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(71) Applicant: **LAVA THERAPEUTICS N.V.** [NL/NL]; Yalelaan 62, 3584 CM Utrecht (NL).

(72) Inventors: **VAN HELDEN, Paula Maria Wilhelmina**; Yalelaan 62, 3584 CM Utrecht (NL). **ROOVERS, Robertus Cornelis**; Yalelaan 62, 3584 CM Utrecht (NL). **LUTJE HULSIK, David**; Yalelaan 62, 3584 CM Utrecht (NL). **VAN DER VLIET, Johannes Jelle**; Yalelaan 62, 3584 CM Utrecht (NL). **ADANG, Anton Egbert Peter**; Yalelaan 62, 3584 CM Utrecht (NL). **DE JONG, Milon**; Yalelaan 62, 3584 CM Utrecht (NL). **SALLER, Manfred**; Yalelaan 62, 3584 CM Utrecht (NL).

(74) Agent: **COOLEY (UK) LLP**; 22 Bishopsgate, London, Greater London EC2N 4BQ (GB).

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(54) Title: NECTIN-4 BINDING AGENTS AND METHODS OF USE

(57) Abstract: Provided herein are Nectin-4 binding agents, multi-specific binding agents thereof, polynucleotides encoding the same, and compositions comprising the same. The present disclosure further provides methods of treating cancer in a subject in need thereof comprising administration of the Nectin-4 binding agents and multi-specific binding agents comprising the same to a subject in need thereof.

WO 2024/200573 A1

NECTIN-4 BINDING AGENTS AND METHODS OF USE

CROSS- REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application 63/492,311, filed March 27, 2023; and EP Application 23164347.9 filed March 27, 2023, and the contents of each are incorporated herein by reference in their entireties.

REFERENCE TO SEQUENCE LISTING

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: LVAT_015_01WO_SeqList_ST26.XML; date recorded: March 25, 2024; file size 264,665 bytes).

FIELD

[0003] The present disclosure provides Nectin-4 binding agents and multi-specific binding agents comprising the same. The present disclosure further provides methods of treating cancer in a subject in need thereof comprising administration of the Nectin-4 binding agents or multi-specific binding agents comprising the same to a subject in need thereof.

BACKGROUND

[0004] Nectin-4, also known as poliovirus receptor-like 4 (PVRL4), is a type 1 transmembrane 66 kDa polypeptide member of the Nectin family. In adults, human Nectin-4 is mainly expressed in the placenta with low expression in other tissues including the skin, bladder, salivary gland, esophagus, breast, stomach, prostate, lung, and trachea. Nectin-4 is involved in forming and maintaining adherens junctions together with cadherins and is a stimulatory co-receptor for the prolactin receptor (Heath and Rosenberg Nature Reviews Urology 18, 93–103 (2021).

[0005] Aberrant expression of Nectin-4 has been observed in several cancer types, including bladder, breast, lung, pancreatic and ovarian cancer. Nectin-4 has been associated with promoting cancer cell proliferation and metastasis via activation of WNT–β-catenin and Rac small G protein in the PI3K–AKT signaling pathway (Siddharth, S. et al. Int. J. Biochem. Cell Biol. 89, 85–94 (2017); Zhang et al. Hum. Pathol. 72:107-116 (2018). Nectin-4 also interacts with the tyrosine kinase receptor ERBB2 to promote its activation, resulting in stimulation of the PI3K–AKT signalling pathway (Kedashiro et al. Sci. Rep. 9, 18997 (2019).

[0006] Nectin-4 targeting antibodies have been described. Enfortumab vedotin is an anti-Nectin-4 antibody linked to the cytotoxic agent monomethyl auristatin E approved for the treatment of urothelial cancer.

[0007] WO 2021/257525 (BioAtla) describes CD3-binding T-cell engaging antibodies that target Nectin-4. Bispecific T-cell engaging antibodies have a tumor target binding specificity and a CD3-directed T-cell binding specificity and thus boost efficacy by re-directing T-cell cytotoxicity to malignant cells, see e.g. Huehls et al. (2015) Immunol Cell Biol 93:290; Ellerman (2019) Methods, 154:102. However, results vary significantly. For example, in one study in which a CD3 binding moiety was combined with binding moieties against 8 different B-cell targets (CD20, CD22, CD24, CD37, CD70, CD79b, CD138 and HLA-DR), it was found that the bispecific antibodies targeting the different tumor targets showed strong variation in their capacity to induce target cell cytotoxicity and that cytotoxicity did not correlate with antigen expression levels. For example, CD3-based bispecific antibodies targeting HLA-DR or CD138 were not able to induce cytotoxicity, in spite of intermediate to high HLA-DR and CD138 expression levels (Engelberts et al. (2020) Ebiomedicine 52:102625). Few T-cell redirecting therapies have reached late-stage clinical development, possibly due to significant toxicity, manufacturing problems, immunogenicity, narrow therapeutic windows and low response rates. In particular, toxicity may occur when the T-cell engager includes a CD3 binding arm and results in uncontrolled and exaggerated immune activation and cytokine release.

SUMMARY

[0008] Thus, while significant progress has been made, there is still a need for novel Nectin-4 targeting agents, such as T-cell engaging Nectin-4 targeting binding agents, that are therapeutically effective against a range of (target) tumor cells yet have acceptable toxicity, as well as good stability, developability and manufacturability properties.

[0009] The present disclosure provides binding agents comprising a Nectin-4 antigen-binding domain. Such binding agents can target cancer cells that overexpress Nectin-4. The present disclosure further provides multi-specific binding agents comprising a Nectin-4 antigen-binding domain and a γδ T cell antigen-binding domain. In some embodiments, such multi-specific binding agents target the tumor cells to activated γδ T cells.

[0010] In some aspects disclosed herein is a single domain antibody that binds to Nectin-4 comprising: (a) a complementarity determining region (CDR)1 comprising an amino acid sequence selected from SEQ ID NOs: 88, 95, 103, 110, 117, 121, 125, 129, and 133; (b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 89, 96, 104, 111, 118, 122, 126, 130, and 134; and (c) a CDR3 comprising an amino acid sequence selected from 90, 97, 105, 112, 119, 123, 127, 131, and 135. In some embodiments, the CDR1, CDR2, and CDR3 sequences are selected from a combination disclosed in **Table 1A**. In some embodiments, the

single domain antibody comprises an amino acid sequence that is at least 90%, or at least 95% identical to any one of SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136. In some embodiments, the single domain antibody comprises SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136. In some embodiments, the single domain antibody consists of SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136. In some embodiments, the CDR1 comprises SEQ ID NO: 88, the CDR2 comprises SEQ ID NO: 89, and the CDR3 comprises SEQ ID NO: 90. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 91, 92, 93, and 94. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 91, 92, 93, and 94. In some embodiments, the CDR1 comprises SEQ ID NO: 95, the CDR2 comprises SEQ ID NO: 96, and the CDR3 comprises SEQ ID NO: 97. In some embodiments, the single antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 98, 99, 100, 101, and 102. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 98, 99, 100, 101, and 102. In some embodiments, the CDR1 comprises SEQ ID NO: 103, the CDR2 comprises SEQ ID NO: 104, and the CDR3 comprises SEQ ID NO: 105. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 106, 107, 108, and 109. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 106, 107, 108, and 109. In some embodiments, the CDR1 comprises SEQ ID NO: 110, the CDR2 comprises SEQ ID NO: 111, and the CDR3 comprises SEQ ID NO: 112. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 113, 114, 115, and 116. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 113, 114, 115, and 116. In some embodiments, the CDR1 comprises SEQ ID NO: 117, the CDR2 comprises SEQ ID NO: 118, and the CDR3 comprises SEQ ID NO: 119. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 120. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of SEQ ID NO: 120. In some embodiments, the CDR1 comprises SEQ ID NO: 121, the CDR2 comprises SEQ ID NO: 122, and the CDR3 comprises SEQ ID NO: 123. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to SEQ

ID NO: 124. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of SEQ ID NO: 124. In some embodiments, the CDR1 comprises SEQ ID NO: 125, the CDR2 comprises SEQ ID NO: 126, and the CDR3 comprises SEQ ID NO: 127. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 128. In some embodiments, the single domain antibody comprises or consist of the amino acid sequence of SEQ ID NO: 128. In some embodiments, the CDR1 comprises SEQ ID NO: 129, the CDR2 comprises SEQ ID NO: 130, and the CDR3 comprises SEQ ID NO: 131. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 132. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of SEQ ID NO: 132. In some embodiments, the CDR1 comprises SEQ ID NO: 133, the CDR2 comprises SEQ ID NO: 134, and the CDR3 comprises SEQ ID NO: 135. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 136. In some embodiments, the single domain antibody comprises or consists of the amino acid sequence of SEQ ID NO: 136. In some embodiments, the CDR1 comprises SEQ ID NO: 95, CDR3 comprises SEQ ID NO: 96, and CDR3 comprises SEQ ID NO: 97. In some embodiments, the single domain antibody comprises an amino acid sequence that is at least 90%, or at least 95% identical to SEQ ID NO: 99. In some embodiments, the single domain antibody comprises SEQ ID NO: 99. In some embodiments, the single domain antibody consists of SEQ ID NO: 99.

[0011] In some aspects disclosed herein is a polynucleotide encoding the single domain antibody of any one of the above aspects or embodiments.

[0012] In some aspects disclosed herein is a vector comprising the polynucleotide of any one of the above aspects or embodiments.

[0013] In some aspects disclosed herein is a host cell comprising the polynucleotide of any one of the above aspects or embodiments or the vector of any one of the above aspects or embodiments.

[0014] In some aspects disclosed herein is a method of producing the single domain antibody of any one of the above aspects or embodiments, comprising: (a) introducing the vector of any one of the above aspects or embodiments into a host cell under conditions permitting expression of the polynucleotide encoding the single domain antibody; and (b) isolating the single domain antibody from the culture supernatant.

[0015] In some aspects disclosed herein is a composition comprising the single domain antibody of any one of the above aspects or embodiments and a pharmaceutically acceptable carrier.

[0016] In some aspects disclosed herein is a binding agent comprising the single domain antibody of any one of the above aspects or embodiments.

[0017] In some aspects disclosed herein is a multi-specific binding agent comprising: (a) a first antigen-binding domain that specifically binds to human Nectin-4; and comprises the single domain antibody of any one of the above aspects or embodiments; and (b) a second antigen-binding domain that specifically binds to an antigen expressed on an immune cell. In some embodiments, the second antigen-binding domain specifically binds to a target antigen selected from CD3, CD2, an αδ T cell receptor, a γδ T cell receptor (e.g., Vδ2 TCR or Vδ1 TCR). In some embodiments, the first and second antigen-binding domains are present in the same polypeptide chain. In some embodiments, the first antigen-binding domain and second antigen-binding domain are covalently linked via a peptide linker. In some embodiments, the first and second antigen-binding domains are present on different polypeptide chains. In some embodiments, the multi-specific binding agent further comprises an Fc domain comprising a first and a second Fc domain monomer. In some embodiments, the multi-specific binding agent comprises: (a) a first polypeptide comprising the first antigen-binding domain that binds to a human Nectin-4 protein and the first Fc domain monomer; and (b) a second polypeptide comprising the second antigen-binding domain that binds to a human Vδ2 T cell receptor and the second Fc domain monomer. In some embodiments, the human Vδ2 T cell receptor is a Vγ9Vδ2 T cell receptor. In some embodiments, the antigen-binding domain that binds to the Vγ9Vδ2 T cell receptor comprises SEQ ID NO: 22. In some embodiments, the antigen-binding domain that binds to the Vγ9Vδ2 T cell receptor consists of SEQ ID NO: 22. In some embodiments, the multi-specific binding agent induces proliferation of Vγ9Vδ2 T cells. In some embodiments, the first and second Fc domain monomers comprise a mutation at position 234 and/or 235 according to the EU numbering system. In some embodiments, the first and second Fc domain monomers comprise an L234F and an L235E substitution. In some embodiments, the first Fc domain monomer comprises a T366W substitution, and the second Fc domain monomer comprises T366S, L368A and Y407V substitutions, or vice versa, according to the EU numbering system; and/or the cysteine residues at position 220 according to the EU numbering system in the first and second Fc domain monomers have been deleted or substituted.

[0018] In some aspects disclosed herein is a multi-specific binding agent comprising (a) a first antigen-binding domain that binds to human Nectin-4, where the first antigen-binding domain comprises a complementarity determining region (CDR)1 comprising SEQ ID NO: 95, a CDR2 comprising SEQ ID NO: 96, and a CDR3 comprising SEQ ID NO: 97; and (b) a second antigen-binding domain that binds to Vδ2, where the second antigen-binding domain comprises a CDR1

comprising SEQ ID NO: 18, a CDR2 comprising SEQ ID NO: 19, and a CDR3 comprising SEQ ID NO: 20. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 99 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first and second antigen-binding domains are comprised in the same polypeptide chain.

[0019] In some aspects disclosed herein is a multi-specific binding agent comprising (a) a first polypeptide comprising (i) a first antigen-binding domain that binds to a human Nectin-4 protein and comprises a complementarity determining region (CDR)1 comprising SEQ ID NO: 95, a CDR2 comprising SEQ ID NO: 96, and a CDR3 comprising SEQ ID NO: 97 and (ii) a first Fc domain monomer; (b) a second polypeptide comprising (i) a second antigen-binding domain that binds to a human Vδ2 T cell receptor and comprises a CDR1 comprising SEQ ID NO: 18, a CDR2 comprising SEQ ID NO: 19, and a CDR3 comprising SEQ ID NO: 20 and (ii) a second Fc domain monomer. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 231 and the second Fc domain monomer comprises SEQ ID NO: 230. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 230 and the second Fc domain monomer comprises SEQ ID NO: 231. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 276. In some embodiments, the second antigen-binding domain comprises SEQ ID NO: 280. In some embodiments, (a) the first polypeptide comprises SEQ ID NO: 276; and (b) the second polypeptide comprises SEQ ID NO: 280.

[0020] In some aspects disclosed herein is a polynucleotide encoding the multi-specific binding agent of any one of the above aspects or embodiments.

[0021] In some aspects disclosed herein is an expression vector comprising the polynucleotide of any one of the above aspects or embodiments.

[0022] In some aspects disclosed herein is a host cell comprising the polynucleotide of any one of the above aspects or embodiments or the expression vector of any one of the above aspects or embodiments. In some embodiments, the cell is a CHO cell.

[0023] In some aspects disclosed herein is a method of producing the multi-specific binding agent of any one of the above aspects or embodiments, comprising culturing the host cell of any one of the above aspects or embodiments under conditions sufficient to express the multi-specific binding agent from the polynucleotide or expression vector therein and purifying the expressed multi-specific binding agent from the culture supernatant.

[0024] In some aspects disclosed herein is a pharmaceutical composition comprising the single domain antibody of any one of the above aspects or embodiments, the binding agent of any one of the above aspects or embodiments, or the multi-specific binding agent of any one of the above aspects or embodiments, and a pharmaceutically-acceptable excipient.

[0025] In some aspects disclosed herein is a method of treating a cancer in a subject in need thereof, comprising administering to the subject the single domain antibody of any one of the above aspects or embodiments, the binding agent of any one of the above aspects or embodiments, or the multi-specific binding agent of any one of the above aspects or embodiments, or the pharmaceutical composition of any one of the above aspects or embodiments. In some embodiments, the subject is human. In some embodiments, the subject is suffering from a bladder cancer, urothelial cell cancer, head and neck cancer, choriocarcinoma, epidermoid carcinoma, or colorectal adenocarcinoma.

[0026] In some aspects disclosed herein is a method of expanding Vγ9Vδ2+ T cells comprising contacting the Vγ9Vδ2+ T cells with single domain antibody of any one of the above aspects or embodiments, the binding agent of any one of the above aspects or embodiments, or the multi-specific binding agent of any one of the above aspects or embodiments, or the pharmaceutical composition of any one of the above aspects or embodiments.

[0027] In some aspects disclosed herein is a method increasing the stability of a protein, wherein the protein is modified at the first position to a non-pyroglutamate forming amino acid. In some embodiments, the non-pyroglutamate forming amino acid is aspartic acid.

BRIEF DESCRIPTION OF THE FIGURES

[0028] FIG. 1A-FIG. 1B show the expression of Nectin-4 (**FIG. 1A**) and percentage of $Vγ9^{+}Vδ2^{+}$ cells out of total $CD3^{+}$ T cells (**FIG. 1B**) in different cancer tissues. (m)CRC = (metastatic) colorectal cancer; ccRCC = clear cell renal cell cancer; GBM = glioblastoma multiforme; AC = adenocarcinoma; SCC = squamous cell carcinoma; H&N = head & neck cancer; UCC = urothelial cell carcinoma.

[0029] FIG. 2A-FIG. 2D show the binding profile of LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185, LV1186, and LV1187 binding agents to CHO cells that were (**FIG. 2A** and **FIG. 2C**) and were not transfected (**FIG. 2B** and **FIG 2D**) with human Nectin-4.

[0030] FIG. 3A-FIG. 3D show the binding profile of LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185, LV1186, and LV1187 binding agents to cynomolgus Nectin-4-transfected CHO cells (FIG. 3A and FIG. 3B) or murine Nectin-4-transfected CHO cells (**FIG. 3C** and **FIG. 3D**).

[0031] FIG. 4A-FIG. 4H show the binding profile of LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185, LV1186, and LV1187 binding agents to BeWo cells (**FIG. 4A-FIG. 4B**), A-431 cells (**FIG. 4C-FIG.4D**), HT-29 cells (**FIG. 4E-FIG.4F**), and U-251 cells (**FIG. 4G-FIG.4H**).

[0032] FIG. 5A-FIG. 5B show the binding profile of multi-specific binding agents comprising LV1181, LV1184, LV1185, or LV1186 with a Vδ2-binding arm to A-431 cells (**FIG. 5A**) and HT-29 cells (**FIG. 5B**).

[0033] FIG. 6 shows the binding profile of multi-specific binding agents comprising LV1181, LV1184, LV1185, or LV1186 with a Vδ2-binding arm to Vγ9Vδ2 T cells.

[0034] FIG. 7A-FIG. 7B show the Vγ9Vδ2 T cell degranulation (**FIG. 7A**) and cytotoxicity (**FIG. 7B**) in A-431 cells expressing Nectin-4 induced by increasing concentrations of multi-specific binding agents comprising LV1181, LV1184, LV1185, or LV1186 with a Vδ2-binding arm in the presence or absence of Vγ9Vδ2 T cells.

[0035] FIG. 8A-FIG 8B show the expression of Nectin-4 in malignant urothelial cell carcinoma (UCC) patient tissue and donor-matched non-malignant bladder tissue. **FIG. 8A** shows Nectin-4 expression as measured by the mean fluorescence intensity (MFI) as expressed by malignant (UCC) and non-malignant bladder tissue. **FIG. 8B** shows the percent Nectin-4 expression of malignant (UCC) and non-malignant bladder tissue.

[0036] FIG. 9A-FIG. 9B show the percent tumor lysis (**FIG. 9A**) and Vγ9Vδ2 T cell degranulation (**FIG. 9B**) in head and neck and urothelial cell cancers and Nectin-4$^-$ tumors that are incubated with Vγ9Vδ2 T cells and with or without LAVA-366.

[0037] FIG. 10 shows the absence of tumor cell lysis when LAVA-366 is incubated with non-malignant Nectin-4$^+$ urothelial tissue.

[0038] FIG. 11 shows the effect of LAVA-366 on tumor cell lysis of bladder cancers when incubated in the absence of additional expanded (allogeneic) Vγ9Vδ2-T cells.

[0039] FIG. 12 illustrates the different CDR numbering systems (Kabat, Chothia, IMGT, and Combined) for the 6H4 VHH.

[0040] FIG. 13 shows a chromatograph that illustrates the retention of pyroglutamate containing and non-pyroglutamate containing multi-specific binding agents in reverse-phase HPLC.

DETAILED DESCRIPTION

Overview

[0041] The present disclosure provides binding agents that specifically bind Nectin-4 and multi-specific binding agents comprising the same, and methods of treating disorders (*e.g.,* cancer) using the binding agents and/or multi-specific binding agents described herein.

Definitions

[0042] The terms used in this specification generally have their ordinary meaning in the art, within the context of this disclosure and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the disclosure and how to make and use them. The scope and meaning of any use of a term will be apparent from the specific context in which the term is used. As such, the definitions set forth herein are intended to provide illustrative guidance in ascertaining particular embodiments of the disclosure, without limitation to particular compositions or biological systems. All publications, patent applications, patents, GenBank or other accession numbers and other references mentioned herein are incorporated by reference in their entirety for all purposes.

[0043] The terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims, unless clearly indicated otherwise.

[0044] The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0045] As used herein, the terms "antibody" and "binding agent" are used interchangeably and refer to a polypeptide or complex of polypeptides comprising at least one antigen-binding domain that specifically binds to a target antigen and includes intact immunoglobulin molecules, antigen-binding fragments thereof, and derivatives of either. Binding agents, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies and humanized antibodies, and antibody fragments provided by any known technique, such as enzymatic cleavage, peptide synthesis and recombinant techniques. In some embodiments a binding agent has the ability to specifically bind to an antigen under typical physiological conditions with a half-life of significant periods of time, such as at least about 30 minutes, at least about one hour, at least about two hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with binding agent binding to the antigen and/or time sufficient for the antibody or binding agent to recruit an effector).

[0046] As used herein, the term "multi-specific" refers to the capability of a binding agent described herein to specifically bind two or more different target antigens or to specifically bind two or more different epitopes on a target antigen.

[0047] Herein, the terms "multi-specific binding agent" and "multi-specific antibody" are used interchangeably and refer to a binding agent comprising two or more antigen-binding domains (e.g., two, three, four, or more), each binding to a different target antigen or to a different epitope on the same target antigen.

[0048] Herein, the terms "bispecific binding agent" and "bispecific antibody" are used interchangeably and refer to a binding agent comprising not more than two antigen-binding domains, each binding to a different target antigen or to a different epitope on the same target antigen.

[0049] Examples of different classes of bispecific binding agents include but are not limited to (i) IgG-like molecules with complementary CH3 domains to force heterodimerization; (ii) recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; (iii) IgG fusion molecules, wherein full length IgG antibodies are fused to extra Fab fragment or parts of Fab fragment; (iv) Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc regions, or parts thereof; (v) Fab fusion molecules, wherein different Fab fragments are fused together, fused to heavy-chain constant-domains, Fc regions or parts thereof; and (vi) scFv-and diabody-based and heavy chain-based antibodies (e.g., domain antibodies, Nanobodies®) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, Nanobodies®) are fused to each other or to another protein or carrier molecule fused to heavy-chain constant-domains, Fc regions or parts thereof.

[0050] Examples of IgG-like molecules with complementary CH3 domains molecules include but are not limited to the Triomab® (Trion Pharma/Fresenius Biotech), the Knobs-into-Holes (Genentech), CrossMAbs (Roche) and the electrostatically-matched (Amgen, Chugai, Oncomed), the LUZ-Y (Genentech, Wranik et al. J. Biol. Chem. 2012, 287(52): 43331-9, doi: 10.1074/jbc.M112.397869. Epub 2012 Nov 1), DIG-body and PIG-body (Pharmabcine, WO2010134666, WO2014081202), the Strand Exchange Engineered Domain body (SEEDbody)(EMD Serono), the Biclonics (Merus, WO2013157953), FcΔAdp (Regeneron), bispecific IgG1 and IgG2 (Pfizer/Rinat), Azymetric scaffold (Zymeworks/Merck,), mAb-Fv (Xencor), bivalent bispecific antibodies (Roche, WO2009080254) and DuoBody® molecules (Genmab).

[0051] Examples of recombinant IgG-like dual targeting molecules include, but are not limited to, Dual Targeting (DT)-Ig (GSK/Domantis, WO2009058383), Two-in-one Antibody (Genentech, Bostrom, et al 2009. Science 323, 1610–1614), Cross-linked Mabs (Karmanos Cancer Center),

mAb2 (F-Star), ZybodiesTM (Zyngenia, LaFleur et al. MAbs. 2013 Mar-Apr;5(2):208-18), approaches with common light chain, κλBodies (NovImmune, WO2012023053), US2020319181 (Merus) and CovX-body® (CovX/Pfizer, Doppalapudi, V.R., et al 2007. Bioorg. Med. Chem. Lett. 17,501–506).

[0052] Examples of IgG fusion molecules include but are not limited to Dual Variable Domain (DVD)-Ig (Abbott), Dual domain double head antibodies (Unilever; Sanofi Aventis), IgG-like Bispecific (ImClone/Eli Lilly, Lewis et al. Nat Biotechnol. 2014 Feb;32(2):191-8), Ts2Ab (MedImmune/AZ, Dimasi et al. J Mol Biol. 2009 Oct 30;393(3):672-92) and BsAb (Zymogenetics, WO2010111625), HERCULES (Biogen Idec), scFv fusion (Novartis), scFv fusion (Changzhou Adam Biotech Inc) and TvAb (Roche).

[0053] Examples of Fc fusion molecules include but are not limited to scFv/Fc Fusions (Academic Institution, Pearce et al Biochem Mol Biol Int. 1997 Sep;42(6):1179), SCORPION (Emergent BioSolutions/Trubion, Blankenship JW, et al. AACR 100th Annual meeting 2009 (Abstract #5465); Zymogenetics/BMS, WO2010111625), Dual Affinity Retargeting Technology (Fc-DARTTM) (MacroGenics) and Dual(ScFv)2-Fab (National Research Center for Antibody Medicine – China).

[0054] Examples of Fab fusion bispecific antibodies include but are not limited to F(ab)2 (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock® (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech).

[0055] Examples of scFv-, diabody-based and domain antibodies include but are not limited to Bispecific T Cell Engager (BiTE®) (Micromet, Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DARTTM) (MacroGenics), Single-chain Diabody (Academic, Lawrence FEBS Lett. 1998 Apr 3;425(3):479-84), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack, WO2010059315) and COMBODY molecules (Epigen Biotech, Zhu et al. Immunol Cell Biol. 2010 Aug;88(6):667-75), dual targeting nanobodies® (Ablynx, Hmila et al., FASEB J. 2010), dual targeting heavy chain only domain antibodies.

[0056] The term “human Nectin-4”, when used herein, refers to the human Nectin-4 protein (UniProt Q96NY8 · NECT4_HUMAN). The sequence of human Nectin-4 is set forth in SEQ ID NO:265.

[0057] As used herein, the term “antigen-binding domain” or “antigen-binding region” refers to a portion of a binding agent that specifically binds a target antigen or target epitope. Antigen-binding domains may comprise variable regions of both the heavy and light chains of an antibody or antigen-binding fragments thereof, including single domain antibodies. Single-domain antibodies may contain only the variable domain of an immunoglobulin chain, i.e. CDR1, CDR2, and CDR3 and framework regions.

[0058] The term "antigen-binding fragment," as used herein refers to a polypeptide comprising at least one complementarity determining region (CDR) that binds to at least one epitope of an antigen of interest (*e.g.*, Nectin-4). In this regard, an antigen-binding fragment may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a variable heavy chain (VH) and/or variable light chain (VL) sequence. Antigen-binding fragments include proteins that comprise a portion of a full length immunoglobulin, generally the antigen-binding or variable region thereof, such as Fab, F(ab')2, Fab', Fd, Fv fragments, minibodies, diabodies, single domain antibodies (dAb, also known as VHH, camelid antibodies, or nanobodies), single-chain variable fragments (scFv), rIgG, antibody mimetics, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment of the required specificity.

