

ANTIGEN BINDING MOLECULES AND METHODS THEREOF I

Field of Invention

The invention relates generally to antigen-binding molecules. In particular, the present disclosure relates to antigen-binding molecules that specifically bind to IgM μ -chain antigen.

Background

Cancer in companion animals is a key concern for both veterinarians and pet owners as it is a major diagnosis as well as leading cause of death in 47% of the dogs and 32% of the cats. Some common types of cancer in companion animals include skin, lymphoma, hemangiosarcoma and mammary cancer. Despite the willingness of pet owners to pay and pursue treatments, cancer therapies for companion animals remain challenging due to lack of targeted oncology drugs for animal use.

Lymphoma is one of the most common cancer type in dogs and cats, along with Mast Cells tumours, Hemangiosarcoma, Melanoma, Osteosarcoma and Mammary Carcinoma. The average survival time without treatment for the most common type of lymphoma is 4 to 6 weeks. The current recommended treatment for lymphoma involves CHOP multidrug chemotherapy: cyclophosphamide, hydroxydaunorubicin (doxorubicin), Oncovin® (vincristine), and prednisone. However, it is uncommon for dogs to be cured of lymphoma although remission can be achieved. Furthermore, reoccurrence of the disease is usually observed within a year post-treatment, with less than 25% of dogs surviving two years.

Although there are moderate overall response post treatments, side effects exist which sometimes resulted in fatal complications. Due to the toxicity of the chemotherapeutic drugs, pet owners handling dogs undergoing treatment have to be protected with chemotherapy-resistant gloves to avoid contact with its faeces, urine, vomit, and saliva.

Accordingly, it is generally desirable to overcome or ameliorate one or more of the above mentioned difficulties.

Summary

Disclosed herein is an antigen-binding molecule that specifically binds to IgM μ -chain antigen.

Disclosed herein is a chimeric molecule comprising an antigen-binding molecule as defined herein and a heterologous moiety.

Disclosed herein is an isolated polynucleotide comprising a nucleic acid sequence encoding the antigen-binding molecule or the chimeric molecule as defined herein.

Disclosed herein is a construct comprising a polynucleotide as defined herein in operable connection with one or more control sequences.

Disclosed herein is a host cell that contains the construct as defined herein.

Disclosed herein is a pharmaceutical composition comprising an antigen-binding molecule or a chimeric molecule as defined herein

Disclosed herein is an antigen-binding molecule, a chimeric molecule or a pharmaceutical composition as defined herein for use as a medicament.

Disclosed herein is a method for reducing or inhibiting proliferation and/or viability of a cancer cell, the method comprising contacting the cancer cell with a therapeutically effective amount of an antigen-binding molecule, a chimeric molecule or a pharmaceutical composition as defined herein.

Disclosed herein is a method of reducing or inhibiting proliferation, survival and/or viability of a cancer cell in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule, a chimeric molecule or a pharmaceutical composition as defined herein to the subject.

Disclosed herein is a method of treating a cancer in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule, a chimeric molecule or a pharmaceutical composition as defined herein to the subject.

Disclosed herein is a method of treating a disease or condition associated with an undesired expression of IgM μ -chain antigen in a subject, wherein the method comprises administering a therapeutically effective amount of an antigen-binding molecule, a chimeric molecule or a pharmaceutical composition as defined herein to the subject.

Disclosed herein is a method of detecting the likelihood of the presence of a cancer in a subject, the method comprising determining the level of IgM μ -chain antigen in a sample obtained from the subject, wherein an increased level of IgM μ -chain antigen as compared to a reference indicates the likelihood of the presence of a cancer in the subject.

Brief Description of the Figures

Figure 1. Isotype of mAb CL-m17A11 is IgG2b

Figure 2. Flow cytometry data showing CL-m17A11 binding to B cell lymphoma (A) CLBL-1, but not to normal canine cells (B) MDCK (C) cf2TH (D) PBMCs. Shaded peaks for the histogram are negative controls and unshaded peaks are CL-m17A11 binding.

Figure 3. Immunohistochemistry staining of normal canine tissue specimens with CL-m17A11, The mAb did not stain most of the normal dog tissues derived from major organs. Positive staining of CLBL-1 cells used as control. Magnification: 2.5X

Figure 4. Identification of the antigen target. CL-m17A11 binds predominantly to a protein band of ~190 kDa (A) Western Blot to detect the protein band that CL-m17A11 binds to. The binding is lost when protein conformation is linearized by reducing agent (B) Immunoprecipitation (IP) of antigen target using Protein G capture of mAb CL-m17A11. Protein bands at ~190 and 80 kDa were detected after incubating Western blot

with CL-m17A11. The bands were reproduced on a parallel SDS-PAGE gel and excised for mass spectrometry.

Figure 5. Validation of antigen target by cross-probing. Commercial antibody from Biorad Goat anti-dog IgM conjugated with HRP was used for validation. Purified canine IgM was used as an immunogen to produce the polyclonal anti-dog IgM. IP samples were run in duplicates on SDS-PAGE and transferred to PVDF blot. Boxed-up bands indicate the IP product after blot was incubated with (A) CL-m17A11 conjugated with Infra-red 800 (B) Goat anti-IgM conjugated with HRP. CL-m17A11 binds to an IgM-like molecule, as suggested by MS data and validated by IP.

Figure 6. Further validation that CL-m17A11 is targeting IgM-like molecule on cell surface. Polyclonal rabbit anti-dog IgM reduced the binding sites available but did not completely blocked binding for (A) CL-m17A11 and (B) vice versa. CL-m17A11 and anti-dog IgM are not likely binding to same epitope on cell surface IgM.

Figure 7. Flow Cytometry of CLBL-1 cells transiently transfected with siRNA. Cells treated with siRNA targeting dog IgM μ -chain (dotted line) were stained for cell surface IgM expression with (A) CL-m17A11 and (B) anti-dog IgM. Cells treated with scrambled siRNA (solid line) were used as negative control. Decreased in cell surface IgM expression was observed.

Figure 8. Binding assays to determine the epitope for CL-m17A11. (A) Periodate assay showed that CL-m17A11 binds to a glycan epitope that is sensitive to the treatment and loss of binding observed after the removal of glycans (B) Cell lysate was subjected to PNGase F treatment for the removal of N-glycans and there was no decreased in mAb binding (C) Beta elimination in 50mM NaOH for O-glycan removal with significant loss of mAb binding (D) Binding is lost after Pronase digestion of lysate. CL-m17A11 binds to an O-linked glycoprotein epitope of ~190kDa protein band (box). uT: untreated samples, T: treated samples.

