or fragments or variants thereof within an individual binding unit each further comprise a C μ 3 domain or fragment or variant thereof, a C μ 2 domain or fragment or variant thereof, a C μ 1 domain or fragment or variant thereof, or any combination thereof.

30 **[0127]** In some embodiments, the binding units of the IgM or IgM-like antibody comprise two light chains. In some embodiments, the binding units of the IgM or IgM-like antibody comprise two fragments of light chains. In some embodiments, the light chains are kappa light chains. In some embodiments, the light chains are lambda light

chains. In some embodiments, the light chains are hybrid kappa and lambda light chains. In some embodiments, each binding unit comprises two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

- 5 IgM antibodies, IgM-like antibodies, and IgM-derived binding molecules with enhanced serum half-life

[0128] Certain IgM-derived multimeric binding molecules, *e.g.*, antibodies or antibody-like molecules provided herein can be modified to have enhanced serum half-life. Exemplary IgM heavy chain constant region mutations that can enhance serum half-life of an IgM-derived binding molecule are disclosed in U.S. Patent No. 10,899,835, which is incorporated by reference herein in its entirety. For example, a variant IgM heavy chain constant region of an IgM-derived binding molecule as provided herein can include an amino acid substitution at an amino acid position corresponding to amino acid S401, E402, E403, R344, and/or E345 of a wild-type human IgM constant region (*e.g.*,
 10 SEQ ID NO: 1 or SEQ ID NO: 2). By “an amino acid corresponding to amino acid S401, E402, E403, R344, and/or E345 of a wild-type human IgM constant region” is meant the amino acid in the sequence of the IgM constant region of any species which is homologous to S401, E402, E403, R344, and/or E345 in the human IgM constant region. In certain embodiments, the amino acid corresponding to S401, E402, E403, R344,
 15 and/or E345 of SEQ ID NO: 1 or SEQ ID NO: 2 can be substituted with any amino acid, *e.g.*, alanine.
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IgM antibodies, IgM-like antibodies, and IgM-derived binding molecules with reduced CDC activity

[0129] Certain IgM-derived multimeric binding molecules, *e.g.*, antibodies or antibody-like molecules as provided herein can be engineered to exhibit reduced complement-dependent cytotoxicity (CDC) activity to cells in the presence of complement, relative to a reference IgM antibody or IgM-like antibody with a corresponding reference human IgM constant region identical, except for the mutations conferring reduced CDC activity. These CDC mutations can be combined with any of the mutations to block N-linked
 25 glycosylation and/or to confer increased serum half-life as provided herein. By
 30 “corresponding reference human IgM constant region” is meant a human IgM constant

region or portion thereof, *e.g.*, a C μ 3 domain, that is identical to the variant IgM constant region except for the modification or modifications in the constant region affecting CDC activity. In certain embodiments, the variant human IgM constant region includes one or more amino acid substitutions, *e.g.*, in the C μ 3 domain, relative to a wild-type human IgM constant region as described, *e.g.*, in U.S. Patent Application Publication No. US 2021-0147567, which is incorporated herein by reference in its entirety. Assays for measuring CDC are well known to those of ordinary skill in the art, and exemplary assays are described *e.g.*, in US Patent Application Publication No. 2021-0147567, which is incorporated by reference herein in its entirety.

10 **[0130]** In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position L310, P311, P313, and/or K315 of SEQ ID NO: 1 (human IgM constant region allele IGHM*03) or SEQ ID NO: 2 (human IgM constant region allele IGHM*04). In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position P311 of SEQ ID NO: 1 or SEQ ID NO: 2. In other embodiments the variant IgM constant region as provided herein contains an amino acid substitution corresponding to the wild-type human IgM constant region at position P313 of SEQ ID NO: 1 or SEQ ID NO: 2. In other embodiments the variant IgM constant region as provided herein contains a combination of substitutions corresponding to the wild-type human IgM constant region at positions P311 of SEQ ID NO: 1 or SEQ ID NO: 2 and/or P313 of SEQ ID NO: 1 or SEQ ID NO: 2. These proline residues can be independently substituted with any amino acid, *e.g.*, with alanine, serine, or glycine. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position K315 of SEQ ID NO: 1 or SEQ ID NO: 2. The lysine residue can be independently substituted with any amino acid, *e.g.*, with alanine, serine, glycine, or aspartic acid. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position K315 of SEQ ID NO: 1 or SEQ ID NO: 2 with aspartic acid. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid

substitution corresponding to the wild-type human IgM constant region at position L310 of SEQ ID NO: 1 or SEQ ID NO: 2. The lysine residue can be independently substituted with any amino acid, *e.g.*, with alanine, serine, glycine, or aspartic acid. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position L310 of SEQ ID NO: 1 or SEQ ID NO: 2 with aspartic acid.

Glyco-modified IgM antibodies, IgM-like antibodies, and IgM-derived binding molecules

- [0131] Human and certain non-human primate IgM constant regions typically include five (5) naturally-occurring asparagine (N)-linked glycosylation motifs or sites. As used herein “an N-linked glycosylation motif” comprises or consists of the amino acid sequence N-X₁-S/T, where N is asparagine, X₁ is any amino acid except proline (P), and S/T is serine (S) or threonine (T). The glycan is attached to the nitrogen atom of the asparagine residue. *See, e.g.*, Drickamer K, Taylor ME (2006), *Introduction to Glycobiology* (2nd ed.). Oxford University Press, USA. N-linked glycosylation motifs occur in the human IgM heavy chain constant regions of SEQ ID NO: 1 or SEQ ID NO: 2 starting at positions 46 (“N1”), 209 (“N2”), 272 (“N3”), 279 (“N4”), and 440 (“N5”). These five motifs are conserved in non-human primate IgM heavy chain constant regions, and four of the five are conserved in the mouse IgM heavy chain constant region. Accordingly, in some embodiments, IgM heavy chain constant regions of a multimeric binding molecule as provided herein comprise 5 N-linked glycosylation motifs: N1, N2, N3, N4, and N5. In some embodiments, at least three of the N-linked glycosylation motifs (*e.g.*, N1, N2, and N3) on each IgM heavy chain constant region are occupied by a complex glycan.
- [0132] In certain embodiments, at least one, at least two, at least three, or at least four of the N- X₁-S/T motifs can include an amino acid insertion, deletion, or substitution that prevents glycosylation at that motif. In certain embodiments, the IgM-derived multimeric binding molecule can include an amino acid insertion, deletion, or substitution at motif N1, motif N2, motif N3, motif N5, or any combination of two or more, three or more, or all four of motifs N1, N2, N3, or N5, where the amino acid insertion, deletion, or substitution prevents glycosylation at that motif. In some embodiment, the IgM constant

region comprises one or more substitutions relative to a wild-type human IgM constant region at positions 46, 209, 272, or 440 of SEQ ID NO: 1 (human IgM constant region allele IGHM*03) or SEQ ID NO: 2 (human IgM constant region allele IGHM*04). *See, e.g.*, PCT Application Publication No. WO 2021/041250, which is incorporated herein
5 by reference in its entirety.

IgA antibodies, IgA-like antibodies, and IgA-derived binding molecules

[0133] IgA plays a critical role in mucosal immunity and comprises about 15% of total immunoglobulin produced. IgA can be monomeric or multimeric, forming primarily
10 dimeric molecules, but can also assemble as trimers, tetramers, and/or pentamers. *See, e.g.*, de Sousa-Pereira, P., and J.M. Woof, *Antibodies* 8:57 (2019).

[0134] In some embodiments, the multimeric binding molecules are dimeric and comprise two bivalent binding units or variants or fragments thereof. In some
15 embodiments, the multimeric binding molecules are dimeric or tetrameric, comprising two or four bivalent binding units or variants or fragments thereof, respectively, and further comprise a J-chain or functional fragment or variant thereof as described herein. In some embodiments, the multimeric binding molecules are dimeric, comprise two bivalent binding units or variants or fragments thereof, and further comprise a J-chain or functional fragment or variant thereof as described herein, where each binding unit comprises two IgA heavy chain constant regions or multimerizing fragments or variants
20 thereof.

[0135] In some embodiments, the multimeric binding molecules are tetrameric and comprise four bivalent binding units or variants or fragments thereof. In some
25 embodiments, the multimeric binding molecules are tetrameric, comprise four bivalent binding units or variants or fragments thereof, and further comprise a J-chain or functional fragment or variant thereof as described herein. In some embodiments, the multimeric binding molecules are tetrameric, comprise four bivalent binding units or variants or fragments thereof, and further comprise a J-chain or functional fragment or variant thereof as described herein, where each binding unit comprises two IgA heavy chain constant regions or multimerizing fragments or variants thereof.

30 [0136] In certain embodiments, the multimeric binding molecule provided by this disclosure is a dimeric binding molecule that includes four IgA heavy chain constant

regions, or multimerizing fragments thereof, each associated with an antigen-binding domain for a total of four antigen-binding domains. As provided herein, a dimeric IgA antibody, IgA-derived binding molecule, or IgA-like antibody includes two binding units and a J-chain, *e.g.*, a modified J-chain comprising a scFv antibody fragment that binds to CD3, or IL-15 and/or the IL-15 receptor- α sushi domain fused thereto as described elsewhere herein. Each binding unit as provided comprises two IgA heavy chain constant regions or multimerizing fragments or variants thereof. In certain embodiments, at least three or all four antigen-binding domains of the multimeric binding molecule bind to the same target antigen. In certain embodiments, at least three or all four binding polypeptides of the multimeric binding molecule are identical.

[0137] A bivalent IgA-derived binding unit includes two IgA heavy chain constant regions, and a dimeric IgA-derived binding molecule includes two binding units. IgA contains the following heavy chain constant domains, C α 1 (or alternatively CA1 or CH1), a hinge region, C α 2 (or alternatively CA2 or CH2), and C α 3 (or alternatively CA3 or CH3), and a C-terminal "tailpiece." Human IgA has two subtypes, IgA1 and IgA2. The human IgA1 constant region typically includes the amino acid sequence SEQ ID NO: 3. The human C α 1 domain extends from about amino acid 6 to about amino acid 98 of SEQ ID NO: 3; the human IgA1 hinge region extends from about amino acid 102 to about amino acid 124 of SEQ ID NO: 3, the human C α 2 domain extends from about amino acid 125 to about amino acid 219 of SEQ ID NO: 3, the human C α 3 domain extends from about amino acid 228 to about amino acid 330 of SEQ ID NO: 3, and the tailpiece extends from about amino acid 331 to about amino acid 352 of SEQ ID NO: 3. The human IgA2 constant region typically includes the amino acid sequence SEQ ID NO: 4. The human C α 1 domain extends from about amino acid 6 to about amino acid 98 of SEQ ID NO: 4; the human IgA2 hinge region extends from about amino acid 102 to about amino acid 111 of SEQ ID NO: 4, the human C α 2 domain extends from about amino acid 113 to about amino acid 206 of SEQ ID NO: 4, the human C α 3 domain extends from about amino acid 215 to about amino acid 317 of SEQ ID NO: 4, and the tailpiece extends from about amino acid 318 to about amino acid 340 of SEQ ID NO: 4.

[0138] Two IgA binding units can form a complex with two additional polypeptide chains, the J-chain (*e.g.*, SEQ ID NO: 7) and the secretory component (precursor, SEQ ID NO: 5, mature, from about amino acid 19 to about amino acid 764 of SEQ ID NO: 5)

to form a bivalent secretory IgA (sIgA)-derived binding molecule as provided herein. The assembly of two IgA binding units into a dimeric IgA-derived binding molecule is thought to involve the C α 3 and tailpiece domains. *See, e.g., Braathen, R., et al., J. Biol. Chem.* 277:42755-42762 (2002). Accordingly, a multimerizing dimeric IgA-derived binding molecule provided in this disclosure typically includes IgA constant regions that include at least the C α 3 and α tailpiece domains. Four IgA binding units can likewise form a tetramer complex with a J-chain. A sIgA antibody can also form as a higher order multimer, *e.g.*, a tetramer.

5 [0139] An IgA heavy chain constant region can additionally include a C α 2 domain or a fragment thereof, an IgA hinge region or fragment thereof, a C α 1 domain or a fragment thereof, and/or other IgA (or other immunoglobulin, *e.g.*, IgG) heavy chain domains, including, *e.g.*, an IgG hinge region. In certain embodiments, a binding molecule as provided herein can include a complete IgA heavy (α) chain constant domain (*e.g.*, SEQ ID NO: 3 or SEQ ID NO: 4), or a variant, derivative, or analog thereof. In some 10 15 embodiments, the IgA heavy chain constant regions or multimerizing fragments thereof are human IgA constant regions.