[0059] The term "immunoglobulin" as used herein is intended to refer to a class of structurally related glycoproteins typically consisting of two pairs of polypeptide chains, one pair of light (L) chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds, although some mammalian species also produce heavy-chain only antibodies or binding agents. The term "immunoglobulin heavy chain", "heavy chain of an immunoglobulin" or "heavy chain" as used herein is intended to refer to one of the chains of an immunoglobulin. A heavy chain is typically comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH) which defines the isotype of the immunoglobulin. The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chain constant region further comprises a hinge region. Within the structure of the immunoglobulin (e.g. IgG), the two heavy chains are inter-connected via disulfide bonds in the hinge region. Equally to the heavy chains, each light chain is typically comprised of several regions: a light chain variable region (VL) and a light chain constant region (CL). Furthermore, the VH and VL regions may be subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. CDR sequences may be determined by use of various methods, e.g. the methods provided by Kabat et al. (1991), "Sequence of protein of immunological interest," fifth edition. NIH publication or Chothia and Lesk (1987) J. Mol. Biol. 196:901. Various methods for CDR determination and amino acid numbering can be compared on www.abysis.org (UCL).

[0060] The constant regions of an immunoglobulin, if present, may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as

effector cells and T cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation.

[0061] The term "single domain antibody," "Nanobody®", "VHH," "VHH antibody," or "VHH domain" as used herein refers to an antigen-binding fragment comprising a single monomeric variable domain (Koch-Nolte, et al, FASEB J., 21 : 3490-3498 (2007)). A single domain antibody comprises three CDRs. Examples of single-domain antibodies are variable fragments of heavy-chain-only antibodies, antibodies that naturally do not comprise light chains, single-domain antibodies derived from conventional antibodies and engineered antibodies. Single-domain antibodies may be derived from any species. For example, naturally occurring VHH molecules can be derived from antibodies raised in Camelidae species, for example in camel, dromedary, llama, alpaca and guanaco. Like an intact immunoglobulin, a single-domain antibody is able to bind selectively to a specific antigen. Single-domain antibodies may contain only the variable domain of an immunoglobulin chain, i.e. CDR1, CDR2 and CDR3 and framework regions.

[0062] The terms "complementarity determining region" or "CDR," as used herein, refer to the amino acid sequences within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering scheme), IMGT (Lefranc et al., IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol. 2003 Jan;27(1):55-77), or a combination thereof.

[0063] The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by an antibody or an antigen-binding fragment thereof and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen.

[0064] Antibody or antigen-binding fragment binding to a target antigen is typically demonstrated by a dissociation constant (K_D). The term "K_D" is intended to refer to the dissociation equilibrium constant of a particular antibody-antigen interaction. The ratio of dissociation rate (k_{off}) to association rate (k_{on}) of an antibody to a monovalent antigen (k_{off}/k_{on}) is the dissociation constant K_D, which is inversely related to affinity. The lower the K_D value, the higher the affinity of the antibody. The value of K_D varies for different complexes of antibody and antigen and depends on

both k_{on} and k_{off}. The dissociation constant K_D for an antibody provided herein can be determined using any method provided herein or any other method well known to those skilled in the art.

[0065] In the context of binding to an antigen, the terms "binds" or "specifically binds" refer to the binding of an binding agent to a predetermined antigen or target (e.g. human Nectin-4 or Vδ2) to which binding typically is with an affinity corresponding to a K_D of about 10^{-6} M or less, e.g. 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less, e.g. when determined as described in the Examples herein. K_D values can for example be determined using for instance surface plasmon resonance (SPR) technology in a BIAcore T200 or bio-layer interferometry (BLI) in an Octet RED96 instrument using the antigen as the ligand and the binding moiety or binding molecule as the analyte. Specific binding means that the antibody binds to the predetermined antigen with an affinity corresponding to a K_D that is at least ten-fold lower, such as at least 100-fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The degree with which the affinity is lower is dependent on the K_D of the binding moiety or binding molecule, so that when the K_D of the binding moiety or binding molecule is very low (that is, the binding moiety or binding molecule is highly specific), then the degree with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000-fold.

[0066] "Polynucleotide" is used interchangeably with "oligonucleotide," and "nucleic acid" herein, and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide, in some embodiments, the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0067] As used herein, the term "host cell" refers to any type of cell that comprises a vector or polynucleotide described herein and is typically used in the manufacturing of proteins (e.g., in the manufacturing of a single domain antibody).

[0068] The term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells.

[0069] The term "subject" as used herein refers to a vertebrate or an invertebrate, and includes mammals, birds, fish, reptiles, and amphibians. Subjects include humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species. Subjects include farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like; and aquatic animals such as fish, shrimp, and crustaceans.

[0070] The terms "first" and "second" antigen-binding domains when used herein do not refer to their orientation / position in the antibody, i.e. they have no meaning with regard to the N- or C-terminus. The terms "first" and "second" only serve to correctly and consistently refer to the two different antigen-binding domains in the claims and the description.

[0071] A "variant binding agent", "variant antibody", "binding agent variant", or "antibody variant" is a binding agent which comprises one or more mutations (substitutions, deletions, or insertions) as compared to a binding agent described herein (referred to as a "parent binding agent" or "parent antibody").

[0072] "% sequence identity", when used herein, refers to the number of identical nucleotide or amino acid positions shared by different sequences (i.e., % identity = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci 4, 11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Nectin-4 Binding Agents

[0073] Nectin-4 is a member of the Nectin superfamily. Nectin-4 is involved in cellular adhesion. Human Nectin-4 is a 510 amino acid protein. The amino acid sequence of wild type human Nectin-4 is provided as SEQ ID NO: 256. Representative Nectin-4 polynucleotide sequences (NCBI Gene ID: 81607) include NM_030916 among others, each of which are available at the Nectin-4 gene page on the NCBI website.

[0074] In some embodiments, the present disclosure provides binding agents comprising at least one antigen-binding domain that specifically binds to human Nectin-4 – referred to herein as Nectin-4 binding agents. In some embodiments, the Nectin-4 binding agent is an immunoglobulin or an antigen-binding fragment thereof. In some embodiments, the Nectin-4 binding agent is a single domain antibody.

[0075] In some embodiments, the Nectin-4 binding agent comprises a first antigen-binding domain comprising a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 88, 95, 103, 110, 117, 121, 125, 129, and 133, a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 89, 96, 104, 111, 118, 122, 126, 130, and 134; and a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 90, 97, 105, 112, 119, 123, 127, 131, and 135. In some embodiments, the Nectin-4 binding agent comprises first antigen-binding domain comprising a CDR1, CDR2, and CDR3 combination selected from **Table 1A, 1B, 1C, or 1D**. In some embodiments, Nectin-4 binding agent comprises first antigen-binding domain comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136. In some embodiments, the Nectin-4 binding agent comprises first antigen-binding domain comprising an amino acid sequence selected from SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136. In some embodiments, the Nectin-4 binding agent comprises a first antigen-binding domain consisting of an amino acid sequence selected from SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136.

[0076] Exemplary CDRs of Nectin-4 binding agents are shown in **Table 1A – Table 1D**.

Table 1A: Exemplary Nectin-4-specific CDR (Kabat) and VHH Sequences

Ref	Domain	Sequence	SEQ ID
LV1184	CDR1	INLMG	88
	CDR2	SISPGGSVRYADSVKG	89
	CDR3	ESERTYYFDS	90
	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWFRQAPA KQRELVTSISPGGSVRYADSVKGRFTISRDVAKNTVDLQMNS LKPEDTAVYYCAAESERTYYFDSWGQGTQVTVSS	91
LV1184_H1	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWFRQAPA KQRELVTSISPGGSVRYADSVKGRFTISRDVAKNTVDLQMNS LRAEDTAVYYCAAESERTYYFDSWGQGTQVTVSS	92
LV1184_H2	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWFRQAPA KQRELVTSISPGGSVRYADSVKGRFTISRDVSKNTVYLQMNS LRAEDTAVYYCAAESERTYYFDSWGQGTQVTVSS	93
LV1184_H3	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWFRQAPA KGLEWVTSISPGGSVRYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAAESERTYYFDSWGQGTQVTVSS	94
LV1181	CDR1	INLMG	95
	CDR2	TITRGGSTNYADSVKG	96
	CDR3	VEPSGMGWRDY	97

Ref	Domain	Sequence	SEQ ID
	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPG KQRELVATITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNS LKPEDTAAYYCVDVEPSGMGWRDYWGQGTQVTVSS	98
LV1181 (E1D)	VHH	DVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPG KQRELVATITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNS LKPEDTAAYYCVDVEPSGMGWRDYWGQGTQVTVSS	99
LV1181_H1	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWHRQAPG KQRELVATITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNS LRAEDTAAYYCVDVEPSGMGWRDYWGQGTQVTVSS	100
LV1181_H2	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWHRQAPG KQRELVATITRGGSTNYADSVKGRFTISRDNGKNTLYLQMNS LRAEDTAAYYCVDVEPSGMGWRDYWGQGTQVTVSS	101
LV1181_H3	VHH	EVQLVESGGGLVQPGGSLRLSCAASGSISSINLMGWHRQAPG KGLEWVATITRGGSTNYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAAYYCVDVEPSGMGWRDYWGQGTLVTVSS	102
LV1185	CDR1	LNIMG	103
	CDR2	TITTGGSTNYADSVRG	104
	CDR3	ELVRRGPTTY	105
	VHH	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITTGGSTNYADSVRGRFTISRDNAEDTVYLQMNS LKPEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	106
LV1185_H1	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITTGGSTNYADSVRGRFTISRDNAEDTVYLQMNS LRAEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	107
LV1185_H2	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITTGGSTNYADSVRGRFTISRDNSEDTVYLQMNS LRAEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	108
LV1185_H3	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITTGGSTNYADSVRGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCTAELVRRGPTTYWGRGTLVTVSS	109
LV1186	CDR1	LNIMG	110
	CDR2	TITSGGSTNYADSVRG	111
	CDR3	ELVRRGPTTY	112
	VHH	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITSGGSTNYADSVRGRFTISRDNAENTVYLQMDS LKPEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	113
LV1186_H1	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITSGGSTNYADSVRGRFTISRDNAENTVYLQMDS LRAEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	114
LV1186_H2	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KQREYVATITSGGSTNYADSVRGRFTISRDNSENTVYLQMDS LRAEDTAVYYCTAELVRRGPTTYWGRGTQVTVSS	115
LV1186_H3	VHH	EVQLVESGGGLVQPGGSLRLSCAASRSTFSLNIMGWYRQAPG KGLEWVATITSGGSTNYADSVRGRFTISRDNSKNTLYLQMDS LRAEDTAVYYCTAELVRRGPTTYWGRGTLVTVSS	116
LV1178	CDR1	RLTMG	117
	CDR2	RVYASGGLTDYADSVKG	118
	CDR3	GLWADMRTMTSTRGY	119
	VHH	EVQLVESGGALVQAGGSLRLSCSVSGLTASRLTMGWFRQAPG KEREFVARVYASGGLTDYADSVKGRFTISRDNAKNTVYLQMN SLEPEDTAVYYCVAGLWADMRTMTSTRGYWGQGTQVTVSS	120

Ref	Domain	Sequence	SEQ ID
LV1183	CDR1	INLMG	121
	CDR2	SITRGGSTWYVDSVKG	122
	CDR3	ESIGSYTFDR	123
	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSIFDINLMGWYRQAPG KLRELVASITRGGSTWYVDSVKGRFIISRDSAKNTVYLQMNS LKPGDTAVYYCAAESIGSYTFDRWGQGTQVTVSS	124
LV1187	CDR1	INVMG	125
	CDR2	GITSGGSRRLADSVKG	126
	CDR3	EAVVGDEPY	127
	VHH	EVQLVESGGGLVQAGGFLRLSCAASRSVFRINVMGWYRQAPG KQRELVAGITSGGSRRLADSVKGRFTISRDNAKNTVYLQMNS LKPEDTAVYYCFAEAVVGDEPYWGQGTQVTVSS	128
LV1179	CDR1	INVMG	129
	CDR2	TITSGGSTNYADSVKG	130
	CDR3	DEIVFRNGYYLPRDY	131
	VHH	EVQLVESGGGWVQAGGSLRLSCTASGSIFSINVMGWYRQAPG KQRELVATITSGGSTNYADSVKGRFTISRDNAKNTVYLQMTS LQPEDTAIYYCTADEIVFRNGYYLPRDYWGQGTQVTVSS	132
LV1180	CDR1	INVMG	133
	CDR2	SITRGGSTNYADSVKG	134
	CDR3	EGDYELGYPPGFDF	135
	VHH	EVQLVESGGGSVQVGGSLRLSCAASGSISTINVMGWYRHPPG NQRELVASITRGGSTNYADSVKGRFTISRDNAKNTVYLQMNS LKPEDTAIYICAAEGDYELGYPPGFDFWGQGTQVTVSS	136

Table 1B: Exemplary Nectin-4-specific CDR Sequences (IMGT)

Ref	Domain	Sequence	SEQ ID
LV1184 LV1184_H1 LV1184_H2 LV1184_H3	CDR1	GSISSINL	137
	CDR2	ISPGGSV	138
	CDR3	AAESERTYYFDS	139
LV1181 LV1181 (E1D) LV1181_H1 LV1181_H2 LV1181_H3	CDR1	GSISSINL	140
	CDR2	ITRGGST	141
	CDR3	VDVEPSGMGWRDY	142
LV1185 LV1185_H1 LV1185_H2 LV1185_H3	CDR1	RSTFSLNI	143
	CDR2	ITTGGST	144
	CDR3	TAELVRRGPTTY	145
LV1186 LV1186_H1 LV1186_H2 LV1186_H3	CDR1	RSTFSLNI	146
	CDR2	ITSGGST	147
	CDR3	TAELVRRGPTTY	148
LV1178	CDR1	GLTASRLT	149
	CDR2	VYASGGLT	150

Ref	Domain	Sequence	SEQ ID
	CDR3	VAGLWADMRTMTSTRGY	151
LV1183	CDR1	GSIFDINL	152
	CDR2	ITRGGST	153
	CDR3	AAESIGSYTFDR	154
LV1187	CDR1	RSVFRINV	155
	CDR2	ITSGGSR	156
	CDR3	FAEAVVGDEPY	157
LV1179	CDR1	GSIFSINV	158
	CDR2	ITSGGST	159
	CDR3	TADEIVFRNGYYLPRDY	160
LV1180	CDR1	GSISTINV	161
	CDR2	ITRGGST	162
	CDR3	AAEGDYELGYPPGFDF	163

Table 1C: Exemplary Nectin-4-specific CDR Sequences (Chothia)

Ref	Domain	Sequence	SEQ ID
LV1184 LV1184_H1 LV1184_H2 LV1184_H3	CDR1	GSISSIN	164
	CDR2	SPGGS	165
	CDR3	ESERTYYFDS	166
LV1181 LV1181 (E1D) LV1181_H1 LV1181_H2 LV1181_H3	CDR1	GSISSIN	167
	CDR2	TRGGS	168
	CDR3	VEPSGMGWRDY	169
LV1185 LV1185_H1 LV1185_H2 LV1185_H3	CDR1	RSTFSLN	170
	CDR2	TTGGS	171
	CDR3	ELVRRGPTTY	172
LV1186 LV1186_H1 LV1186_H2 LV1186_H3	CDR1	RSTFSLN	173
	CDR2	TSGGS	174
	CDR3	ELVRRGPTTY	175
LV1178	CDR1	GLTASRL	176
	CDR2	YASGGL	177
	CDR3	GLWADMRTMTSTRGY	178
LV1183	CDR1	GSIFDIN	179
	CDR2	TRGGS	180
	CDR3	ESIGSYTFDR	181
LV1187	CDR1	RSVFRIN	182
	CDR2	TSGGS	183
	CDR3	EAVVGDEPY	184
LV1179	CDR1	GSIFSIN	185

Ref	Domain	Sequence	SEQ ID
	CDR2	TSGGS	186
	CDR3	DEIVFRNGYYLPRDY	187
LV1180	CDR1	GSISTIN	188
	CDR2	TRGGS	189
	CDR3	EGDYELGYPPGFDF	190

Table 1D: Exemplary Nectin-4-specific CDR Sequences (Combined)

Ref	Domain	Sequence	SEQ ID
LV1184 LV1184_H1 LV1184_H2 LV1184_H3	CDR1	GSISSINLMG	191
	CDR2	SISPGGSVRYADSVKG	192
	CDR3	AAESERTYYFDS	193
LV1181 LV1181 (E1D) LV1181_H1 LV1181_H2 LV1181_H3	CDR1	GSISSINLMG	194
	CDR2	TITRGGSTNYADSVKG	195
	CDR3	VDVEPSGMGWRDY	196
LV1185 LV1185_H1 LV1185_H2 LV1185_H3	CDR1	RSTFSLNIMG	197
	CDR2	TITTGGSTNYADSVRG	198
	CDR3	TAELVRRGPTTY	199
LV1186 LV1186_H1 LV1186_H2 LV1186_H3	CDR1	RSTFSLNIMG	200
	CDR2	TITSGGSTNYADSVRG	201
	CDR3	TAELVRRGPTTY	202
LV1178	CDR1	GLTASRLTMG	203
	CDR2	RVYASGGLTDYADSVKG	204
	CDR3	VAGLWADMRTMTSTRGY	205
LV1183	CDR1	GSIFDINLMG	206
	CDR2	SITRGGSTWYVDSVKG	207
	CDR3	AAESIGSYTFDR	208
LV1187	CDR1	RSVFRINVMG	209
	CDR2	GITSGGSRRLADSVKG	210
	CDR3	FAEAVVGDEPY	211
LV1179	CDR1	GSIFSINVMG	212
	CDR2	TITSGGSTNYADSVKG	213
	CDR3	TADEIVFRNGYYLPRDY	214
LV1180	CDR1	GSISTINVMG	215
	CDR2	SITRGGSTNYADSVKG	216
	CDR3	AAEGDYELGYPPGFDF	217

[0077] One of skill in the art will recognize that there are many numbering systems known in the art to define the CDR sequences of an antibody, including AbM, Kabat, Chothia, and IMGT

systems. An overview of these systems is provided in Dondelinger et al., Understanding the Significance and Implications of Antibody Numbering and Antigen-Binding Surface/Residue Definition. Front Immunol. 2018 Oct 16;9:2278. CDR sequences of the Nectin-4-specific antigen-binding domains as defined by Kabat, IMGT, and Chothia are provided in **Tables 1A, 1B, and 1C** above. The description of the binding agents herein refers to the CDRs as defined by the Kabat numbering system. However, one of skill in the art is able to correlate a CDR sequence defined by one numbering system to the same CDR sequence as defined by another system.

[0078] In some embodiments, a combination of CDR numbering systems may be used. In such embodiments, CDR sequences of a given binding agent as determined by multiple numbering systems (*e.g.*, a CDR1 sequence as determined by the Kabat, IMGT, and Chothia numbering systems) are compiled into a single sequence that encompasses the entirety of each of the CDR amino acid ranges in the variable region. As an illustrative example, CDRs for the 6H4 VHH described in **Tables 1A**, **1B**, and **1C** are shown in **Fig. 12**. The N- and C-terminal ranges for each CDR region are indicated by dashed lines. The combined CDR sequences covering the entirety of these ranges are shown in the last row. Illustrative combined CDRs for Nectin-4 binding agents are shown in **Table 1D**.

[0079] As an illustrative example, a 6H4 VHH comprising a CDR1 amino acid sequence comprising SEQ ID NO: 27, a CDR2 amino acid sequence comprising SEQ ID NO: 28, and a CDR3 amino acid sequence comprising SEQ ID NO: 29 as defined by the Kabat numbering system will be understood by one of skill in the art to be the equivalent of (1) a 6H4 VHH comprising a CDR1 amino acid sequence comprising SEQ ID NO: 46, a CDR2 amino acid sequence comprising SEQ ID NO: 47, and a CDR3 amino acid sequence comprising SEQ ID NO: 48 as defined by the IMGT numbering system; (2) a 6H4 VHH comprising a CDR1 amino acid sequence comprising SEQ ID NO: 64, a CDR2 amino acid sequence comprising SEQ ID NO: 65, and a CDR3 amino acid sequence comprising SEQ ID NO: 66 as defined by the Chothia numbering system; and (3) a 6H4 VHH comprising a CDR1 amino acid sequence comprising SEQ ID NO: 85, a CDR2 amino acid sequence comprising SEQ ID NO: 86, and a CDR3 amino acid sequence comprising SEQ ID NO: 87.

[0080] In some embodiments, the first antigen-binding domain comprise one or more framework regions (FR). In some embodiments, the binding agent disclosed herein are of the format FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. Exemplary framework regions are shown in **Table 2** below. In some embodiments, the first antigen-binding domain comprises an FR1 amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 234, 238, 242, and 246. In some embodiments, the

first antigen-binding domain comprises an FR1 amino acid sequence that comprises or consists of SEQ ID NOs: 234, 238, 242, and 246. In some embodiments, the first antigen-binding domain comprises an FR2 amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 235, 239, 243, and 247. In some embodiments, the first antigen-binding domain comprises an FR2 amino acid sequence that comprises or consists of SEQ ID NOs: 235, 239, 243, and 247. In some embodiments, the first antigen-binding domain comprises an FR3 amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 236, 240, 244, and 248. In some embodiments, the first antigen-binding domain comprises an FR3 amino acid sequence that comprises or consists of SEQ ID NOs: 236, 240, 244, and 248. In some embodiments, the first antigen-binding domain comprises an FR4 amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 237, 241, 245, and 249. In some embodiments, the first antigen-binding domain comprises an FR4 amino acid sequence that comprises or consists of SEQ ID NOs: 237, 241, 245, and 249. In some embodiments, the first antigen-binding domain comprises or consists of, from N' to C' terminus, SEQ ID NOs: 234, 88, 235, 89, 236, 90, and 238. In some embodiments, the first antigen-binding domain comprises or consists of, from N' to C' terminus, SEQ ID NOs: 238, 95, 239, 96, 240, 97, and 241. In some embodiments, the first antigen-binding domain comprises or consists of, from N' to C' terminus, SEQ ID NOs: 242, 103, 243, 104, 244, 105, and 245. In some embodiments, the first antigen-binding domain comprises or consists of, from N' to C' terminus, SEQ ID NOs: 234, 110, 235, 111, 236, 112, and 238.

Table 2: Exemplary Framework Regions

Ref	Domain	Sequence	SEQ ID
LV1184	FR1	EVQLVESGGGLVQAGGSLRLSCAASGSISS	234
	FR2	WFRQAPAKQRELVT	235
	FR3	RFTISRDVAKNTVDLQMNSLKPEDTAVYYCAA	236
	FR4	WGQGTQVTVSS	237
LV1181	FR1	EVQLVESGGGLVQAGGSLRLSCAASGSISS	238
	FR2	WHRQAPGKQRELVA	239
	FR3	RFTISRDNGKNTVYLQMNSLKPEDTAAYYCVD	240
	FR4	WGQGTQVTVSS	241
LV1185	FR1	EVQLVESGGGLVQAGGSLRLSCAASRSTFS	242
	FR2	WYRQAPGKQREYVA	243
	FR3	RFTISRDNAEDTVYLQMNSLKPEDTAVYYCTA	244

Ref	Domain	Sequence	SEQ ID
	FR4	WGRGTQVTVSS	245
LV1186	FR1	EVQLVESGGGLVQAGGSLRLSCAASRSTFS	246
	FR2	WYRQAPGKQREYVA	247
	FR3	RFTISRDNAENTVYLQMDSLKPEDTAVYYCTA	248
	FR4	WGRGTQVTVSS	249

[0081] In some embodiments, the binding agent described herein comprises a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 88, a CDR2 amino acid sequence comprising SEQ ID NO: 89, and a CDR3 amino acid sequence comprising SEQ ID NO: 90. In some embodiments, one or more of CDR1, CDR2, or CDR3 comprise one or two amino acid modification (e.g., a substitution, insertion, or deletion). In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 88, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 89, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 90, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 88, a CDR2 amino acid sequence consisting of SEQ ID NO: 89, and a CDR3 amino acid sequence consisting of SEQ ID NO: 90.

[0082] In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 91. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 91. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 91. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 92. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 92. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 92. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 93. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 93. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 93. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 94. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 94. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 94.

[0083] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 95, a CDR2 amino acid sequence comprising SEQ ID NO: 96, and a CDR3 amino acid sequence comprising SEQ ID NO: 97. In some embodiments, one or more of CDR1, CDR2, or CDR3 comprise one or two amino acid modification (e.g., a substitution, insertion, or deletion). In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 95, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 96, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 97, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 95, a CDR2 amino acid sequence consisting of SEQ ID NO: 96, and a CDR3 amino acid sequence consisting of SEQ ID NO: 97.

[0084] In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 98. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 98. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 98. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 99. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 99. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 99. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 100. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 100. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 100. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 101. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 101. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 101. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 102. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 102. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 102.

[0085] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 103, a CDR2

amino acid sequence comprising SEQ ID NO: 104, and a CDR3 amino acid sequence comprising SEQ ID NO: 105. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 103, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 104, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 105, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 103, a CDR2 amino acid sequence consisting of SEQ ID NO: 104, and a CDR3 amino acid sequence consisting of SEQ ID NO: 105.

[0086] In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 106. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 106. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 106. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 107. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 107. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 107. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 108. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 108. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 108. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 109. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 109. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 109.

[0087] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 110, a CDR2 amino acid sequence comprising SEQ ID NO: 111, and a CDR3 amino acid sequence comprising SEQ ID NO: 112. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 110, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 111, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 112, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 110, a CDR2 amino acid

sequence consisting of SEQ ID NO: 111, and a CDR3 amino acid sequence consisting of SEQ ID NO: 112.

[0088] In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 113. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 113. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 113. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 114. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 114. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 114. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 115. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 115. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 115. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 116. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 116. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 116.

[0089] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 117, a CDR2 amino acid sequence comprising SEQ ID NO: 118, and a CDR3 amino acid sequence comprising SEQ ID NO: 119. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 117, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 118, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 119, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 117, a CDR2 amino acid sequence consisting of SEQ ID NO: 118, and a CDR3 amino acid sequence consisting of SEQ ID NO: 119. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 120. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 120. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 120.

[0090] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 121, a CDR2

amino acid sequence comprising SEQ ID NO: 122, and a CDR3 amino acid sequence comprising SEQ ID NO: 123. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 121, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 122, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 123, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 121, a CDR2 amino acid sequence consisting of SEQ ID NO: 122, and a CDR3 amino acid sequence consisting of SEQ ID NO: 123. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 124. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 124. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 124.

[0091] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 125, a CDR2 amino acid sequence comprising SEQ ID NO: 126, and a CDR3 amino acid sequence comprising SEQ ID NO: 127. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 125, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 126, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 127, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 125, a CDR2 amino acid sequence consisting of SEQ ID NO: 126, and a CDR3 amino acid sequence consisting of SEQ ID NO: 127. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 128. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 128. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 128.

[0092] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 129, a CDR2 amino acid sequence comprising SEQ ID NO: 130, and a CDR3 amino acid sequence comprising SEQ ID NO: 131. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 129, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 130, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 131, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain

comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 129, a CDR2 amino acid sequence consisting of SEQ ID NO: 130, and a CDR3 amino acid sequence consisting of SEQ ID NO: 131. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 132. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 132. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 132.

[0093] In some embodiments, the binding agents described herein comprise a first antigen-binding domain comprising a CDR1 amino acid sequence comprising SEQ ID NO: 133, a CDR2 amino acid sequence comprising SEQ ID NO: 134, and a CDR3 amino acid sequence comprising SEQ ID NO: 135. In some embodiments, CDR1 comprises the amino acid sequence of SEQ ID NO: 133, wherein one or two amino acids are modified. In some embodiments, CDR2 comprises the amino acid sequence of SEQ ID NO: 134, wherein one or two amino acids are modified. In some embodiments, CDR3 comprises the amino acid sequence of SEQ ID NO: 135, wherein one or two amino acids are modified. In some embodiments, the first antigen-binding domain comprises a CDR1 amino acid sequence consisting of SEQ ID NO: 133, a CDR2 amino acid sequence consisting of SEQ ID NO: 134, and a CDR3 amino acid sequence consisting of SEQ ID NO: 135. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 136. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 136. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 136.