Figure 9. Sequence of variable region of CL-c17A11 (A) V_H (B) V_L. Underlined sequences depict the CDR regions

Figure 10. ADC assay showing the cytotoxic effect of mAb CL-m17A11 and CL-c17A11 conjugated to monomethyl auristatin E (MMAE). Comparable cytotoxic effect observed in CLBL-1 cells for both mouse and canine CL-17A11 mAbs 72 hrs after spiking in culture. Detection with Cell-Titre-GLO (CTG) kit from Promega and chemilluminescence was recorded with a Tecan Spectrophotometer.

Figure 11. Characterization of tumours formed in mice treated with PBS (negative control). (A) Tumours formed were well-vascularized (B) Expression level of the cell surface protein that CL-m17A11 binds to was present on more than 95% of tumour cells. Controls for experiment were included; cLy11A12 (negative control) and CD20 (positive control).

Figure 12. Treatment of mice inoculated with 2×10^6 CLBL-1 cells. Intraperitoneal doses were given on Day 1, 7 and 14 (Dose 1Q3W). (A) Mice treated with CL-m17A11-MMAE did not have tumor formation (B) Tumor formation (if any) was delayed or not present when mice were treated with higher dosage of CL-m17A11 (naked antibody) (C) Survival of mice in the different treatment groups. CL-m17A11-MMAE was efficacious to inhibit CLBL-1 cells and prevented B lymphoma tumour growth in mice for more than 90 days. cLy-11A12 was used as an isotype control.

Figure 13. Characterization of tumours formed in mice treated with PBS (negative control). (A) Tumours formed were well-vascularized (B) Expression level of the cell surface protein that CL-c17A11 binds to was present on more than 95% of tumour cells.

Figure 14. Dose escalation studies of CL-c17A11-MMAE on immunodeficient mice. Groups of mice were inoculated subcutaneously with 2×10^6 CLBL-1 cells. Intravenous doses (arrows) were given once a week for 3 weeks to randomized mice when tumours reached 100-150mm³. (A) Tumours regression observed for mice treated with both doses of CL-c17A11-MMAE (8 and 15 mg/kg) as early as after first dose. (B) Survival of mice in the different treatment groups. The groups of mice were observed for more than 30 days and no relapse was observed.

Detailed Description

The present disclosure teaches antigen-binding molecules that specifically bind to IgM μ -chain antigen.

Disclosed herein is an antigen-binding molecule that specifically binds to IgM μ -chain antigen.

Without being bound by theory, the inventors have developed a chimeric (canine constant region genes-mouse variable region genes) IgGb monoclonal antibody (mAb) to target B cell lymphoma. The antibody binds to an antigen present on the cell surface of B cell lymphoma and tissues from xenograft but not on normal canine cells. Several characterization assays were performed to determine the identity of the antigen target. In cell-based assay, the naked mAb was observed to slow cell growth. When the antibody was conjugated to a toxin such as Monomethyl auristatin E (MMAE), it demonstrated highly-specific cytotoxicity. Tumour regression was achieved in immunocompromised mice when treated with the conjugated monoclonal antibody.

In one embodiment, the IgM μ -chain antigen is a canine IgM μ -chain antigen. The canine IgM μ -chain antigen may have a protein accession number of E2RE31.

By “antigen-binding molecule” is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity. Representative antigen-binding molecules that are useful in the practice of the present invention include antibodies and their antigen-binding fragments. The term “antigen-binding molecule” includes antibodies and antigen-binding fragments of antibodies.

In an embodiment, the antigen-binding molecule, as described herein, is conjugated to another molecule or moiety, including functional moieties (*e.g.*, toxins), detectable moieties (*e.g.*, fluorescent molecules, radioisotopes), small molecule drugs and polypeptides.

The term “antibody”, as used herein, is understood to mean any antigen-binding

molecule or molecular complex comprising at least one complementarity determining region (CDR) that binds specifically to, or interacts specifically with, the target antigen. The term “antibody” includes full-length immunoglobulin molecules comprising two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (which may be abbreviated as HCVR, V_H or V_H) and a heavy chain constant region. The heavy chain constant region typically comprises three domains - C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (which may be abbreviated as LCVR, V_L, V_K, V_K or V_L) and a light chain constant region. The light chain constant region will typically comprise one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, also referred to as framework regions (FR). Each V_H and V_L typically comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In some embodiments, the FRs of the antigen-binding molecules described herein may be identical to the FR of germline sequences of the target species (*i.e.*, the species to which the antigen-binding molecules or antigen-binding fragments thereof, as described herein, will be administered). In some embodiments, the FR may be naturally or artificially modified. Whilst it is generally desirable that each of the FR sequences are identical to FR sequences derived from immunoglobulin molecules of the target species, including to minimize an immune response being raised against the binding molecule upon administration to a subject of the target species, in some embodiments, the antigen-binding molecule, or antigen-binding fragment thereof, may comprise one or more amino acid residues across one or more of its FR sequences that would be foreign at a corresponding position in one or more FR from the target species.

An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ ,

and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known to a person skilled in the art.

As used herein, the term “complementarity determining regions” (CDRs; *i.e.*, CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable domain the presence of which are necessary for antigen binding. Each variable domain typically has three CDR regions identified as CDR1, CDR2 and CDR3. Each complementarity determining region may comprise amino acid residues from a “complementarity determining region” as defined for example by Kabat (*i.e.*, about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a “hypervariable loop” (*i.e.*, about residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). In some instances, a complementarity determining region can include amino acids from both a CDR region defined according to Kabat and a hypervariable loop.

An “antigen-binding site” refers to the site, *i.e.*, one or more amino acid residues, of an antigen binding molecule which provides interaction with the antigen. For example, the antigen binding site of an antibody comprises amino acid residues from the complementarity determining regions (CDRs). A native immunoglobulin molecule typically has two antigen binding sites, a Fab molecule typically has a single antigen binding site. An antigen-binding site of an antigen-binding molecule described herein typically binds specifically to an antigen and more particularly to an epitope of the antigen.

The present disclosure extends to antigen-binding molecules that bind specifically to IgM μ -chain of any species. The IgM μ -chain may be human IgM μ -chain or canine IgM μ -chain. In an embodiment, the IgM μ -chain is canine IgM μ -chain. In another embodiment, the IgM μ -chain is human IgM μ -chain. The present disclosure extends to antigen binding molecules that bind specifically to native IgM μ -chain (*i.e.*, naturally-occurring IgM μ -chain), as well as to variants thereof. Such variants may include IgM

μ -chain molecules that differ from a naturally-occurring (wild-type) molecule by one or more amino acid substitutions, deletions and / or insertions. Variant IgM μ -chain molecules of this type may be naturally-occurring or synthetic (*e.g.*, recombinant) forms. It is to be understood, however, that in one embodiment, the antigen-binding molecules described herein bind specifically to a native form of IgM μ -chain, whether of a human or non-human species (such as canine IgM μ -chain).