[0140] In certain embodiments each binding unit of a multimeric binding molecule as provided herein includes two IgA heavy chain constant regions or multimerizing fragments or variants thereof, each including at least an IgA C α 3 domain and an IgA 20 tailpiece domain. In certain embodiments the IgA heavy chain constant regions can each further include an IgA C α 2 domain situated N-terminal to the IgA C α 3 and IgA tailpiece domains. For example, the IgA heavy chain constant regions can include amino acids 125 to 353 of SEQ ID NO: 3 or amino acids 113 to 340 of SEQ ID NO: 4. In certain embodiments the IgA heavy chain constant regions can each further include an IgA or 25 IgG hinge region situated N-terminal to the IgA C α 2 domains. For example, the IgA heavy chain constant regions can include amino acids 102 to 353 of SEQ ID NO: 3 or amino acids 102 to 340 of SEQ ID NO: 4. In certain embodiments the IgA heavy chain constant regions can each further include an IgA C α 1 domain situated N-terminal to the IgA hinge region.

30 [0141] In some embodiments, each binding unit of an IgA antibody, IgA-like antibody, or other IgA-derived binding molecule comprises two light chains. In some embodiments, each binding unit of an IgA antibody, IgA-like antibody, or other IgA-

derived binding molecule comprises two fragments light chains. In some embodiments, the light chains are kappa light chains. In some embodiments, the light chains are lambda light chains. In some embodiments the light chains are chimeric kappa-lambda light chains. In some embodiments, each binding unit comprises two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

Modified and/or Variant J-chains

[0142] In certain embodiments, the multimeric binding molecule, *e.g.*, antibody or antibody-like molecule provided herein comprises a J-chain or functional fragment or variant thereof. In certain embodiments, the multimeric binding molecule provided herein is a pentameric IgM antibody or IgM antibody-like molecule and comprises a J-chain or functional fragment or variant thereof. In certain embodiments, the multimeric binding molecule provided herein is a dimeric IgA antibody or IgA antibody-like molecule and comprises a J-chain or functional fragment or variant thereof. In some embodiments, the multimeric binding molecule can comprise a naturally occurring J-chain, such as a mature human J-chain sequence (*e.g.*, SEQ ID NO: 7). In some embodiments, the multimeric binding molecule can comprise a functional fragment or functional variant of a naturally occurring J-chain.

[0143] In certain embodiments, the J-chain of a pentameric an IgM or IgM-like antibody or a dimeric IgA or IgA-like antibody as provided herein can be modified, *e.g.*, by introduction of a heterologous moiety, or two or more heterologous moieties, *e.g.*, polypeptides, without interfering with the ability of the IgM or IgM-like antibody or IgA or IgA-like antibody to assemble and bind to its binding target(s). *See* U.S. Patent Nos. 9,951,134, 10,975,147, 10,400,038, and 10,618,978, and U.S. Patent Application Publication No. US-2019-0185570, each of which is incorporated herein by reference in its entirety. Accordingly, IgM or IgM-like antibodies or IgA or IgA-like antibodies as provided herein, including bispecific or multispecific IgM or IgM-like antibodies or IgA or IgA-like antibodies as described elsewhere herein, can include a modified J-chain or functional fragment or variant thereof that further includes a heterologous moiety, *e.g.*, a heterologous polypeptide, introduced into the J-chain or fragment or variant thereof. In certain embodiments heterologous moiety can be a peptide or polypeptide fused in frame

or chemically conjugated to the J-chain or fragment or variant thereof. For example, the heterologous polypeptide can be fused to the J-chain or functional fragment or variant thereof. In certain embodiments, the heterologous polypeptide is fused to the J-chain or functional fragment or variant thereof via a linker, *e.g.*, a peptide linker consisting of
5 least 5 amino acids, but typically no more than 25 amino acids. In certain embodiments, the peptide linker consists of GGGGS (SEQ ID NO: 9), GGGGSGGGGS (SEQ ID NO: 10), GGGGSGGGGSGGGGS (SEQ ID NO: 11), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 12), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 13). In certain embodiments the heterologous moiety can be a chemical moiety conjugated to
10 the J-chain. Heterologous moieties to be attached to a J-chain can include, without limitation, a binding moiety, *e.g.*, an antibody or antigen-binding fragment thereof, *e.g.*, a single chain Fv (scFv) molecule, a stabilizing peptide that can increase the half-life of the IgM or IgM-like antibody, or a chemical moiety such as a polymer or a cytotoxin. In some embodiments, the heterologous moiety comprises a stabilizing peptide that can
15 increase the half-life of the binding molecule, *e.g.*, human serum albumin (HSA) or an HSA binding molecule.

[0144] In some embodiments, a modified J-chain includes a J-chain-associated antigen-binding domain, *e.g.*, a polypeptide capable of specifically binding to a target antigen. In certain embodiments, a J-chain-associated antigen-binding domain can be an antibody or
20 an antigen-binding fragment thereof, as described elsewhere herein. In certain embodiments the J-chain-associated antigen-binding domain can be a single chain Fv (scFv) antigen-binding domain or a single-chain antigen-binding domain derived, *e.g.*, from a camelid or condricthoid antibody. The J-chain-associated antigen-binding domain can be introduced into the J-chain at any location that allows the binding of the J-chain-associated antigen-binding domain to its binding target without interfering with J-chain
25 function or the function of an associated IgM or IgA antibody. Insertion locations include but are not limited to at or near the C-terminus, at or near the N-terminus or at an internal location that, based on the three-dimensional structure of the J-chain, is accessible. In certain embodiments, the J-chain-associated antigen-binding domain can
30 be introduced into the mature human J-chain of SEQ ID NO: 7 between cysteine residues 92 and 101 of SEQ ID NO: 7. In a further embodiment, the J-chain-associated antigen-binding domain can be introduced into the human J-chain of SEQ ID NO: 7 at or near a

glycosylation site. In a further embodiment, the J-chain-associated antigen-binding domain can be introduced into the human J-chain of SEQ ID NO: 7 within about 10 amino acid residues from the C-terminus, or within about 10 amino acids from the N-terminus. As described elsewhere herein, this disclosure provides a multimeric, bispecific binding molecule comprising a modified J-chain, where the modified J-chain comprises a J-chain-associated antigen binding domain that specifically binds to an immune effector cell, *e.g.*, a T cell such as a CD4⁺ T cell or a CD8⁺ cytotoxic T cell or an NK cell.

5 [0145] In some embodiments, a modified J-chain can further include an immune stimulatory agent (ISA), *e.g.*, cytokine, *e.g.*, interleukin-2 (IL-2) or interleukin-15 (IL-15), or a receptor-binding fragment or variant thereof, which in certain embodiments can be associated, either via binding or covalent attachment, with part of its receptor, *e.g.*, the sushi domain of IL-15 receptor- α . Such ISAs are described in detail in PCT Publication No. WO 2021/030688, which is incorporated herein by reference in its entirety.

15 [0146] In certain embodiments, the J-chain of an IgM antibody, IgM-like antibody, IgA antibody, IgA-like antibody, or IgM-or IgA- derived binding molecule as provided herein is a variant J-chain that comprises one or more amino acid substitutions that can alter, *e.g.*, the serum half-life of an IgM antibody, IgM-like antibody, IgA antibody, IgA-like antibody, or IgM-or IgA- derived binding molecule provided herein. For example, 20 certain amino acid substitutions, deletions, or insertions can result in the IgM-derived binding molecule exhibiting an increased serum half-life upon administration to a subject animal relative to a reference IgM-derived binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the variant J-chain, and is administered using the same method to the same animal species. In certain 25 embodiments the variant J-chain can include one, two, three, or four single amino acid substitutions, deletions, or insertions relative to the reference J-chain.

[0147] In some embodiments, the multimeric binding molecule can comprise a variant J-chain sequence, such as a variant sequence described herein with reduced glycosylation or reduced binding to one or more polymeric Ig receptors (*e.g.*, pIgR, Fc alpha-mu receptor (Fc α μ R), or Fc mu receptor (Fc μ R)). *See, e.g.*, U.S. Patent No. 10,899,835, 30 which is incorporated herein by reference in its entirety. In certain embodiments, the variant J-chain can comprise an amino acid substitution at the amino acid position

corresponding to amino acid Y102 of the mature wild-type human J-chain (SEQ ID NO: 7). By “an amino acid corresponding to amino acid Y102 of the mature wild-type human J-chain” is meant the amino acid in the sequence of the J-chain of any species which is homologous to Y102 in the human J-chain. See U.S. Patent No. 10,899,835, which is incorporated herein by reference in its entirety. The position corresponding to Y102 in SEQ ID NO: 7 is conserved in the J-chain amino acid sequences of at least 43 other species. *See* FIG. 4 of U.S. Patent No. 9,951,134, which is incorporated by reference herein. Certain mutations at the position corresponding to Y102 of SEQ ID NO: 7 can inhibit the binding of certain immunoglobulin receptors, *e.g.*, the human or murine Fc $\alpha\mu$ receptor, the murine Fc μ receptor, and/or the human or murine polymeric Ig receptor (pIg receptor) to an IgM pentamer comprising the mutant J-chain. IgM antibodies, IgM-like antibodies, and IgM-derived binding molecules comprising a mutation at the amino acid corresponding to Y102 of SEQ ID NO: 7 have an improved serum half-life when administered to an animal than a corresponding antibody, antibody-like molecule or binding molecule that is identical except for the substitution, and which is administered to the same species in the same manner. In certain embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 7 can be substituted with any amino acid. In certain embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 7 can be substituted with alanine (A), serine (S) or arginine (R). In a particular embodiment, the amino acid corresponding to Y102 of SEQ ID NO: 7 can be substituted with alanine. In a particular embodiment the J-chain or functional fragment or variant thereof is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 8, a J chain referred to herein as “J*”.

[0148] Wild-type J-chains typically include one N-linked glycosylation site. In certain embodiments, a variant J-chain or functional fragment thereof of a multimeric binding molecule as provided herein includes a mutation within the asparagine(N)-linked glycosylation motif N-X₁-S/T, *e.g.*, starting at the amino acid position corresponding to amino acid 49 (motif N6) of the mature human J-chain (SEQ ID NO: 7) or J* (SEQ ID NO: 8), where N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, and where the mutation prevents glycosylation at that motif. As demonstrated in U.S. Patent No. 10,899,835, mutations preventing glycosylation at this site can result in the multimeric binding molecule as provided herein, exhibiting an increased serum

half-life upon administration to a subject animal relative to a reference multimeric binding molecule that is identical except for the mutation or mutations preventing glycosylation in the variant J-chain, and is administered in the same way to the same animal species.

5 **[0149]** For example, in certain embodiments the variant J-chain or functional fragment thereof of a binding molecule comprising a J-chain as provided herein can include an amino acid substitution at the amino acid position corresponding to amino acid N49 or amino acid S51 of SEQ ID NO: 7 or SEQ ID NO: 8, provided that the amino acid corresponding to S51 is not substituted with threonine (T), or where the variant J-chain
10 comprises amino acid substitutions at the amino acid positions corresponding to both amino acids N49 and S51 of SEQ ID NO: 7 or SEQ ID NO: 8. In certain embodiments, the position corresponding to N49 of SEQ ID NO: 7 or SEQ ID NO: 8 is substituted with any amino acid, *e.g.*, alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D). In a particular embodiment, the position corresponding to N49 of SEQ ID NO: 7 or
15 SEQ ID NO: 8 can be substituted with alanine (A). In another embodiment, the position corresponding to N49 of SEQ ID NO: 7 or SEQ ID NO: 8 can be substituted with aspartic acid (D). In some embodiments, the position corresponding to S51 of SEQ ID NO: 7 or SEQ ID NO: 8 is substituted with alanine (A) or glycine (G). In some
20 embodiments, the position corresponding to S51 of SEQ ID NO: 7 or SEQ ID NO: 8 is substituted with alanine (A).

Multimeric bispecific or multispecific anti-CD123 binding molecules with a modified J-chain that binds to an immune effector cell.

[0150] This disclosure provides a multimeric, bispecific or multispecific binding molecule for use in treating cancers, *e.g.*, hematologic cancers, *e.g.*, acute myeloid
25 Leukemia (AML), where the binding molecule is bispecific and targets CD123 (IL-3R α) on cancer cells with high avidity, while also targeting an immune effector cell, *e.g.*, a CD4⁺ or CD8⁺ T cell or an NK cell via a single antigen-binding domain, thereby facilitating effector cell-mediated killing of the cancer cells while at the same time minimizing excessive release of cytokines. In certain embodiments the multimeric,
30 bispecific, anti-CD123 binding molecule is an anti-CD123 x anti-CD3 binding molecule.