[0094] In some embodiments, one or more amino acid substitutions are introduced into a Nectin-4 binding agent described herein. In some embodiments, the one or more amino acid substitutions is a conservative amino acid substitution. The phrase "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., supra). Examples of conservative mutations include amino acid substitutions of amino acids within the sub-groups above, for example, lysine for arginine and vice versa such that a positive charge may be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained; serine for threonine such that a free -OH can be

maintained; and glutamine for asparagine such that a free -NH2 can be maintained. Examples of amino acid classes are provided in **Table 3**, **Table 4**, and **Table 5** below.

Table 3: Amino acid residue classifications

Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)
Aliphatic Uncharged Residues	Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

Table 4: Alternative amino acid classifications

1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M
6	F	Y	W

Table 5: Alternative Physical and Functional Amino acid classifications

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	Q, T, K, S, G, N, D, E, and R

[0095] In some embodiments, the one or more amino acid substitutions is a non-conservative amino acid substitution. "Non-conservative substitutions" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the binding agent (i.e., does not interfere with binding to the target antigen). The non-conservative amino acid substitution may enhance the biological activity of the

functional variant, such that the biological activity of the functional variant is increased as compared to the parent binding agent.

[0096] In some embodiments, the binding agents described herein are chimeric antibodies, wherein the variable region is derived from a non-human species (e.g. derived from rodents) and the constant region is derived from a different species, such as human. Chimeric antibodies may be generated by genetic engineering. Chimeric monoclonal antibodies for therapeutic applications are developed to reduce antibody immunogenicity.

[0097] In some embodiments, the binding agents described herein are humanized immunoglobulins or antigen-binding fragments thereof. A "humanized" immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a "donor," and the human immunoglobulin providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences.

[0098] A "humanized antibody" is an antibody comprising a humanized light chain and/or a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen-binding or other immunoglobulin functions. Humanized immunoglobulins can be constructed by means of genetic engineering (for example, see U.S. Patent No. 5,585,089).

[0099] In some embodiments, the Nectin-4 binding agents described herein have a binding affinity (K_D) for human Nectin-4 of less than 10^{-7} M, such as approximately less than 10^{-8} M, less than 10^{-9} M, or less than 10^{-10} M when determined by surface plasmon resonance (SPR) using recombinant human Nectin-4 as the analyte and the Nectin-4 binding agent as the ligand. In some embodiments, the binding agent binds to human Nectin-4 with an affinity that is at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, 4.5-, 5.0-, 6.0-, 7.0-, 8.0-, 9.0-, or 10.0-fold or greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein).

[0100] Methods of testing binding agents for the ability to bind a target antigen are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., infra, U.S. Patent Application Publication No. 2002/0197266 Al, and U.S. Patent No. 7,338,929).

[0101] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to recombinant Nectin-4 protein with a K_D of less than 10 nM, for example less than 5 nM, less than 2 nM, less than 1 nM or less than 0.5 nM, as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein.

[0102] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to recombinant Nectin-4 protein with a K_D of between 10 nM and 1 nM, such as between 5 nM and 1 nM as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein.

[0103] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to BeWo cells with an EC50 of less than 0.5 nM, when tested as described in Example 3 herein. In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to A-431 cells with an EC50 of less than 0.5 nM, when tested as described in Example 3 herein. In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to HT-29 cells with an EC50 of less than 2 nM, when tested as described in Example 3 herein.

[0104] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of binding to Vγ9Vδ2 T cells with an EC50 of less than 5 nM, such as less than 2 nM, less than 1 nM, or less than 0.5 nM when tested as described in Example 9 herein.

[0105] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein capable of binding cynomolgus Nectin-4 and/or murine Nectin-4.

[0106] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of mediating killing of Nectin-4-expressing tumor cells, such as BeWo, A-431, or HT-29 cells, by Vγ9Vδ2 T cells.

[0107] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of inducing killing of A-431 cells through

activation of Vγ9Vδ2 T cells with an EC50 value of 100 pM or less, such as 20 pM or less, e.g. 10 pM or less, or even 7 pM or less, or 5 pM or less when tested as described in Example 10 herein.

[0108] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are not capable of mediating killing of Nectin-4-negative cells, such as Nectin-4 negative human cells.

[0109] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are capable of mediating killing of Nectin-4-positive cells, such as Nectin-4-positive tumor cells, by Vγ9Vδ2 T cells.

[0110] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are:

(a) capable of binding to recombinant human Nectin-4 with a K_D of less than 10 nM, for example less than 5 nM, less than 2 nM, less than 1 nM or less than 0.5 nM, as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein; and

(b) capable of inducing killing of A-431 cells through activation of Vγ9Vδ2 T cells with an EC_{50} value of 10 pM or less, or even 7 pM or less, or 5 pM or less when tested as described in Example 10 herein.

[0111] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein are:

(a) capable of binding to recombinant Nectin-4 protein with a K_D of between 10 nM and 1 nM, such as between 5 nM and 1 nM as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein; and

(b) capable of inducing killing of A-431 cells through activation of Vγ9Vδ2 T cells with an EC_{50} value of 10 pM or less, or even 7 pM or less, or 5 pM or less when tested as described in Example 10 herein.

[0112] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein have all of the following properties:

(a) capable of mediating killing BeWo, A-431 and HT-29 cells, by Vγ9Vδ2 T cells,

(b) capable of inducing killing of A-431 cells through activation of Vγ9Vδ2 T cells with an EC_{50} value of 100 pM or less, such as 20 pM or less, e.g. 10 pM or less, or even 7 pM or less, or 5 pM or less when tested as described in Example 10 herein.

(c) capable of binding human Nectin-4, cynomolgous Nectin-4 and murine Nectin-4, and

(d) capable of binding to recombinant human Nectin-4 with a K_D of less than 10 nM, for example less than 5 nM, less than 2 nM, less than 1 nM or less than 0.5 nM, as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein.

[0113] In some embodiments, the Nectin-4 binding agents and multi-specific binding agents comprising the same described herein have all of the following properties:

(a) capable of mediating killing BeWo, A-431 and HT-29 cells, by Vγ9Vδ2 T cells,

(b) capable of inducing killing of A-431 cells through activation of Vγ9Vδ2 T cells with an EC_{50} value of 100 pM or less, such as 20 pM or less, e.g. 10 pM or less, or even 7 pM or less, or 5 pM or less when tested as described in Example 10 herein,

(c) capable of binding human Nectin-4, cynomolgous Nectin-4 and murine Nectin-4, and

(d) capable of binding to recombinant Nectin-4 protein with a K_D of between 10 nM and 1 nM, such as between 5 nM and 1 nM as determined by Bio-Layer Interferometry according to the methods described in Example 5 herein.

[0114] In the context of the present invention, “capable of competing” or “able to compete” or “competes” refers to any detectably significant reduction in the propensity for a particular binding molecule (e.g., a Nectin-4 binding agent) to bind a particular binding partner (e.g. Nectin-4) in the presence of another molecule (e.g. a different Nectin-4 binding agent) that binds the binding partner. Typically, competition means that saturating binding of the first binding agent to the antigen (Nectin-4) blocks or prevents binding of the second binding agent, as determined by, e.g., biolayer interferometry (BLI) as described in Example 6 herein using sufficient amounts of the two (or more) competing binding agents. Additional methods for determining binding specificity by competitive inhibition may be found in for instance Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988), Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc, and Wiley InterScience N. Y., (1992, 1993), and Muller, Meth. Enzymol. 92, 589-601 (1983).

[0115] In one embodiment, the binding agent or multi-specific comprising the same of the present disclosure binds to the same epitope on Nectin-4 as Nectin-4 binding agents LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185 LV1186 and/or LV1187 and/or to the same epitope on Vδ2 as the 5C8 or 6H4 binding agents (WO 2015/156673) or the variants of 5C8 set forth in SEQ ID NOs: 8, 9, 13, 17, 21, 22, and 26. There are several methods available for mapping the binding agent binding epitopes on target antigens known in the art, including but not limited to: crosslinking coupled mass spectrometry, allowing identification of peptides that are part of the epitope, and X-ray crystallography identifying individual residues on the antigen that form the

epitope. Epitope residues can be determined as being all amino acid residues with at least one atom less than or equal to 5 Å from the antibody. 5 Å was chosen as the epitope cutoff distance to allow for atoms within a van der Waals radius plus a possible water-mediated hydrogen bond. Next, epitope residues can be determined as being all amino acid residues with at least one atom less than or equal to 8 Å. Less than or equal to 8 Å is chosen as the epitope cutoff distance to allow for the length of an extended arginine amino acid. Crosslinking coupled mass spectrometry begins by binding the antibody and the antigen with a mass labeled chemical crosslinker. Next the presence of the complex is confirmed using high mass MALDI detection. Because after crosslinking chemistry the Ab/Ag complex is extremely stable, many various enzymes and digestion conditions can be applied to the complex to provide many different overlapping peptides. Identification of these peptides is performed using high resolution mass spectrometry and MS/MS techniques. Identification of the crosslinked peptides is determined using mass tag linked to the cross-linking reagents. After MS/MS fragmentation and data analysis, peptides that are crosslinked and are derived from the antigen are part of the epitope, while peptides derived from the binding agent are part of the paratope. All residues between the most N- and C-terminal crosslinked residue from the individual crosslinked peptides found are considered to be part of the epitope or paratope.

Multi-specific Binding Agents

[0116] In some embodiments, the Nectin-4 binding agent is a multi-specific binding agent. In some embodiments, the multi-specific binding agent comprises a first antigen-binding domain that specifically binds to human Nectin-4 described above and a second antigen-binding domain that specifically binds to a second target antigen or epitope (*e.g.*, a bispecific binding agent). In some embodiments, the multi-specific binding agent comprises a first antigen-binding domain that specifically binds to human Nectin-4, a second antigen-binding domain that specifically binds to a second target antigen or epitope, and a third antigen-binding domain that specifically binds to a third target antigen or epitope.

[0117] In some embodiments, the multi-specific binding agent is in a VHH-Fc format, i.e. the binding agent comprises two or more single-domain antigen-binding domains that are linked to each other via a human Fc domain dimer. In this format, each single-domain antigen-binding domain is fused to an Fc region polypeptide and the two fusion polypeptides form a dimeric bispecific binding agent via disulfide bridges in the hinge region. Such constructs typically do not contain full, or any, CH1 or light chain sequences. **Figure 12B** of WO 2006/064136 provides an illustration of an example of this embodiment.

[0118] In some embodiments, the multi-specific binding agents provided herein comprise a first antigen-binding domain that specifically binds to human Nectin-4 and a second antigen-binding domain that specifically binds to an antigen expressed on an immune cell. The term "immune cell" refers to cells involved in mounting innate and adaptive immune responses, including but not limited to lymphocytes (such as T-cells and B-cells), natural killer (NK) cells, NKT cells, macrophages, monocytes, eosinophils, basophils, neutrophils, dendritic cells, and mast cells. In some embodiments, the immune cell is a T cell, such as a CD4+ T cell, a CD8+ T cell (also referred to as a cytotoxic T cell or CTL), or a γδ T cell. In some embodiments, the immune cell is a γδ T cell.

[0119] In some embodiments, the second antigen-binding domain specifically binds to a target antigen selected from CD3, CD2, αδ T cell receptors, γδ T cell receptors (e.g., Vδ2 TCR or Vδ1 TCR), CD56, CD16, CD19, and CD20.

[0120] In some embodiments, the second antigen-binding domain specifically binds to a γδ T cell receptor. Antigen-binding domains that specifically bind to Vδ1+ T cells are known in the art. See *e.g.*, WO 2021/032963. Antigen-binding domains that specifically bind to Vδ2+ T cell receptors are known in the art. See *e.g.*, WO 2015/156673, WO 2022/008646, WO 2022/122973, and WO 2023/242319, each disclosing antigen-binding domains that specifically bind to Vδ2 and each incorporated herein by reference.

[0121] In some embodiments, the second antigen-binding domain specifically binds to human Vδ2. In some embodiments, the multi-specific binding agents provided herein are capable of binding the Vδ2 chain of a Vγ9Vδ2-TCR. "Capable of binding the Vδ2 chain of a Vγ9Vδ2-TCR" means that the binding agent disclosed herein or multi-specific binding agent comprising the same can bind the Vδ2 chain as a separate molecule and/or as part of a Vγ9Vδ2-TCR. This can be referred to as a Vδ2 binding agent. However, the binding agent or multi-specific binding agent comprising the same will not bind to the Vγ9 chain as a separate molecule. The term "human Vδ2", when used herein, refers to the rearranged δ2 chain of the Vγ9Vδ2-T cell receptor (TCR). UniProtKB – A0JD36 (A0JD36_HUMAN) gives an example of a variable TRDV2 sequence. The sequence of human Vδ2 is set forth in SEQ ID NO:266. Exemplary Vγ9Vδ2 TCR antigen-binding domain sequences are shown in **Table 6A, Table 6B, Table 6C, and Table 6D.**

Table 6A: Exemplary Vδ2 TCR-specific CDR (Kabat) and VHH Sequences

Reference	Component	Sequence	SEQ ID
5C8	CDR1	NYAMG	1
	CDR2	AISWSGGSTSYADSVKG	2
	CDR3	QFSGADYGFGRLGIRGYEYDY	3

Reference	Component	Sequence	SEQ ID
	VHH	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYAMGWF RQAPGKEREFVAAISWSGGSTSYADSVKGRFTISRDN AKNTVYLQMNSPKPEDTAIYYCAAQFSGADYGFGRLG IRGYEYDYWGQGTQVTVSS	4
5C8 var1 5C8 var2	CDR1	NYAMS	5
	CDR2	AISWSGGSTSYADSVKG	6
	CDR3	QFSGADYGFGRLGIRGYEYDY	7
	VHH (var1)	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADYGFGRLG IRGYEYDYWGQGTQVTVSS	8
	VHH (var2)	EVQLLESGGGLVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADYGFGRLG IRGYEYDYWGQGTLVTVSS	9
5C8 var1 (Y105F)	CDR1	NYAMS	10
	CDR2	AISWSGGSTSYADSVKG	11
	CDR3	QFSGADFGFGRLGIRGYEYDY	12
	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADFGFGRLG IRGYEYDYWGQGTQVTVSS	13
5C8 var1 (Y105S)	CDR1	NYAMS	14
	CDR2	AISWSGGSTSYADSVKG	15
	CDR3	QFSGADSGFGRLGIRGYEY	16
	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADSGFGRLG IRGYEYDYWGQGTQVTVSS	17
5C8 var1 (Y105F-R109A)	CDR1	NYAMS	18
	CDR2	AISWSGGSTSYADSVKG	19
	CDR3	QFSGADFGFGALGIRGYEYDY	20
	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADFGFGALG IRGYEYDYWGQGTQVTVSS	21
5C8 var1 (E1D-Y105F-R109A)	VHH	DVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADFGFGALG IRGYEYDYWGQGTQVTVSS	22
5C8 var1 (Y105F-G108A)	CDR1	NYAMS	23
	CDR2	AISWSGGSTSYADSVKG	24
	CDR3	QFSGADFGFARLGIRGYEYDY	25
	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWF RQAPGKEREFVSAISWSGGSTSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAQFSGADFGFARLG IRGYEYDYWGQGTQVTVSS	26
6H4	CDR1	NYGMG	27
	CDR2	GISWSGGSTDYADSVKG	28
	CDR3	VFSGAETAYYPSDDYDY	29
	VHH	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWF RQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSD DYDYWGQGTQVTVSS	30

Table 6B: Exemplary Vδ2 TCR-specific CDR Sequences (IMGT)

Reference	Component	Sequence	SEQ ID
5C8 5C8 var1 5C8 var2	CDR1	GRPFSNYA	31
	CDR2	ISWSGGST	32
	CDR3	AAQFSGADYGFGRLGIRGYEYDY	33
5C8 var1 (Y105F)	CDR1	GRPFSNYA	34
	CDR2	ISWSGGST	35
	CDR3	AAQFSGADFGFGRLGIRGYEYDY	36
5C8 var1 (Y105S)	CDR1	GRPFSNYA	37
	CDR2	ISWSGGST	38
	CDR3	AAQFSGADSGFGRLGIRGYEYDY	39
5C8 var1 (Y105F-R109A) 5C8 var1 (E1D-105F-R109A)	CDR1	GRPFSNYA	40
	CDR2	ISWSGGST	41
	CDR3	AAQFSGADFGFGALGIRGYEYDY	42
5C8 var1 (Y105F-G108A)	CDR1	GRPFSNYA	43
	CDR2	ISWSGGST	44
	CDR3	AAQFSGADFGFARLGIRGYEYDY	45
6H4	CDR1	GRPFSNYG	46
	CDR2	ISWSGGST	47
	CDR3	AAVFSGAETAYYPSDDYDY	48

Table 6C: Exemplary Vδ2 TCR-specific CDR Sequences (Chothia)

Reference	Component	Sequence	SEQ ID
5C8 5C8 var1 5C8 var2	CDR1	GRPFSNY	49
	CDR2	SWSGGS	50
	CDR3	QFSGADYGFGRLGIRGYEYDY	51
5C8 var1 (Y105F)	CDR1	GRPFSNY	52
	CDR2	SWSGGS	53
	CDR3	QFSGADFGFGRLGIRGYEYDY	54
5C8 var1 (Y105S)	CDR1	GRPFSNY	55
	CDR2	SWSGGS	56
	CDR3	QFSGADSGFGRLGIRGYEYDY	57
5C8 var1 (Y105F-R109A) 5C8 var1 (E1D-105F-R109A)	CDR1	GRPFSNY	58
	CDR2	SWSGGS	59
	CDR3	QFSGADFGFGALGIRGYEYDY	60
5C8 var1 (Y105F-G108A)	CDR1	GRPFSNY	61
	CDR2	SWSGGS	62
	CDR3	QFSGADFGFARLGIRGYEYDY	63
6H4	CDR1	GRPFSNY	64
	CDR2	SWSGGS	65
	CDR3	VFSGAETAYYPSDDYDY	66

Table 6D: Exemplary Vδ2 TCR-specific CDR Sequences (Combined)

Reference	Component	Sequence	SEQ ID
5C8	CDR1	GRPFSNYAMG	67
	CDR2	AISWSGGSTSYADSVKG	68

Reference	Component	Sequence	SEQ ID
	CDR3	AAQFSGADYGFGRLGIRGYEYDY	69
5C8 var1 5C8 var2	CDR1	GRPFSNYAMS	70
	CDR2	AISWSGGSTSYADSVKG	71
	CDR3	AAQFSGADYGFGRLGIRGYEYDY	72
5C8 var1 (Y105F)	CDR1	GRPFSNYAMS	73
	CDR2	AISWSGGSTSYADSVKG	74
	CDR3	AAQFSGADFGFGRLGIRGYEYDY	75
5C8 var1 (Y105S)	CDR1	GRPFSNYAMS	76
	CDR2	AISWSGGSTSYADSVKG	77
	CDR3	AAQFSGADSGFGRLGIRGYEYDY	78
5C8 var1 (Y105F-R109A) 5C8 var1 (E1D-105F-R109A)	CDR1	GRPFSNYAMS	79
	CDR2	AISWSGGSTSYADSVKG	80
	CDR3	AAQFSGADFGFGALGIRGYEYDY	81
5C8 var1 (Y105F-G108A)	CDR1	GRPFSNYAMS	82
	CDR2	AISWSGGSTSYADSVKG	83
	CDR3	AAQFSGADFGFARLGIRGYEYDY	84
6H4	CDR1	GRPFSNYGMG	85
	CDR2	GISWSGGSTDYADSVKG	86
	CDR3	AAVFSGAETAYYPSDDYDY	87

[0122] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 88, a CDR2 of SEQ ID NO: 89, and a CDR3 of SEQ ID NO: 90; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from **Table 6A** (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 91, 92, 93, and 94, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises a sequence selected from SEQ ID NOs: 91, 92, 93, and 94, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of a sequence selected from SEQ ID NOs: 91, 92, 93, and 94, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0123] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96, and a CDR3 of SEQ ID NO: 97; and (ii) a second antigen-binding domain that binds to Vδ2

and comprises a combination of CDR sequences selected from **Table 6A** (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 98, 99, 100, 101, and 102, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises a sequence selected from SEQ ID NOs: 98, 99, 100, 101, and 102, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of a sequence selected from SEQ ID NOs: 98, 99, 100, 101, and 102, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0124] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 103, a CDR2 of SEQ ID NO: 104, and a CDR3 of SEQ ID NO: 105; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 106, 107, 108, and 109, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises a sequence selected from SEQ ID NOs: 106, 107, 108, and 109, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of a sequence selected from SEQ ID NOs: 106, 107, 108, and 109, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0125] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 110, a CDR2 of SEQ ID NO: 111, and a CDR3 of SEQ ID NO: 112; and (ii) a second antigen-binding domain that binds to

Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 113, 114, 115, and 116, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises a sequence selected from SEQ ID NOs: 113, 114, 115, and 116, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of a sequence selected from SEQ ID NOs: 113, 114, 115, and 116, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0126] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 117, a CDR2 of SEQ ID NO: 118, and a CDR3 of SEQ ID NO: 119; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical SEQ ID NO: 120, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 120, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 120, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0127] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 121, a CDR2 of SEQ ID NO: 122, and a CDR3 of SEQ ID NO: 123; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding

domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical SEQ ID NO: 124, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 124, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 124, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0128] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 125, a CDR2 of SEQ ID NO: 126, and a CDR3 of SEQ ID NO: 127; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical SEQ ID NO: 128, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 128, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 128, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0129] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 129, a CDR2 of SEQ ID NO: 130, and a CDR3 of SEQ ID NO: 131; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical SEQ ID NO: 132, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 132, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 132, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0130] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain that binds to Nectin-4 and comprises a CDR1 of SEQ ID NO: 133, a CDR2 of SEQ ID NO: 134, and a CDR3 of SEQ ID NO: 135; and (ii) a second antigen-binding domain that binds to Vδ2 and comprises a combination of CDR sequences selected from Table 6A (*e.g.*, the CDR combinations for any of the described VHHs). In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical SEQ ID NO: 136, and the second antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 136, and the second antigen-binding domain comprises a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 136, and the second antigen-binding domain consists of a sequence selected from SEQ ID NOs: 4, 8, 9, 13, 17, 21, 22, 26, and 30.

[0131] In some embodiments, any of the multi-specific binding agents disclosed herein are modified to reduce or eliminate the formation of pyroglutamic acid at the N-terminus of the modified multi-specific binding agents as compared to the unmodified multi-specific binding agents of this disclosure. In some embodiments, the glutamate residue (E) or glutamine (Q) residue at the first position is modified to an aspartic acid residue (D). In some embodiments, any of the multi-specific binding agents of the present disclosure may comprise an aspartic acid (D) residue in the first position.

[0132] In some embodiments, the Nectin-4 binding agent, or multi-specific binding agent comprising the same, comprises an aspartic acid or a glutamic acid residue at the N-terminal amino acid position and elutes earlier in reversed-phase HPLC as described in Example 15, than a Nectin-4 binding agent or multi-specific binding agent comprising the same that comprises a pyroglutamate residue at the N-terminal amino acid position.

[0133] In some embodiments, the Vδ2 binding agent, or multi-specific binding agent comprising the same, comprises an aspartic acid or a glutamic acid residue at the N-terminal amino acid position and elutes earlier in reversed-phase HPLC as described in Example 15, than a Nectin-4 binding agent or multi-specific binding agent comprising the same that comprises a pyroglutamate residue at the N-terminal amino acid position.

[0134] In some embodiments, the second antigen-binding domain specifically binds to human Vγ9. In some embodiments, the multi-specific binding agents provided herein are capable of binding the Vγ9 chain of a Vγ9Vδ2-TCR. "Capable of binding the Vγ9 chain of a Vγ9Vδ2-TCR" means that the binding agent disclosed herein or multi-specific binding agent comprising the same can bind the Vγ9 chain as a separate molecule and/or as part of a Vγ9Vδ2-TCR. However, the binding agent or multi-specific binding agent comprising the same will not bind to the Vδ2 chain as a separate molecule. The term "human Vγ9", when used herein, refers to the rearranged Vγ9 chain of the Vγ9Vδ2-T cell receptor (TCR). UniProtKB – Q99603 (TRGV9_HUMAN) gives an example of a variable Vγ9 sequence. Exemplary Vγ9Vδ2 TCR antigen-binding domain sequences are known in the art, see e.g. WO 2015/156673.

[0135] In some embodiments, the multi-specific binding agents provided herein are able to active Vγ9Vδ2-T cells. "Able to activate Vγ9Vδ2-T cells" in the context of the present disclosure refers to activation of Vγ9Vδ2-T cells in the presence of a multi-specific binding agent, such as a bispecific binding agent, of the disclosure in the presence of a target cell expressing Nectin-4. Preferably the activation of the Vγ9Vδ2-T cells is measurable through gene-expression and/or (surface) marker expression (e.g., activation markers, such as CD25, CD69, or CD107a) and/or secretory protein (e.g., cytokines or chemokines) profiles. In some embodiments, the multi-specific binding agent is able to induce activation (e.g. upregulation of CD69 and/or CD25 expression) resulting in degranulation (marked by an increase in CD107a expression; see e.g. Fig. 7A) and cytokine production (e.g. TNF, IFN-γ) by Vγ9Vδ2-T cells. Preferably, the multi-specific binding agent that is used is able to increase CD107a expression on Vγ9Vδ2-T cells to at least 10%, more preferably at least 20%, more preferably at least 40%, most preferably at least 90%, when used in an assay as described in Example 10 herein, wherein e.g. 10% means that 10% of the total number of cells is positive for CD107a. In another embodiment, the number of cells positive for CD107a is increased 1.5-fold, such as 2-fold, e.g. 5-fold, in the presence of a multi-specific binding agent of the disclosure.

Fc domains

[0136] In some embodiments, the present disclosure provides a Nectin-4 binding agent or multi-specific binding agent comprising the same, further comprising an Fc domain. In some embodiments, the Fc domain comprises a first and a second Fc domain monomer.

[0137] As used herein, "Fc domain" or "Fc region" describes the minimum region (in the context of a larger polypeptide) or smallest protein folded structure (in the context of an isolated protein) that can bind to or be bound by an Fc receptor (FcR). The Fc domain is the fragment of an antibody which would be typically generated after digestion with papain, and generally includes the two CH2-CH3 regions of an immunoglobulin and a hinge region. As used herein, "Fc domain monomer" describes the single chain protein that, when associated with another Fc domain monomer, forms a functional Fc domain. The association of two Fc domain monomers creates one Fc domain. When two Fc domain monomers associate, the resulting Fc domain has Fc receptor binding activity. Thus, an Fc domain is a dimeric structure that can bind an Fc receptor. Unless otherwise noted, all references herein to an "Fc domain" are to be understood as referring to a dimeric Fc domain, in which each Fc domain monomer comprises the referenced mutation. The Fc domain mediates the effector functions of antibodies with cell surface receptors called Fc receptors and proteins of the complement system.

[0138] It will be understood that Fc domain as used herein includes the polypeptides comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc domain refers to the last two constant region immunoglobulin domains (CH2, CH3) of IgG and optionally the flexible hinge N-terminal to these domains. Unless otherwise noted, all references to amino acid positions in Fc domains and Fc domain monomers are according to the EU index as set forth in Kabat (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.). It is noted that polymorphisms have been observed at a number of Fc domain positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the sequences provided herein and sequences in the art may exist.

[0139] The Fc domain may be derived from any of a variety of different antibody isotypes, including but not limited to, a wild-type or modified IgG1, IgG2, IgG3, IgG4. IgA, IgE, or IgM. In some embodiments, the Fc domain is derived from a human IgG1.