The terms “antigen-binding fragment”, “antigen-binding portion”, “antigen-binding domain” and “antigen-binding site” are used interchangeably herein to refer to a part of an antigen-binding molecule that participates in antigen-binding. These terms include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex.

Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, one-armed antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small

modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression “antigen-binding fragment” as used herein.

An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H , V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H - C_H1 ; (ii) V_H - C_H2 ; (iii) V_H - C_H3 ; (iv) V_H - C_H1 - C_H2 ; (v) V_H - C_H1 - C_H2 - C_H3 ; (vi) V_H - C_H2 - C_H3 ; (vii) V_H - C_L ; (viii) V_L - C_H1 ; (ix) V_L - C_H2 ; (x) V_L - C_H3 ; (xi) V_L - C_H1 - C_H2 ; (xii) V_L - C_H1 - C_H2 - C_H3 ; (xiii) V_L - C_H2 - C_H3 ; and (xiv) V_L - C_L . In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present disclosure may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)). A multispecific antigen-binding molecule will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antigen-binding molecule format, including bispecific antigen-binding molecule formats, may be adapted for use in the context of an antigen-binding

fragment of an antibody of the present disclosure using routine techniques available in the art.

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antigen binding molecule to antigen. The variable domains of the heavy chain and light chain (V_H and V_L , respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, *e.g.*, Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity.

The term “constant domains” or “constant region” as used herein denotes the sum of the domains of an antibody other than the variable region. The constant region is not directly involved in binding of an antigen, but exhibits various immune effector functions.

In one embodiment, the antigen-binding molecule or antigen-binding fragment thereof is modified for compatibility with the target species. Thus, in an embodiment, the antigen-binding molecule or antigen-binding fragment thereof is humanized or caninized.

By “humanized” is meant that the antigen-binding molecule comprises an amino acid sequence that is compatible with humans, such that the amino acid sequence is unlikely to be seen as foreign by the immune system of a human subject. In an embodiment, the humanized antigen-binding molecule comprises one or more immunoglobulin framework regions derived from one or more human immunoglobulin molecules. In some embodiments, all of the framework regions of the humanized antigen-binding molecule will be derived from one or more human immunoglobulin molecules. The humanized antibody may optionally comprise an immunoglobulin heavy chain constant region derived from a human immunoglobulin molecule.

By “caninized” is meant that the antigen-binding molecule comprises an amino acid sequence that is compatible with canine, such that the amino acid sequence is unlikely to be seen as foreign by the immune system of a canine subject. In an embodiment, the caninized antigen-binding molecule comprises one or more immunoglobulin framework

regions derived from one or more canine immunoglobulin molecules. In some embodiments, all of the framework regions of the caninized antigen-binding molecule will be derived from one or more canine immunoglobulin molecules. The caninized antibody may optionally comprise an immunoglobulin heavy chain constant region derived from a canine immunoglobulin molecule.

It is to be understood that the present disclosure also extends to antigen-binding molecules that are compatible with species other than human and canine. In this context, the antigen-binding molecules can be referred to as "speciesized", referring to the target species to which the molecule will be administered.

The phrase "specifically binds" or "specific binding" refers to a binding reaction between two molecules that is at least two times the background and more typically more than 10 to 100 times background molecular associations under physiological conditions. When using one or more detectable binding agents that are proteins, specific binding is determinative of the presence of the protein, in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antigen-binding molecule binds to a particular antigenic determinant, thereby identifying its presence. Specific binding to an antigenic determinant under such conditions requires an antigen-binding molecule that is selected for its specificity to that determinant. This selection may be achieved by subtracting out antigen-binding molecules that cross-react with other molecules. A variety of immunoassay formats may be used to select antigen-binding molecules (*e.g.*, immunoglobulins) such that they are specifically immunoreactive with a particular antigen. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, *e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Methods of determining binding affinity and specificity are also well known in the art (see, for example, Harlow and Lane, *supra*); Friefelder, "Physical Biochemistry: Applications to biochemistry and molecular biology" (W.H. Freeman and Co. 1976)).

"Affinity" or "binding affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antigen-binding

molecule) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair *e.g.*, an antigen-binding molecule. The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d), which is the ratio of dissociation and association rate constants (k_{off} and k_{on} , respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by common methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

The terms “polypeptide”, “peptide”, or “protein” are used interchangeably herein to designate a linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The amino acid residues are usually in the natural “L” isomeric form. However, residues in the “D” isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide

As used herein, the term “modified antibody” includes synthetic forms of antibodies which are altered such that they are not naturally occurring, *e.g.*, antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as domain deleted antibodies or minibodies); multispecific forms of antibodies (*e.g.*, bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen; heavy chain molecules joined to scFv molecules and the like. ScFv molecules are known in the art and are described, *e.g.*, in U.S. Pat. No. 5,892,019. In addition, the term “modified antibody” includes multivalent forms of antibodies (*e.g.*, trivalent, tetravalent, etc., antibodies that bind to three or more copies of the same antigen).

The antigen-binding molecule may comprise a heavy chain variable (VH) region comprising the VHCDR1 amino acid sequence K A S G Y S F T D Y N I Y (SEQ ID NO: 1), the VHCDR2 amino acid sequence F F D P H N G N T T Y N Q K F K (SEQ ID NO: 2) and the VHCDR3 amino acid sequence E G Y L Y A M D Y (SEQ ID NO: 3). The antigen-binding molecule may comprise a light chain variable (VL) region comprising the VLCDR1 amino acid sequence R S S Q S I E H S N G N T Y L E (SEQ

ID: 4), the VLCDR2 amino acid sequence F K V S N R F S (SEQ ID NO: 5) and the VLCDR3 amino acid sequence F Q G S H V P P T (SEQ ID NO: 6).

In one embodiment, the antigen-binding molecule comprises:

- a) a heavy chain variable (VH) region comprising the VHCDR1 amino acid sequence K A S G Y S F T D Y N I Y (SEQ ID NO: 1), the VHCDR2 amino acid sequence F F D P H N G N T T Y N Q K F K (SEQ ID NO: 2) and the VHCDR3 amino acid sequence E G Y L Y A M D Y (SEQ ID NO: 3); and
- b) a light chain variable (VL) region comprising the VLCDR1 amino acid sequence R S S Q S I E H S N G N T Y L E (SEQ ID: 4), the VLCDR2 amino acid sequence F K V S N R F S (SEQ ID NO: 5) and the VLCDR3 amino acid sequence F Q G S H V P P T (SEQ ID NO: 6).

The antigen-binding molecule may comprise a) a VH region comprising an amino acid sequence having at least 70% (including at least 71% to 99% and all integer percentages therebetween) sequence identity to:

Q I Q L Q Q S G P E L V K P G A S V K V S C K A S G Y S F T D Y N I Y W V K
Q S H G K S L E W I G F F D P H N G N T T Y N Q K F K G K A T L T V D K S S
S T A F M H L N S L T S E D S A V Y Y C A R E G Y L Y A M D Y W G Q G T S
V T V S S (SEQ ID NO: 7).