[0151] Accordingly, the disclosure provides a multimeric, bispecific or multispecific binding molecule comprising two IgA or IgA-like or five IgM or IgM-like bivalent binding units and a modified J-chain, where the modified J-chain includes at least a wild-type J-chain or a functional fragment or variant thereof and a J-chain-associated antigen-binding domain that specifically binds to an immune effector cell. Each binding unit comprises two antibody heavy chains, each comprising an IgA, IgA-like, IgM, or IgM-like heavy chain constant region or multimerizing fragment thereof (as described elsewhere herein) and at least a heavy chain variable region (VH) portion of a binding unit-associated antigen-binding domain. At least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or all ten of the binding unit-associated antigen-binding domains specifically bind to CD123. A binding molecule as provided herein can induce immune effector cell-dependent killing of cells, *e.g.*, cancer cells, expressing CD123.

[0152] In certain embodiments, the modified J-chain of the binding molecule provided herein includes a variant of a wild-type J-chain or fragment thereof, where the variant includes one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J-chain that can affect serum half-life of the binding molecule; and wherein the binding molecule exhibits an increased serum half-life upon administration to an animal relative to a reference binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the J-chain, and is administered in the same way to the same animal species. For example, in certain embodiments the J-chain is a variant human J-chain that comprises the amino acid sequence SEQ ID NO: 8 (“J*”).

[0153] In certain embodiments, the J-chain-associated antigen-binding domain of the provided binding molecule comprises an antibody or fragment thereof. In certain embodiments the antibody fragment is a single chain Fv (scFv). The scFv can be fused or chemically conjugated to the J-chain or fragment or variant, *e.g.*, J*. In certain embodiments, the scFv is fused to the J-chain via a peptide linker *e.g.*, SEQ ID NOs: 9-13. As noted elsewhere in the disclosure, the scFv can be fused to J-chain or fragment or variant thereof in any way so long as the function of the J-chain, *i.e.*, to assemble with IgM, IgM-like, IgA, or IgA-like binding units to form a dimer or a pentamer, is not affected. For example, the scFv can be fused to the N-terminus of the J-chain or fragment

or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

[0154] The immune effector cell bound by the antigen binding domain of the modified J-chain can be any immune effector cell confers a beneficial effect when associated with a cancer cell targeted by CD123, for example mediating cell-based killing of the CD123+ cancer cell. In certain embodiments the immune effector cell can be, without limitation, a T cell, *e.g.*, a CD4+ T cell, a CD8+ T cell, an NKT cell, or a $\gamma\delta$ T cell, a B cell, a plasma cell, a macrophage, a dendritic cell, or a natural killer (NK) cell. In certain embodiments the immune effector cell is a T cell, *e.g.*, a CD4+ or CD8+ T cell. In certain embodiments the immune effector cell is a CD8+ cytotoxic T cell. In certain embodiments the immune effector cell is an NK cell.

[0155] Where the immune effector cell is a T cell, for example a CD8+ T cell, the J-chain-associated antibody or fragment thereof, *e.g.*, scFv, can specifically bind to the T cell surface antigen CD3, *e.g.*, CD3 ϵ . In certain embodiments the anti-CD3 scFv comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises the VH complementarity-determining regions VHCDR1, VHCDR2, and VHCDR3 and the VL comprises the VL complementarity-determining regions VLCDR1, VLCDR2, and VLCDR3, wherein the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise, respectively, the amino acid sequences SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25; SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41; SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49; SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57; SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 64, and SEQ ID NO: 65; or SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73; with zero, one, or two amino acid substitutions. In some embodiments, the scFv comprises the VH and VL amino acid sequences SEQ ID NO: 18 and SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 38, SEQ ID NO: 42 and SEQ ID NO:

46, SEQ ID NO: 50 and SEQ ID NO: 54, SEQ ID NO: 58 and SEQ ID NO: 62, or SEQ ID NO: 66 and SEQ ID NO: 70, respectively.

[0156] In certain other embodiments, the immune effector cell is an NK cell, and the J-chain-associated antibody or fragment thereof, *e.g.*, scFv can specifically bind to CD16 or CD56. Many CD16 and CD56 scFv are known, such as those disclosed in U.S. Patent Nos. 9,035,026, 9,701,750, 10,730,941, 11,001,633, McCall et al., 1999. Mol Immunol. 7:433-445.

[0157] A modified J-chain of a multimeric, bispecific, anti-CD123 binding molecule, *e.g.*, an anti-CD123 x anti-CD3 binding molecule as provided herein can be further modified to include additional heterologous moieties attached to the J-chain. Exemplary moieties are described, *e.g.*, in U.S. Patent No. 9,951,134, and in U.S. Patent Application Publication Nos. US 2019-0185570 and US Patent No. 10,618,978, and in PCT Publication No. WO2021/030688, all of which are incorporated herein by reference in their entireties. In certain embodiments, the modified J-chain of a multimeric, bispecific anti-CD123 binding molecule, *e.g.*, an anti-CD123 x anti-CD3 binding molecule as provided herein can further include an immune stimulatory agent ("ISA") fused or chemically conjugated to the J-chain or fragment or variant thereof. For example, the ISA can include a cytokine or receptor-binding fragment or variant thereof. In a particular embodiment, a J-chain-associated ISA can include (a) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), and (b) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I ("R"), wherein the J-chain or fragment or variant thereof and at least one of I and R, or both I and R, are associated as a fusion protein, and wherein I and R can associate to function as the ISA. In certain embodiments, the ISA can be fused to the J-chain via a peptide linker.

Polynucleotides, Vectors, and Host Cells

[0158] The disclosure further provides a polynucleotide, *e.g.*, an isolated, recombinant, and/or non-naturally occurring polynucleotide, that includes a nucleic acid sequence that encodes an antigen-binding domain as provided herein or a polypeptide subunit of an antibody or antibody-like molecule, *e.g.*, a dimeric, hexameric, or pentameric antibody or antibody-like molecule as provided herein. By "polypeptide subunit" is meant a portion

of an antibody or antibody-like molecule, binding unit, or antigen-binding domain that can be independently translated. Examples include, without limitation, an antibody variable domain, *e.g.*, a VH or a VL, a J chain, including modified J-chains as provided herein, a secretory component, a single chain Fv, an antibody heavy chain, an antibody
5 light chain, an antibody heavy chain constant region, an antibody light chain constant region, and/or any fragment, variant, or derivative thereof.

[0159] In certain embodiments, the polynucleotide comprising a nucleic acid sequence that encodes a polypeptide subunit of a binding molecule described herein. In some
10 embodiments, the polynucleotide encodes a polypeptide subunit comprising a heavy chain constant region and at least an antibody VH portion of the binding domain of the binding molecule. In some embodiments, the polynucleotide encodes a polypeptide subunit comprising the heavy chain of the binding molecule. In some embodiments, the polynucleotide encodes a polypeptide subunit comprises a VH comprising HCDR1, HCDR2, and HCDR3 regions, wherein the HCDR1, HCDR2, and HCDR3 comprise,
15 respectively, the amino acid sequences of SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 81, or SEQ ID NO: 82.

[0160] In some embodiments, the polynucleotide encodes a polypeptide subunit comprising a light constant region and at least an antibody VL portion of the binding domain of the binding molecule. In some embodiments, the polynucleotide encodes a
20 polypeptide subunit comprising the light chain of the binding molecule. In some embodiments, the polynucleotide encodes a polypeptide subunit comprises a VL comprising LCDR1, LCDR2, and LCDR3 regions, wherein the LCDR1, LCDR2, and LCDR3 comprise, respectively, the amino acid sequences of SEQ ID NO: 79 or SEQ ID NO: 83.

25 **[0161]** In certain embodiments, the polypeptide subunit can include an IgM heavy chain constant region or IgM-like heavy chain constant region or multimerizing fragment thereof, or an IgA heavy chain constant region or IgA-like heavy chain constant region or multimerizing fragment thereof, which is fused to an antigen-binding domain or a subunit thereof, *e.g.*, to the VH portion of an antigen-binding domain or the VL portion
30 of an antigen binding domain, all as provided herein. In certain embodiments the polynucleotide can encode a polypeptide subunit that includes a human IgM heavy chain constant region, a human IgM-like heavy chain constant region, a human IgA heavy

chain constant region, a human IgA-like heavy chain constant region, or multimerizing fragment thereof, *e.g.*, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, any of which is associated with, *e.g.*, fused to an antigen-binding domain or subunit thereof, *e.g.*, the C-terminal end of a VH.

5 **[0162]** To form the antigen-binding domains or the variable regions of antibodies that specifically bind to CD123, the provided polynucleotides can be inserted into expression vector templates, *e.g.*, for a monomeric antibody, *e.g.*, an IgG antibody, or for IgM and/or IgA structures, thereby creating monomeric antibodies comprising a single binding unit, or multimeric antibodies or multimerizing fragments or derivatives thereof
10 having at least two bivalent binding units. In brief, nucleic acid sequences encoding the heavy and light chain variable domain sequences can be synthesized or amplified from existing molecules and inserted into vectors in the proper orientation and in frame such that upon expression, the vector will yield a full length heavy or light chain. Vectors useful for these purposes are known in the art. Such vectors can also comprise enhancer
15 and other sequences needed to achieve expression of the desired chains. Multiple vectors or single vectors can be used. These vectors are transfected into host cells and then the chains are expressed and purified. Upon expression the chains form fully functional multimeric binding molecules, as has been reported in the literature. The fully assembled multimeric binding molecules can then be purified by standard methods. The expression
20 and purification processes can be performed at commercial scale, if needed.

[0163] The disclosure further provides a composition comprising two or more polynucleotides, where the two or more polynucleotides collectively can encode an antigen-binding domain or an antibody or antibody-like molecule, *e.g.*, a monomeric, dimeric, hexameric, or pentameric antibody as described herein. In certain embodiments
25 the composition can include a polynucleotide encoding an IgG, IgM, and/or IgA heavy chain or fragment thereof, *e.g.*, a human IgG, IgM, or IgA heavy chain as described above, where the IgG, IgM, and/or IgA heavy chain comprises at least the provided VH of a CD123 antigen-binding domain as provided herein, and a polynucleotide encoding a light chain or fragment thereof, *e.g.*, a human kappa or lambda light chain that comprises
30 at least the provided VL of a CD123 antigen-binding domain as provided herein. A polynucleotide composition as provided can further include a polynucleotide encoding a J chain, *e.g.*, a human J chain, or a fragment, variant, or derivative thereof. In certain

embodiments the polynucleotides making up a composition as provided herein can be situated on two, three, or more separate vectors, *e.g.*, expression vectors. Such vectors are provided by the disclosure. In certain embodiments, two or more of the polynucleotides making up a composition as provided herein can be situated on a single vector, *e.g.*, an expression vector. Such a vector is provided by the disclosure.

[0164] In certain embodiments, this disclosure provides a composition comprising two, three, or more polynucleotides as provided herein, where the polynucleotides together can encode an anti-CD123 binding molecule, *e.g.*, a multimeric, bispecific anti-CD123 binding molecule, *e.g.*, an anti-CD123 x anti-CD3 binding molecule as provided herein.

10 In certain embodiments the polynucleotides can be situated on separate vectors. In certain embodiments two or more of the polynucleotides can be situated on the same vector. Such vectors are likewise provided by the disclosure.

[0165] The disclosure further provides a host cell, *e.g.*, a prokaryotic or eukaryotic host cell, that includes a polynucleotide or two or more polynucleotides encoding an anti-CD123 binding molecule, *e.g.*, a multimeric, bispecific, anti-CD123 binding molecule, *e.g.*, an anti-CD123 x anti-CD3 binding molecule as provided herein, or any subunit thereof, a polynucleotide composition as provided herein, or a vector or two, three, or more vectors that collectively encode the binding molecule as provided herein, or any subunit thereof.

20 [0166] In a related embodiment, the disclosure provides a method of producing a multimeric binding molecule as provided by this disclosure, where the method comprises culturing a host cell as provided herein and recovering the multimeric binding molecule.

Methods of Use

[0167] The disclosure further provides a method of treating a disease or disorder, *e.g.*, cancer or other malignancy, *e.g.*, a hematologic cancer or malignancy, in a subject in need of treatment, comprising administering to the subject a therapeutically effective amount of an anti-CD123 antibody or antigen-binding fragment or derivative thereof, *e.g.*, an anti-CD123 x anti-CD3 antibody as provided herein. By "therapeutically effective dose or amount" or "effective amount" is intended an amount of the binding molecule that when administered brings about a positive response, *e.g.*, killing of tumor cells, in the subject.