[0140] There are many known polymorphs for the IgG1 Fc domain, including the "DEL" polymorph and the "EEM" polymorph. The DEL polymorph comprises the amino acids D-E-L at positions 356, 357, and 358, respectively (e.g., SEQ ID NO: 220). The EEM polymorph comprises the amino acids E-E-M at positions 356, 357, and 358, respectively (e.g., SEQ ID NO: 219). Two binding agents that are otherwise identical except for the presence of a DEL Fc domain or an

EEM Fc domain are expected to demonstrate similar properties in terms of ligand binding and therapeutic efficacy. In some embodiments, the Fc domain is a DEL Fc domain. In some embodiments of the technology, the Fc domain is an EEM Fc domain.

[0141] In some embodiments, the Fc domain is a variant Fc domain that forms a variant Fc domain with a desirable property, such as increased half-life, compared to naturally occurring (wild-type) Fc sequences. As used herein, a "variant Fc domain" refers to a non-naturally occurring Fc domain, for example an Fc domain comprising one or more non-naturally occurring amino acid residues, one or more amino acid substitutions relative to a wild-type human constant domain, or one or more amino acid deletion, addition and/or modification.

[0142] The serum half-life of binding agents comprising Fc domains may be increased by increasing the binding affinity of the Fc domain for FcRn. In some embodiments, the Fc domain variant has enhanced serum half-life relative to a comparable molecule. In a particular embodiment, the Fc domain variant comprises at least one amino acid substitution at one or more positions selected from the group consisting of M252Y, S254T and T256E (referred to herein as "YTE"; e.g., SEQ ID NO: 221 and 222). In another embodiment, the Fc domain variant comprises a Y at position 252 (referred to herein as "Y", e.g., SEQ ID NO: 223 and 224). Optionally, the Fc domain variant may comprise a non-naturally occurring amino acid residue at additional and/or alternative positions known to one skilled in the art (see, e.g., U.S. Pat. Nos. 5,624,821; 6,277,375; 6,737,056; PCT Patent Publications WO 01/58957; WO 02/06919; WO 04/016750; WO 04/029207; WO 04/035752; WO 04/074455; WO 04/099249; WO 04/063351; WO 05/070963; WO 05/040217, WO 05/092925, and WO 06/020114).

[0143] Examples for means to extend serum half-life of the binding agents of the disclosure include peptides, proteins or domains of proteins, which are fused or otherwise attached to the binding agents. The group of peptides, proteins or protein domains includes peptides binding to other proteins with preferred pharmacokinetic profile in the human body such as serum albumin (see WO 2009/127691). As used herein, the term "human serum albumin" refers to the albumin protein present in human blood plasma. Human serum albumin is the most abundant protein in the blood. It constitutes about half of the blood serum protein. In some embodiments, a human serum albumin has the sequence of UniProt ID NO: P02768.

[0144] An alternative concept of such half-life extending peptides includes peptides binding to the neonatal Fc receptor (FcRn, see WO 2007/098420), which can also be used in the polypeptides and binding agents of the present disclosure. The concept of attaching larger domains of proteins or complete proteins includes e.g. the fusion of human serum albumin, variants or mutants of human serum albumin (see WO 2011/051489, WO 2012/059486, WO 2012/150319, WO

2013/135896, WO 2014/072481, WO 2013/075066) or domains thereof as well as the fusion of constant region of immunoglobulins (Fc domains) and variants thereof, as described herein. Such variants of Fc domains may be optimized/modified in order to allow the desired pairing of dimers or multimers, to abolish Fc receptor binding (e.g., the Fcγ receptor), to enhance binding to FcRn, or for other reasons. A further concept known in the art to extend the half-life of small protein compounds in the human body is the pegylation of those compounds such as the polypeptide or binding agent of the present disclosure.

[0145] In an exemplary embodiment, the Fc domain monomer allows assembly of two or more polypeptide chains in a covalent manner, for example by disulfide linking between cysteine residues. In this way the Fc domain monomer acts as a dimerization domain, allowing assembly of two polypeptide chains to form a dimer. In some embodiments, such dimers comprise two polypeptides, each polypeptide including an antigen-binding domain described herein linked to an Fc domain monomer, thereby forming a bivalent binding agent. In some embodiments, such dimers comprise two polypeptides, one polypeptide comprising an antigen-binding domain described herein linked to an Fc domain monomer, and one polypeptide comprising an Fc domain monomer, thereby forming a monovalent binding agent.

[0146] The Fc domain may be a heterodimer comprising two Fc monomers, wherein the first antigen-binding domain is fused to the first Fc monomer and the second antigen-binding domain is fused to the second Fc monomer and wherein the first and second Fc monomers comprise asymmetric amino acid mutations that favor the formation of heterodimers over the formation of homodimers (see e.g., Ridgway et al. (1996) 'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng 9:617). The CH3 regions of the Fc monomers may comprise said asymmetric amino acid mutations, for example the first Fc polypeptide may comprise a T366W substitution, and the second Fc polypeptide may comprise T366S, L368A and Y407V substitutions, or vice versa, wherein the amino acid positions correspond to human IgG1 according to the EU numbering system. Furthermore, the cysteine residues at position 220 in the first and second Fc polypeptides may have been deleted or substituted, wherein the amino acid position corresponds to human IgG1 according to the EU numbering system.

[0147] Furthermore, the first and/or second Fc monomers may contain mutations that render the antibody inert, i.e. unable to, or having reduced ability to, mediate Fc effector functions (e.g., Fc-mediated cross-linking of FcRs). The inert Fc domain may in addition not be able to bind C1q and/or one or more Fc receptors. In some embodiments, the first and second Fc monomers may comprise a mutation at position 234 and/or 235, for example the first and second Fc monomer

may comprise an L234F and an L235E substitution, wherein the amino acid positions correspond to human IgG1 according to the EU numbering system. In some embodiments, the first and second Fc monomers may comprise an L234A and L235A substitution. In some embodiments, the first and second Fc domain monomers may further comprise a P329G substitution and/or a D265A substitution.

[0148] Exemplary Fc domain monomer sequences are shown in **TABLE 7**. Hinge sequences are shown in bold and italicized text; DEL and EEM polymorphisms are shown in boxed text; mutations relative to WT IgG1 are shown in bold and underlined text.

Table 7: Fc domain sequences

Fc domain	AA Sequence	SEQ ID:
WT IgG1 (EEM)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[EEM]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	219
WT IgG1 (DEL)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	220
IgG1 – YTE (EEM)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[EEM]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	221
IgG1 – YTE (DEL)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	222
IgG1 – Y (EEM)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTL**Y**ISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[EEM]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	223
IgG1 – Y (DEL)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTL**Y**ISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY	224

Fc domain	AA Sequence	SEQ ID:
	KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	
IgG1 L234F L235E (Effector)	***EPKSDKTHTCPPCP***APE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSL**T**CLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	225
IgG1 T366W (Knob)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSL**W**CLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	226
IgG1 T366S L368A Y407V (Hole)	***EPKSDKTHTCPPCP***APELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSL**S**C**A**VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	227
IgG1 Effector+Knob	***AAASDKTHTCPPCP***APE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSL**W**CLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG	228
IgG1 Effector+Hole	***AAASDKTHTCPPCP***APE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSL**S**C**A**VKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG	229
IgG1 (Effector+Knob) + K	***AAASDKTHTCPPCP***APE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSL**W**CLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	230
IgG1 (Effector+Hole) +K	***AAASDKTHTCPPCP***APE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSR[DEL]TKNQVSL**S**C**A**VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	231

[0149] In some embodiments, the Fc domains described in **Table 7** comprise a wild type IgG1 hinge (*e.g.*, SEQ ID NO: 232 – EPKSDKTHTCPPCP). In some embodiments, the Fc domains

described in **Table 7** comprise a modified IgG1 hinge (*e.g.*, SEQ ID NO: 233 – AAASDKTHTCPPCP). In some embodiments, the Fc domain sequences may further comprise a lysine (K) residue at the C terminus.

Linkers

[0150] In some embodiments, the multi-specific binding agents disclosed herein comprise one or more linkers. As used herein, the term "linker" refers to a short stretch of amino acids used to connect two functional domains (*e.g.*, antigen-binding domains or Fc domains) together in a polypeptide chain. For example, in some embodiments of the multi-specific binding agents of the disclosure, the antigen-binding domains are linked together on a polypeptide chain via one or more peptide linkers. Peptide linkers can also be used to attach other domains (such as half-life extension domains) to the multi-specific binding agents of the disclosure.

[0151] Suitable linkers are known in the art and include, for example, peptide linkers containing flexible amino acid residues such as glycine, alanine, and serine. In some embodiments, a linker can contain motifs, e.g., multiple or repeating motifs, of GA, GS, GG, GGA, GGS, GGG, GGGA (SEQ ID NO: 250), GGGS (SEQ ID NO: 251), GGGG (SEQ ID NO: 252), GGGGA (SEQ ID NO: 253), GGGGS (SEQ ID NO: 254), GGGGG (SEQ ID NO: 255), GGAG (SEQ ID NO: 256), GGSG (SEQ ID NO: 257), AGGG (SEQ ID NO: 258), or SGGG (SEQ ID NO: 259).

Polynucleotides

[0152] In some embodiments, the present disclosure provides a polynucleotide encoding any one of the Nectin-4 binding agents described herein. In some embodiments, the polynucleotide encodes a polypeptide sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a polynucleotide selected from the group consisting of SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136.

[0153] Methods of determining sequence similarity or identity between two or more nucleic acid sequences or amino acid sequences are known in the art. For example, sequence similarity or identity may be determined using the local sequence identity algorithm of Smith & Waterman, Adv. Appl. Math. 2, 482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch J Mol. Biol. 48, 443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA 85, 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux et al. Nucl. Acid Res. 12, 387-395 (1984), or by inspection.

[0154] Another suitable algorithm is the BLAST algorithm, described in Altschul et al. J. Mol. Biol. 215, 403-410, (1990) and Karlin et al. Proc. Natl. Acad. Sci. USA 90, 5873-5787 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul et al. Methods in Enzymology, 266, 460-480 (1996); blast.wustl/edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are optionally set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. Further, an additional useful algorithm is gapped BLAST as reported by Altschul et al, (1997) Nucleic Acids Res. 25, 3389-3402. Unless otherwise indicated, percent identity is determined herein using the algorithm available at the internet address: blast.ncbi.nlm.nih.gov/Blast.cgi.

[0155] In some embodiments, polynucleotide sequences described herein have been codon-optimized for improved expression. Without wishing to be bound by theory, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

[0156] The polynucleotides provided by the present disclosure may be not naturally occurring or may have a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques, such as those described in Sambrook et al., supra. The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al., supra, and Ausubel et al. supra. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-flurouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil,

dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopenenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladen ine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids provided by the present disclosure can be purchased from companies, such as integrated DNA Technologies (Coralville, IA, USA).

[0157] In some embodiments, the polynucleotides described herein comprise a nucleic acid sequence encoding a signal peptide at the 5' end of the nucleic acid sequence encoding the binding agents described herein. In some embodiments, the signal peptide facilitates secretion of the encoded protein from a host cell (*e.g.*, secretion of the Nectin-4 binding agents or multi-specific binding agents comprising the same). A person of skill in the art will recognize that signal peptides are cleaved during intracellular transport such that the mature proteins do not express the signal peptide. Signal peptides suitable for use according to the present disclosure are known in the art, for example signal peptides derived from GMCSF, the Igκ -chain, CD8α, CD33, or TPA.

Vectors

[0158] In some embodiments, the present disclosure provides a vector comprising a polynucleotide encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same.

[0159] In some embodiments, the vector comprising a polynucleotide encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same is a viral vector. Illustrative viral vectors include, but are not limited to, adeno- associated virus (AAV), retrovirus (e.g., lentivirus), herpes simplex virus, adenovirus, and vaccinia virus. AAV (rAAV) vectors are typically composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). In particular embodiments, the rAAV comprises ITRs and capsid sequences isolated from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In some embodiments, the ITR sequences of one AAV serotype and the capsid sequences of a different AAV serotype may be used to create a chimeric rAAV. In some embodiments, engineering and selection methods can be applied to AAV capsids to make them more likely to transduce cells of interest. Construction of rAAV vectors, production, and purification thereof have been disclosed,

e.g., in U.S. Patent Nos. 9,169,494; 9,169,492; 9,012,224; 8,889,641; 8,809,058; and 8,784,799, each of which is incorporated by reference herein, in its entirety.

[0160] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus. Illustrative lentiviruses include, but are not limited to, HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In various embodiments, a lentiviral vector contemplated herein comprises one or more LTRs, and one or more of the following accessory elements: a cPPT/FLAP, a Psi packaging signal, an export element, polyA sequences, and may optionally comprise a WPRE or HPRE, an insulator element, a selectable marker, and a cell suicide gene. In particular embodiments, lentiviral vectors contemplated herein may be integrative or non-integrating or integration defective lentivirus (e.g., having an integrase that lacks the capacity to integrate the viral genome into the genome of the host cells. Integration-incompetent viral vectors have been described in patent application WO 2006/010834, which is herein incorporated by reference in its entirety.

[0161] In particular embodiments, expression of heterologous sequences in viral vectors is increased by incorporating post-transcriptional regulatory elements, efficient polyadenylation sites, and optionally, transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements can increase expression of a heterologous polynucleotide at the protein, e.g., woodchuck hepatitis vims posttranscriptional regulatory element (WPRE; Zufferey et al., 1999, J. Virol., 73:2886); the posttranscriptional regulatory element present in hepatitis B vims (HPRE) (Huang el al, Mol. Cell. Biol., 5:3864); and the like (Liu el al, 1995, Genes Dev., 9: 1766).

[0162] In some embodiments, the vector comprising a polynucleotide encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same described herein is a plasmid. Numerous suitable plasmid expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other plasmid vector may be used so long as it is compatible with the host cell. Depending on the cell type utilized, any of a number of suitable transcription and translation

control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) Methods in Enzymology, 153:516-544).

[0163] In some embodiments, a polynucleotide sequence encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element may be functional in either a eukaryotic cell (e.g., a mammalian cell) or a prokaryotic cell (e.g., bacterial or archaeal cell). In some embodiments, a polynucleotide sequence encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same is operably linked to multiple control elements that allow expression of the polynucleotide in both prokaryotic and eukaryotic cells. Depending on the cell type utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) Methods in Enzymology, 153:516-544).

[0164] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression. The expression vector may also include nucleotide sequences encoding protein tags (e.g., 6xHis tag, hemagglutinin tag, green fluorescent protein, etc.) that are fused to the Nectin-4 binding agent or multi-specific binding agent comprising the same, thus resulting in a chimeric polypeptide.

[0165] In some embodiments, a polynucleotide sequence encoding a Nectin-4 binding agent or multi-specific binding agent comprising the same is operably linked to an inducible or a constitutive promoter.

[0166] A number of transfection techniques are generally known in the art (see, e.g., Graham et al., Virology, 52: 456-467 (1973); Sambrook et al., supra; Davis et al., Basic Methods in Molecular Biology, Elsevier (1986); and Chu et al., Gene, 13: 97 (1981). Transfection methods include calcium phosphate co-precipitation (see, e.g., Graham et al., supra), direct micro injection into cultured cells (see, e.g., Capecchi, Cell, 22: 479-488 (1980)), electroporation (see, e.g., Shigekawa et al., BioTechniques, 6: 742-751 (1988)), liposome mediated gene transfer (see, e.g., Mannino et al., BioTechniques, 6: 682-690 (1988)), lipid mediated transduction (see, e.g., Feigner

et al., Proc. Natl. Acad. Sci. USA, 84: 7413-7417 (1987)), and nucleic acid delivery using high velocity microprojectiles (see, e.g., Klein et al., Nature, 327: 70-73 (1987)).

[0167] In an embodiment, the vectors provided by the present disclosure can be prepared using standard techniques described in, for example, Sambrook et al., supra, and Ausubel et al., supra. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell, Replication systems can be derived, e.g., from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0168] The vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based. The expression vector may comprise restriction sites to facilitate cloning.

[0169] The vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0170] The vectors can be designed for either transient expression, for stable expression, or for both, of the polynucleotides comprised therein. Also, the vectors can be made for constitutive expression or for inducible expression of the polynucleotides comprised therein.

Pharmaceutical Compositions

[0171] In some embodiments, the present disclosure provides compositions comprising the Nectin-4 binding agents and/or multi-specific binding agents described herein. In some embodiments, the present disclosure provides pharmaceutical compositions comprising the Nectin-4 binding agents and/or multi-specific binding agents described herein and a pharmaceutically-acceptable excipient.

[0172] Typically, formulations include all physiologically acceptable compositions including derivatives and/or prodrugs, solvates, stereoisomers, racemates, or tautomers thereof with any physiologically acceptable carriers, diluents, and/or excipients. A “therapeutic composition” or “pharmaceutical composition” (used interchangeably herein) is a composition capable of being administered to a subject for the treatment of a particular disease or disorder.

[0173] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0174] As used herein "pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen- free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations. Except insofar as any conventional media and/or agent is incompatible with the agents of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[0175] "Pharmaceutically acceptable salt" includes both acid and base addition salts. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid,

lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, ptoluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

[0176] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0177] Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0178] Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, Pa., 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990).

Method of producing Nectin-4 binding agents

[0179] In some embodiments, the present disclosure provides a method of producing the Nectin-4 binding agents and multi-specific binding agents comprising the same comprising introducing a polynucleotide encoding the Nectin-4 binding agent or a vector comprising the same into a host cell, culturing the host cell under conditions suitable for expression of the Nectin-4 binding agent, and purifying the expressed Nectin-4 binding agent from the culture supernatant.

[0180] Exemplary host cells suitable for use in the manufacture of the binding agents described herein are known in the art, for example, a host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DK5α E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the expression vector, the host cell may be a prokaryotic cell, e.g., a DH5α cell. For the purposes of producing a binding agent, the host cell may be a mammalian cell. In some embodiments, the present disclosure provides a host cell comprising a polynucleotide described herein or vector comprising the same.

[0181] Suitable methods of making antibodies, including single domain antibodies, are known in the art. For instance, standard hybridoma methods are described in, e.g., Köhler and Milstein, Eur. J. Immunol. 5, 511-519 (1976), Harlow and Lane (eds.), Antibodies: A Laboratory Manual, CSH Press (1988), and C.A. Janeway et al. (eds,), Immumobiology, 5th Ed., Garland Publishing, New York, NY (2001). Alternatively, other methods, such as EBV-hybridoma methods (Haskard and Archer, J. Immunol. Methods, 74(2), 361-67 (1984), and Roder et al., Methods Enzymol., 121, 140-67 (1986)), and bacteriophage vector expression systems (see, e.g., Huse et al, Science, 246, 1275-81 (1989)) are known in the art. Further, methods of producing antibodies in non-human animals are described in, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, U.S. Patent Application Publication No. 2002/0197266, and U.S. Patent No. 7,338,929).

[0182] Antibodies can be produced by transgenic mice that are transgenic for specific heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., supra.

[0183] Methods for generating humanized antibodies an antigen-binding fragments thereof are well known in the art and are described in detail in, for example, Janeway et al., supra, U.S. Patents 5,225,539, 5,585,089 and 5,693,761, European Patent No. 0239400 Bl, and United Kingdom Patent No. 2188638. Humanized antibodies can also be generated using the antibody

resurfacing technology described in U.S. Patent 5,639,641 and Pedersen et al., J. Mol. Biol., 235, 959-973 (1994).

[0184] Multi-specific binding agents of the invention are typically produced recombinantly, i.e. by expression of polynucleotides encoding the multi-specific binding agents in suitable host cells, followed by purification of the produced recombinant multi-specific binding agent from the cell culture. Polynucleotides can be produced by standard molecular biological techniques well-known in the art. The polynucleotides are typically introduced into the host cell using an expression vector. Suitable nucleic acid constructs and expression vectors are known in the art. Host cells suitable for the recombinant expression of antibodies are well-known in the art, and include CHO (Chinese Hamster Ovary), HEK-293, Expi293F, PER-C6, NS/0 and Sp2/0 cells.

[0185] Accordingly, in a further embodiment, the invention relates to a polynucleotide encoding a binding agent of the invention, such as multi-specific binding agent according to the invention. In one embodiment, the polynucleotide is a DNA construct. In another embodiment, the polynucleotide is an RNA construct. The polynucleotide typically further comprises a promoter.

[0186] In a further embodiment, the invention relates to an expression vector comprising a polynucleotide encoding a multi-specific binding agent according to the invention. The expression vector typically further comprises a promoter.

[0187] In a further embodiment, the invention relates to a host cell, such as a non-human host cell, for example a CHO cell, comprising one or more polynucleotides encoding a multi-specific binding agent according to the invention or an expression vector comprising a polynucleotide encoding a multi-specific binding agent according to the invention.

Method of Treatment

[0188] In some embodiments, the present disclosure provides a method of treating cancer in a subject in need thereof comprising administering a Nectin-4 binding agent described herein, a multi-specific binding agent comprising the same, or a composition comprising the same.

[0189] In some embodiments, treatment comprises delivering an effective amount of a Nectin-4 binding agent or multi-specific binding agent comprising the same, or a composition thereof, to a subject in need thereof. In some embodiments, treating refers to the treatment of a cancer in a mammal, e.g., in a human, including (a) inhibiting the cancer, i.e., arresting cancer development or preventing cancer progression; (b) relieving the cancer, i.e., causing regression of the cancer state or relieving one or more symptoms of the cancer; and (c) curing the cancer, i.e., remission of one or more cancer symptoms. In some embodiments, treatment may refer to a short-term (e.g., temporary and/or acute) and/or a long-term (e.g., sustained) reduction in one or more

cancer symptoms. In some embodiments, treatment results in an improvement or remediation of the symptoms of the cancer. The improvement is an observable or measurable improvement, or may be an improvement in the general feeling of well-being of the subject.

[0190] An "effective amount" or "an amount effective to treat" refers to a dose that is adequate to prevent or treat cancer in an individual. In some embodiments, an effective amount is also one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. The effective amount of a Nectin-4 binding agent or multi-specific binding agent, or a composition comprising the same administered to a particular subject will depend on a variety of factors, several of which will differ from patient to patient including the disorder being treated and the severity of the disorder; activity of the specific agent(s) employed; the age, body weight, general health, sex and diet of the patient; the timing of administration, route of administration; the duration of the treatment; the ability of the binding agent or multi-specific binding agent comprising the same to elicit a desired response in the individual; drugs used in combination; the judgment of the prescribing physician; and like factors known in the medical arts. The size of the dose will also be determined by the existence, nature, and extent of any adverse side- effects that might accompany the administration of a particular active, and the desired physiological effect. It will be appreciated by one of skill in the art that various diseases or disorders could require prolonged treatment involving multiple administrations, perhaps using various rounds of administration.

[0191] The cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia (AML), alveolar rhabdomyosarcoma, B-cell chronic lymphoproliferative disorders, bladder cancer (e.g., bladder carcinoma), blastic plasmacytoid dendritic cell neoplasm, bone cancer, brain cancer (e.g., medulloblastoma), breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, head and neck cancer (e.g., head and neck squamous cell carcinoma), Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer (e.g., non-small cell lung carcinoma and lung adenocarcinoma), lymphoma, mesothelioma, mastocytoma, melanoma, multiple myeloma, myelodysplastic syndrome, nasopharynx cancer, non-Hodgkin lymphoma, B-cell chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), Burkitt's lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer,

small intestine cancer, soft tissue cancer, solid tumors, synovial sarcoma, gastric cancer, testicular cancer, thyroid cancer, and ureter cancer.

[0192] Exemplary expression of Nectin-4 in certain isolated patient samples are shown in **FIG. 1A** In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is acute myeloid leukemia. In some embodiments, the cancer is a head and neck cancer. In some embodiments, the cancer is a bladder cancer. In some embodiments, the bladder cancer is a urothelial cancer. In some embodiments, the cancer is choriocarcinoma. In some embodiments, the cancer is epidermoid carcinoma. In some embodiments, the cancer is colorectal adenocarcinoma.

[0193] In some embodiments, the cancer is characterized by the expression of Nectin-4. For example, in some embodiments, the cancer is selected from metastatic colorectal cancer, clear cell renal cell cancer, glioblastoma multiforme, adenocarcinoma, squamous cell carcinoma, head & neck cancer, primary colorectal cancer, esophagus carcinoma, and urothelial cell carcinoma.

[0194] In some embodiments, the cancer is selected from bladder cancer (such as bladder urothelial cancer), breast cancer (such as triple negative breast cancer), renal cancer, prostate cancer, ovarian cancer, esophageal cancer, head and neck cancer, lung cancer, pancreatic cancer, gastric cancer, thyroid cancer, colorectal cancer, cholangiocarcinoma and uterine corpus endometrial carcinoma.

[0195] "Treatment" or "treating" refers to the administration of an effective amount of a binding agent or multi-specific binding agent comprising the same according to the present disclosure with the purpose of easing, ameliorating, arresting, eradicating (curing) or preventing symptoms or disease states.

[0196] In some embodiments, the subject may be a neonate, a juvenile, or an adult. Of particular interest are mammalian subjects. Mammalian species that may be treated with the present methods include canines and felines; equines; bovines; ovines; etc. and primates, particularly humans. Animal models, particularly small mammals (e.g. mice, rats, guinea pigs, hamsters, rabbits, etc.) may be used for experimental investigations.

[0197] In some embodiments, the present disclosure provides methods of expanding Vγ9Vδ2 T cells comprising contacting the Vγ9Vδ2 T cells with a Nectin-4 binding agent described herein, a multi-specific binding agent comprising the same, or a composition comprising the same. In some embodiments, the contacting is in vitro, in vivo, or ex vivo. Expansion of Vγ9Vδ2 T cells may be measured by any method known in the art (e.g., labeling with a suitable agent such as carboxyfluorescein succinimidyl ester followed by quantification of fluorescence by e.g., flow cytometry).

[0198] Administration of the Nectin-4 binding agent or multi-specific binding agent, or of a composition comprising the same, can occur by injection, irrigation, inhalation, consumption, electro-osmosis, hemodialysis, iontophoresis, and other methods known in the art. In some embodiments, administration route is local or systemic. In some embodiments administration route is intraarterial, intracranial, intradermal, intraduodenal, intramammary, intrameningeal, intraperitoneal, intrathecal, intratumoral, intravenous, intravitreal, ophthalmic, parenteral, spinal, subcutaneous, ureteral, urethral, vaginal, intramuscular, inhalation, oral, or intrauterine.

[0199] In some embodiments, the administration route is by infusion (e.g., continuous or bolus). Examples of methods for local administration, that is, delivery to the site of injury or disease, include through an Ommaya reservoir, e.g. for intrathecal delivery (See e.g., US Patent Nos. 5,222,982 and 5,385,582, incorporated herein by reference); by bolus injection, e.g. by a syringe, e.g. into a joint; by continuous infusion, e.g. by cannulation, such as with convection (See e.g., US Patent Application Publication No. 2007-0254842, incorporated herein by reference); or by implanting a device upon which the binding agents have been reversibly affixed (see e.g. US Patent Application Publication Nos. 2008-0081064 and 2009-0196903, incorporated herein by reference). In some embodiments, the administration route is by topical administration or direct injection. In some embodiments, the binding agents described herein may be provided to the subject alone or with a suitable substrate or matrix.

[0200] In some embodiments, at least 0.01 μg/kg of the Nectin-4 binding agent or multi-specific binding agent, or of a composition comprising the same, is administered to a subject. In some embodiments, between about 0.01 μg/kg and about 100mg/kg, between about 0.1 μg/kg and about 50 mg/kg, between about 0.01 mg/kg and about 20 mg/kg, or between about 0.01 mg/kg and about 10 mg/kg of the Nectin-4 binding agent or multi-specific binding agent, or of a composition comprising the same, is administered to a subject. In some embodiments, at least about 0.01 mg/kg, at least about 0.03 mg/kg, at least about 0.05 mg/kg, at least about 0.1 mg/kg, about 0.3 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 3 mg/kg, about 5 mg/kg, about 8 mg/kg, or about 10 mg/kg of the Nectin-4 binding agent or multi-specific binding agent, or of a composition comprising the same, is administered to the subject. In some embodiments, at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11.0, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or at least 500 nM of the Nectin-4 binding

agent or multi-specific binding agent, or of a composition comprising the same, is administered to a subject.