The antigen binding molecule may comprise a VL region comprising an amino acid sequence having at least 70% (including at least 71% to 99% and all integer percentages therebetween) sequence identity to:

D V L M T Q T P L S L P V S L G D Q A S I S C R S S Q S I E H S N G N T Y L E
W Y L Q K P G Q A P K L L I F K V S N R F S G V P D R F S G S G S G T D F T L
K I S R M E A E D L G V Y Y C F Q G S H V P P T F G G G T K L E I K R A D A
A P T V S (SEQ ID NO: 8).

In one embodiment, the antigen-binding molecule comprises:

- a) a VH region comprising an amino acid sequence having at least 70% (including at least 71% to 99% and all integer percentages therebetween) sequence identity to:
- Q I Q L Q Q S G P E L V K P G A S V K V S C K A S G Y S F T D Y N I Y W V K
Q S H G K S L E W I G F F D P H N G N T T Y N Q K F K G K A T L T V D K S S

S T A F M H L N S L T S E D S A V Y Y C A R E G Y L Y A M D Y W G Q G T S
V T V S S (SEQ ID NO: 7); and

b) a VL region comprising an amino acid sequence having at least 70% (including at least 71% to 99% and all integer percentages therebetween) sequence identity to:

D V L M T Q T P L S L P V S L G D Q A S I S C R S S Q S I E H S N G N T Y L E
W Y L Q K P G Q A P K L L I F K V S N R F S G V P D R F S G S G S G T D F T L
K I S R M E A E D L G V Y Y C F Q G S H V P P T F G G G T K L E I K R A D A
A P T V S (SEQ ID NO: 8).

In one embodiment, the antigen-binding molecule comprises

- a) a heavy chain variable region (VH) as defined herein comprising at least 70% sequence identity to at least one region other than a CDR of the VH amino acid sequence set forth in SEQ ID NO:7 (e.g., to at least one framework region, such as 1, 2, 3 or 4 framework regions, of the VH), and
- b) a light chain variable region (VL) as defined herein comprising at least 70% sequence identity to at least one region other than a CDR of the VL amino acid sequence set forth in SEQ ID NO:8 (e.g., to at least one framework region, such as 1, 2, 3 or 4 framework regions, of the VL).

In one embodiment, the antigen-binding molecule comprises

- a) a VH as defined herein which is distinguished from the VH amino acid sequence set forth in SEQ ID NO:7 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4 or 5) amino acids in at least one region other than a CDR of the VH amino acid sequence set forth in SEQ ID NO:7 (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the VH), and
- b) a VL as defined in (1) which is distinguished from the VL amino acid sequence set forth in SEQ ID NO:8 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4 or 5) amino acids in at least one region other than a CDR of the VL amino acid sequence set forth in SEQ ID NO:8 (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the VL).

The antigen-binding molecule may comprise a canine IgGB constant region. The antigen-binding molecule may comprise a heavy chain constant region comprising an

amino acid sequence having at least 70% (or at least 75%, 80%, 85%, 90% or 95%) sequence identity to:

TTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSV
LQSSGLYSLSSMVTVPSSRWSETFTCNVAHPASKTKVDKPVPKRENGRVPRP
PDCPKCPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISW
FVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKAL
PSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEWQS
NGQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALH
NHYTQESLSHSPGK (SEQ ID NO: 9).

The antigen-binding molecule may comprise a light chain constant region comprising an amino acid sequence having at least 70% (or at least 75%, 80%, 85%, 90% or 95%) sequence identity to:

DAQPAVYLFQSPDQLHTGSASVVCLLNSFYPKDINVKKVKVDGVIQDTGIQES
VTEQDKDSTYLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFRSECQRVD
(SEQ ID NO: 10).

The term “sequence identity” as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G and I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The antigen-binding molecule as defined herein may comprise one or more conservative amino acid substitutions.

A “conservative amino acid substitution” is to be understood as meaning a substitution in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined

in the art, which can be generally sub-classified as shown in the table "*Amino Acid Classification*", below:

AMINO ACID SUB-CLASSIFICATION

Sub-classes	Amino acids
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine
Residues that influence chain orientation	Glycine and Proline

Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying its activity.

Conservative substitutions are also shown in the table below (*EXEMPLARY AND*

PREFERRED AMINO ACID SUBSTITUTIONS). Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. After the substitutions are introduced, the variants can be screened for their ability to bind specifically to NGF using methods known to persons skilled in the art, including those methods described elsewhere herein.

EXEMPLARY AND PREFERRED AMINO ACID SUBSTITUTIONS

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleu	Leu
Leu	Norleu, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala, Norleu	Leu

The antigen-binding fragment may include recombinant, monoclonal, polyclonal, chimeric, humanised, bispecific or heteroconjugate antibodies. It may include a

chimeric antigen receptor (CAR), a single variable domain, a domain antibody, antigen binding fragments, an immunologically effective fragment, a single chain Fv, a single chain antibody, a univalent antibody lacking a hinge region, a minibody, or a diabody.

In one embodiment, the antigen-binding fragment is an antibody or antigen-binding fragment thereof.

In one embodiment, the antibody or antigen binding fragment thereof is a full-length antibody, a substantially intact antibody, a Fab fragment, a scFab, a Fab', a single chain variable fragment (scFv) or a one-armed antibody.

In one embodiment, the antibody or antigen binding fragment thereof is a full-length antibody and comprises a canine constant region.

In one embodiment, the antibody or antigen-binding molecule thereof is caninized or chimerized.

Disclosed herein is a chimeric molecule comprising an antigen-binding molecule as defined herein and a heterologous moiety.

As used herein, a "chimeric" molecule is one which comprises one or more unrelated types of components or contain two or more chemically distinct regions which can be conjugated to each other, fused, linked, translated, attached via a linker, chemically synthesized, expressed from a nucleic acid sequence, etc. For example, a peptide and a nucleic acid sequence, a peptide and a detectable label, unrelated peptide sequences, and the like. In embodiments in which the chimeric molecule comprises amino acid sequences of different origin, the chimeric molecule includes (1) polypeptide sequences that are not found together in nature (*i.e.*, at least one of the amino acid sequences is heterologous with respect to at least one of its other amino acid sequences), or (2) amino acid sequences that are not naturally adjoined. For example, a "chimeric" antibody" as used herein refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

In one embodiment, the heterologous moiety is a detectable moiety, a half-life extending moiety or a therapeutic moiety.