[0168] In certain embodiments the cancer to be treated can be any cancer in which the malignant cells express or over-express CD123. For example, the cancer can be acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), B-cell acute lymphoblastic leukemia (B-cell ALL), classical Hodgkin's lymphoma, hairy cell leukemia, chronic lymphocytic leukemia (CLL), systemic mastocytosis, or plasmacytoid dendritic cell leukemia.

[0169] Effective doses of compositions for treatment of cancer vary depending upon many different factors, including means of administration, target site, physiological state of the subject, whether the subject is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the subject is a human, but non-human mammals including transgenic mammals can also be treated.

[0170] The subject to be treated can be any mammal in need of treatment, in certain embodiments, the subject is a human subject.

[0171] In its simplest form, a preparation to be administered to a subject is an anti-CD123 antibody or antigen-binding fragment of derivative thereof, *e.g.*, an anti-CD123 x anti-CD3 antibody as provided herein, administered in conventional dosage form, which can be combined with a pharmaceutical excipient, carrier or diluent as described elsewhere herein.

[0172] The compositions of the disclosure can be administered by any suitable method, *e.g.*, parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

25 Pharmaceutical Compositions and Administration Methods

[0173] Methods of preparing and administering an anti-CD123 antibody or antigen-binding fragment or derivative thereof, *e.g.*, an anti-CD123 x anti-CD3 antibody as provided herein to a subject in need thereof are well known to or are readily determined by those skilled in the art in view of this disclosure. The route of administration of can be, for example, intratumoral, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal,

intramuscular, subcutaneous, rectal, or vaginal administration. While these forms of administration are contemplated as suitable forms, another example of a form for administration would be a solution for injection, for intratumoral, intravenous, or intraarterial injection or drip. A suitable pharmaceutical composition can comprise a
5 buffer (*e.g.e.g.*, acetate, phosphate, or citrate buffer), a surfactant (*e.g.e.g.*, polysorbate), optionally a stabilizer agent (*e.g.e.g.*, human albumin), etc.

[0174] As discussed herein, an anti-CD123 antibody or antigen-binding fragment or derivative thereof, *e.g.*, an anti-CD123 x anti-CD3 antibody as provided herein can be administered in a pharmaceutically effective amount for the treatment of a subject in
10 need thereof. In this regard, it will be appreciated that the disclosed antibodies or antigen-binding fragments or derivatives thereof can be formulated to facilitate administration and promote stability of the active agent. Pharmaceutical compositions accordingly can comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives, and the like. A pharmaceutically
15 effective amount of a antibody or antigen-binding fragment or derivative thereof as provided herein means an amount sufficient to achieve effective binding to a target and to achieve a therapeutic benefit. Suitable formulations are described in Remington's Pharmaceutical Sciences, *e.g.*, 21st Edition (Lippincott Williams & Wilkins) (2005).

[0175] Certain pharmaceutical compositions provided herein can be orally administered
20 in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions, or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

25 [0176] The amount of an anti-CD123 antibody or antibody-like molecule, *e.g.*, an anti-CD123 x anti-CD3 antibody, disclosed herein that can be combined with carrier materials to produce a single dosage form will vary depending, *e.g.*, upon the subject treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion.
30 Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0177] In keeping with the scope of the present disclosure, an anti-CD123 antibody or antibody-like molecule, *e.g.*, an anti-CD123 x anti-CD3 antibody, as provided herein can be administered to a subject in need of therapy in an amount sufficient to produce a therapeutic effect. An anti-CD123 antibody or antibody-like molecule, *e.g.*, an anti-CD123 x anti-CD3 antibody, as provided herein can be administered to the subject in a conventional dosage form prepared by combining the antibody or antibody-like molecule of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. The form and character of the pharmaceutically acceptable carrier or diluent can be dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

[0178] This disclosure also provides for the use of an anti-CD123 antibody or antibody-like molecule, *e.g.*, an anti-CD123 x anti-CD3 antibody, as provided herein in the manufacture of a medicament for treating, preventing, or managing cancer or other malignancy. The disclosure also provides for an anti-CD123 antibody or antibody-like molecule, *e.g.*, an anti-CD123 x anti-CD3 binding molecule as provided herein for use in treating, preventing, or managing cancer.

[0179] This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Green and Sambrook, ed. (2012) *Molecular Cloning A Laboratory Manual* (4th ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) *Molecular Cloning: A Laboratory Manual*, (Cold Springs Harbor Laboratory, NY); D. N. Glover and B.D. Hames, eds., (1995) *DNA Cloning 2d Edition* (IRL Press), Volumes 1-4; Gait, ed. (1990) *Oligonucleotide Synthesis* (IRL Press); Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1985) *Nucleic Acid Hybridization* (IRL Press); Hames and Higgins, eds. (1984) *Transcription And Translation* (IRL Press); Freshney (2016) *Culture Of Animal Cells*, 7th Edition (Wiley-Blackwell); Woodward, J., *Immobilized Cells And Enzymes* (IRL Press) (1985); Perbal (1988) *A Practical Guide To Molecular Cloning*; 2d Edition (Wiley-Interscience); Miller and Calos eds. (1987) *Gene Transfer Vectors For Mammalian Cells*, (Cold Spring Harbor Laboratory); S.C. Makrides (2003) *Gene Transfer and Expression in Mammalian Cells* (Elsevier Science); Methods in

Enzymology, Vols. 151-155 (Academic Press, Inc., N.Y.); Mayer and Walker, eds. (1987) Immunochemical Methods in Cell and Molecular Biology (Academic Press, London); Weir and Blackwell, eds.; and in Ausubel *et al.* (1995) Current Protocols in Molecular Biology (John Wiley and Sons).

5 **[0180]** General principles of antibody engineering are set forth, *e.g.*, in Strohl, W.R., and L.M. Strohl (2012), Therapeutic Antibody Engineering (Woodhead Publishing). General principles of protein engineering are set forth, *e.g.*, in Park and Cochran, eds. (2009), Protein Engineering and Design (CDC Press). General principles of immunology are set forth, *e.g.*, in: Abbas and Lichtman (2017) Cellular and Molecular Immunology 9th
10 Edition (Elsevier). Additionally, standard methods in immunology known in the art can be followed, *e.g.*, in Current Protocols in Immunology (Wiley Online Library); Wild, D. (2013), The Immunoassay Handbook 4th Edition (Elsevier Science); Greenfield, ed. (2013), Antibodies, a Laboratory Manual, 2d Edition (Cold Spring Harbor Press); and
15 Ossipow and Fischer, eds., (2014), Monoclonal Antibodies: Methods and Protocols (Humana Press).

[0181] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0182] The following examples are offered by way of illustration and not by way of limitation.

20 Exemplary Embodiments

[0183] Among the provided embodiments are:

[0184] Embodiment 1. An antibody or antigen-binding fragment or derivative thereof comprising an antigen-binding domain that specifically binds to CD123, wherein the antigen-binding domain comprises a heavy chain variable region (VH) and light chain
25 variable region (VL), wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79, SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83.

[0185] Embodiment 2. The antibody or fragment or derivative thereof of embodiment 1,
30 wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79.

- [0186]** Embodiment 3. The antibody or fragment or derivative thereof of embodiment 1 or embodiment 2, which is a multimeric antibody comprising five, six, or two bivalent binding units and ten, twelve, or four antigen-binding domains wherein at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123; wherein each binding unit comprises two heavy chains each comprising an IgM or IgA constant region or a multimerizing fragment or variant thereof, and wherein at least one two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy chain constant regions of the multimeric antibody is associated with a copy of the VH.
- [0187]** Embodiment 4. The antibody or fragment or derivative thereof of embodiment 1 or embodiment 2, which comprises a single bivalent binding unit comprising two antigen-binding domains wherein at least one antigen-binding domain specifically binds to CD123, wherein the binding unit comprises two heavy chains each comprising a heavy chain constant region or fragment or variant thereof, and wherein at least one heavy chain constant region or fragment or variant thereof of the binding unit is associated with a copy of the VH.
- [0188]** Embodiment 5. The antibody or fragment or derivative thereof of embodiment 4, wherein the heavy chains comprise IgG heavy chain constant regions or fragments or variants thereof.
- [0189]** Embodiment 6. The antibody or fragment or derivative thereof of embodiment 1 or embodiment 2, which is an Fv fragment, a single-chain Fv fragment (scFv), or a disulfide-linked Fv fragment (sdFv).
- [0190]** Embodiment 7. The antibody or fragment or derivative thereof of any one of embodiments 1 to 6, which is multispecific.
- [0191]** Embodiment 8. The antibody or fragment or derivative thereof of embodiment 7, which is bispecific.
- [0192]** Embodiment 9. The antibody or fragment or derivative thereof of embodiment 7 or embodiment 8, which can bind CD3.
- [0193]** Embodiment 10. The antibody or fragment or derivative thereof of any one of embodiments 1 to 9, which can specifically bind to human CD123.
- [0194]** Embodiment 11. The antibody or fragment or derivative thereof of embodiment 10, which specifically binds to human CD123 with an affinity characterized by a

dissociation constant K_D no greater than 500 nM, 100 nM, 50.0 nM, 40.0 nM, 30.0 nM, 20.0 nM, 10.0 nM, 9.0 nM, 8.0 nM, 7.0 nM, 6.0 nM, 5.0 nM, 4.0 nM, 3.0 nM, 2.0 nM, 1.0 nM, 0.50 nM, 0.10 nM, 0.050 nM, 0.01 nM, 0.005 nM, or 0.001 nM.

[0195] Embodiment 12. A multimeric antibody comprising five, six, or two bivalent
5 binding units and ten, twelve, or four antigen-binding domains wherein at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123; wherein the antigen-binding domain comprises a heavy chain variable region (VH) and light chain variable region (VL), wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79,
10 SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83, wherein each binding unit comprises two heavy chains each comprising an IgM or IgA constant region or a multimerizing fragment or variant thereof, and wherein at least three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy
15 chain constant regions of the multimeric antibody is associated with a copy of the VH.

[0196] Embodiment 13. The multimeric antibody of embodiment 12, wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79.

[0197] Embodiment 14. The multimeric antibody of embodiment 12 or embodiment 13,
20 which is pentameric or hexameric and comprises five or six bivalent IgM binding units, wherein each binding unit comprises a $C_{\mu 4}$ domain and a μ -tail piece (μ tp) domain or multimerizing fragment or variant thereof.

[0198] Embodiment 15. The multimeric antibody of embodiment 14, wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each further
25 comprise a $C_{\mu 1}$ domain, a $C_{\mu 2}$ domain, a $C_{\mu 3}$ domain, or any combination thereof.

[0199] Embodiment 16. The multimeric antibody of any one of embodiments 12 to 15, wherein each IgM heavy chain constant region is a human IgM constant region or multimerizing variant or fragment thereof, comprising the amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, or a multimerizing variant or fragment thereof.

30 [0200] Embodiment 17. The multimeric antibody of embodiment 15 or embodiment 16, comprising a variant human IgM constant region, wherein the multimeric antibody has

reduced CDC activity relative to a multimeric antibody comprising IgM heavy chain constant regions comprising the amino acid sequence SEQ ID NO: 1 or SEQ ID NO: 2.

[0201] Embodiment 18. The multimeric antibody of embodiment 17, wherein each variant human IgM constant region comprises an amino acid substitution corresponding to position P311 of SEQ ID NO: 1 or SEQ ID NO: 2, an amino acid substitution corresponding to position P313 of SEQ ID NO: 1 or SEQ ID NO: 2, or amino acid substitutions corresponding to positions P311 and P313 of SEQ ID NO: 1 or SEQ ID NO: 2.

[0202] Embodiment 19. The multimeric antibody of any one of embodiments 15 to 18, wherein each IgM heavy chain constant region or multimerizing variant or fragment thereof is a variant human IgM constant region with one or more single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more single amino acid substitutions, deletions, or insertions; and wherein the multimeric antibody exhibits increased serum half-life upon administration to a subject animal relative to multimeric antibody comprising the reference IgM heavy chain constant regions, which is administered in the same way to the same animal species.

[0203] Embodiment 20. The multimeric antibody of embodiment 19, wherein the variant IgM heavy chain constant regions comprise amino acid substitutions at one or more amino acid positions corresponding to amino acid E345, S401, E402, or E403 of SEQ ID NO: 1 or SEQ ID NO: 2.