[0201] The number of administrations of treatment to a subject may vary. In some embodiments, administration to the subject may be a one-time event. In some embodiments, such treatment may require an on-going series of repeated treatments. In some embodiments, multiple administrations may be required before an effect is observed. The exact protocols depend upon the disease or condition, the stage of the disease and parameters of the individual subject being treated.

[0202] In some embodiments, the effective amount of a Nectin-4 binding agent, a multi-specific binding agent comprising the same, or a composition comprising the same may be the amount required to result in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more fold decrease in tumor mass or volume, decrease in the number of tumor cells, or decrease in the number of metastases. In some embodiments, the effective amount of a Nectin-4 binding agent, a multi-specific binding agent comprising the same, or a composition comprising the same may be the amount required to achieve an increase in life expectancy, an increase in progression-free or disease-free survival, or amelioration of various physiological symptoms associated with the disease being treated.

[0203] In some embodiments, the methods of treatment provided herein further comprise the administration of one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered prior to, simultaneously with, or after the Nectin-4 binding agent or multi-specific binding agent comprising the same, or composition comprising the same described herein. In some embodiments, the additional therapeutic agent is IL-2 or IL-15.

[0204] In some embodiments, the additional therapeutic agent is IL-2. In some embodiments, the IL-2 is Aldesleukin/Proleukin. Other suitable forms of IL-2 include NKTR-214 (Bempegaldesleukin/Nektar), ALK 4230 (nemvaleukin/Aalkermes-Reliant), SAR444245 – THOR 707/Synthorx/Sanofi) and XTX-202 (Xilio). In some embodiments, the IL-2 is a variant IL-2. In some embodiments, the variant IL-2 binds to the IL-2Rbeta and gamma chains, but does not bind or demonstrates reduced/abrogated binding to the IL-2Ralpha chain (e.g. NL201 from Neoleukin, Silva et al. De novo design of potent and selective mimics of IL-2 and IL-15. Nature 565, 186–191 (2019), and MDNA11 from Medicenna). In some embodiments, the IL-2 and/or variants thereof is pegylated.

[0205] In some embodiments, the additional therapeutic agent is IL-15. Suitable forms of IL-15 are known in the art. See e.g., Int J Mol Sci. 2022 Jul; 23(13): 7311, which is herein incorporated by reference in its entirety. In some embodiments, the IL-15 is a heterodimer of IL-15 and IL-15

receptor alpha (hetIL-15), which is also referred to as NIZ985. In some embodiments, the IL-15 is N-803, formerly known as ALT-803, which is an IL-15 variant complexed with a human IL-15Rα sushi domain-Fc fusion protein. In some embodiments, the IL-15 is SOT101, also referred to as Nanrilkefusp alfa, which is a human fusion protein comprising the cytokine IL-15 and the high-affinity binding sushi+ domain of IL-15 receptor alpha (IL-15Rα). In some embodiments, the IL-15 is NKTR-255, which is a polyethylene glycol-conjugate of rhIL-15. In some embodiments, the IL-15 and/or variants thereof is pegylated.

Exemplary Multi-specific Binding Agents

[0206] In some embodiments, the present disclosure provides a multi-specific binding agent comprising a first antigen-binding domain that binds to Nectin-4 and a second antigen-binding domain that binds to Vδ2. In some embodiments, the first and second antigen-binding domains are comprised in the same polypeptide chain. In some embodiments, the multi-specific binding agent comprises the following format, from N' to C' terminus: first antigen-binding domain – linker – second antigen-binding domain. In some embodiments, the multi-specific binding agent comprises the following format, from N' to C' terminus: second antigen-binding domain – linker – first antigen-binding domain. In some embodiments, the linker is a glycine-serine linker. In some embodiments, the linker is G_4S and comprises SEQ ID NO: 254.

[0207] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain comprising a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96 and a CDR3 of SEQ ID NO: 97 and (ii) a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2 and a CDR3 of SEQ ID NO: 3. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 98 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 4. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 98 and the second antigen-binding domain comprises SEQ ID NO: 4. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 98 and the second antigen-binding domain consists of SEQ ID NO: 4. In some embodiments, the multi-specific binding agent comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 281. In some embodiments, the multi-specific binding agent comprises SEQ ID NO: 281. In some

embodiments, the multi-specific binding agent consists of SEQ ID NO: 281. Such a multi-specific binding agent is referred to herein as LAVA-366.

[0208] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain comprising a CDR1 of SEQ ID NO: 88, a CDR2 of SEQ ID NO: 89 and a CDR3 of SEQ ID NO: 90 and (ii) a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2 and a CDR3 of SEQ ID NO: 3. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 91 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 4. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 91 and the second antigen-binding domain comprises SEQ ID NO: 4. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 91 and the second antigen-binding domain consists of SEQ ID NO: 4. In some embodiments, the multi-specific binding agent comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 282. In some embodiments, the multi-specific binding agent comprises SEQ ID NO: 282. In some embodiments, the multi-specific binding agent consists of SEQ ID NO: 282. Such a multi-specific binding agent is referred to herein as LAVA-367.

[0209] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain comprising a CDR1 of SEQ ID NO: 103, a CDR2 of SEQ ID NO: 104 and a CDR3 of SEQ ID NO: 105 and (ii) a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2 and a CDR3 of SEQ ID NO: 3. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 106 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 4. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 106 and the second antigen-binding domain comprises SEQ ID NO: 4. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 106 and the second antigen-binding domain consists of SEQ ID NO: 4. In some embodiments, the multi-specific binding agent comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 283. In some embodiments, the multi-specific binding agent comprises SEQ ID NO: 283. In some embodiments, the multi-specific binding agent consists of SEQ ID NO: 283. Such a multi-specific binding agent is referred to herein as LAVA-368.

[0210] In some embodiments, the multi-specific binding agent comprises (i) a first antigen-binding domain comprising a CDR1 of SEQ ID NO: 110, a CDR2 of SEQ ID NO: 111 and a CDR3 of SEQ ID NO: 112 and (ii) a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2 and a CDR3 of SEQ ID NO: 3. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 113 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 4. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 113 and the second antigen-binding domain comprises SEQ ID NO: 4. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 113 and the second antigen-binding domain consists of SEQ ID NO: 4. In some embodiments, the multi-specific binding agent comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 284. In some embodiments, the multi-specific binding agent comprises SEQ ID NO: 284. In some embodiments, the multi-specific binding agent consists of SEQ ID NO: 284. Such a multi-specific binding agent is referred to herein as LAVA-369.

[0211] Exemplary embodiments of such single chain multi-specific binding agents are provided in **TABLE 8**.

Table 8: Exemplary single chain multi-specific binding agents.

Bispecific agent	Format	Amino acid sequence	SEQ ID
LV1181 x Vδ2 (LAVA-366)	LV1181 - G_4S – 5C8	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPGK QRELVATITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNSLK PEDTAAYYCVDVEPSGMGWRDYWGQGTQVTVSSGGGGSEVQLV ESGGGLVQAGGSLRLSCAASGRPFSNYAMGWFRQAPGKEREFV AAISWSGGSTSYADSVKGRFTISRDNAKNTVYLQMNSPKPEDT AIYYCAAQFSGADYGFGRLGIRGYEYDYWGQGTQVTVSS	281
LV1184 x Vδ2 (LAVA-367)	LV1184 - G_4S – 5C8	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWFRQAPAK QRELVTSISPGGSVRYADSVKGRFTISRDVAKNTVDLQMNSLK PEDTAVYYCAAESERTYYFDSWGQGTQVTVSSGGGGSEVQLVE SGGGLVQAGGSLRLSCAASGRPFSNYAMGWFRQAPGKEREFVA	282

Bispecific agent	Format	Amino acid sequence	SEQ ID
		AISWSGGSTSYADSVKGRFTISRDNAKNTVYLQMNSPKPEDTA IYYCAAQFSGADYGFGRLGIRGYEYDYWGQGTQVTVSS	
LV1185 x Vδ2 (LAVA-368)	LV1185 - G_4S – 5C8	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGK QREYVATITTGGSTNYADSVRGRFTISRDNAEDTVYLQMNSLK PEDTAVYYCTAELVRRGPTTYWGRGTQVTVSSGGGGSEVQLVE SGGGLVQAGGSLRLSCAASGRPFSNYAMGWFRQAPGKEREFVA AISWSGGSTSYADSVKGRFTISRDNAKNTVYLQMNSPKPEDTA IYYCAAQFSGADYGFGRLGIRGYEYDYWGQGTQVTVSS	283
LV1186 x Vδ2 (LAVA-369)	LV1186 - G_4S – 5C8	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGK QREYVATITSGGSTNYADSVRGRFTISRDNAENTVYLQMDSLK PEDTAVYYCTAELVRRGPTTYWGRGTQVTVSSGGGGSEVQLVE SGGGLVQAGGSLRLSCAASGRPFSNYAMGWFRQAPGKEREFVA AISWSGGSTSYADSVKGRFTISRDNAKNTVYLQMNSPKPEDTA IYYCAAQFSGADYGFGRLGIRGYEYDYWGQGTQVTVSS	284

[0212] In some embodiments the multi-specific binding agent further comprises one or more Fc domains. In such embodiments, the multi-specific binding agent comprises a first and a second polypeptide, wherein the first polypeptide comprises a first antigen-binding domain that binds to Nectin-4 and a first Fc domain monomer, and wherein the second polypeptide comprises a second antigen-binding domain and a second Fc domain monomer. The Fc domain monomers associate to form a dimeric multi-specific binding agent. In some embodiments, the Fc domain monomers comprise one or more mutations to facilitate dimerization and/or reduce effector function.

[0213] Exemplary embodiments of such first and second polypeptides are provided below in **TABLE 9.** Antigen-binding domains are shown in underlined text. Hinges are shown in italicized and bolded text. Fc domains are shown in regular text. For all of the Nectin-4 VHH – Fc fusion proteins, the Fc domain corresponds to SEQ ID NO: 229 or 231 (*i.e.*, effector mutations + hole mutations, w/ or w/o a C-terminal lysine). For all of the Vδ2 VHH – Fc fusions proteins, the Fc domain corresponds to SEQ ID NO: 228 or 230 (*i.e.*, effector mutations + knob mutations, w/ or w/o a C-terminal lysine).

Table 9: Exemplary VHH-Fc Binding Agents

Reference	AA Sequence	SEQ ID:
LV1181-IgG1 (Effector+ Knob)	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPGKQRELVA TITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAAYYCVDV EPSGMGWRDYWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG	267

Reference	AA Sequence	SEQ ID:
LV1181-IgG1 (Effector+ Knob) +K	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPGKQRELVA TITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAAYYCVDV EPSGMGWRDYWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK	268
LV1184-IgG1 (Effector+ Knob)	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWFRQAPAKQRELVT SISPGGSVRYADSVKGRFTISRDVAKNTVDLQMNSLKPEDTAVYYCAAE SERTYYFDSWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG	269
LV1184-IgG1 (Effector+ Knob) +K	EVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWFRQAPAKQRELVT SISPGGSVRYADSVKGRFTISRDVAKNTVDLQMNSLKPEDTAVYYCAAE SERTYYFDSWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	270
LV1185-IgG1 (Effector+ Knob)	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGKQREYVA TITTGGSTNYADSVRGRFTISRDNAEDTVYLQMNSLKPEDTAVYYCTAE LVRRGPTTYWGRGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG	271
LV1185-IgG1 (Effector+ Knob) +K	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGKQREYVA TITTGGSTNYADSVRGRFTISRDNAEDTVYLQMNSLKPEDTAVYYCTAE LVRRGPTTYWGRGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	272
LV1186-IgG1 (Effector+ Knob)	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGKQREYVA TITSGGSTNYADSVRGRFTISRDNAENTVYLQMDSLKPEDTAVYYCTAE LVRRGPTTYWGRGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG	273
LV1186-IgG1	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNIMGWYRQAPGKQREYVA TITSGGSTNYADSVRGRFTISRDNAENTVYLQMDSLKPEDTAVYYCTAE	274

Reference	AA Sequence	SEQ ID:
(Effector+ Knob) +K	LVRRGPTTYWGRGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	
LV1181-E1D- IgG1 (Effector+ Knob)	DVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPGKQRELVA TITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAAYYCVDV EPSGMGWRDYWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG	275
LV1181-E1D- IgG1 (Effector+ Knob) +K	DVQLVESGGGLVQAGGSLRLSCAASGSISSINLMGWHRQAPGKQRELVA TITRGGSTNYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAAYYCVDV EPSGMGWRDYWGQGTQVTVSS***AAASDKTHTCPPCP***APEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK	276
5C8var1-(105F-R109A) IgG1 (Effector+ hole)	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWFRQAPGKEREFVS AISWSGGSTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA QFSGADFGFGALGIRGYEYDYWGQGTQVTVSS***AAASDKTHTCPPCP***APE FEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG	277
5C8var1-(105F-R109A) IgG1 (Effector+ hole) + K	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWFRQAPGKEREFVS AISWSGGSTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA QFSGADFGFGALGIRGYEYDYWGQGTQVTVSS***AAASDKTHTCPPCP***APE FEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK	278
5C8 var1 (E1D-105F-R109A)-IgG1 (Effector+ hole)	DVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWFRQAPGKEREFVS AISWSGGSTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA QFSGADFGFGALGIRGYEYDYWGQGTQVTVSS***AAASDKTHTCPPCP***APE FEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG	279
5C8 var1 (E1D-105F-R109A)-	DVQLLESGGGSVQPGGSLRLSCAASGRPFSNYAMSWFRQAPGKEREFVS AISWSGGSTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA QFSGADFGFGALGIRGYEYDYWGQGTQVTVSS***AAASDKTHTCPPCP***APE FEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV	280

Reference	AA Sequence	SEQ ID:
IgG1 (Effector+ hole) +K	EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK	

[0214] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 88, a CDR2 of SEQ ID NO: 89 and a CDR3 of SEQ ID NO: 90 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6 and a CDR3 of SEQ ID NO: 7 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 91 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 91 and the second antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 91 and the second antigen-binding domain consists of SEQ ID NO: 8. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 270 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 278. In some embodiments, the first polypeptide comprises SEQ ID NO: 270 and the second polypeptide comprises SEQ ID NO: 278. In some embodiments, the first polypeptide consists of SEQ ID NO: 270 and the second polypeptide consists of SEQ ID NO: 278. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 269 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

SEQ ID NO: 277. In some embodiments, the first polypeptide comprises SEQ ID NO: 269 and the second polypeptide comprises SEQ ID NO: 277. In some embodiments, the first polypeptide consists of SEQ ID NO: 269 and the second polypeptide consists of SEQ ID NO: 277.

[0215] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96 and a CDR3 of SEQ ID NO: 97 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6 and a CDR3 of SEQ ID NO: 7 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 98 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 98 and the second antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 98 and the second antigen-binding domain consists of SEQ ID NO: 8. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 268 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 278. In some embodiments, the first polypeptide comprises SEQ ID NO: 268 and the second polypeptide comprises SEQ ID NO: 278. In some embodiments, the first polypeptide consists of SEQ ID NO: 268, referred to herein as LAVA-387, and the second polypeptide consists of SEQ ID NO: 278, referred to herein as LAVA-349. Such a multi-specific binding agent is referred to herein as LAVA-1387. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 267 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least

97%, at least 98%, or at least 99% identical to SEQ ID NO: 277. In some embodiments, the first polypeptide comprises SEQ ID NO: 267 and the second polypeptide comprises SEQ ID NO: 277. In some embodiments, the first polypeptide consists of SEQ ID NO: 267 and the second polypeptide consists of SEQ ID NO: 277.

[0216] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 103, a CDR2 of SEQ ID NO: 104 and a CDR3 of SEQ ID NO: 105 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6 and a CDR3 of SEQ ID NO: 7 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 106 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 106 and the second antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 106 and the second antigen-binding domain consists of SEQ ID NO: 8. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 272 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 278. In some embodiments, the first polypeptide comprises SEQ ID NO: 272 and the second polypeptide comprises SEQ ID NO: 278. In some embodiments, the first polypeptide consists of SEQ ID NO: 278 and the second polypeptide consists of SEQ ID NO: 278. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 271 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

SEQ ID NO: 277. In some embodiments, the first polypeptide comprises SEQ ID NO: 271 and the second polypeptide comprises SEQ ID NO: 277. In some embodiments, the first polypeptide consists of SEQ ID NO: 271 and the second polypeptide consists of SEQ ID NO: 277.

[0217] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 110, a CDR2 of SEQ ID NO: 111 and a CDR3 of SEQ ID NO: 112 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6 and a CDR3 of SEQ ID NO: 7 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 113 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 113 and the second antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 113 and the second antigen-binding domain consists of SEQ ID NO: 8. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 274 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 278. In some embodiments, the first polypeptide comprises SEQ ID NO: 274 and the second polypeptide comprises SEQ ID NO: 278. In some embodiments, the first polypeptide consists of SEQ ID NO: 274 and the second polypeptide consists of SEQ ID NO: 278. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 273 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 277. In some embodiments, the first polypeptide comprises SEQ ID NO: 273 and

the second polypeptide comprises SEQ ID NO: 277. In some embodiments, the first polypeptide consists of SEQ ID NO: 273 and the second polypeptide consists of SEQ ID NO: 277.

[0218] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96 and a CDR3 of SEQ ID NO: 97 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6 and a CDR3 of SEQ ID NO: 7 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 99 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 99 and the second antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 99 and the second antigen-binding domain consists of SEQ ID NO: 8. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 276 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 278. In some embodiments, the first polypeptide comprises SEQ ID NO: 276 and the second polypeptide comprises SEQ ID NO: 278. In some embodiments, the first polypeptide consists of SEQ ID NO: 276 and the second polypeptide consists of SEQ ID NO: 278. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 275 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 277. In some embodiments, the first polypeptide comprises SEQ ID NO: 275 and

the second polypeptide comprises SEQ ID NO: 277. In some embodiments, the first polypeptide consists of SEQ ID NO: 275 and the second polypeptide consists of SEQ ID NO: 277.

[0219] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 88, a CDR2 of SEQ ID NO: 89 and a CDR3 of SEQ ID NO: 90 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 18, a CDR2 of SEQ ID NO: 19 and a CDR3 of SEQ ID NO: 20 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 91 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 91 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 91 and the second antigen-binding domain consists of SEQ ID NO: 22. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 270 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 280. In some embodiments, the first polypeptide comprises SEQ ID NO: 270 and the second polypeptide comprises SEQ ID NO: 280. In some embodiments, the first polypeptide consists of SEQ ID NO: 270 and the second polypeptide consists of SEQ ID NO: 280.In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 269 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 279. In some embodiments, the first polypeptide comprises SEQ ID NO: 269 and

the second polypeptide comprises SEQ ID NO: 279. In some embodiments, the first polypeptide consists of SEQ ID NO: 269 and the second polypeptide consists of SEQ ID NO: 279.

[0220] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96 and a CDR3 of SEQ ID NO: 97 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 18, a CDR2 of SEQ ID NO: 19 and a CDR3 of SEQ ID NO: 20 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 98 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 98 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 98 and the second antigen-binding domain consists of SEQ ID NO: 22. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 268 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 280. In some embodiments, the first polypeptide comprises SEQ ID NO: 268 and the second polypeptide comprises SEQ ID NO: 280. In some embodiments, the first polypeptide consists of SEQ ID NO: 268 and the second polypeptide consists of SEQ ID NO: 280. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 267 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 279. In some embodiments, the first polypeptide comprises SEQ ID NO: 267 and

the second polypeptide comprises SEQ ID NO: 279. In some embodiments, the first polypeptide consists of SEQ ID NO: 267 and the second polypeptide consists of SEQ ID NO: 279.

[0221] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 103, a CDR2 of SEQ ID NO: 104 and a CDR3 of SEQ ID NO: 105 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 18, a CDR2 of SEQ ID NO: 19 and a CDR3 of SEQ ID NO: 20 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 106 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 106 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 106 and the second antigen-binding domain consists of SEQ ID NO: 22. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 272 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 280. In some embodiments, the first polypeptide comprises SEQ ID NO: 272 and the second polypeptide comprises SEQ ID NO: 280. In some embodiments, the first polypeptide consists of SEQ ID NO: 272 and the second polypeptide consists of SEQ ID NO: 280. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 271 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 279. In some embodiments, the first polypeptide comprises SEQ ID NO: 271 and

the second polypeptide comprises SEQ ID NO: 279. In some embodiments, the first polypeptide consists of SEQ ID NO: 271 and the second polypeptide consists of SEQ ID NO: 279.

[0222] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 110, a CDR2 of SEQ ID NO: 111 and a CDR3 of SEQ ID NO: 112 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 18, a CDR2 of SEQ ID NO: 19 and a CDR3 of SEQ ID NO: 20 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 113 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 113 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 113 and the second antigen-binding domain consists of SEQ ID NO: 22. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 274 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 280. In some embodiments, the first polypeptide comprises SEQ ID NO: 274 and the second polypeptide comprises SEQ ID NO: 280. In some embodiments, the first polypeptide consists of SEQ ID NO: 274 and the second polypeptide consists of SEQ ID NO: 280. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 273 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 279. In some embodiments, the first polypeptide comprises SEQ ID NO: 273 and

the second polypeptide comprises SEQ ID NO: 279. In some embodiments, the first polypeptide consists of SEQ ID NO: 273 and the second polypeptide consists of SEQ ID NO: 279.

[0223] In some embodiments, the multi-specific binding agent comprises (i) a first polypeptide comprising a first antigen-binding domain that binds Nectin-4 comprising a CDR1 of SEQ ID NO: 95, a CDR2 of SEQ ID NO: 96 and a CDR3 of SEQ ID NO: 97 and a first Fc domain monomer; and (ii) a second polypeptide comprising a second antigen-binding domain comprising a CDR1 of SEQ ID NO: 18, a CDR2 of SEQ ID NO: 19 and a CDR3 of SEQ ID NO: 20 and a second Fc domain monomer. In some embodiments, the first antigen-binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 99 and the second antigen-binding domain comprises an amino acid sequence that this at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22. In some embodiments, the first antigen-binding domain comprises SEQ ID NO: 99 and the second antigen-binding domain comprises SEQ ID NO: 22. In some embodiments, the first antigen-binding domain consists of SEQ ID NO: 99 and the second antigen-binding domain consists of SEQ ID NO: 22. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 228 or 230 and the second Fc domain monomer comprises SEQ ID NO: 229 or 231. In some embodiments, the first Fc domain monomer comprises SEQ ID NO: 229 or 231 and the second Fc domain monomer comprises SEQ ID NO: 228 or 230. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 276 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 280. In some embodiments, the first polypeptide comprises SEQ ID NO: 276 and the second polypeptide comprises SEQ ID NO: 280. In some embodiments, the first polypeptide consists of SEQ ID NO: 276, referred to herein as LAVA-427 and the second polypeptide consists of SEQ ID NO: 280, referred to herein as LAVA-448. Such a multi-specific binding agent is referred to herein as LAVA-1427.

[0224] In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 275 and the second polypeptide comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

SEQ ID NO: 279. In some embodiments, the first polypeptide comprises SEQ ID NO: 275 and the second polypeptide comprises SEQ ID NO: 279. In some embodiments, the first polypeptide consists of SEQ ID NO: 275 and the second polypeptide consists of SEQ ID NO: 279.

EXAMPLES

Example 1: Generation of anti-Nectin-4 binding agents

[0225] Lama glama (2 animals) were immunized with 4 injections of HepG2 cells (day 0, 14, 28 and 35) followed by 2 injections of human Nectin-4 protein (Sino Biologics) on days 49 and 63. Serum was collected before and after immunization and sera were tested for the presence of anti-Nectin-4 antibodies by ELISA.

[0226] After confirmation of a Nectin-4 specific immune response, a whole blood sample was collected for peripheral blood lymphocyte (PBL) isolation and phage libraries were constructed. In brief: total RNA was extracted from PBLs, transcribed into cDNA, purified, and used as a template for immunoglobulin heavy-chain-encoding cDNA amplification. The resultant cDNA was run on an agarose gel and the cDNA encoding heavy-chain-only immunoglobulin (~ 700 bp) were excised from the gel. The purified cDNA and used as a template for a nested PCR that introduced an appropriate 5' flanking restriction site and amplified only binding agent-encoding cDNA. The purified PCR product was digested with appropriate restriction enzymes, after which the digested products were ligated in frame with geneIII into the pQ81 phagemid vector.

[0227] The ligated library was transformed into TG1 E. coli by electroporation for display on filamentous bacteriophage. Two consecutive rounds of selection were performed, followed by screening single clones for binding to the recombinant captured antigen. Those clones that scored positive for binding were sequenced and a selection of clones having a different sequence were then tested for Nectin-4 binding by ELISA. Nine different clones were identified and termed: LV1178 (SEQ ID NO: 120), LV1179 (SEQ ID NO: 132), LV1180 (SEQ ID NO: 136), LV1181 (SEQ ID NO: 98), LV1183 (SEQ ID NO: 124), LV1184 (SEQ ID NO: 91), LV1185 (SEQ ID NO: 106), LV1186 (SEQ ID NO: 113), and LV1187 (SEQ ID NO: 128).

Example 2: Binding of Nectin-4 binding agents to human, cynomolgus monkey, and mouse Nectin-4

[0228] Protein sequences of human (Uniprot number Q96NY8), cynomolgus monkey (Uniprot number A0A2K5WES1) and mouse (Uniprot number Q8R007) Nectin-4 were reverse-translated into cDNA and then codon-optimized for expression in human cells. Regulatory elements were added to each cDNA, namely an N-terminal Kozak sequence and C-terminal stop codon

(including BamH1 and Age1 restriction sites for cloning) and the cDNA was made into a synthetic gene. cDNAs were then cloned into a suitable vector and the sequences were verified. Expression of the proteins was performed by transient transfection of the resulting plasmids in CHO-K1 cells. Expression of human/cynomolgus/mouse Nectin-4 was confirmed using commercially available control antibodies (Nectin-4-AF647 [R&D systems] and Nectin-4 Antibody, anti-human, APC, REAfinity [Miltenyi]) in flow cytometry.

[0229] Cells from each transfection were seeded in 96F CellBIND plates (Corning) and cultured overnight at 37°C/5% CO_2 in a humidified atmosphere and subsequently incubated with serial dilutions of each binding agent, ranging from 0.032 to 500 nM for one hour at 37°C/5% CO_2. Plates were washed and cells were incubated with HRP-labeled rabbit anti-camelid VHH cocktail (GenScript) for one hour at 37°C/5% CO_2. After washing, 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added, followed by 20 minute incubation at room temperature. The reaction was stopped by addition of stop buffer (1M H_2SO_4) and the optical densities were measured with a multi-mode plate reader Spectramax iD5, at wavelengths 450 nm and 610 nm.

[0230] **FIG. 2A-FIG. 2D** show that all the binding agents bound dose-dependently to human Nectin-4 expressing CHO-K1 cells, and did not bind to non-transfected CHO-K1 cells. Based on the strength of their binding, Nectin-4 binding agents were divided in three groups:

(a) Strong binders: LV1181 (SEQ ID NO: 98), LV1184 (SEQ ID NO: 91), LV1185 (SEQ ID NO: 106), LV1186 (SEQ ID NO: 113) and LV1187 (SEQ ID NO: 128);

(b) Intermediate binders LV1178 (SEQ ID NO: 120) and LV1183 (SEQ ID NO: 124); and

(c) Weak binders LV1179 (SEQ ID NO: 132) and LV1180 (SEQ ID NO: 136).