Detectable moieties contemplated by the present invention include for example any species known in the art that is appropriate for diagnostic detection, including in vitro detection and in vivo imaging. The detectable moiety may be, for example, a fluorophore, a radionuclide reporter, a metal-containing nanoparticle or microparticle, an ultrasound contrast agent (e.g., a nanobubble or microbubble) or an optical imaging dye. This also includes contrast particles visible in magnetic resonance imaging (MRI) and magnetic particle imaging (MPI). Fluorophores can be detected and/or imaged, for example, by fluorescence polarization, fluorescence-activated cell sorting and fluorescence microscopy, which may or may not be in combination with electrospray ionization-mass spectrometry (ESI-MS) detection, as well as fluorescence emission computed tomography (FLECT) imaging. Radionuclide reporters can be detected and imaged by radionuclide (nuclear) detection, such as, for example, single-photon emission computed tomography (SPECT), positron emission tomography (PET) or scintigraphic imaging. Metal-containing nanoparticles or microparticles may be detected using optical imaging, including MRI, which is typically used with paramagnetic nanoparticles or microparticles, and MPI, which is generally used with superparamagnetic particles. Ultrasound contrast agents can be detected using ultrasound imaging including contrast-enhanced ultrasound (CEU).

The detectable label may also be an enzyme-substrate label. The enzyme may generally catalyze a chemical alteration of the chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a chemical alteration of the chromogenic substrate that can be measured using the various techniques. For example, the example may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light that can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-

dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as urease and xanthine oxidase), lactoperoxidase, microperoxidase, and the like.

Examples of enzyme-substrate combinations include, for example:

- 1) Horseradish peroxidase (HRPO) utilizes hydrogen peroxide to oxidize a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));
- 2) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and
- 3) β -D-galactosidase (β -D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β -D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl- β -D-galactosidase.

In another embodiment of the invention, the antigen-binding molecule need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the antigen-binding molecule. The antigen-binding molecule of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, immunohistochemistry and immunoprecipitation assays.

In one embodiment, the chimeric molecule comprises at least one heterologous moiety that is a "half-life extending moiety". Half-life extending moieties, can comprise, for example, (i) XTEN polypeptides; (ii) Fc; (iii) albumin, (iv) albumin binding polypeptide or fatty acid, (v) the C-terminal peptide (CTP) of the 13 subunit of human chorionic gonadotropin, (vi) PAS; (vii) HAP; (viii) transferrin; (ix) polyethylene glycol (PEG); (x) hydroxyethyl starch (HES), (xi) polysialic acids (PSAs); (xii) a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor; (xiii) low complexity peptides; (xiv) or any combinations thereof. In some embodiments, the half-life extending moiety comprises an Fc region. In other embodiments, the half-life extending moiety comprises two Fc regions fused by a linker. Exemplary heterologous moieties also include, e.g., FcRn binding moieties (e.g., complete Fc regions or portions thereof which bind to FcRn), single chain Fc regions

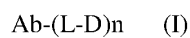
(scFc regions, e.g., as described in U.S. Publ. No. 20080260738, WO 2008/012543 and WO 2008/1439545), or processable scFc regions. In some embodiments, a heterologous moiety can include an attachment site for a non-polypeptide moiety such as polyethylene glycol (PEG), hydroxyethyl starch (HES),

In one embodiment, the therapeutic moiety is a toxin. The toxin may, for example, be auristatin E, mertansine (DM-1), saporin, Exatecan (DX8951), gemcitabine, irinotecan, etoposide, vinblastine, pemetrexed, docetaxel, paclitaxel, platinum agents (for example, cisplatin, oxaliplatin or carboplatin), vinorelbine, capecitabine, mitoxantrone, ixabepilone, eribulin, 5-fluorouracil, trifluridine or tipiracil). The toxin may be auristatin, saporin or mertansine (DM1). In one embodiment, the toxin is Monomethyl auristatin E (MMAE). In one embodiment, the toxin is saporin. In one embodiment, the toxin is mertansine (DM1). In one embodiment, the toxin is Exatecan (DX8951).

In one embodiment, the antigen-binding molecule is joined to the therapeutic moiety via a linker. The antigen-binding molecule may be conjugated via a protease-cleavable maleimidocaproyl valine citrulline (MC-vc-PAB) linker.

In one embodiment, the antigen-binding molecule is conjugated to monomethyl auristatin E (MMAE) via a maleimidocaproyl valine citrulline (MC-vc-PAB) linker. In one embodiment, the linker-toxin combination has the chemical formula of $C_{58}H_{94}N_{10}O_{12}$ with a chemical name of L-Valinamide, N-methyl-N-[[[4-[[L-valyl-N5-(aminocarbonyl)-L-ornithyl]amino]phenyl] methoxy]carbonyl]-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl] amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl. The conjugation may be performed by a maleimide-cysteine based method by first reducing the mAb inter-chain disulfide bonds with TCEP at 37°C and then linking the maleimide moiety of the drug to the reduce cysteines. Drug Antibody Ratio (DAR) may be analysed on hydrophobic interaction chromatography (HIC) . The DAR ratio may, for example, be between 3 and 4.

In embodiment, there is provided an antigen-binding molecule or antigen-drug conjugate (ADC) of the following formula (I):



or a pharmaceutically acceptable salt thereof,

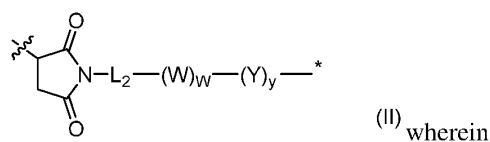
wherein

Ab is an antibody or antibody fragment thereof as defined herein:

L is a linker;

D is a cytotoxin.

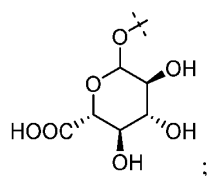
In an embodiment, there is provided an ADC wherein L is a linker of the following formula (II):



L2 is cycloalkylene-carbonyl, (C2-C6)alkyl or (C2-C6)alkyl-carbonyl;

W is an amino acid unit; w is an integer comprised of 0 to 5;

Y is PAB-carbonyl with PAB being



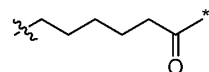
x can be H or

y is 0 or 1;

the asterisk indicates the point of attachment to D; and

the wavy line indicates the point of attachment to Ab.