[0204] Embodiment 21. The multimeric antibody of any one of embodiments 15 to 20, wherein the IgM heavy chain constant regions or multimerizing variant or fragment thereof each comprise one or more single amino acid substitutions corresponding to amino acid positions N46, N209, N272, or N440 of SEQ ID NO: 1 or SEQ ID NO: 2, and wherein the one or more single amino acid substitutions prevent asparagine (N)-linked glycosylation.

[0205] Embodiment 22. The multimeric antibody of any one of embodiments 12 to 21, wherein each heavy chain constant region or multimerizing fragment or variant thereof is associated with a copy of the VH.

[0206] Embodiment 23. The multimeric antibody of any one of embodiments 12 to 22, wherein each binding unit further comprises two light chains each comprising a light

chain constant region or fragment or variant thereof, and wherein at least three, four, five, six, seven eight, nine, ten, eleven, or twelve light chain constant regions or fragments or variants thereof is/are associated with a copy of the VL.

5 [0207] Embodiment 24. The multimeric antibody of embodiment 23, wherein each light chain constant region or fragment or variant thereof is associated with a copy of the VL.

[0208] Embodiment 25. The multimeric antibody of any one of embodiments 14 to 20, which is pentameric, and further comprises a J chain, or fragment thereof, or variant thereof.

10 [0209] Embodiment 26. The multimeric antibody of embodiment 12 or embodiment 13, which is dimeric and comprises two bivalent IgA binding units and a J chain or fragment or variant thereof, wherein each binding unit comprises a C α 3 domain and an α -tail piece (α tp) domain.

15 [0210] Embodiment 27. The multimeric antibody of embodiment 26, wherein the J-chain or fragment or variant thereof is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 7 or a fragment thereof, or a variant thereof.

[0211] Embodiment 28. The multimeric antibody of embodiment 26 or embodiment 27, wherein the IgA heavy chain constant regions or multimerizing fragments or variants thereof each further comprise a C α 1 domain, a C α 2 domain, an IgA hinge region, or any combination thereof.

20 [0212] Embodiment 29. The multimeric antibody of embodiment 28, wherein the IgA heavy chain constant regions or multimerizing fragments or variants thereof are IgA1 heavy chain constant regions or multimerizing fragments or variants thereof.

[0213] Embodiment 30. The multimeric antibody of embodiment 29, wherein the IgA heavy chain constant regions comprise SEQ ID NO: 3.

25 [0214] Embodiment 31. The multimeric antibody of embodiment 28, wherein the IgA heavy chain constant regions or multimerizing fragments or variants thereof are IgA2 heavy chain or multimerizing fragments or variants thereof.

[0215] Embodiment 32. The multimeric antibody of embodiment 31, wherein the IgA heavy chain constant regions comprise SEQ ID NO: 4.

30 [0216] Embodiment 33. The multimeric antibody of any one of embodiments 25 to 32, further comprising a secretory component, or fragment or variant thereof.

- [0217] Embodiment 34. The multimeric antibody of any one of embodiments 12 to 33, which is multispecific.
- [0218] Embodiment 35. The multimeric antibody of embodiment 34, which is bispecific.
- 5 [0219] Embodiment 36. The multimeric antibody of embodiment 34 or embodiment 35, which can bind CD3.
- [0220] Embodiment 37. The multimeric antibody of embodiment 25, wherein the J-chain or fragment or variant thereof is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 7 or a fragment thereof, or a variant thereof.
- 10 [0221] Embodiment 38. The multimeric antibody of embodiment 37, wherein the variant J-chain or fragment thereof is a variant J-chain comprising an amino acid substitution at the amino acid position corresponding to amino acid Y102 of SEQ ID NO: 7, and wherein an IgM antibody comprising the variant J-chain exhibits an increased serum half-life upon administration to an animal relative to a reference IgM
- 15 antibody that is identical except for the amino acid substitution in the J-chain, and is administered in the same way to the same animal species.
- [0222] Embodiment 39. The multimeric antibody of embodiment 38, wherein the amino acid corresponding to Y102 of SEQ ID NO: 7 is substituted with alanine (A).
- [0223] Embodiment 40. The multimeric antibody of embodiment 39, wherein the
- 20 variant J-chain comprises the amino acid sequence SEQ ID NO: 8.
- [0224] Embodiment 41. The multimeric antibody of any one of embodiments 25 to 40, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, wherein the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof.
- 25 [0225] Embodiment 42. The multimeric antibody of embodiment 41, wherein the heterologous moiety is a heterologous polypeptide fused to the J-chain or fragment or variant thereof.
- [0226] Embodiment 43. The multimeric antibody of embodiment 42, wherein the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a
- 30 peptide linker comprising at least 5 amino acids, but no more than 25 amino acids.
- [0227] Embodiment 44. The multimeric antibody of embodiment 42 or embodiment 43, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or

fragment or variant thereof, to the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof, wherein the heterologous polypeptides fused to both the N-terminus and C-terminus can be the same or different.

5 **[0228]** Embodiment 45. The multimeric antibody of any one of embodiments 42 to 44, wherein the heterologous polypeptide is an antibody antigen-binding domain, or a subunit thereof.

[0229] Embodiment 46. The multimeric antibody of embodiment 45, wherein the antibody antigen-binding domain comprises a scFv fragment.

10 **[0230]** Embodiment 47. The multimeric antibody of any one of embodiments 44 to 46, wherein the heterologous polypeptide binds to CD3.

[0231] Embodiment 48. The multimeric antibody of embodiment 46 or embodiment 47, wherein the antibody antigen-binding domain binds to CD3 and comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises
15 VH complementarity-determining regions VHCDR1, VHCDR2, and VHCDR3 and the VL comprises VL complementarity-determining regions VLCDR1, VLCDR2, and VLCDR3, wherein the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise, respectively, the amino acid sequences SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; SEQ
20 ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25; SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41; SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49; SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57; SEQ
25 ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 64, and SEQ ID NO: 65; or SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73.

[0232] Embodiment 49. The multimeric antibody of embodiment 48, wherein the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 comprise,
30 respectively, the amino acid sequences SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

- [0233] Embodiment 50. The multimeric antibody of embodiment 48, wherein the antibody antigen-binding domain comprises VH and VL amino acid sequences at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 18 and SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 38, SEQ ID NO: 42 and SEQ ID NO: 46, SEQ ID NO: 50 and SEQ ID NO: 54, SEQ ID NO: 58 and SEQ ID NO: 62, or SEQ ID NO: 66 and SEQ ID NO: 70, respectively.
- [0234] Embodiment 51. The multimeric antibody of embodiment 50, wherein the antibody antigen-binding domain comprises the VH and VL amino acid sequences SEQ ID NO: 18 and SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 38, SEQ ID NO: 42 and SEQ ID NO: 46, SEQ ID NO: 50 and SEQ ID NO: 54, SEQ ID NO: 58 and SEQ ID NO: 62, or SEQ ID NO: 66 and SEQ ID NO: 70, respectively.
- [0235] Embodiment 52. The multimeric antibody of embodiment 51, wherein the antibody antigen-binding domain comprises the VH and VL amino acid sequences SEQ ID NO: 26 and SEQ ID NO: 30, respectively.
- [0236] Embodiment 53. The multimeric antibody of any one of embodiments 12 to 52, which can specifically bind to human CD123.
- [0237] Embodiment 54. The multimeric antibody of embodiment 53, which specifically binds to human CD123 with an affinity characterized by a dissociation constant KD no greater than 500 nM, 100 nM, 50.0 nM, 40.0 nM, 30.0 nM, 20.0 nM, 10.0 nM, 9.0 nM, 8.0 nM, 7.0 nM, 6.0 nM, 5.0 nM, 4.0 nM, 3.0 nM, 2.0 nM, 1.0 nM, 0.50 nM, 0.10 nM, 0.050 nM, 0.01 nM, 0.005 nM, or 0.001 nM.
- [0238] Embodiment 55. A composition comprising the antibody or fragment or derivative thereof of any one of embodiments 1 to 11 or the multimeric antibody of any one of embodiments 12 to 54.
- [0239] Embodiment 56. A polynucleotide comprising a nucleic acid sequence that encodes the antibody or fragment or derivative thereof of any one of embodiments 1 to 11 or the multimeric antibody of any one of embodiments 12 to 54 or a subunit thereof.
- [0240] Embodiment 57. A vector comprising the polynucleotide of embodiment 56.
- [0241] Embodiment 58. A host cell comprising the vector of embodiment 57.
- [0242] Embodiment 59. A method of producing the antibody or fragment or derivative thereof of any one of embodiments 1 to 11 or the multimeric antibody of any one of

embodiments 12 to 54, comprising culturing the host cell of embodiment 58, and recovering the antibody or fragment or derivative thereof or the multimeric antibody.

[0243] Embodiment 60. A method of treating cancer comprising administering to a subject in need of treatment an effective amount of the antibody or fragment or derivative thereof of any one of embodiments 1 to 11 or the multimeric antibody of any one of embodiments 12 to 54.

[0244] Embodiment 61. The method of embodiment 60, wherein the subject is human.

[0245] Embodiment 62. The method of embodiment 60 or embodiment 61, wherein the cancer is a hematological cancer.

[0246] Embodiment 63. The method of embodiment 62, wherein the hematological cancer is acute myeloid leukemia (AML).

Examples

Example 1: Humanization of Anti-CD123 Antibody

[0247] The sequences of anti-CD123 antibody 32716 are described in Du (2007) J Immunother 30:607-13. A homology model of 32716 was generated in BioLuminate based on crystal structure of mouse Ab 2H4 (PDB 5YWF). The homology of 32716 to Ab 2H4 was determined using sequence alignment to be the following: VH identity 81%, VL identity 71%, VH framework identity 91%, VL framework identity 80%, additionally the CDR length was identical for 5/6 CDRs (except Light chain CDR1).

[0248] Two human antibodies were chosen as framework acceptors: 4D9Q with VH Framework identity of 75% (**Fig. 1A**) and VK framework identity of 70% (**Fig. 1B**) and 4NWT with VH framework identity 65% (**Fig. 1C**) and VK framework identity of 80% (**Fig. 1D**). Several humanized sequences were designed based on each framework **FIGS. 1A-D**. The parental 32716 VH and VL and 6 combinations of humanized 32716 VH and VL sequences were cloned into human IgG formats according to standard cloning protocols as described in **Table 2**. Human IgG constructs were synthesized, expressed, and purified through commercial vendors (ATUM and Celltheon).

Table 2: Anti-CD123 antibodies

Antibody	VH SEQ ID NO	VH Name	VL SEQ ID NO	VL Name
32716	74	32716-VH	75	32716-VL
h32716-1-1	76	h32716-VH1	79	h32716-VL1
h32716-2-1	77	h32716-VH2	79	h32716-VL1
h32716-3-1	78	h32716-VH3	79	h32716-VL1
h32716-4-2	80	h32716-VH4	83	h32716-VL2
h32716-5-2	81	h32716-VH5	83	h32716-VL2
h32716-6-2	82	h32716-VH6	83	h32716-VL2

Example 2: Biolayer Interferometry Affinity Measurement Assay of humanized 32716 IgG Antibodies

[0249] The binding affinities of parental 32716 IgG and humanized variants with recombinant human CD123 protein (CD123)-His Acrobio# ILA-H52H6) were determined by BLI on an Octet-384 (Sartorius/Fortebio, NY, USA) using Anti-penta His biosensors (Sartorius). PBST (1xPBS+ 1% BSA+ 0.05%Tween-20) buffer was used as Antibody/CD123 dilution and sensor hydration buffer. The experiment followed a five-step sequential assay at 24°C. First, biosensors were hydrated for 10 minutes. Samples and buffer were applied in 384-well plate. After initial baseline for 60 s, sensors were loaded with 7nM hu IL-3R alpha (CD123)-His Acrobio# ILA-H52H6. The biosensors were dipped into PBST for 20 s to reach baseline, then incubated for 420 s with 2-fold serial diluted anti-CD123 antibodies starting at 10 nM for association, followed by 900 s in PBST for dissociation. Results were analyzed by ForteBio Data Analysis software 9.0 using 1:1 global fit model.

[0250] Binding of parental 32716 IgG, and the humanized variants is shown in **FIGS. 2A-G**, respectively. The K_D , k_{on} , and k_{dis} are shown in **Table 3**. Humanized IgG antibodies have retained affinity to CD123 within 1.5-fold of original mouse antibody. h32716-3-1 IgG displayed slightly better affinity compared to the original murine IgG antibody.