[0231] All the Nectin-4 binding agents showed cross reactivity to CHO-K1 cells expressing cynomolgus monkey Nectin-4 (**FIG. 3A-FIG. 3B**). Again, the three groups of strong, intermediate, and weak binding of the Nectin-4 binding agents were observed. The Nectin-4 binding agents that had 'strong binding' and 'weak binding' also cross-reacted to murine Nectin-4. However, the Nectin-4 binding agents with 'intermediate binding' did not cross-react with murine Nectin-4 (**FIG. 3C-FIG. 3D**).

Example 3: Binding of the Nectin-4 binding agents to tumor cell lines

[0232] Based on the results in Example 2, the binders were further characterized . Using flow cytometry, binding of the selected Nectin-4 binding agents to Nectin-4 endogenously expressed on human tumor cell lines was determined. Nectin-4 expression on tumor cell lines BeWo (Sigma), A-431 (ATCC), and HT-29 (ATCC) was confirmed using commercially available Nectin-

4 monoclonal antibodies (Nectin-4-AF647 [R&D systems] and Nectin-4 Antibody, anti-human, APC, REAfinity [Miltenyi]). The U251 cell line was used as a Nectin-4-negative cell line.

[0233] Cells were incubated with a concentration range of each of the binding agents, ranging from 0.032 to 100 nM, and the binding of the Nectin-4 binding agent was detected using a rabbit anti-camelid iFluor 647-labeled antibody (Genscript). Staining was visualized using a FACS Celesta (Becton and Dickinson).

[0234] **FIG. 4A-FIG. 4F** show that the LV1181 and LV1184 Nectin-4 binding agents had strong binding to Nectin-4 expressing tumor cells; the LV1185, LV1186, and LV1187 Nectin-4 binding agents showed less, but still detectable binding to Nectin-4 expressing tumor cells; and the LV1178, LV1179 and LV1180 Nectin-4 binding agents showed very limited binding to Nectin-4 expressing tumor cells. EC50s for the LV1181 and LV1184 Nectin-4 binding agents to BeWo (**FIG. 4A-FIG. 4B**), A431(**FIG. 4C-FIG. 4D**) and HT-29 (**FIG. 4E-FIG. 4F**) cells were determined by curve fitting using GraphPad-Prism software and are shown in **Table 10**; EC50s for binding of the other Nectin-4 binding agents could not be determined due to incomplete binding curves.

Table 10: EC50 values for the LV1181 and LV1184 Nectin-4 binding agents to tumor cells

Tumor cell line	EC50 LV1181 (nM)	EC50 LV1184 (nM)
BeWo	0.4	0.3
A431	0.33	0.24
HT-29	1.5	1.2

Example 4: Synthetic gene synthesis, production, and purification of Nectin-4xVδ2 multi-specific binding agents

[0235] The sequences of the four Nectin-4 binding agents that showed strongest binding to tumor cells that endogenously express Nectin-4 (LV1181, LV1184, LV1185, and LV1186) were then re-formatted into multi-specific binding agents with a Vδ2 binding agent (5C8) in the orientation: N' Nectin-4 VHH - linker - Vδ2 VHH C'. The linker between the two binding agent 'heads' was a glycine(G)-serine(S) stretch with the sequence G_4S (SEQ ID NO: 254). Full amino acid IDs for these bispecific binding agents are provided below in **Table 11**.

Table 11: Amino acid sequences of bispecific binding agents.

Bispecific agent	Format	SEQ ID
LV1181 x Vδ2	LV1181 - G_4S – 5C8	281
LV1184 x Vδ2	LV1184 - G_4S – 5C8	282
LV1185 x Vδ2	LV1185 - G_4S – 5C8	283
LV1186 x Vδ2	LV1186 - G_4S – 5C8	284

[0236] The cDNAs encoding these proteins were made by synthetic gene assembly and cloned into a proprietary expression vector. HEK293E-253 cells were transiently transfected with the expression vectors to express the encoded proteins. After 6 days, the proteins were then purified from the conditioned cell culture supernatant using HiTrap Fibro PrismA columns according to the supplier's protocol. After elution, proteins were buffer-exchanged to PBS. The purified proteins (Nectin-4 x Vδ2 multi-specific binding agents) were always >95% pure as determined by SDS-PAGE analysis using Coomassie staining and contained very low levels of endotoxin (<0.5 EU/mg).

Example 5: Affinity determination of the Nectin-4 binding agents and Nectin-4xVδ2 multi-specific binding agents to human Nectin-4 using biolayer interferometry (BLI)

[0237] To determine the kinetics of binding of the Nectin-4 binding agents and multi-specific binding agents to human Nectin-4, recombinant purified human Nectin-4-Fc fusion protein (Acro Biosystems) was loaded onto anti-human IgG Fc Capture biosensors (using a concentration of 2.5 µg/mL) for an Octet Red96e (Sartorius) instrument. The sensors were then dipped in different concentrations of the various binding agents and multi-specific binding agents, which were diluted in 10x kinetic buffer (10xKB) provided by the supplier. From the obtained sensorgrams, the kinetic association- and dissociation rate constants were determined by curve fitting. The binding agents tested were LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185, LV1186, and LV1187. The multi-specific binding agents tested were LV1181xVδ2, LV1184xVδ2, LV1185xVδ2, and LV1186xVδ2. **Table 12** shows the binding affinities (K_D) of the binding agents determined in 2 independent runs. **Table 13** shows the kinetic parameters of binding of the Nectin-4xVδ2 multi-specific binding agents for human Nectin-4 determined in 2 independent runs. ND means Not Determined

Table 12: Binding affinities (K_D) of the Nectin-4 VHHs determined by BLI

Binding agent	KD (nM) Run 1	KD (nM) Run 2
LV1178	ND	8.02
LV1179	12.86	7.33
LV1180	10.43	7.8
LV1181	0.8	0.76
LV1183	5.7	7.98
LV1184	0.51	0.51
LV1185	3.61	2.34

Binding agent	KD (nM) Run 1	KD (nM) Run 2
LV1186	3.93	3.94
LV1187	5.2	6.09

Table 13: Binding affinities and kinetics of Nectin-4xVδ2 multi-specific binding agents determined by BLI

Multi-specific binding agent	Run number	K_D (nM)	K_{on} (1/Ms)	K_{dis} (1/s)
LV1181xVδ2	1	0.26	1.04E+06	2.66E-04
	2	0.37	8.18E+05	3.00E-04
LV1184xVδ2	1	0.07	9.73E+05	6.33E-05
	2	0.18	8.44E+05	1.49E-04
LV1185xVδ2	1	2.0	6.42E+05	1.27E-03
	2	2.4	5.93E+05	1.44E-03
LV1186xVδ2	1	2.3	7.31E+05	1.65E-03
	2	2.6	6.60E+05	1.70E-03

Example 6: Competition for binding to human Nectin-4 determination

[0238] Using BLI, competition for binding to human Nectin-4 between the Nectin-4 binding agents was determined. Anti-human IgG Fc Capture sensors were loaded with human Nectin-4 Fc fusion protein as described above. The nine different Nectin-4 binding agents identified in the previous examples (LV1178, LV1179, LV1180, LV1181, LV1183, LV1184, LV1185, LV1186, and LV1187) were used in concentrations based on the affinities as indicated above. A fixed amount of the first binding agent was allowed to bind to the Nectin-4 loaded sensor, after which the sensor was exposed to a mixture of the same amount of first binding agent and a fixed amount of the second binding agent, followed by dissociation in 10X KB.

[0239] From the obtained sensorgrams, it was concluded that all nine different VHHs competed with each other for binding to human Nectin-4. Results are shown in **Table 14**, where "+" shows competitive bindingand blank cells were not tested.

Table 14: High Affinity Nectin-4 Binding Agents Bind the Same Region on Nectin-4

	Second Association								
First association	**LV1178**	**LV1179**	**LV1180**	**LV1181**	**LV1183**	**LV1184**	**LV1185**	**LV1186**	**LV1187**
LV1178	+	+	+						
LV1179	+	+	+						
LV1180	+	+	+						
LV1181	+	+	+	+	+	+	+	+	+

LV1183	+	+	+	+	+	+	+	+	+
LV1184	+	+	+	+	+	+	+	+	+
LV1185	+	+	+	+	+	+	+	+	+
LV1186	+	+	+	+	+	+	+	+	+
LV1187	+	+	+	+	+	+	+	+	+

Example 7: Binding of Nectin-4xVδ2 multi-specific binding agents to tumor cell lines

[0240] Binding of the Nectin-4xVδ2 multi-specific binding agents to Nectin-4 endogenously expressed on human tumor cell lines A-431 and HT-29 was determined by flow cytometry as described above.

[0241] FIG. 5A-FIG. 5B show that both the LV1181xVδ2 and the LV1184xVδ2 multi-specific binding agents bound to both cell lines with high affinity, whereas both the LV1185xVδ2 and the LV1186xVδ2 multi-specific binding agents bound to the tumor cell lines with lower affinity. See **Table 15**.

Table 15: EC_{50} of Nectin-4xVδ2 multi-specific binding agents to Nectin-4-expressing cancer cells

Multi-specific Binding agent	EC50 (nM) A-431 cells	EC50 (nM) HT-29 cells
LV1181xVδ2	0.75	1.2
LV1184xVδ2	0.46	0.87
LV1185xVδ2	4.4	5.9
LV1186xVδ2	9.5	20

Example 8: PBMC isolation and generation of human donor-derived Vγ9Vδ2-T cell cultures

[0242] Buffy coats were obtained from blood supply service Sanquin and used for isolation of peripheral blood mononuclear cells (PBMC). PBMC were isolated using Lymphoprep™ density gradient centrifugation. Vγ9Vδ2-T cells were then isolated from healthy donor-derived PBMC by magnetic-activated cell sorting (MACS) using a FITC-labeled anti-TCR Vδ2 mouse monoclonal antibody (Mab) in combination with goat anti-mouse IgG microbeads. Purified Vγ9Vδ2-T cells were stimulated every seven days with a feeder cell mix consisting of irradiated PBMC from two (other) healthy donors and an Epstein Barr Virus transformed B cell line (JY) resuspended in Roswell Park Memorial Institute (RPMI) medium supplemented with 10 IU/mL IL-7, 10 ng/mL IL-15 and 50 ng/ml PHA. Expanded Vγ9Vδ2-T cell cultures between 12 and 15 days after the last stimulation and were tested for purity (and only used when >90% pure) before being used for experiments.

Example 9: Binding of Nectin-4xVδ2 multi-specific binding agents to Vγ9Vδ2 T cells

[0243] Binding of the Nectin-4xVδ2 multi-specific binding agents to Vγ9Vδ2 T cells was determined by flow cytometry as described above. **FIG. 6** shows that all 4 Nectin-4xVδ2 multi-specific binding agents bound to Vγ9Vδ2 T cells with similar EC50s (See **Table 15**).

Table 15: EC_{50} of Nectin-4xVδ2 multi-specific binding agents to Vγ9Vδ2 T cells

Multi-specific Binding agent	EC50 (nM) Vγ9Vδ2 T cells
LV1181xVδ2	0.98
LV1184xVδ2	1.3
LV1185xVδ2	1.6
LV1186xVδ2	1.6

Example 10: Vγ9Vδ2-T cell degranulation and cytotoxicity of A-431 cells induced by Nectin-4xVδ2 multi-specific binding agents

[0244] To test Vγ9Vδ2-T cell activation and tumor cytotoxicity, A-431 tumor target cells and Vγ9Vδ2 T cells were mixed in a 1:1 ratio in culture medium with labeled anti-CD107a antibody. This mixture was seeded in 96-well culture plates (with 50,000 target/Vγ9Vδ2 T cells per well) and serial dilutions of Nectin-4xVδ2 multi-specific binding agents were added. Tumor cells and Vγ9Vδ2-T cells alone with, and without, the highest concentration of Nectin-4xVδ2 multi-specific binding agents were also prepared as controls. After incubation for 24 hours at 37°C/5% CO_2, cells were resuspended, washed, and transferred to 96-well U bottom FACS plates. Remaining adherent cells were detached using trypsin/0.5% EDTA, washed and transferred to the corresponding wells of the FACS plates. The plates were washed and the cells were resuspended in FACS buffer (PBS/1% BSA) and incubated with labeled anti-CD3 (Biolegend) and anti-Vγ9 (Beckman Coulter) antibodies for 30 minutes at 4°C. Cells were then washed and incubated with labeled-7-AAD. Staining was visualized using a FACS Celesta (Becton and Dickinson).

[0245] FIG.7A-FIG. 7B show that all four Nectin-4xVδ2 multi-specific binding agents induced similar levels of Vγ9Vδ2 T cell degranulation as determined by CD107a expression on the T cells (**FIG. 7A**). Surprisingly, despite differences in binding to the tumor cells as described above (See Example 7), all four Nectin-4xVδ2 multi-specific binding agents induced the same level of lysis of A431 cells with similar EC50s (**FIG. 7B**).

Example 11: Humanization of the anti-Nectin-4 binding agents

[0246] The amino acid sequence of the llama-derived anti-Nectin-4 binding agents (LV1181, LV1184, LV1185 and LV1186) were aligned to the human V gene database and the closest

human germline match was found to be IGHV3-66*01. Based on sequence differences in the framework regions between the human and llama-derived sequence, for each anti-Nectin-4 VHH three humanized variants (SEQ ID NOs: 92, 93, 94, 100, 101, 102, 107, 108, 109, 114, 115, and 116) were designed.

Example 12: Expression of Nectin-4 in Cancers

[0247] **FIG. 1A** shows Nectin-4 expression on freshly isolated patient tumor samples. **FIG. 8A** and **FIG. 8B** shows the Nectin-4 expression of urothelial cell carcinoma (UCC) and non-malignant bladder tissue as measured by the mean fluorescence index (MFI) (**FIG. 8A**) and as the percentage of Nectin-4 positive cells in urothelial cell carcinoma and non-malignant tissue (**FIG. 8B**). Non-malignant tissue was taken by pathologist from part of the bladder without macroscopic tumor tissue. (m)CRC = (metastatic) colorectal cancer; ccRCC = clear cell renal cell cancer; GBM = glioblastoma multiforme; AC = adenocarcinoma; SCC = squamous cell carcinoma; H&N = head & neck cancer; UCC = urothelial cell carcinoma.

Example 13: Nectin-4xVδ2 multi-specific binding agents induce Vγ9Vδ2T cell degranulation and tumor cell lysis in Nectin-4+ head and neck and bladder cancers.

[0248] Experiments were performed to evaluate the activity of LAVA-366 against head and neck cancers, urothelial cell carcinomas and malignant tumors that were Nectin-4$^-$. Malignant urothelial cell carcinoma cells (UCC), head and neck cancer cells, or cancer cells from Nectin-4 negative tumors were incubated with allogeneic expanded Vγ9Vδ2-T cells at a ratio of effector:target 1:2, and then further incubated overnight with 10 nM of LAVA-366. LAVA-366 comprises the LV1181 Nectin-4 VHH and the 5C8 Vδ2 VHH and the LAVA-366 sequence comprises SEQ ID NO: 281. Control conditions without LAVA-366 were also included.

[0249] As seen in **Fig. 9A-FIG. 9B,** there was an increase in both Vγ9Vδ2 T cell degranulation (as measured by percent increase in CD107a expression (**FIG. 9B**)) and tumor cell lysis (**FIG. 9A**) in tumor sample with allogeneic Vγ9Vδ2 T cells in the presence of LAVA-366 as opposed to the tumor samples that were only incubated with allogeneic Vγ9Vδ2 T cells. Accordingly, in Nectin-4$^-$ tissue there was no tumor cell lysis, and only minimal T cell degranulation in the presence of allogeneic Vγ9Vδ2 T cells and LAVA-366.

[0250] In urothelial cell carcinomas the presence of LAVA-366 lead to an increase in tumor cell lysis even without the presence of added expanded Vγ9Vδ2-T cells (**FIG. 11**).

[0251] In non-malignant Nectin-4$^+$ urothelial cells (EpCAM$^+$), there was no change in tumor cell lysis (**FIG. 10**) when the cells were incubated overnight with Vγ9Vδ2 T cells and either with or without 10 nM of LAVA-366.

Example 14: In vivo testing of the Nectin-4xVδ2 multi-specific binding agents

[0252] The anti-tumor effect of the multi-specific binding agents are tested in vivo. Mice are inoculated with head and neck or urothelial cell carcinoma. The mice are then split into different groups: 1) further inoculation with Vγ9Vδ2-T cells; 2) further inoculation with Vγ9Vδ2-T cells + 0.1 mg/kg the LV1181 (E1D) x 5C8 var1 (E1D-105F-R109A) multi-specific binding agent (LAVA-1427, comprising SEQ ID NOs: 276 and 280); 3) further inoculation with Vγ9Vδ2-T cells + 0.5 mg/kg LAVA-1427; 4) further inoculation with Vγ9Vδ2-T cells + 1 mg/kg LAVA-1427; 5) further inoculation with Vγ9Vδ2-T cells + 2 mg/kg LAVA-1427; 6) 0.1 mg/kg LAVA-1427; 7) 0.5 mg/kg LAVA-1427; 8) 1 mg/kg LAVA-1427; 9) 2 mg/kg LAVA-1427; and 10) vehicle. Mouse survival is analyzed. After 6 weeks mice are euthanized and tumor volume and mass are measured.

Example 15: Modifications to binding agents to prevent pyroglutamate formation

[0253] Pyroglutamate formation at the first amino acid position in proteins increases heterogeneity and can reduce the stability of the proteins at 2-8℃, therefore requiring storage at -20℃ as opposed to storage at 2-8℃. Therefore, a multi-specific binding agent was generated wherein aspartic acid, which cannot form pyroglutamate, was substituted for glutamic acid at the first N-terminal amino acid position. Pyroglutamination increases the hydrophobicity of a protein. Therefore, the presence of pyroglutamination was determined by reverse phase-HPLC, as this technique separates proteins based on hydrophobicity. More hydrophilic protein species elute at shorter retention times and more hydrophobic species elute at longer retention times.

[0254] Briefly, Chinese hamster ovary (CHO) cells were transfected with vectors coding either LAVA-1387 (forms pyroglutamate) or LAVA-1427 (cannot form pyroglutamate) and subsequently cultured in liquid medium for protein expression. The proteins were then purified and analyzed by reversed phase-HPLC. Protein samples were loaded onto a liquid chromatography system equipped with a reversed-phase column. Organic solvents were used for binding and eluting of proteins. Results are shown in **FIG. 13.** The main species of LAVA-1387 and LAVA-1427 exhibit essentially identical hydrophobicities as demonstrated by their highly similar retention times. However, a significant part of LAVA-1387 (black trace) carries the N-terminal modification pyroglutamate seen as the peak in the hydrophobic region. The N-terminal aspartate as present in LAVA-1427 (gray trace) cannot undergo such a modification since ring formation is hampered by the shorter sidechains. Consequently, no hydrophobic pyroglutamation peak is detected for LAVA-1427.

CLAIMS

1. A single domain antibody that binds to Nectin-4 comprising:

a) a complementarity determining region (CDR)1 comprising an amino acid sequence selected from SEQ ID NOs: 88, 95, 103, 110, 117, 121, 125, 129, and 133;

b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 89, 96, 104, 111, 118, 122, 126, 130, and 134; and

c) a CDR3 comprising an amino acid sequence selected from 90, 97, 105, 112, 119, 123, 127, 131, and 135.

2. The single domain antibody of claim 1, wherein the CDR1, CDR2, and CDR3 sequences are selected from a combination disclosed in **Table 1A**.

3. The single domain antibody of any one of claims 1-2, comprising an amino acid sequence that is at least 90%, or at least 95% identical to any one of SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136.

4. The single domain antibody of any one of claims 1-3, comprising SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136.

5. The single domain antibody of any one of claims 1-4, consisting of SEQ ID NOs: 91, 92, 93, 94, 98, 99, 100, 101, 102, 106, 107, 108, 109, 113, 114, 115, 116, 120, 124, 128, 132, and 136.

6. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 88, the CDR2 comprises SEQ ID NO: 89, and the CDR3 comprises SEQ ID NO: 90.

7. The single domain antibody of claim 6, comprising an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 91, 92, 93, and 94.

8. The single domain antibody of claim 6 or 7, comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 91, 92, 93, and 94.

9. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 95, the CDR2 comprises SEQ ID NO: 96, and the CDR3 comprises SEQ ID NO: 97.

10. The single domain antibody of claim 9, comprising an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 98, 99, 100, 101, and 102.

11. The single domain antibody of claim 9 or 10, comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 98, 99, 100, 101, and 102.

12. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 103, the CDR2 comprises SEQ ID NO: 104, and the CDR3 comprises SEQ ID NO: 105.

13. The single domain antibody of claim 12, comprising an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 106, 107, 108, and 109.

14. The single domain antibody of claim 12 or 13, comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 106, 107, 108, and 109.

15. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 110, the CDR2 comprises SEQ ID NO: 111, and the CDR3 comprises SEQ ID NO: 112.

16. The single domain antibody of claim 15, comprising an amino acid sequence that is at least 90% or at least 95% identical to any one of SEQ ID NOs: 113, 114, 115, and 116.

17. The single domain antibody of claim 15 or 16, comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 113, 114, 115, and 116.

18. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 117, the CDR2 comprises SEQ ID NO: 118, and the CDR3 comprises SEQ ID NO: 119.

19. The single domain antibody of claim 18, comprising an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 120.

20. The single domain antibody of claim 18 or 19, comprising or consisting of the amino acid sequence of SEQ ID NO: 120.

21. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 121, the CDR2 comprises SEQ ID NO: 122, and the CDR3 comprises SEQ ID NO: 123.

22. The single domain antibody of claim 21, comprising an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 124.

23. The single domain antibody of claim 21 or 22, comprising or consisting of the amino acid sequence of SEQ ID NO: 124.

24. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 125, the CDR2 comprises SEQ ID NO: 126, and the CDR3 comprises SEQ ID NO: 127.

25. The single domain antibody of claim 24, comprising an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 128.

26. The single domain antibody of claim 24 or 25, comprising or consisting of the amino acid sequence of SEQ ID NO: 128.

27. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 129, the CDR2 comprises SEQ ID NO: 130, and the CDR3 comprises SEQ ID NO: 131.

28. The single domain antibody of claim 27, comprising an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 132.

29. The single domain antibody of claim 27 or 28, comprising or consisting of the amino acid sequence of SEQ ID NO: 132.

30. The single domain antibody of claim 1 or claim 2, wherein the CDR1 comprises SEQ ID NO: 133, the CDR2 comprises SEQ ID NO: 134, and the CDR3 comprises SEQ ID NO: 135.

31. The single domain antibody of claim 30, comprising an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 136.

32. The single domain antibody of claim 30 or 31, comprising or consisting of the amino acid sequence of SEQ ID NO: 136.

33. The single domain antibody of any one of claims 1-2, wherein the CDR1 comprises SEQ ID NO: 95, CDR3 comprises SEQ ID NO: 96, and CDR3 comprises SEQ ID NO: 97.

34. The single domain antibody of any one of claims 1-2 or 6, comprising an amino acid sequence that is at least 90%, or at least 95% identical to SEQ ID NO: 99.

35. The single domain antibody of any one of claims 1-2 or 33-34, comprising SEQ ID NO: 99.

36. The single domain antibody of any one of claims 1-2 or 33-35, consisting of SEQ ID NO: 99.

37. A polynucleotide encoding the single domain antibody of any one of claims 1-36.

38. A vector comprising the polynucleotide of claim 37.

39. A host cell comprising the polynucleotide of claim 37 or the vector of claim 38.

40. A method of producing the single domain antibody of any one of claims 1-36, comprising:
    a. introducing the vector of claim 38 into a host cell under conditions permitting expression of the polynucleotide encoding the single domain antibody; and
    b. isolating the single domain antibody from the culture supernatant.

41. A composition comprising the single domain antibody of any one of claims 1-36 and a pharmaceutically acceptable carrier.

42. A binding agent comprising the single domain antibody of any one of claims 1-36.

43. A multi-specific binding agent comprising:
    a. a first antigen-binding domain that specifically binds to human Nectin-4; and comprises the single domain antibody of any one of claims 1-36; and
    b. a second antigen-binding domain that specifically binds to an antigen expressed on an immune cell.

44. The multi-specific binding agent of claim 43, wherein the second antigen-binding domain specifically binds to a target antigen selected from CD3, CD2, an αδ T cell receptor, a γδ T cell receptor (e.g., Vδ2 TCR or Vδ1 TCR).

45. The multi-specific binding agent of claim 43 or claim 44, wherein the first and second antigen-binding domains are present in the same polypeptide chain.

46. The multi-specific binding agent of any one of claims 43-45, wherein the first antigen-binding domain and second antigen-binding domain are covalently linked via a peptide linker.

47. The multi-specific binding agent of claim 43 or claim 44, wherein the first and second antigen-binding domains are present on different polypeptide chains.

48. The multi-specific binding agent of any one of claims 43-44 or 47, further comprising an Fc domain comprising a first and a second Fc domain monomer.

49. The multi-specific binding agent of claim 48, comprising:

a. a first polypeptide comprising the first antigen-binding domain that binds to a human Nectin-4 protein and the first Fc domain monomer; and
b. a second polypeptide comprising the second antigen-binding domain that binds to a human Vδ2 T cell receptor and the second Fc domain monomer.

50. The multi-specific binding agent of claim 49, wherein the human Vδ2 T cell receptor is a Vγ9Vδ2 T cell receptor.

51. The multi-specific binding agent of any one of claims 49-50, wherein the antigen-binding domain that binds to the Vγ9Vδ2 T cell receptor comprises SEQ ID NO: 22.

52. The multi-specific binding agent of any one of claims 49-51, wherein the antigen-binding domain that binds to the Vγ9Vδ2 T cell receptor consists of SEQ ID NO: 22.

53. The multi-specific binding agent of any one of claims 50-52, wherein the multi-specific binding agent induces proliferation of Vγ9Vδ2 T cells.

54. The multi-specific binding agent of any one of claims 49-53, wherein the first and second Fc domain monomers comprise a mutation at position 234 and/or 235 according to the EU numbering system.

55. The multi-specific binding agent of claim 54, wherein the first and second Fc domain

monomers comprise an L234F and an L235E substitution.

56. The multi-specific binding agent of any one of claims 49-55,

wherein the first Fc domain monomer comprises a T366W substitution, and the second Fc domain monomer comprises T366S, L368A and Y407V substitutions, or vice versa, according to the EU numbering system; and/or

wherein the cysteine residues at position 220 according to the EU numbering system in the first and second Fc domain monomers have been deleted or substituted.

57. A multi-specific binding agent comprising

a) a first antigen-binding domain that binds to human Nectin-4, where the first antigen-binding domain comprises a complementarity determining region (CDR)1 comprising SEQ ID NO: 95, a CDR2 comprising SEQ ID NO: 96, and a CDR3 comprising SEQ ID NO: 97; and

b) a second antigen-binding domain that binds to Vδ2, where the second antigen-binding domain comprises a CDR1 comprising SEQ ID NO: 18, a CDR2 comprising SEQ ID NO: 19, and a CDR3 comprising SEQ ID NO: 20.

58. The multi-specific binding agent of claim 57, wherein the first antigen-binding domain comprises SEQ ID NO: 99 and the second antigen-binding domain comprises SEQ ID NO: 22.

59. The multi-specific binding agent of any one of claims 57 or 58, wherein the first and second antigen-binding domains are comprised in the same polypeptide chain.

60. A multi-specific binding agent comprising

a) a first polypeptide comprising (i) a first antigen-binding domain that binds to a human Nectin-4 protein and comprises a complementarity determining region (CDR)1 comprising SEQ ID NO: 95, a CDR2 comprising SEQ ID NO: 96, and a CDR3 comprising SEQ ID NO: 97 and (ii) a first Fc domain monomer;

b) a second polypeptide comprising (i) a second antigen-binding domain that binds to a human Vδ2 T cell receptor and comprises a CDR1 comprising SEQ ID NO: 18, a CDR2 comprising SEQ ID NO: 19, and a CDR3 comprising SEQ ID NO: 20 and (ii) a second Fc domain monomer.