In one embodiment, there is provided an ADC wherein L2 is of the following formula:

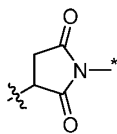


wherein

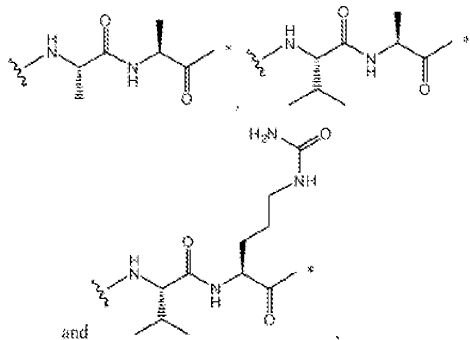
the asterisk indicates the point of attachment to (W)_w; and

the wavy line indicates the point of attachment to the nitrogen atom of the maleimide moiety of formula:

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In one embodiment, $w = 0$, or $w = 2$ and then $(W)_w$ is selected from:

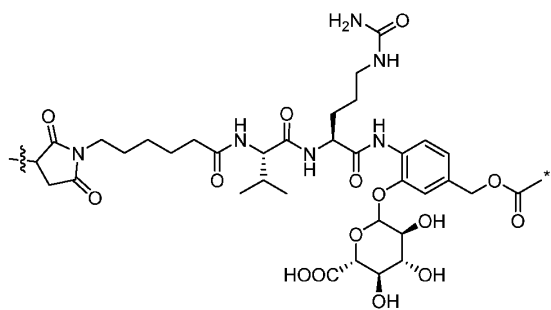


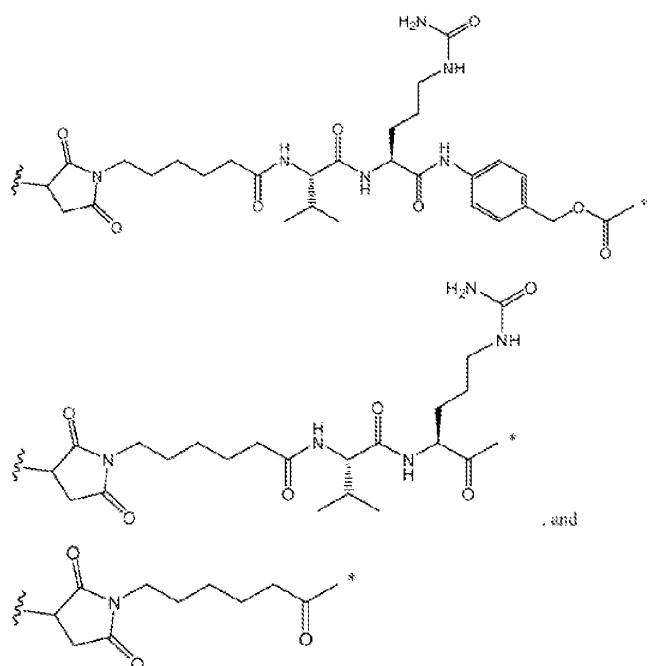
wherein

the asterisk indicates the point of attachment to $(Y)_y$; and

the wavy line indicates the point of attachment to L_2 .

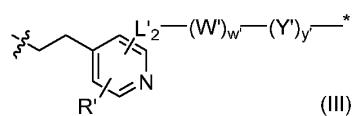
In one embodiment, there is provided an ADC wherein L is selected from:





wherein the asterisk indicates the point of attachment to D, and the wavy line indicates the point of attachment to Ab.

In another embodiment the invention relates to an ADC wherein L is a linker of the following formula (III):



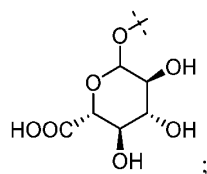
wherein

L'₂ is cycloalkylene-carbonyl, (C2-C6)alkylene, or (C2-C6)alkylene-carbonyl;

W' is an amino acid unit;

w' is an integer of 0 to 5;

Y' is PAB-carbonyl with PAB being

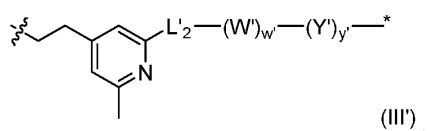


x can be H or

y' is 0 or 1;

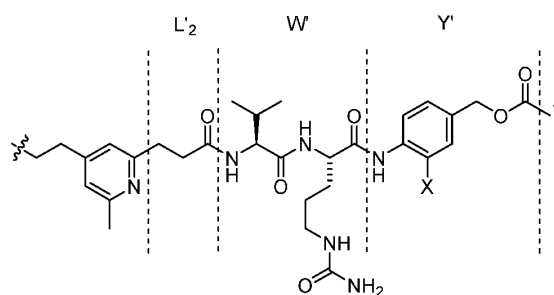
R' is C₁-C₃ alkenyl or H.

In an embodiment the compound of formula (III) is a compound of formula (III'):

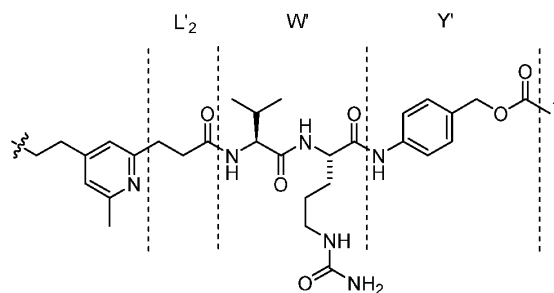


In an embodiment the compound of formula (III') is characterised with L₂' being C₂ alkylene carbonyl and w' being 2.

In an embodiment the linker compound of formula (III') is:

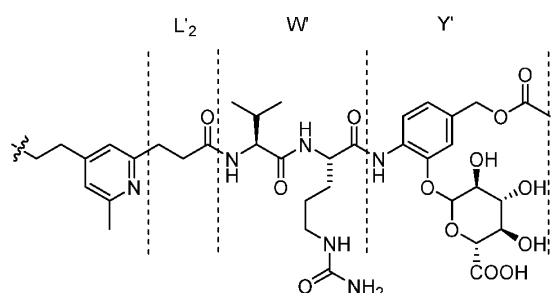


In another embodiment, the linker compound of formula (III') is:



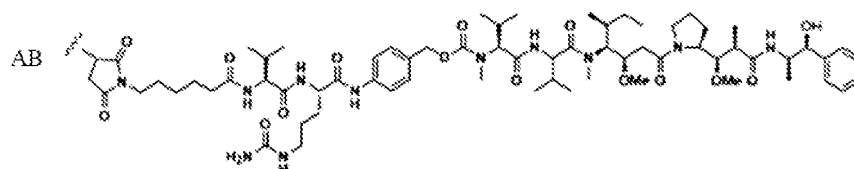
In another embodiment, the linker compound of formula (III') is:

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The present linkers may be synthesized using amide bond coupling. Many methods exist for amide synthesis. Some methods, but not limited to, are described in Montalbetti, Christian A. G. N (*Tetrahedron* 61(46), **2005**, 10827-10852). Alternatively, the linkers may be synthesized using standard stepwise addition of one or more residues using, for example, a peptide or protein synthesizer. Alternatively, other methods that may be used for amide formation includes, but not limited to, Beckmann rearrangement, Schmidt reaction, Nitrile hydrolysis, Willgerodt-Kindler reaction, Passerini reaction, Ugi reaction, Bodroux reaction, Chapman rearrangement, Leuckart amide synthesis, Ritter reaction, Ester aminolysis, Schotten-Baumann reaction, ruthenium based catalysis of alcohol and amine, or Photolytic addition of formamide to olefins.