Table 3: Binding Kinetics Results of humanized IgG antibodies

Antibody	KD [nM] $\pm$ Stdev	KD Error	kon(1/Ms)	kdis(1/s)	R ²
32716 IgG	0.44 $\pm$ 0.09	1.2 E-11	7.1 $\pm$ 1.4 E+05	3.0 $\pm$ 0.2 E-04	0.9974
h32716-1-1 IgG	0.36 $\pm$ 0.08	8.5 E-12	9.4 $\pm$ 0.4 E+05	4.0 $\pm$ 0.16 E-04	0.994
h32716-2-1 IgG	0.45 $\pm$ 0.1	1.2 E-11	1.1 $\pm$ 0.18 E+06	4.9 $\pm$ 0.6 E-04	0.9904
h32716-3-1 IgG	0.25 $\pm$ 0.03	7.7 E-12	1.2 $\pm$ 0.4 E+06	3.1 $\pm$ 0.9 E-04	0.9908
h32716-4-2 IgG	0.60 $\pm$ 0.06	1.3 E-11	7.7 $\pm$ 1.4 E+05	4.6 $\pm$ 0.5 E-04	0.9899
h32716-5-2 IgG	0.71 $\pm$ 0.15	1.5 E-11	7.0 $\pm$ 0.8 E+05	4.5 $\pm$ 0.4 E-04	0.9943
h32716-6-2 IgG	0.33 $\pm$ 0.13	7.0 E-12	1.1 $\pm$ 0.7 E+05	2.0 $\pm$ 0.5 E-04	0.9982

Example 3: Binding to a CD123 expressing cell line

[0251] To assess the ability of IgG antibodies to bind CD123 on CHO cells expressing the human CD123, a binding assay was performed. CHO cells expressing human CD123 were detached from flask using trypsin. Cells (1×10^5) were pipetted into wells in a round bottom 96 well plate, washed with FACS Stain Buffer (BD Pharmingen Catalog #554656) and pre-incubated with Fc Block (BD, #564220) for 10 minutes at room temperature followed by incubation at 4°C with serial dilutions of 32716 or h32716 IgG antibodies for 30 minutes. Cells were washed twice and stained with a mouse anti human kappa antibody conjugated with Alexa647 (Southern Biotech, clone SB81a). Cells were analyzed by flow cytometry. The results are shown in **FIG. 3**. Mean fluorescence intensity (MFI) values were analyzed with GraphPad Prism using a 4-parameter logistic model. Data is shown in **Table 4**. All humanized IgG antibodies of 32716 anti-CD123 bound with similar affinities and intensities.

Table 4. Binding of humanized IgG antibodies to CD123 expressing CHO cells

Antibody	Max binding intensity (MFI)	HillSlope	IC ₅₀ (nM)
32716 IgG	910000	2.2	11
h32716-1-1 IgG	950000	2.0	7.4
h32716-2-1 IgG	930000	1.7	11

h32716-3-1 IgG	940000	1.9	10
h32716-4-2 IgG	980000	2.4	6.8
h32716-5-2 IgG	1100000	2.2	7.2
h32716-6-2 IgG	1000000	1.9	11

Example 4: Humanized 32716 anti-CD123 conversion to IgM and binding kinetics to CD123

- [0252] To generate the IgM humanized constructs, the VH and VL regions of two of the humanized anti-CD123 sequences were incorporated into IgM with a modified J chain comprising a CD3-binding scFv (SEQ ID NO: 84) to form bispecific IgM antibodies according to standard cloning protocols. The IgM antibody constructs were expressed in Expi293 or CHO cells. The IgM antibodies were purified according to methods described in Keyt, B., *et al. Antibodies*: 9:53, doi: 10.3390/antib9040053 (2020). The IgM antibodies assembled as pentamers with a J-chain.
- 10 [0253] The binding affinities of CD123XCD3 IgM antibodies 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM with recombinant human CD123 protein (CD123, Fc-Fusion (IgG1) Avi-Tag, Biotin-Labeled, BPS Bioscience Cat.100068-2) were determined by BLI on an Octet-384 (Sartorius/Fortebio, NY, USA) using Anti-human Fc (AFC) biosensors (Sartorius Cat#185064) as described in Example 2.
- 15 [0254] Binding of parental 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM to CD123 is shown in **FIG. 4A-C**, respectively. The K_D , k_{on} , and k_{dis} are shown in **Table 5**. Humanized IgM antibodies have retained affinity to CD123 within 1.5-fold of original mouse antibody.

Table 5: Binding kinetics of humanized IgM antibodies

Antibody	K_D [M]	k_{on} (1/Ms)	k_{dis} (1/s)	R^2
32716 IgM	<1.0E-12	4.10E+06	<1.0E-07	0.9933
h32716-1-1 IgM	<1.0E-12	3.90E+06	<1.0E-07	0.9947
h32716-4-2 IgM	4.43E-12	3.82E+06	1.69E-05	0.996

Example 5: IgM Antibody Specificity Measured by ELISA

[0255] The specificities of the Anti-CD123xCD3 IgM antibodies 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM for human CD123 and CD3 ϵ were measured in ELISA assays as follows. 96-well white polystyrene ELISA plates (Pierce 15042) were coated with 100 μ L per well of 0.2 μ g/mL recombinant human CD123 protein (Sino Biological 10518-H08H-50) or 0.5 μ g/ml recombinant human CD3 ϵ protein (Acro Biosystems, CDE-H5256-100) overnight at 4 °C. Plates were then washed 5 times with 0.05% PBS-Tween and blocked with 2% BSA-PBS. After blocking, 100 μ L of serial dilutions of CD123xCD3 IgM antibodies, standards, and controls were added to the wells and incubated at room temperature for 2 hours. The plates were then washed 10 times and incubated with HRP conjugated mouse anti-human kappa (Southern Biotech, 9230-05. 1:6000 diluted in 2% BSA-PBS) for 30 min. After 10 final washes using 0.05% PBS-Tween, the plates were read out using SuperSignal chemiluminescent substrate (ThermoFisher, 37070). Luminescent data were collected on an EnVision plate reader (Perkin-Elmer) and analyzed with GraphPad Prism using a 4-parameter logistic model. Binding of the IgM bispecific antibodies to CD123 is shown in **FIG. 5A** and binding to CD3 ϵ is shown in **FIG. 5B**.

Example 6: IgM Antibody Binding to MV411 AML cell line

[0256] To assess the ability of the Anti-CD123xCD3 IgM antibodies 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM to bind CD123 on AML cells expressing the CD123 protein, a binding assay was performed. MV411 cells were washed with FACS Stain Buffer (BD Pharmingen Catalog #554656) and pre-incubated with Fc Block (BD, 564220) for 10 minutes at room temperature. 5×10^4 cells were stained with serial dilutions of IgM antibodies for 30 minutes at 4°C. Cells were washed twice, then stained for 30 minutes at 4°C with 5 μ g/mL anti-human IgM-PE labeled secondary antibody (SB, clone SA-DA4). Cells were washed twice, resuspended in FACS Stain Buffer, and acquired by flow cytometry. The results are shown in **FIG. 6**.

Example 7: Humanized 32716 IgM bispecific antibodies retain T Cell-Directed AML Cell Killing potency

[0257] In order to demonstrate the ability of the Anti-CD123xCD3 IgM antibodies 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM to kill target cells in the presence of human T-cells, we performed co-culture experiments. 7 x 10³ tumor cells KG1a and MV4-11 (expressing firefly luciferase) were co-cultured with T cells at 7:1 Effector to target (E:T) ratios in the presence of serial dilutions of anti-CD123xCD3 IgM antibodies in 100 µL total volume of AIM-V cell culture medium (GIBCO, #12055091) supplemented with 3% heat-inactivated fetal bovine serum (FBS, HyClone, #SH3007103HI) per well on a 96 round bottom tissue culture plate. After 72 hours of incubation at 37 °C in a 5% CO₂ incubator, 50 µl of supernatant was removed and frozen at -80 °C for later cytokine release analysis. 50 µl of luciferase substrate *e.g.*, ONE-Glo EX Luciferase Assay System, Promega was added to the wells. The plates were shaken briefly to mix the reagents, and luciferase luminescent signal was measured on an EnVision plate reader (Perkin-Elmer). The data was then analyzed with GraphPad Prism to determine the EC₅₀. Representative dose response curves for KG1a and MV411 are shown in **FIG. 7A-B** and max killing percentage and EC₅₀ values are shown in **Table 6**.

Table 6: in vitro killing potency of anti-CD123xCD3 IgM antibodies

Antibody	KG1a		MV411	
	max killing (%)	EC ₅₀ (pM)	max killing (%)	EC ₅₀ (pM)
32716 IgM	99.03	13.36	100.2	0.3337
h32716-1-1 IgM	99.98	7.833	100.8	0.2809
h32716-4-2 IgM	97.84	17.47	100	0.9878

Example 8: Humanization Effects on IgM Stability and Aggregation

[0258] Equal concentrations of 32716 IgM, h32716-1-1 IgM, and h32716-4-2 IgM were formulated in the same buffer solution. An initial (t=0) percentage of high molecular weight aggregates (%HMW), *i.e.*, molecules with molecular masses greater than

pentameric IgM with J-chain, was determined by size exclusion high performance liquid chromatography (HPLC). Aliquots of each antibody were exposed to 3 or 5 cycles of freezing and thawing, wherein the aliquot was stored at -80 °C for 2-20 hours and 2 hours at 25 °C. Other aliquots were stored at 4 °C or 40 °C for one week. The %HMW of all aliquots was measured after treatment. The results are shown in FIG. 8.

[0259] h32716-1-1 IgM showed a better stability profile and a lower tendency to aggregate compared to 32716 IgM and h32716-4-2 IgM.

Example 9: *In Vivo* Treatment with Humanized 32716

[0260] 8-week-old female MHC -/- NSG mice were purchased from The Jackson Laboratory. The mice were humanized by engrafting 10×10^6 healthy human donor derived peripheral blood mononuclear cells (PBMCs) per mouse. 10 days post human PBMC engraftment, 5×10^6 MV4-11-gfp-luc tumor cells, mixed with Matrigel (1:1 with 1x phosphate buffered saline (PBS)), were implanted subcutaneously in the right flank of the mice. One day post tumor implant, mice were dosed with vehicle intravenously (i.v.) every third day for a total of 8 doses, 0.1 mg/kg of anti-CD123XCD3 IgG #1 (comprising the CD123 VH and VL of SEQ ID NOs: 85 and 86 and a CD3 scFv of SEQ ID NO: 87) i.v. every third day for 5 doses, 5 mg/kg h32716-1-1 IgM antibody i.v. every third day for 8 doses, or 15 mg/kg h32716-1-1 IgM antibody i.v. every third day for 8 doses. (n=10 animals/group).

[0261] Average tumor volumes over time through day 75 are shown in FIG. 9A. Individual tumor volumes on day 75 are shown in FIG. 9B. Individual tumor volumes over time through day 75 for vehicle, anti-CD123XCD3 IgG #1 treatment, 5 mg/kg h32716-1-1 IgM antibody, and 15 mg/kg h32716-1-1 IgM antibody are shown in FIGS. 10A-D, respectfully.

[0262] Treatment with h32716-1-1 IgM antibody at 5mg/kg significantly reduced the tumor volume compared to vehicle treatment. On day 75 of the study, 7 out of 10 mice were tumor free in the h32716-1-1 IgM antibody at 5mg/kg group, and 6 out of 10 mice were tumor free in the h32716-1-1 IgM antibody at 15mg/kg group.

Example 10: CD123xCD3 IgM Inhibits *In Vitro* Human AML Colony Formation

[0263] To test the effect of the CD123xCD3 IgM on the growth of multiple myeloma cells *in vitro*, a colony formation assay was used. Frozen bone marrow mononuclear cells from four distinct acute myeloid leukemia (AML) donors were thawed and resuspended at 0.5×10^6 cells/mL in a liquid-based medium containing cytokines (IL-3, GM-CSF and SCF) and plated into wells of a 12-well plate. To each well, the test antibody was added at 7 distinct concentrations (150, 50, 10, 2, 0.4, 0.08, 0.06 $\mu\text{g/mL}$). Additionally, a daunorubicin control was evaluated at 50, 10, and 1 nM. A well containing just the solvent control was also included. The 12 well plate was incubated in a humidified incubator at 37 °C, 5% CO₂ for 72 hours. Following the incubation, the cells within each well were dispersed carefully by pipetting. Four hundred μL of cells (and medium) were removed from each well and added to 4.0 mL of methylcellulose containing IL-3, GM-CSF and SCF. The tubes of methylcellulose were vortexed to ensure equal distribution of cells throughout the matrix. Triplicate cultures in 35 mm dishes were set up for each condition. The replicate dishes were placed at 37°C, 5% CO₂ for a total of 14-16 days, after which the resultant colonies were evaluated and enumerated based on morphology. The effector cell to T cell ratios (E:T) used for each donor are shown in **FIG. 11**.