61. The multi-specific binding agent of claim 60, wherein the first Fc domain monomer comprises SEQ ID NO: 231 and the second Fc domain monomer comprises SEQ ID NO: 230.

62. The multi-specific binding agent of claim 60, wherein the first Fc domain monomer comprises SEQ ID NO: 230 and the second Fc domain monomer comprises SEQ ID NO: 231.

63. The multi-specific binding agent of any one of claims 60-62, wherein the first antigen-binding domain comprises SEQ ID NO: 276.

64. The multi-specific binding agent of any one of claims 60-63, wherein the second antigen-binding domain comprises SEQ ID NO: 280.

65. The multi-specific binding agent of any one of claims 60-64, wherein
a) the first polypeptide comprises SEQ ID NO: 276; and
b) the second polypeptide comprises SEQ ID NO: 280.

66. A polynucleotide encoding the multi-specific binding agent of any one of claims 43-65.

67. An expression vector comprising the polynucleotide of claim 66.

68. A host cell comprising the polynucleotide of claim 66 or the expression vector of claim 67.

69. The host cell of claim 68, wherein the cell is a CHO cell.

70. A method of producing the multi-specific binding agent of any one of claims 43-65, comprising culturing the host cell of claim 68 or 69 under conditions sufficient to express the multi-specific binding agent from the polynucleotide or expression vector therein and purifying the expressed multi-specific binding agent from the culture supernatant.

71. A pharmaceutical composition comprising the single domain antibody of any one of claims 1-36, the binding agent of claim 42, or the multi-specific binding agent of any one of claims 43-65, and a pharmaceutically-acceptable excipient.

72. A method of treating a cancer in a subject in need thereof, comprising administering to the subject the single domain antibody of any one of claims 1-36, the binding agent of claim 42, or the multi-specific binding agent of any one of claims 43-65, or the pharmaceutical composition of claim 71.

73. The method of claim 72, wherein the subject is human.

74. The method of any one of claims 72-73, wherein the subject is suffering from a bladder cancer, urothelial cell cancer, head and neck cancer, choriocarcinoma, epidermoid carcinoma, or colorectal adenocarcinoma.

75. A method of expanding Vγ9Vδ2+ T cells comprising contacting the Vγ9Vδ2+ T cells with single domain antibody of any one of claims 1-36, the binding agent of claim 42, or the multi-specific binding agent of any one of claims 43-65, or the pharmaceutical composition of claim 71.

76. A method increasing the stability of a protein, wherein the protein is modified at the first position to a non-pyroglutamate forming amino acid.

77. The method of claim 76, wherein the non-pyroglutamate forming amino acid is aspartic acid.

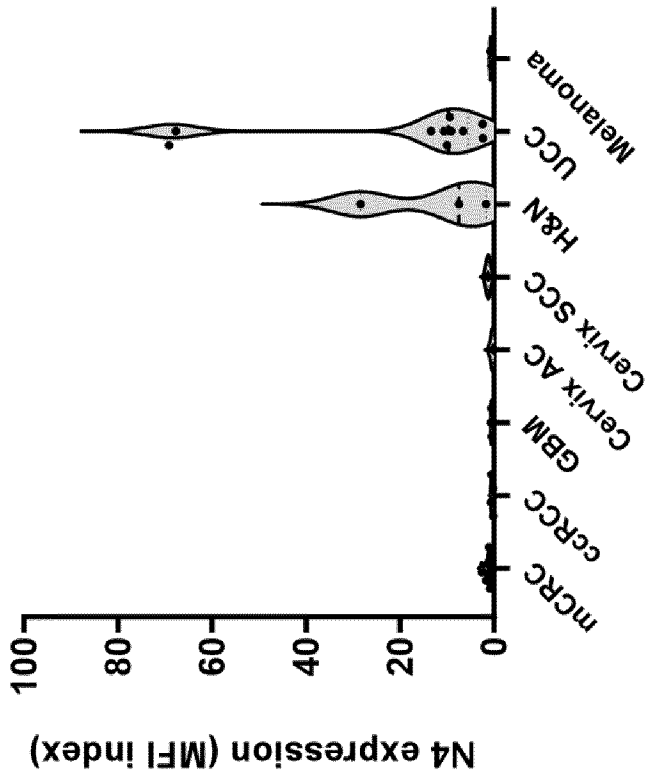

N4 expression (MFI index)
100
80
60
40
20
0
mCRC
ccRCC
GBM
Cervix AC
Cervix SCC
H&N
UCC
Melanoma

FIG. 1A

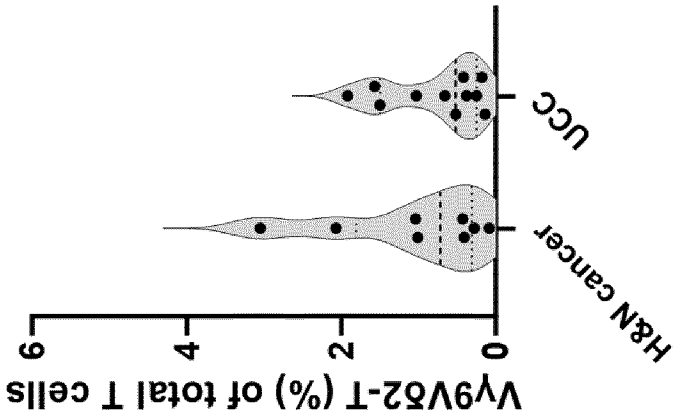

Vγ9Vδ2-T (%) of total T cells
6
4
2
0
H&N cancer
UCC

FIG. 1B

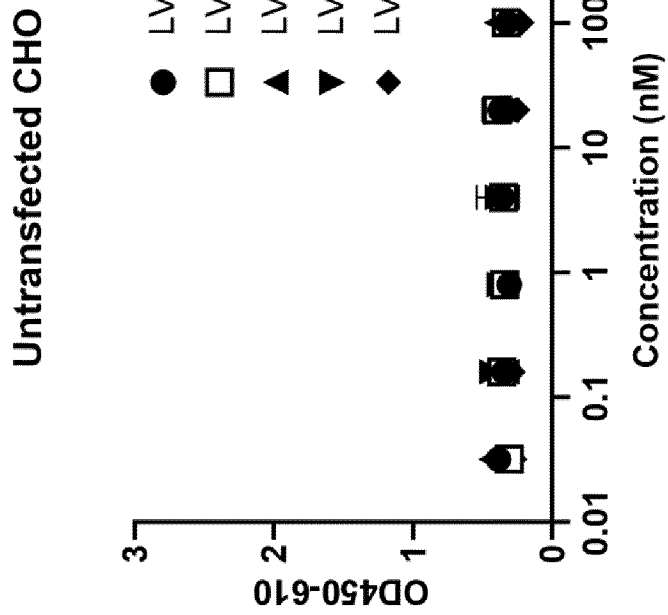

Untransfected CHO
LV1178
LV1179
LV1180
LV1181
LV1183
OD450-610
0
1
2
3
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 2B

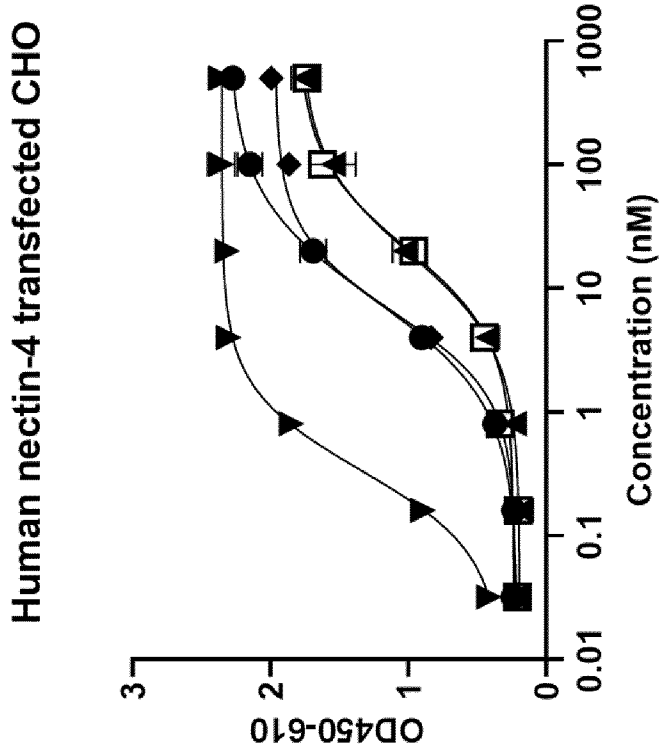

Human nectin-4 transfected CHO
OD450-610
0
1
2
3
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 2A

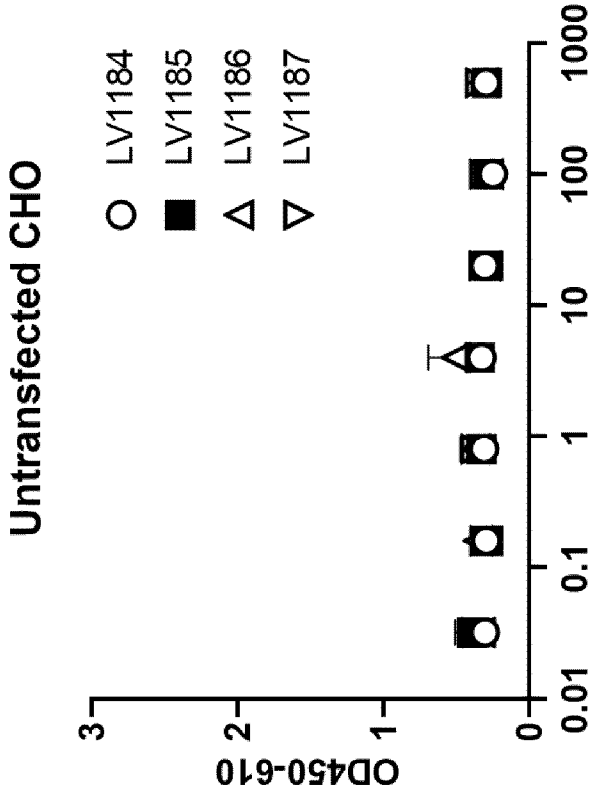

Untransfected CHO
LV1184
LV1185
LV1186
LV1187
OD450-610
0
1
2
3
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 2D

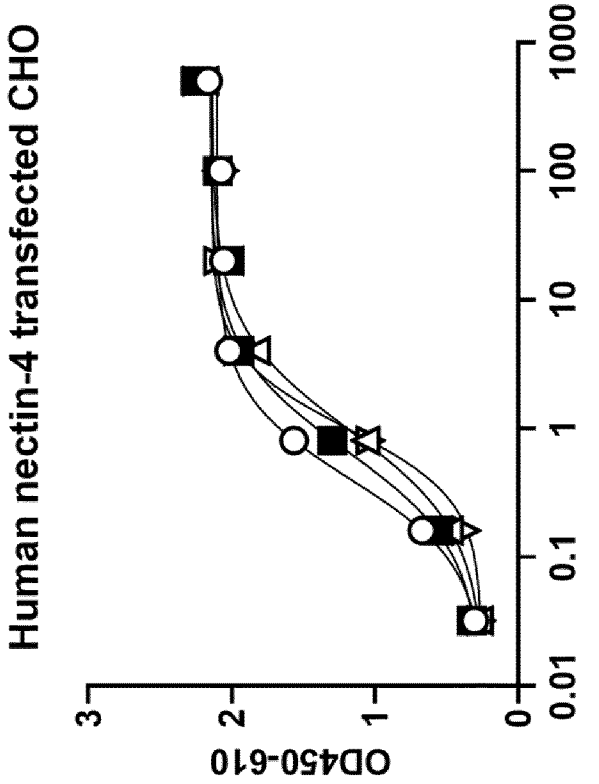

Human nectin-4 transfected CHO
OD450-610
0
1
2
3
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 2C

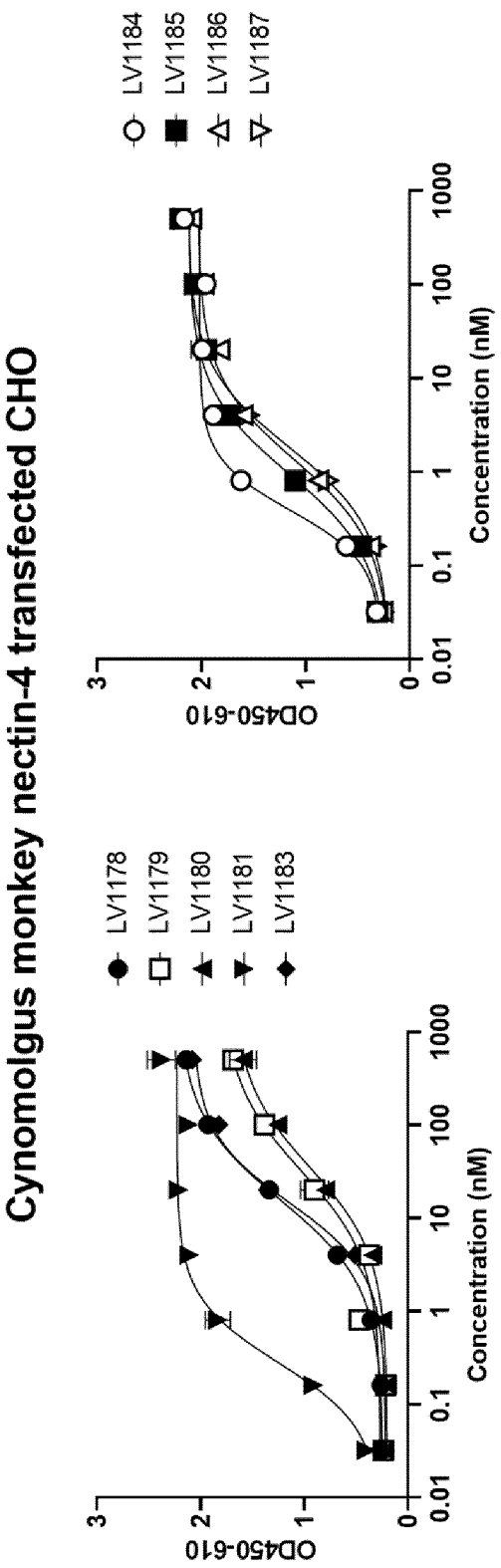

Cynomolgus monkey nectin-4 transfected CHO
LV1178
LV1179
LV1180
LV1181
LV1183
OD450-610
Concentration (nM)
0.01
0.1
1
10
100
1000
0
1
2
3

FIG. 3A

LV1184
LV1185
LV1186
LV1187
OD450-610
Concentration (nM)
0.01
0.1
1
10
100
1000
0
1
2
3

FIG. 3B

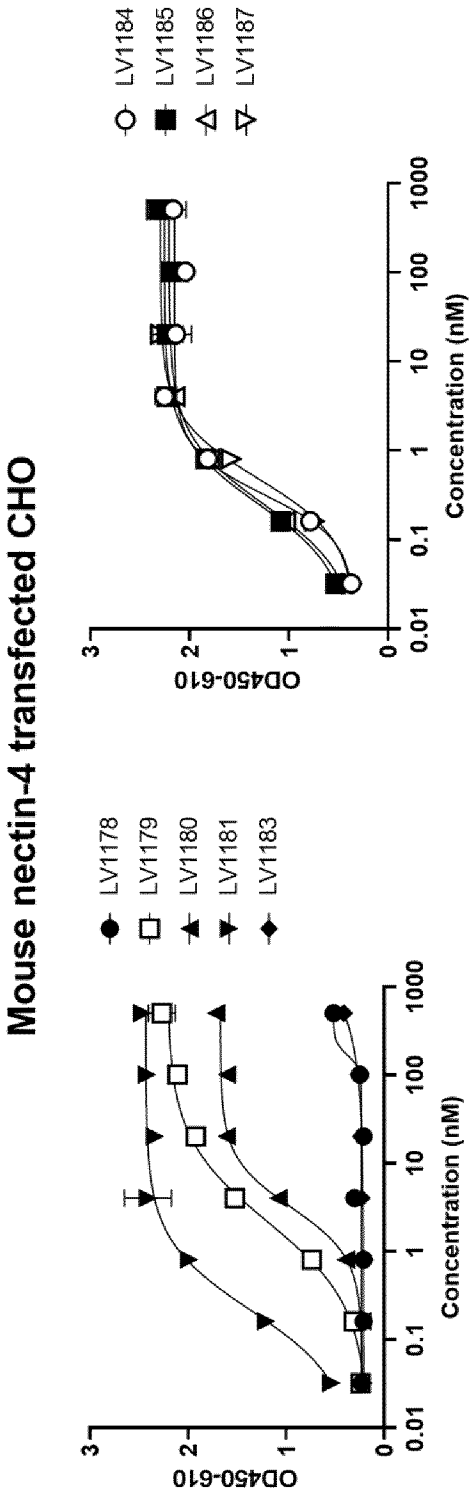

Mouse nectin-4 transfected CHO
LV1178
LV1179
LV1180
LV1181
LV1183
OD450-610
Concentration (nM)
0
1
2
3
0.01
0.1
1
10
100
1000
LV1184
LV1185
LV1186
LV1187
OD450-610
Concentration (nM)

FIG. 3C

FIG. 3D

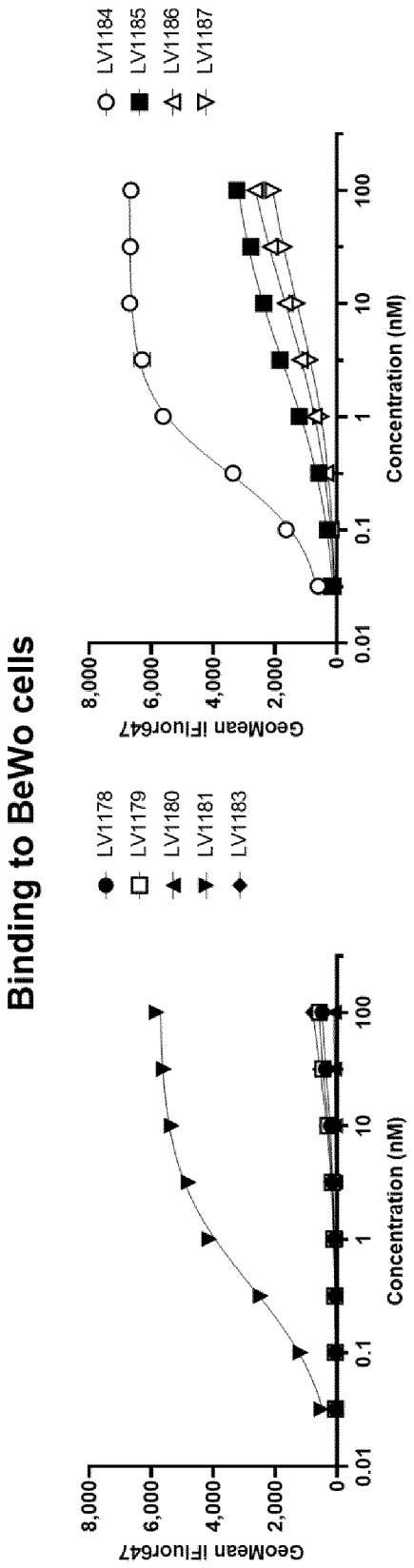

Binding to BeWo cells
LV1178
LV1179
LV1180
LV1181
LV1183
LV1184
LV1185
LV1186
LV1187
GeoMean IFluor647
8,000
6,000
4,000
2,000
0
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4A

FIG. 4B

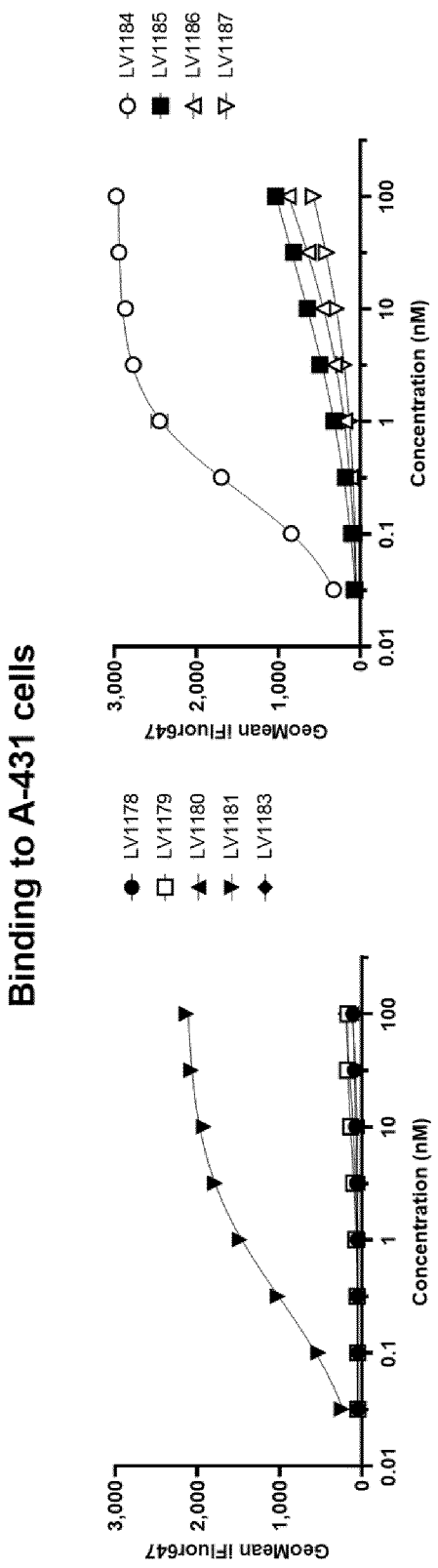

Binding to A-431 cells
LV1178
LV1179
LV1180
LV1181
LV1183
LV1184
LV1185
LV1186
LV1187
GeoMean iFluor647
3,000
2,000
1,000
0
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4C

FIG. 4D

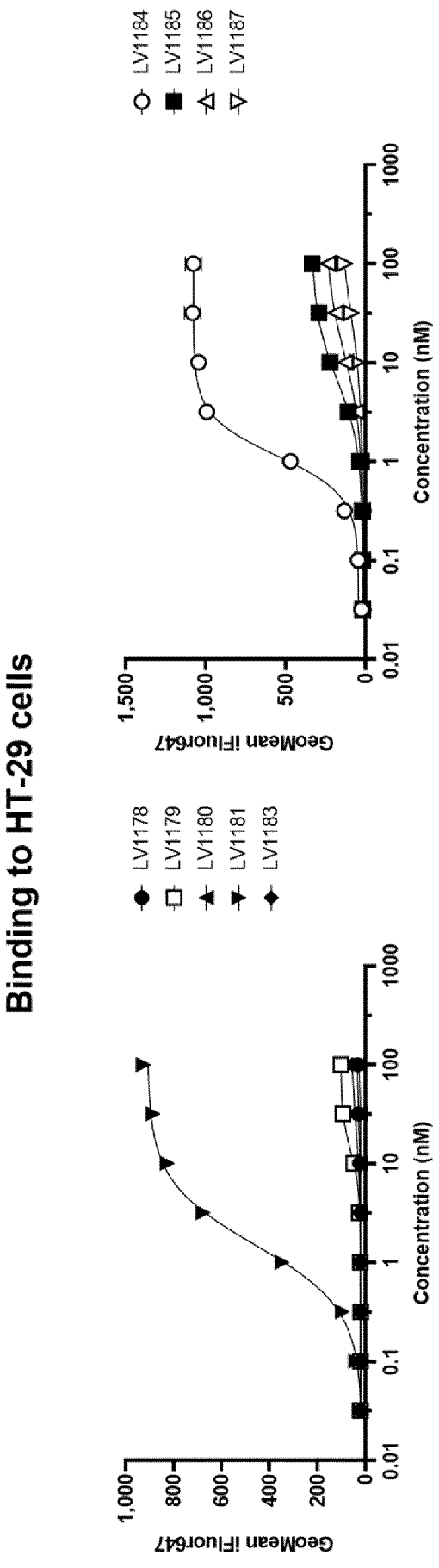

Binding to HT-29 cells
LV1178
LV1179
LV1180
LV1181
LV1183
GeoMean iFluor647
1,000
800
600
400
200
0
0.01
0.1
1
10
100
1000
Concentration (nM)
LV1184
LV1185
LV1186
LV1187
GeoMean iFluor647
1,500
1,000
500
0
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 4E

FIG. 4F

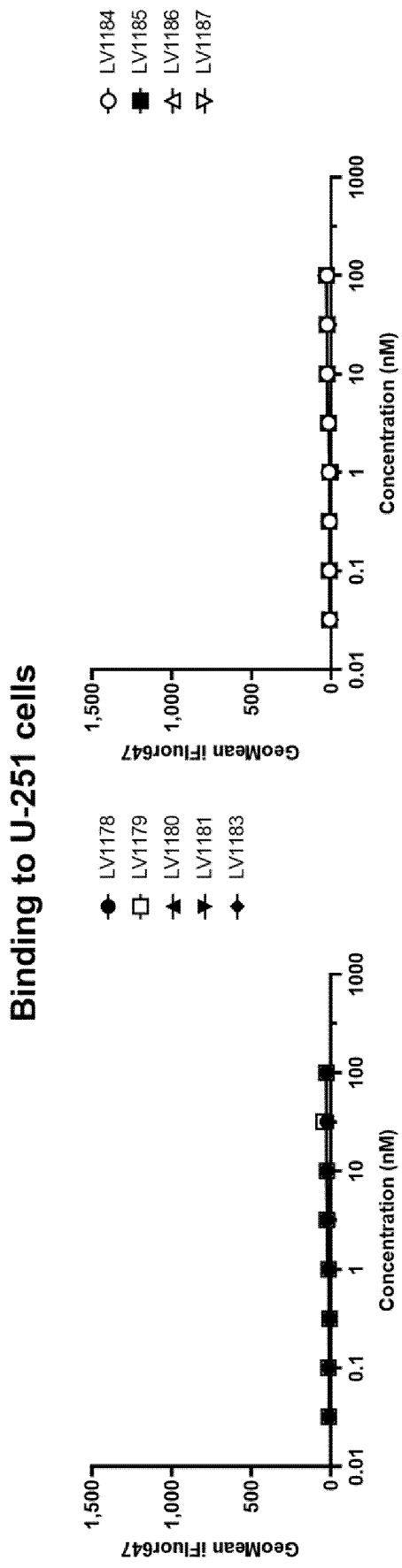

Binding to U-251 cells
LV1178
LV1179
LV1180
LV1181
LV1183
GeoMean iFluor647
1,500
1,000
500
0
0.01
0.1
1
10
100
1000
Concentration (nM)
LV1184
LV1185
LV1186
LV1187
GeoMean iFluor647
1,500
1,000
500
0
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 4G