In one embodiment, the antigen-binding molecule or antigen-drug conjugate (ADC) has the following structure:



In one embodiment, the chimeric molecule is an Antibody Drug Conjugate (ADC).

In one embodiment, the antigen-binding molecule or chimeric molecule as defined herein is capable of being internalized into a cell. This makes the antigen-binding molecule or chimeric molecule suitable for delivering a therapeutic moiety into a cell.

Disclosed herein is an isolated polynucleotide comprising a nucleic acid sequence encoding the antigen-binding molecule as defined herein, or the chimeric molecule as defined herein.

The term “polynucleotide” or “nucleic acid” are used interchangeably herein to refer to a polymer of nucleotides, which can be mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

Also disclosed herein is a vector that comprises a nucleic acid encoding the antigen-binding molecule as described herein.

By “vector” is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extrachromosomal element, a mini-chromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are well known to those of skill in the art.

Disclosed herein is a construct comprising a polynucleotide as defined herein in operable connection with one or more control sequences.

The term “construct” refers to a recombinant genetic molecule including one or more isolated nucleic acid sequences from different sources. Thus, constructs are chimeric molecules in which two or more nucleic acid sequences of different origin are assembled into a single nucleic acid molecule and include any construct that contains (1) nucleic acid sequences, including regulatory and coding sequences that are not found together in nature (i.e., at least one of the nucleotide sequences is heterologous with respect to at least one of its other nucleotide sequences), or (2) sequences encoding parts of functional RNA molecules or proteins not naturally adjoined, or (3) parts of promoters that are not naturally adjoined. Representative constructs include any recombinant nucleic acid molecule such as a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, or linear or circular single stranded or double stranded DNA or RNA nucleic acid molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where one or more nucleic acid molecules have been operably linked. Constructs of the present invention will generally include the necessary elements to direct expression of a nucleic acid sequence of interest that is also contained in the construct, such as, for example, a target nucleic acid sequence or a modulator nucleic acid sequence. Such elements may include control elements or regulatory sequences such as a promoter that is operably linked to (so as to direct transcription of) the nucleic acid sequence of interest, and often includes a polyadenylation sequence as well. Within certain embodiments of the invention, the construct may be contained within a vector. In addition to the components of the construct, the vector may include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome of a host cell. Two or more constructs can be contained within a single nucleic acid molecule, such as a single vector, or can be contained within two or more separate nucleic acid molecules, such as two or more separate vectors. An “expression construct” generally includes at least a control sequence operably linked to a nucleotide sequence of interest. In this manner, for example, promoters in operable connection with the nucleotide sequences to be expressed are provided in expression constructs for expression in an organism or part thereof including a host cell. For the practice of the

present invention, conventional compositions and methods for preparing and using constructs and host cells are well known to one skilled in the art, see for example, *Molecular Cloning: A Laboratory Manual*, 3rd edition Volumes 1, 2, and 3. J. F. Sambrook, D. W. Russell, and N. Irwin, Cold Spring Harbor Laboratory Press, 2000.

By “control element”, “control sequence”, “regulatory sequence” and the like, as used herein, mean a nucleic acid sequence (e.g., DNA) necessary for expression of an operably linked coding sequence in a particular host cell. The control sequences that are suitable for prokaryotic cells for example, include a promoter, and optionally a cis-acting sequence such as an operator sequence and a ribosome binding site. Control sequences that are suitable for eukaryotic cells include transcriptional control sequences such as promoters, polyadenylation signals, transcriptional enhancers, translational control sequences such as translational enhancers and internal ribosome binding sites (IRES), nucleic acid sequences that modulate mRNA stability, as well as targeting sequences that target a product encoded by a transcribed polynucleotide to an intracellular compartment within a cell or to the extracellular environment.

Disclosed herein is a host cell that contains the construct as defined herein.

The terms “host”, “host cell”, “host cell line” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells”, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the antigen binding molecules of the present invention. Host cells include cultured cells, *e.g.*, mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

Disclosed herein is a pharmaceutical composition comprising an antigen-binding molecule as defined herein or a chimeric molecule as defined herein.

By “pharmaceutically acceptable carrier” is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient(s), its use in the pharmaceutical compositions is contemplated.

The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Suitable pharmaceutical compositions may be administered intravenously, subcutaneously or intramuscularly. In some embodiments, the compositions are in the form of injectable or infusible solutions. A preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In specific embodiments, the pharmaceutical composition is administered by intravenous infusion or injection. In other embodiments, the pharmaceutical composition is administered by intramuscular or subcutaneous injection.

The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject invention, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives can also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and will preferably be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin and/or by the maintenance of the required particle size. In specific embodiments, an agent of the present disclosure may be conjugated to a vehicle for cellular delivery. In these embodiments, the agent may be encapsulated in a suitable vehicle to either aid in the delivery of the agent to target cells, to increase the stability of the agent, or to minimize

potential toxicity of the agent. As will be appreciated by a skilled artisan, a variety of vehicles are suitable for delivering an agent of the present disclosure. Non-limiting examples of suitable structured fluid delivery systems may include nanoparticles, liposomes, microemulsions, micelles, dendrimers and other phospholipid-containing systems. Methods of incorporating agents of the present disclosure into delivery vehicles are known in the art. Although various embodiments are presented below, it will be appreciated that other methods known in the art to incorporate an antigen-binding molecule, as described herein, into a delivery vehicle are contemplated.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. An antigen-binding molecule of the present disclosure can be administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of modified polypeptide or antigen in the patient. Alternatively, the antigen-binding molecule can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.

It may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Dosages and therapeutic regimens of the antigen-binding molecule can be determined by a skilled artisan. In certain embodiments, the antigen-binding molecule is administered by injection (*e.g.*, subcutaneously or intravenously) at a dose of about 0.01 to 40 mg/kg, *e.g.*, 0.01 to 0.1 mg/kg, *e.g.*, about 0.1 to 1 mg/kg, about 1 to 5 mg/kg,

about 5 to 25 mg/kg, about 10 to 40 mg/kg. The dosing schedule can vary from *e.g.*, once a week to once every 2, 3, or 4 weeks.

It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Disclosed herein is an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein for use as a medicament.