[0264] **FIG. 11** shows *in vitro* colony formation of multiple myeloma cells from four different donors, following treatment with h32716-1-1. Treatment with h32716-1-1 reduced colony formation for all donors tested.

Example 11: Pharmacokinetic Properties of CD123XCD3 IgM Antibodies

[0265] Pharmacokinetic parameters were measured for various CD123XCD3 IgM antibodies in an *in vivo* mouse model as follows. Balb/c mice were injected with 5 mg/kg of h32716, h32716-4-2, or h32716-1-1 via intravenous bolus administration. Blood samples were collected at 8 time points total for each antibody, with 2 mice per time point. A sandwich ELISA assay was used to measure the plasma concentration of each antibody at each time point. Quality metrics were verified on all ELISAs, and PK parameters, including $t_{1/2}$, clearance (CL), area under the concentration curve (AUC), and maximum concentration (C_{max}) were derived using nonlinear curve fitting techniques

(WinNonLin, Phoenix Software). A plot of concentrations over time is shown in **FIG.**

12. PK parameters are presented in **Table 7.**

Table 7: PK Parameters

Parameter	Unit	32716	h32716-1-1	h32716-4-2
Dose	mg/kg	5	5	5
CL	mL/day/kg	129.5	162	171.6
t _{1/2}	hours	6.9	10.5	15.5
V _{ss}	mL/kg	298.7	103.3	126.6
C _{max}	ug/mL	100.5	76.2	61.9
AUC _{inf}	days*ug/mL	38.6	30.8	29.1
%Extrap	%	0.21	1.02	2.55

5 Example 12: CD123xCD3 IgM Induces Lower Levels of Cytokines From T-Cells

TDCC assay method

[0266] MV4-11 leukemia cells were labeled with a PKH26 red fluorescent tag using the PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich, Cat#MINI26-1kt) per manufacturer's instructions. Briefly, 5×10^6 MV4-11 cells were washed with serum free RPMI-1640 medium (Gibco, REF22400-071). After centrifugation, 0.25 ml of diluent C was added to the cell pellet and was gently pipetted to resuspend. Immediately prior to staining, 4 μ l of the PKH26 ethanolic dye solution was added to 1.5 ml of Diluent C (2X) in a polypropylene centrifuge tube and mixed well to disperse, which resulted in a 2X Dye Solution. 0.25 ml of 2X Dye Solution was rapidly added to 0.25 ml of 2X Cell Suspension, and the sample was immediately mixed. The sample was incubated 5 min and 0.5 ml of heat inactivated FBS was added to stop the staining. The cells were centrifuged and resuspended in culture medium to make final concentration of 2.5×10^5 /ml. Human peripheral blood mononuclear cells (PBMCs) (IQ Biosciences, IQB-PBMC103) were washed and resuspend in culture medium at 2×10^6 /ml. 80 μ l of MV4-11 cells, 100 μ l of PBMCs, and 20 μ l of 5x serial diluted testing antibodies were added

in each well with highest final concentration of 5 nM. The plates were incubated for 48 and 72 hours. FACS analysis was performed to determine the number of live tumor cells.

MSD assay Method

- 5 **[0267]** Cytokine levels from supernatants harvested from MV4-11 cell TDCC assay at 48 and 72 hours were measured with MSD Proinflammatory Panel 1 (human) Kit (V-PLEX, Cat# K15049D-2). Briefly, TDCC media was diluted 25 times, 5 times or 2 times with Diluent 2. Detection antibody solution was prepared by adding 60 μ L of SULFO-TAG anti-human IFN- γ , IL-6, or IL-10 to 2.4 mL of Diluent 3. The plate was
- 10 washed three times with 200 μ L/well of wash buffer and then incubated with 50 μ L/well of diluted samples or calibrators at room temperature with shaking for 2 hours. After washing three times with 200 μ L/well of wash buffer, the plate was incubated with 25 μ L of detection antibody solution at room temperature with shaking for 2 hours. The plate was then washed three times with 200 μ L/well of wash buffer. 150 μ L of 2X Read Buffer
- 15 T was added to each well of the plate and the plate was analyzed on an MSD instrument.
- [0268]** The calibration curves used to calculate analyte concentrations were established by fitting the signals from calibrators to a 4-parameter logistic model with a $1/Y^2$ weighting. Analyte concentrations were determined from the ECL signals by back-fitting to the calibration curve. The calculations to establish calibration curves and determine
- 20 concentrations were carried out using the MSD DISCOVERY WORKBENCH analysis software.
- [0269]** The number of live cells, amount of IFN γ , IL-6, and IL-10 after 48 hours of TDCC with either CD123xCD3 IgG #1 or h32716-1-1 are shown in **FIGS. 13A-13D**, respectively and after 72 hours of TDCC either CD123xCD3 IgG #1 or h32716-1-1 are
- 25 shown in **FIGS. 14A-14D**, respectively. At concentrations that resulted in equal levels of cell killing, h32716-1-1 resulted in the production of much lower levels of cytokines compared to CD123xCD3 IgG #1.

Table 8: Sequences of exemplary CD3 binders

SP34 VH e.g., WO2015095392					
SEQ ID	VH				
18	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKD RFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLLTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
19	TYAMN	20	RIRSKYNNYATYYADSVK D	21	HGNFGNSYVSWFAY
SEQ ID	VL				
22	QAVVTQESALTTSPGETVTILTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSL IGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
23	RSSTGAVTTSNYAN	24	GTNKRAP	25	ALWYSNLWV
WO2018208864					
SEQ ID	VH				
26	EVQLLES ~~GGGLVQPGGSLRL~~ SCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKD RFTISRDDSKSTLYLQME ~~SLRA~~ EDTAVYYCVRHANFGAGYVSWFAH ~~HW~~ QGTLLTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
27	TYAMN	28	RIRSKYNNYATYYADSVK D	29	HANFGAGYVSWFAH
SEQ ID	VL				
30	QTVVTQEP ~~SLSV~~ SPGGTVTLTC ~~GS~~ SSTGAVTTSNYANWVQQT ~~PGQAPR~~ GLIGGT ~~DKRAP~~ GVPDRFSGSL LGDKAALTITGAQ ~~A~~ EDEADYYCALWYSN ~~HW~~ VFGGGTKLTVL				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
31	GSSTGAVTTSNYAN	32	GTDKRAP	33	ALWYSNHWV
WO2018208864					
SEQ ID	VH				
34	EVQLLES ~~GGGLVQPGGSLRL~~ SCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKD RFTISRDDSKSTLYLQME ~~SLRA~~ EDTAVYYCVRHANFGAGYVSWFAH ~~HW~~ QGTLLTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
35	TYAMN	36	RIRSKYNNYATYYADSVK D	37	HANFGAGYVSWFAH
SEQ ID	VL				
38	QTVVTQEP ~~SLSV~~ SPGGTVTLTC ~~GS~~ SSTGAVTTSNYANWVQQT ~~PGQAPR~~ GLIGGT ~~DKRAP~~ GVPDRFSGSL LGDKAALTITGAQ ~~A~~ EDEADYYCALWYS ~~DLW~~ VFGGGTKLTVL				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
39	GSSTGAVTTSNYAN	40	GTDKRAP	41	ALWYSDLWV
WO2018208864					
SEQ ID	VH				
42	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYMHVVRQAPGQRLEWMGWIDLENANTIYDAKFQGRV TITRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYDVWGQGTLLTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
43	DYYMH	44	WIDLENANTIYDAKFQ G	45	DAYGRYFYDV
SEQ ID	VL				

46	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYSRRTFGGGTKVEIK				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
47	KSSQSLLNARTGKN YLA	48	WASTRES	49	KQSYSRRT
WO2018208864					
SEQ ID	VH				
50	QVQLVQSGAEVKKPGASVKVSCASGFNIKDYIMHWVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTITRDTASASTAYMELSSLRSEDTAVYYCARDAYGRYFYDVWGQGTlVTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
51	DYIMH	52	WIDLENANTVYDAKFQG	53	DAYGRYFYDV
SEQ ID	VL				
54	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVEIK				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
55	KSSQSLLNARTGKN YLA	56	WASTRES	57	KQSYFRRT
WO2018208864					
SEQ ID	VH				
58	QVQLVQSGAEVKKPGASVKVSCASGFNIKDYIMHWVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTITRDTASASTAYMELSSLRSEDTAVYYCARDAYGQYFYDVWGQGTlVTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
59	DYIMH	60	WIDLENANTVYDAKFQG	61	DAYGQYFYDV
SEQ ID	VL				
62	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVEIK				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
63	KSSQSLLNARTGKN YLA	64	WASTRES	65	TQSYFRRT
US5834597A					
SEQ ID	VH				
66	QVQLVQSGAEVKKPGASVKVSCASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYDYDGFAYWGQGTlVTVSS				
SEQ ID	HCDR1 Sequence	SEQ ID	HCDR2 Sequence	SEQ ID	HCDR3 Sequence
67	SYTMH	68	YINPRSGYTHYNQKLKD	69	SAYDYDGFAY
SEQ ID	VL				
70	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQWSSNPPTFGGGTKVEIK				
SEQ ID	LCDR1 Sequence	SEQ ID	LCDR2 Sequence	SEQ ID	LCDR3 Sequence
71	SASSSVSYMN	72	DTSKLAS	73	QQWSSNPPT

Table 9: Other sequences in disclosure

SEQ ID	Nickname (source)	Sequence
1	Human IgM Constant region IMGT allele IGHM*03 (GenBank: pir S37768)	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDIS STRGFPSVLRGGKYAATSQVLLPSKDVMOGTDEHVCKVQHPNGNKEKNVP LPVIAELPPKVSFVPPRDGFFGNPRKSKLICQATGFSRQIQVSWLREGK QVSGSVTTDQVQAEAKESGPTYKVTSTLTIKESDWLSQSMFTCRVDHRL TFQQNASSMCPVDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTYYDSV TISWTRQNGEAVKTHTNISSEHPNATFSAVGEASICEDDWNSGERFTCTVT HTDLPSPLKQTI SRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFS PADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTG ETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
2	Human IgM Constant region IMGT allele IGHM*04 (GenBank: sp P01871.4)	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDIS STRGFPSVLRGGKYAATSQVLLPSKDVMOGTDEHVCKVQHPNGNKEKNVP LPVIAELPPKVSFVPPRDGFFGNPRKSKLICQATGFSRQIQVSWLREGK QVSGSVTTDQVQAEAKESGPTYKVTSTLTIKESDWLQSMFTCRVDHRL TFQQNASSMCPVDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTYYDSV TISWTRQNGEAVKTHTNISSEHPNATFSAVGEASICEDDWNSGERFTCTVT HTDLPSPLKQTI SRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFS PADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTG ETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
3	Human IgA1 heavy chain constant region, e.g., amino acids 144 to 496 of GenBank AIC59035.1	ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFPPQEPLSVTWSESGQGV TAR NFPFSQDASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVP VPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLGSEANLTCTLTGLR DASGVTFWTTPSSGKSAVQGPPELDLCGCYSVSSVLPGCAEPWNHKGFTTC TAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGF SPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTFFAVTSILRVAEDWK KGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY
4	Human IgA2 heavy chain constant region, e.g., amino acids 1 to 340 of GenBank P01877.4	ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFPPQEPLSVTWSESGQNV TAR NFPFSQDASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNSSQDVTVP VPPPPPCCHPRLSLHRPALEDLLGSEANLTCTLTGLRDASGATFTWTPSS GKSAVQGPPELDLCGCYSVSSVLPGCAQPWNHGETFCTAAHPELKTPLTA NITKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGS QELPREKYLTWASRQEPSQGTTFYAVTSILRVAEDWKKGETFSCMVGHEA LPLAFTQKTIDRMAGKPTHINVSVMMAEADGTCY
5	Precursor Human Secretory Component	MLLFVLTCLLAVFPAISTKSPIFGPEEVNSVEGNSVSITCYPPPTSVNRHT RKYWCRQGARGGCITLISSEGYVSSKYAGRANLTNFPENGTFVNVIAQLSQ DDSGRYKCGLGINSRGLSFDVSLEVSQGPGLLNDTKVYTVDLGRVTINCP FKTENAQKRKSLYKQIGLYPVLVIDSSGYVNPNTGRIRLDIQGTGQLLFS VVINQLRLSDAGQYLCQAGDDSNKKNADLQVLKPEPELVYEDLRGSVTF HCALGPEVANVAKFLCRQSSGENCDVVNTLGKRAPAFEGRIILLNPQDKDG SFSVVITGLRKEDAGRYLCGAHSDGQLQEGSPIQAWQLFVNEESTIPRST VVKGVAGGSVAVLCPYNRKESKSIKYWCLWEGAQNGRCPLLDVSEGWVKAQ YEGRLSLLEEPNGTFTVILNQLTSRDAGFYWCLTNGDTLWRTTVEIKIE GEPNLKVPGNVTAVLGETLKVPCFPCFESSYKEYWCKWNNTGCQALPSQD EGPSKAFVNCDENSRLVSLTLNLVTRADEGWYWCQGHFYGETAAVYVA VEERKAAGSRDVS LAKADAAPDEKVLDSGFREIENKAIQDPRLFAEEKAVA DTRDQADGSRASVDSGSSEEQGGSSRALVSTLVPLGLVLAVGAVGVARA RHRKNVDRVSI RSYRTDISMSDFENSREFGANDNMGASSITQETSLGGKEE FVATTESTTTETKEPKAKRSSKEEAEMAYKD FLLQSSTVAEEAQDGPQEA
6	Precursor Human J Chain	MKNHLLFWGVLAVFIKAVHVKAQEDERIVLDNKKCARITSRIIRSEDP NEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELD

		NQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMOVETALTP DACYPD
7	Mature Human J Chain	QEDERIVLVDNKCKCARITSRIIRSSSEDPNEDIVERNIRIIVPLNNRENIS DPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCY TYDRNKCYTAVVPLVYGGETKMOVETALTPDACYPD
8	J Chain Y102A mutation	QEDERIVLVDNKCKCARITSRIIRSSSEDPNEDIVERNIRIIVPLNNRENIS DPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCA TYDRNKCYTAVVPLVYGGETKMOVETALTPDACYPD
9	"5" Peptide linker	GGGGS
10	"10" Peptide linker	GGGSGGGGS
11	"15" Peptide linker	GGGSGGGSGGGGS
12	"20" Peptide linker	GGGSGGGSGGGSGGGGS
13	"25" Peptide Linker	GGGSGGGSGGGSGGGSGGGGS
14	human CD123 isoform 1 precursor NCBI Reference Sequence: NP_002174.1	MVLLWLTLILLALPCLLQTKEDPNPPITNLRMKAKAQQLTWDLNRNVTDIE CVKDADYSMPAVNNSYCQFGAISLCEVTNYTVRVANPPFSTWILFPENSGK PWAGAENLTCWIHDVDFLSCSWAVGPGAPADVQYDLYLVNANRRQQYECLH YKTDAGQTRIGCRFDDISRLSSGSQSSHILVRGRSAAFGIPCTDKFVVSQ IEILTPPNMTAKCNKTHSFMHWKMRSHFNKFRYELQIQKRMQPVITEQVR DRTSFQLLNPGTYTVQIRARERVYEFLSAWSTPQRFECDOEEGANTRAWRT SLLIAGLTLLALVCVFVICRRLVLMQRLFPRI PHMKDPIGDSFQNDKLVVW EAGKAGLEECLVTEVQVVQKT
15	human CD123 isoform 2 precursor NCBI Reference Sequence: NP_001254642.1	MVLLWLTLILLALPCLLQTKEGGKPWAGAENLTCWIHDVDFLSCSWAVGPG APADVQYDLYLVNANRRQQYECLHYKTDAGQTRIGCRFDDISRLSSGSQSS HILVRGRSAAFGIPCTDKFVVSQIEILTPPNMTAKCNKTHSFMHWKMRSH FNKFRYELQIQKRMQPVITEQVRDRTSFQLLNPGTYTVQIRARERVYEFL SAWSTPQRFECDOEEGANTRAWRTSLLIAGLTLLALVCVFVICRRLVLMQRL LFPRI PHMKDPIGDSFQNDKLVVWEAGKAGLEECLVTEVQVVQKT
16	Cyno CD123 GenBank: EHH61867.1	MTLLWLTLILLVATPCLLRKEDPNAPIRNLRMKEKAQQLMWDLNRNVTDVE CIKGTDYSPAMNNSYCQFGAISLCEVTNYTVRVASPPFSTWILFPENSGT PRAGAENLTCWVHDVDFLSCSWVVGPAAPADVQYDLYLVNPNNSHEQYRCLH YKTDARGTQIGCRFDDIAPLSRGSQSSHILVRGRSAAVSIPTDKFVVSQ IERLTPPNMTGECNETHSFMHWKMKSHFNKFRYELRIQKRMQPVITEQVR DRTSFQLLNPGTYTVQIRARETVYEFLSAWSTPQRFECDOEEGASSRAWRT SLLIAGLTLLALLCVFLICRRLVLMQRLFPRI PHMKDPIGDTFQQDKLVVW EAGKAGLEECLVSEVQVVEKT
17	Mouse CD123 NCBI Reference Sequence: NP_032395.1	MAANLWLILGLASHSSDLAAVREAPPTAVTTPIQNLHIDPAHYTLSDWPA PGADITTGAFCKRGRDIFVWADPGLARCSFQSLSLCHVTNFTVFLGKDRV AGSIQFPDDDDGHEAAQDLRCWVHEGQLSCQWERGPKATGDVHYRMFWR DVR LGPAHNRECPHYHSLDVNTAGPAPHGGHEGCTLDLDTVLGSTPNSPDL VPQVTITVNGSGRAGPVPCMDNTVDLQRAEVLAPPTLTVECNSEAHARWV ARNRFHHGLLGYTLQVNQSSRSEPQEYNVSI PHFWVPNAGAISFRVKSRS E VYPRKLSSWSEAWGLVCPPEVMPVKLTALVTSVATVLGAGLVAAGLLLWWRK SLLYRLCPPIPRRLPLAGEMVWWEPALEDCEVTPVTD
84	anti-CD3 + J Chain Y102A mutation	EVQLLESGGGLVQPGGSLRLSCAASGFTFTDYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQMESLRADTA VYYCVRHANFGAGYVSWFAHWGQGLTVTVSSGGGSGGGSGGGGSQ TVVTQEPLSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQT PGQAPRG LIGGTDKRAPGV PDRFSGSL LGDKAALTITGAQAEDEADYYCALWYS NHWVFGGGTKLTVLGGGSGGGSGGGSGGGSGQEDERIVLVDNKCKCARI TSRIIRSSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHL

		SDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCATYDRNKCYTA VVPLVYGGETKMOVETALTPDACYPD
85	CD123 VH of CD123XCD3 IgG#1 US 9856327 B2	QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYIMKWVKQSHGKSLEW MGDIIPSNGATFYNQKFKGKATLTVDRSTSTAYMELSSLRSEDVAVY YCARSHLLRASWFAYWGQGTLLVTVSS
86	CD123 VH of CD123XCD3 IgG#1 US 9856327 B2	DFVMTQSPDSLAVSLGERATINCKSSQSLNLTGNQKNYLTWYQQKPG QPPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISLQAEDVAVYYC QNDYSYPYTFGGGTKLEIK
87	CD3 scFv of CD123XCD3 IgG#1 US 9856327 B2	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEW VGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTA VYYCVRHGNFGDSYVSWFAYWGQGTLLVTVSSGKPGSGKPGSGKPGSG KPGSQAVVTQEPSTLVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPG KSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYC ALWYSNHWVFGGGTKLTVL

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment or derivative thereof comprising an antigen-binding domain that specifically binds to CD123, wherein the antigen-binding domain comprises a heavy chain variable region (VH) and light chain variable region (VL), wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79, SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83.

2. The antibody or fragment or derivative thereof of claim 1, which comprises a single bivalent binding unit comprising two antigen-binding domains wherein at least one antigen-binding domain specifically binds to CD123, wherein the binding unit comprises two heavy chains each comprising a heavy chain constant region or fragment or variant thereof, and wherein at least one heavy chain constant region or fragment or variant thereof of the binding unit is associated with a copy of the VH.

3. A multimeric antibody comprising five, six, or two bivalent binding units and ten, twelve, or four antigen-binding domains wherein at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen-binding domains specifically binds to CD123;

wherein the antigen-binding domain comprises a heavy chain variable region (VH) and light chain variable region (VL), wherein the VH and VL comprise, respectively, the amino acid sequences SEQ ID NO: 76 and SEQ ID NO: 79, SEQ ID NO: 77 and SEQ ID NO: 79, SEQ ID NO: 78 and SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 83, SEQ ID NO: 81 and SEQ ID NO: 83, and SEQ ID NO: 82 and SEQ ID NO: 83,

wherein each binding unit comprises two heavy chains each comprising an IgM or IgA constant region or a multimerizing fragment or variant thereof, and wherein at least three, four, five, six, seven, eight, nine, ten, eleven, or twelve heavy chain constant regions of the multimeric antibody is associated with a copy of the VH.

4. The multimeric antibody of claim 3, wherein each heavy chain constant region or multimerizing fragment or variant thereof is associated with a copy of the VH.

5. The multimeric antibody of claim 4, wherein each binding unit further comprises two light chains each comprising a light chain constant region or fragment or variant

thereof, and wherein at least three, four, five, six, seven eight, nine, ten, eleven, or twelve light chain constant regions or fragments or variants thereof is/are associated with a copy of the VL.

6. The multimeric antibody of claim 3, which is pentameric or hexameric and comprises five or six bivalent IgM binding units, wherein each binding unit comprises a C μ 4 domain and a μ -tail piece (μ tp) domain or multimerizing fragment or variant thereof.

7. The multimeric antibody of claim 6, wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each further comprise a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof.

8. The multimeric antibody of claim 7, wherein each IgM heavy chain constant region is a human IgM constant region or multimerizing variant or fragment thereof, comprising the amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, or a multimerizing variant or fragment thereof.

9. The multimeric antibody of any one of claims 3 to 8, which is pentameric, and further comprises a J chain, or fragment thereof, or variant thereof.

10. The multimeric antibody of claim 3, which is dimeric and comprises two bivalent IgA binding units and a J chain or fragment or variant thereof, wherein each binding unit comprises a C α 1 domain, a C α 2 domain, an IgA hinge region, a C α 3 domain and an α -tail piece (α tp) domain.

11. The multimeric antibody of claim 9 or claim 10, wherein the J-chain or fragment or variant thereof is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 7 or a fragment thereof, or a variant thereof.

12. The multimeric antibody of claim 11, wherein the J-chain or fragment thereof is a variant J-chain comprising an amino acid substitution at the amino acid position corresponding to amino acid Y102 of SEQ ID NO: 7, and wherein an IgM antibody comprising the variant J-chain exhibits an increased serum half-life upon administration to an animal relative to a reference IgM antibody that is identical except for the amino acid substitution in the J-chain, and is administered in the same way to the same animal species.

13. The multimeric antibody of claim 12, wherein the amino acid position corresponding to amino acid Y102 of SEQ ID NO: 7 is substituted with alanine (A), and wherein the variant J-chain comprises the amino acid sequence SEQ ID NO: 8.

14. The multimeric antibody of any one of claims 9 to 13, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, wherein the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof.

15. The multimeric antibody of claim 14, wherein the heterologous moiety is a heterologous polypeptide fused to the J-chain or fragment or variant thereof.

16. The multimeric antibody of claim 15, wherein the heterologous polypeptide is an antibody antigen-binding domain, or a subunit thereof.

17. The multimeric antibody of claim 16, wherein the antibody antigen-binding domain comprises a scFv fragment.

18. The multimeric antibody of claim 17, wherein the antibody antigen-binding domain binds to CD3.

19. A composition comprising the antibody or fragment or derivative thereof of claim 1 or the multimeric antibody of any one of claims 3 to 18.

20. A polynucleotide comprising a nucleic acid sequence that encodes the antibody or fragment or derivative thereof of claim 1 or the multimeric antibody of any one of claims 3 to 18 or a subunit thereof.

21. A vector comprising the polynucleotide of claim 20.

22. A host cell comprising the vector of claim 21.

23. A method of producing the antibody or fragment or derivative thereof of claim 1 or the multimeric antibody of any one of claims 3 to 18, comprising culturing the host cell of claim 22, and recovering the antibody or fragment or derivative thereof or the multimeric antibody.

24. A method of treating cancer comprising administering to a subject in need of treatment an effective amount of the antibody or fragment or derivative thereof of claim 1 or the multimeric antibody of any one of claims 3 to 18.

25. The method of claim 24, wherein the subject is human.

26. The method of claim 24 or claim 25, wherein the cancer is a hematological cancer.

27. The method of claim 26, wherein the hematological cancer is acute myeloid leukemia (AML).