FIG. 4H

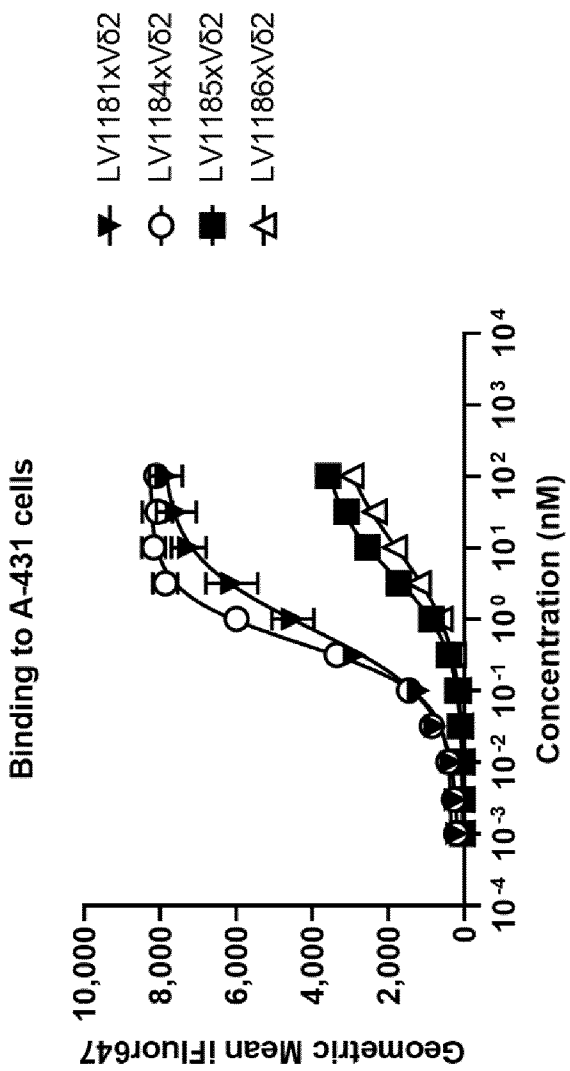

Binding to A-431 cells
Geometric Mean iFluor647
10,000
8,000
6,000
4,000
2,000
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4}
Concentration (nM)
LV1181xVδ2
LV1184xVδ2
LV1185xVδ2
LV1186xVδ2

FIG. 5A

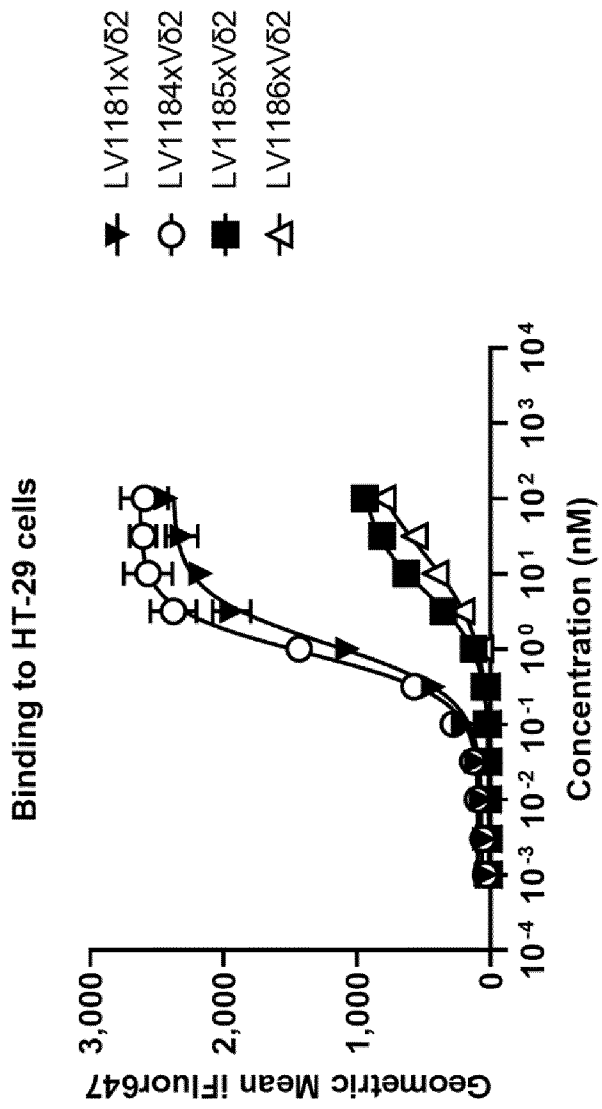

Binding to HT-29 cells
Geometric Mean iFluor647
3,000
2,000
1,000
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4}
Concentration (nM)
LV1181xVδ2
LV1184xVδ2
LV1185xVδ2
LV1186xVδ2

FIG. 5B

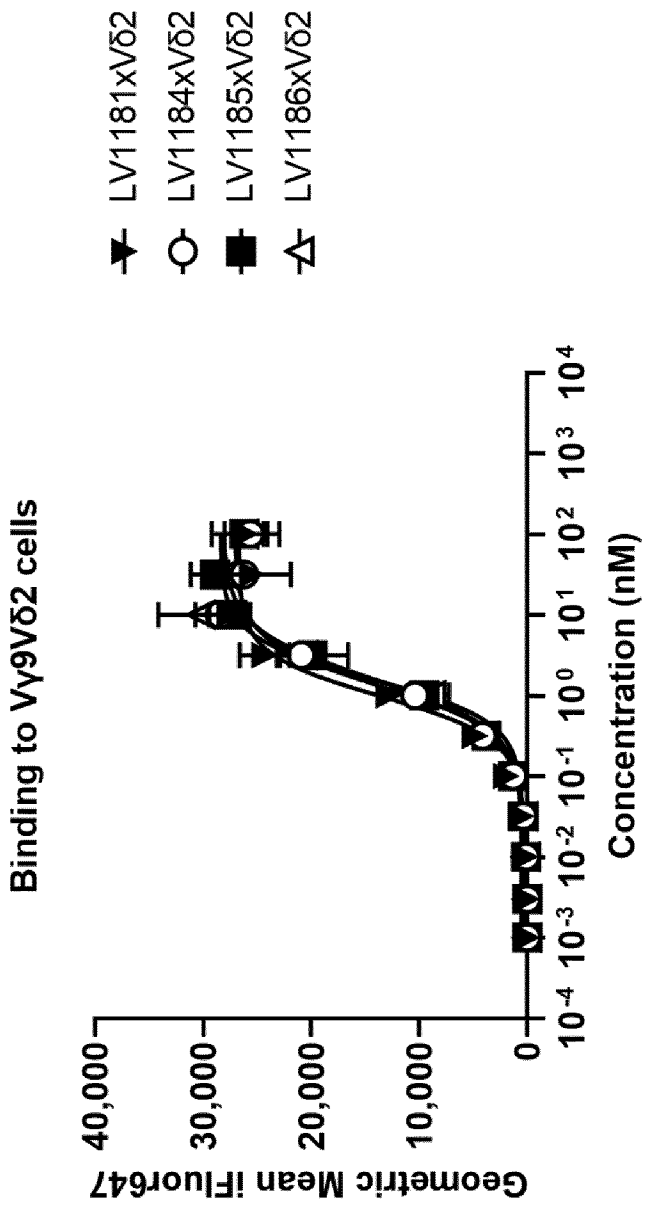
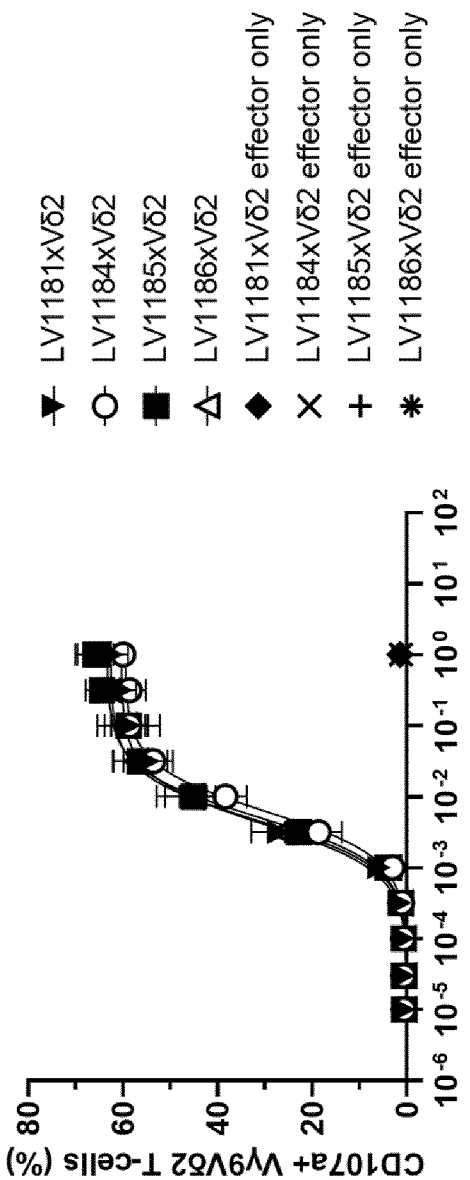
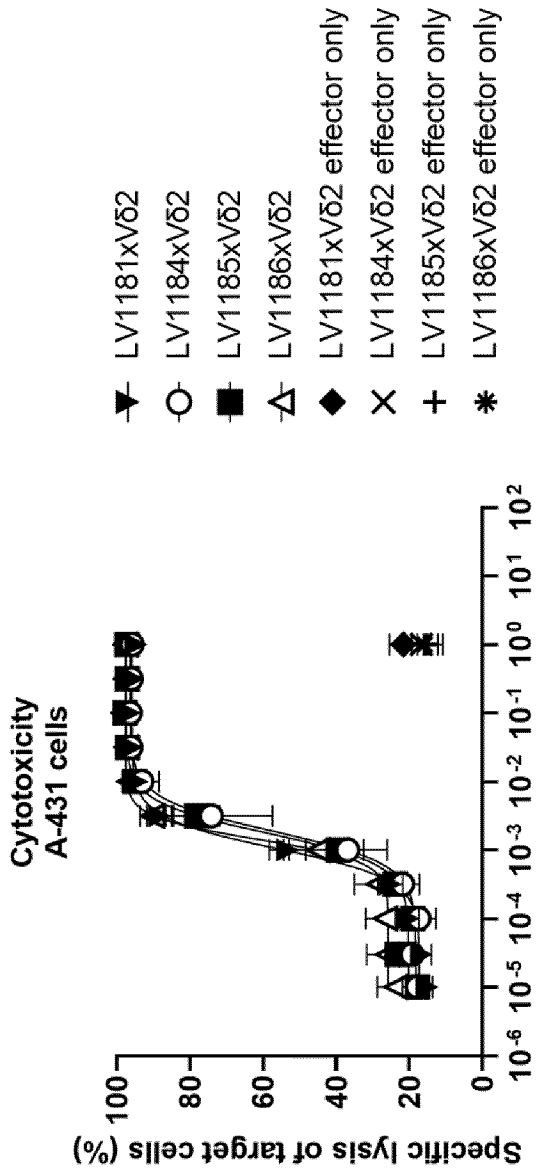

Binding to Vγ9Vδ2 cells
LV1181xVδ2
LV1184xVδ2
LV1185xVδ2
LV1186xVδ2
Geometric Mean iFluor647
40,000
30,000
20,000
10,000
0
10-4 10-3 10-2 10-1 100 101 102 103 104
Concentration (nM)

FIG. 6



	LV1181xVδ2	LV1184xVδ2	LV1185xVδ2	LV1186xVδ2
EC50	0.0041	0.0062	0.0052	0.0045

FIG. 7A



	LV1181xVδ2	LV1184xVδ2	LV1185xVδ2	LV1186xVδ2
EC50	0.0011	0.0019	0.0018	0.0014

FIG. 7B

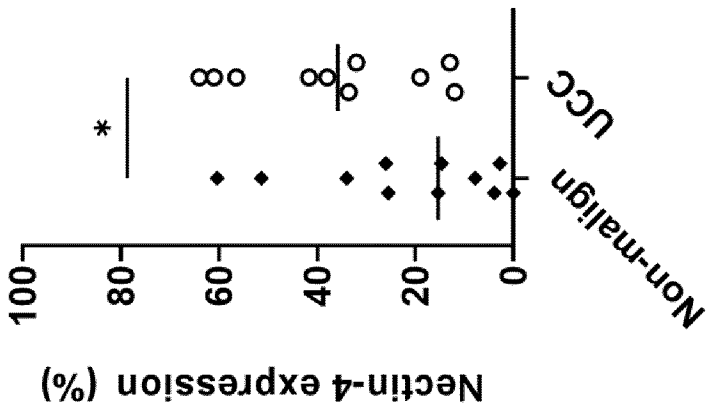

Nectin-4 expression (%)
100
80
60
40
20
0
*
Non-malign
UCC

FIG. 8B

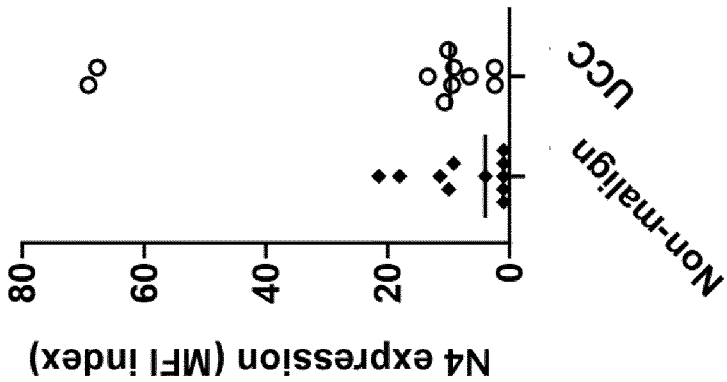

N4 expression (MFI index)
80
60
40
20
0
Non-malign
UCC

FIG. 8A

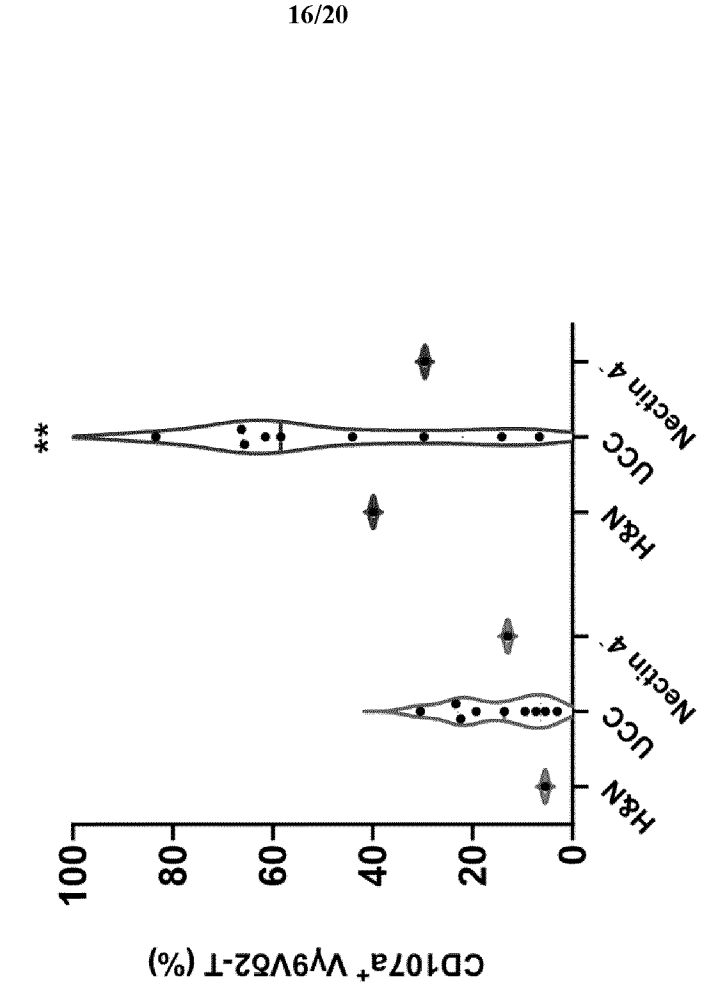

Tumor + Vγ9Vδ2-T cells
Tumor + Vγ9Vδ2-T cells + 10 nM LAVA-366
CD107a+ Vγ9Vδ2-T (%)
0
20
40
60
80
100
**
H&N
UCC
Nectin 4-

FIG. 9B

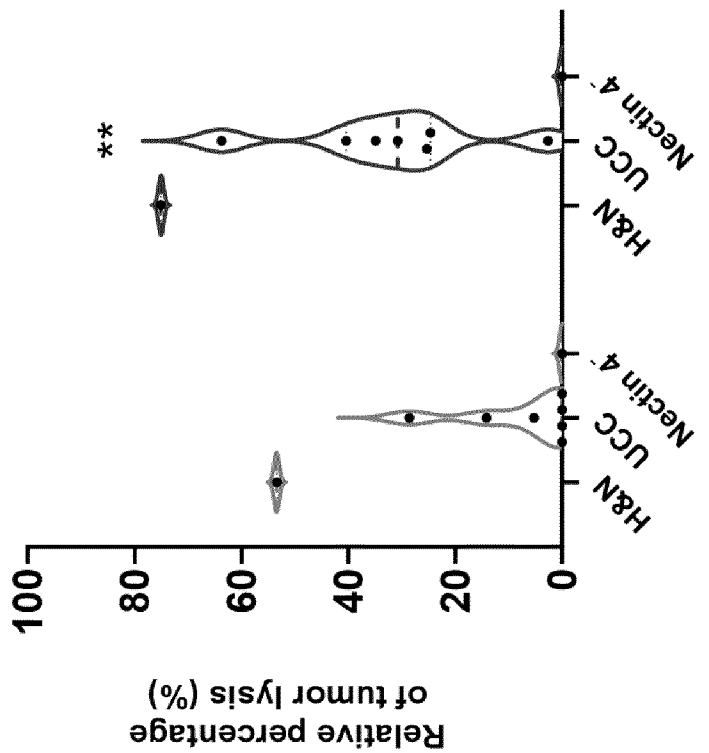

Relative percentage of tumor lysis (%)
0
20
40
60
80
100
**
H&N
UCC
Nectin 4-

FIG. 9A

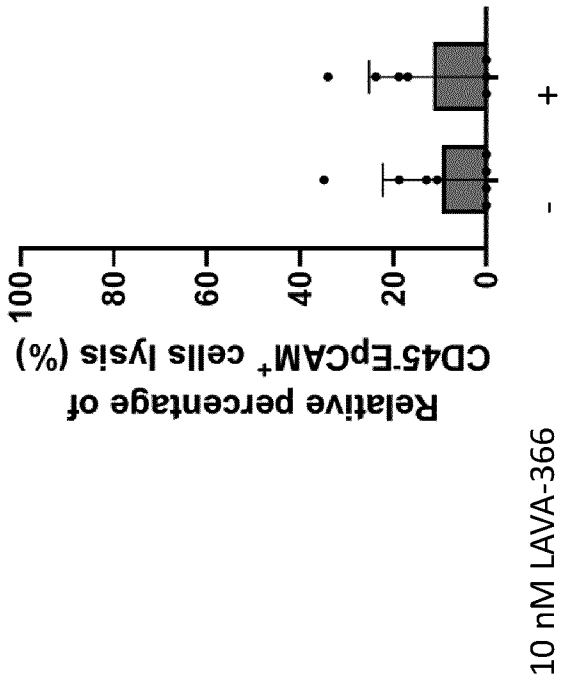

Relative percentage of
CD45-EpCAM+ cells lysis (%)
100
80
60
40
20
0
-
+
10 nM LAVA-366

FIG. 10

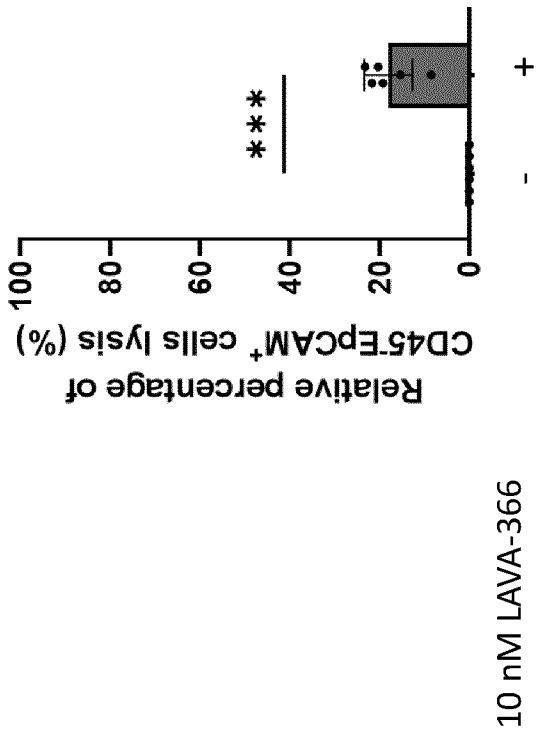

Relative percentage of
CD45⁻EpCAM⁺ cells lysis (%)
100
80
60
40
20
0
***
-
+
10 nM LAVA-366

FIG. 11

System	6H4 CDR Annotations
Kabat	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSS
IMGT	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSS
Chothia	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSS
Combined	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYGMGWFRQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSDDYDYWGQGTQVTVSS

FIG. 12

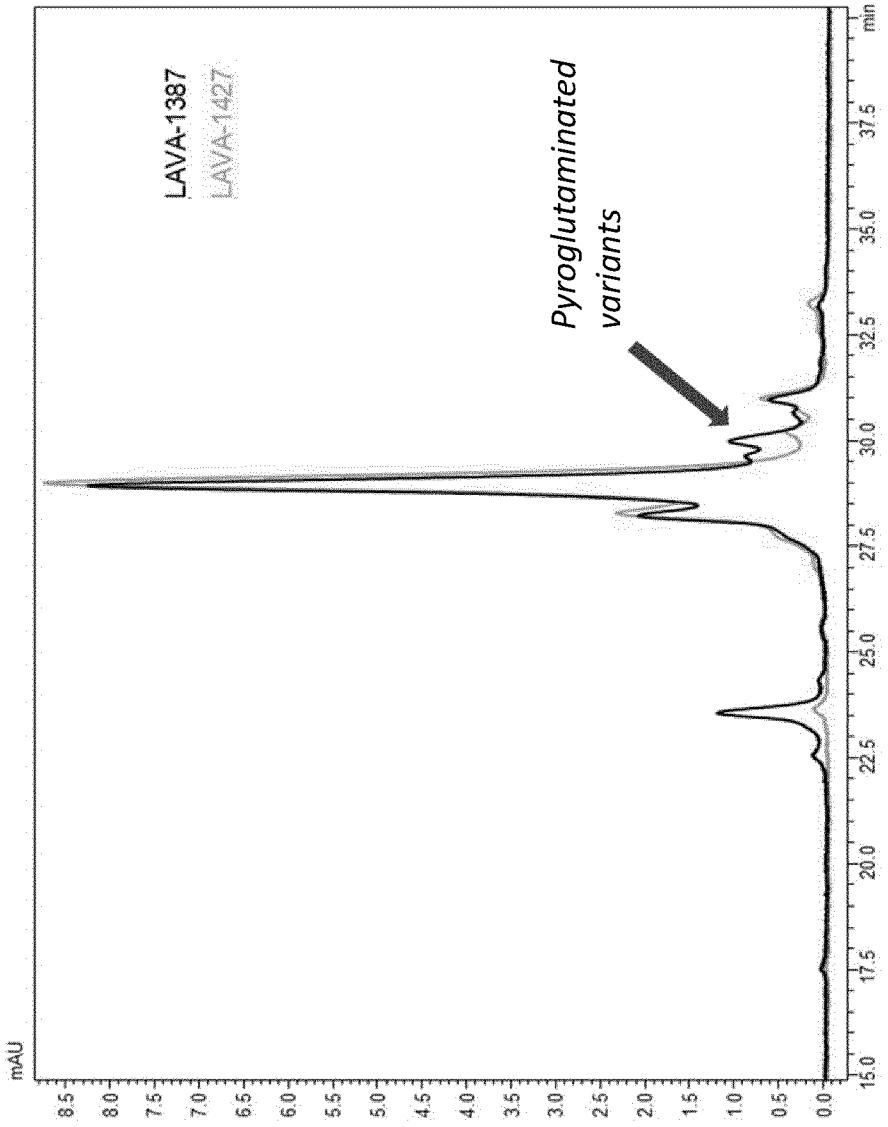

LAVA-1387
LAVA-1427
Pyroglutaminated variants
mAU
min
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/058365

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/058365

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
6-8(completely); 1-5, 37-56, 66-75(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/058365

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 114 702 589 A (BOJI BIOMEDICAL TECH HANGZHOU CO LTD) 5 July 2022 (2022-07-05)	1-8, 37-48, 71-74
Y	claims 6-7, 12-14 examples 4, 6 paragraphs [0005], [0080] - [0081], [0087] - [0091] table 4	49-56, 66-71,75
	- - - - -	
X	CN 114 671 953 A (BORGI BIOMEDICAL SCIENCE AND TECH HANGZHOU LIMITED COMPANY) 28 June 2022 (2022-06-28)	1-8, 37-48, 71-74
Y	claims 6-7, 14 examples 4, 6 paragraph [0005] table 4	49-56, 66-71,75
	- - - - -	
	-/- -	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2024	26/08/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Colling-Ziarko, L

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/058365

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 114 702 588 A (BOJI BIOMEDICAL TECH HANGZHOU CO LTD) 5 July 2022 (2022-07-05)	1-8, 37-48, 71-74
Y	claims 6-7, 14 examples 4, 6 paragraph [0005] table 4	49-56, 66-71,75

X	WO 2022/056304 A1 (TCR2 THERAPEUTICS INC [US]) 17 March 2022 (2022-03-17)	1-8, 37-48, 71-74
Y	paragraphs [0486] - [0489], [0564]; claims 141-146	49-56, 66-71

X	WO 2023/278480 A1 (ELPIS BIOPHARMACEUTICALS [US]) 5 January 2023 (2023-01-05)	1-8, 37-48, 71-74
Y	figure 2 page 17, lines 23-25 example 7 claims 44-46	49-56, 66-71,75

Y	WO 2022/008646 A1 (LAVA THERAPEUTICS N V [NL]) 13 January 2022 (2022-01-13) claims 1-2, 32-34 table 1; sequences 5, 17-19 page 24, paragraph 3 - page 25, paragraph 3 example 14	49-56, 66-71,75

A	DE BRUIN RENÉE C. G. ET AL: "A bispecific nanobody approach to leverage the potent and widely applicable tumor cytolytic capacity of V[gamma]9V[delta]2-T cells", ONCOIMMUNOLOGY , vol. 7, no. 1 2 January 2018 (2018-01-02), page e1375641, XP055937322, DOI: 10.1080/2162402X.2017.1375641 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5739573/pdf/koni-07-01-1375641.pdf abstract	49-56, 66-75

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/058365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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CN 114671953 A	28-06-2022	CN 114671953 A	28-06-2022
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		WO 2023278480 A1	05-01-2023
WO 2022008646 A1	13-01-2022	AU 2021305396 A1	09-02-2023
		BR 112023000269 A2	28-03-2023
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		CN 116323667 A	23-06-2023
		EP 4178981 A1	17-05-2023
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		JP 2023532807 A	31-07-2023
		KR 20230042035 A	27-03-2023
		US 2023272110 A1	31-08-2023
		WO 2022008646 A1	13-01-2022

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 6-8(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 88, CDR2: SEQ ID NO:89 and CDR3: ID NO: 90.

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2. claims: 9-11, 33-36, 57-65(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 95, CDR2: SEQ ID NO:96 and CDR3: ID NO: 97.

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3. claims: 12-14(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 103, CDR2: SEQ ID NO: 104 and CDR3: ID NO: 105.

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4. claims: 15-17(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 110, CDR2: SEQ ID NO: 111 and CDR3: ID NO: 112.

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5. claims: 18-20(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 117, CDR2: SEQ ID NO: 118 and CDR3: ID NO: 119.

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6. claims: 21-23(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 121, CDR2: SEQ ID NO: 122 and CDR3: ID NO: 123.

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7. claims: 24-26(completely); 1-5, 37-56, 66-75(partially)

   A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 125, CDR2: SEQ ID NO: 126 and CDR3: ID NO:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

127.

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8. claims: 27-29(completely); 1-5, 37-56, 66-75(partially)

A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 129, CDR2: SEQ ID NO: 130 and CDR3: ID NO: 131.

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9. claims: 30-32(completely); 1-5, 37-56, 66-75(partially)

A single domain antibody binding Nectin-4 and related multispecific antibodies, defined through CDR sequences CDR1: SEQ ID NO: 133, CDR2: SEQ ID NO: 134 and CDR3: ID NO: 135.

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10. claims: 76, 77

A method increasing the stability of a protein, wherein the protein is modified at the first position to a non-pyroglutamate forming amino acid, i.e, aspartic acid.