Disclosed herein is a method for reducing or inhibiting proliferation and/or viability of a cancer cell, the method comprising contacting the cancer cell with a therapeutically effective amount of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized in part by unregulated cell growth. As used herein, the term "cancer" refers to non-metastatic and metastatic cancers, including early stage and late stage cancers. By "non-metastatic" is meant a cancer that remains at the primary site and has not penetrated into the lymphatic or blood vessel system or to tissues other than the primary site. The term "metastatic cancer" refers to cancer that has spread or is capable of spreading from one part of the body to another. Generally, a non-metastatic cancer is any cancer that is a Stage 0, I, or II cancer, and occasionally a Stage III cancer. A metastatic cancer, on the other hand, is usually a stage IV cancer.

The term "cancer" includes but is not limited to, breast cancer, large intestinal cancer, lung cancer, small cell lung cancer, gastric (stomach) cancer, liver cancer, blood cancer, bone cancer, pancreatic cancer, skin cancer, head and/or neck cancer, cutaneous or intraocular melanoma, uterine sarcoma, ovarian cancer, rectal or colorectal cancer, anal cancer, colon cancer, fallopian tube carcinoma, endometrial carcinoma, cervical cancer,

vulval cancer, squamous cell carcinoma, vaginal carcinoma, Hodgkin's disease, non-Hodgkin's lymphoma, esophageal cancer, small intestine cancer, endocrine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue tumor, urethral cancer, penile cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphoma, bladder cancer, kidney cancer, ureter cancer, renal cell carcinoma, renal pelvic carcinoma, CNS tumor, glioma, astrocytoma, glioblastoma multiforme, primary CNS lymphoma, bone marrow tumor, brain stem nerve gliomas, pituitary adenoma, uveal melanoma (also known as intraocular melanoma), testicular cancer, oral cancer, pharyngeal cancer or a combination thereof.

In one embodiment, the cancer cell is a solid or haematological cancer cell.

The term “haematological cancer” may refer to one or more of leukemia, lymphoma, Chronic Myeloproliferative Disorders, Langerhans Cell Histiocytosis, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplasia Syndromes, Myelodysplastic/Myeloproliferative Neoplasms or a combination thereof. In some embodiments, leukemia is any one or more of Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Hairy Cell Leukemia (HCL) or a combination thereof. In some embodiments, lymphoma is any one or more of AIDS-Related Lymphoma, Cutaneous T-Cell Lymphoma, Hodgkin Lymphoma, Mycosis Fungoides, Non-Hodgkin Lymphoma, Primary Central Nervous System Lymphoma, Sezary Syndrome, T-Cell Lymphoma, Cutaneous, Waldenstrom Macroglobulinemia, B cell lymphoma or a combination thereof.

In one embodiment, the cancer cell is a lymphoma cell. In one embodiment, the lymphoma cell is a B lymphoma cell.

In one embodiment, the subject is suffering from a lymphoma. The subject may be suffering from B cell lymphoma.

The term “solid cancer” may refer to one or more of breast cancer, large intestinal cancer, lung cancer, small cell lung cancer, gastric (stomach) cancer, liver cancer, bone cancer, pancreatic cancer, skin cancer, head and/or neck cancer, cutaneous or intraocular

melanoma, uterine sarcoma, ovarian cancer, rectal or colorectal cancer, anal cancer, colon cancer, fallopian tube carcinoma, endometrial carcinoma, cervical cancer, vulval cancer, squamous cell carcinoma, vaginal carcinoma, esophageal cancer, small intestine cancer, endocrine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue tumor, urethral cancer, penile cancer, prostate cancer, bladder cancer, kidney cancer, ureter cancer, renal cell carcinoma, renal pelvic carcinoma, CNS tumor, glioma, astrocytoma, glioblastoma multiforme, primary CNS lymphoma, bone marrow tumor, brain stem nerve gliomas, pituitary adenoma, uveal melanoma (also known as intraocular melanoma), testicular cancer, oral cancer, pharyngeal cancer, sarcomas or a combination thereof.

In one embodiment, the cancer is a metastatic cancer. The cancer may be a refractory or a relapsed cancer.

Disclosed herein is a method of reducing or inhibiting proliferation, survival and/or viability of a cancer cell in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein to the subject.

Disclosed herein is a method of treating a cancer in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein to the subject.

Disclosed herein is a method of treating a disease or condition associated with an undesired expression of IgM μ -chain antigen in a subject, wherein the method comprises administering a therapeutically effective amount of an antigen-binding molecule as defined herein, a chimeric molecule according as defined herein or a pharmaceutical composition as defined herein to the subject.

The term "treating" as used herein may refer to (1) delaying the appearance of one or more symptoms of the condition; (2) inhibiting the development of the condition or one or more symptoms of the condition; (3) relieving the condition, *i.e.*, causing regression

of the condition or at least one or more symptoms of the condition; and/or (4) causing a decrease in the severity of the condition or of one or more symptoms of the condition.

The terms "treating", "treatment" and the like, are used interchangeably herein to mean relieving, reducing, alleviating, ameliorating or otherwise inhibiting the condition, including one or more symptoms of the condition. The terms "prevent", "preventing", "prophylaxis", "prophylactic", "preventative" and the like are used interchangeably herein to mean preventing or delaying the onset of the condition, or the risk of developing the condition.

The terms "subject", "patient", "host" or "individual" used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates (*e.g.*, humans, monkeys and apes, and includes species of monkeys such as from the genus *Macaca* (*e.g.*, cynomolgus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets (species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (*e.g.*, mice rats, guinea pigs), lagomorphs (*e.g.*, rabbits, hares), bovinos (*e.g.*, cattle), ovines (*e.g.*, sheep), caprines (*e.g.*, goats), porcines (*e.g.*, pigs), equines (*e.g.*, horses), canines (*e.g.*, dogs), felines (*e.g.*, cats), avians (*e.g.*, chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars etc.), marine mammals (*e.g.*, dolphins, whales), reptiles (snakes, frogs, lizards etc.), and fish. In one embodiment, the subject is a canine subject. In another embodiment, the subject is a human subject.

The methods as disclosed herein may comprises the administration of a "therapeutically effective amount" of an agent (*e.g.* an antigen-binding molecule, a chimeric molecule, a polynucleotide, a construct, a vector, a host cell or a pharmaceutical composition) to a subject. As used herein the term "therapeutically effective amount" includes within its meaning a non-toxic but sufficient amount of an agent or compound to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of

the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact “effective amount”. However, for any given case, an appropriate “effective amount” may be determined by one of ordinary skill in the art using only routine experimentation.

In one embodiment, there is provided an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein for use in reducing or inhibiting proliferation, survival and/or viability of a cancer cell in a subject.

In one embodiment, there is provided the use of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein in the manufacture of a medicament for reducing or inhibiting proliferation, survival and/or viability of a cancer cell in a subject.

In one embodiment, there is provided an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein for use in treating a cancer in a subject.

In one embodiment, there is provided the use of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein in the manufacture of a medicament for treating a cancer in a subject.

In one embodiment, there is provided an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein for use in treating a disease or condition associated with an undesired expression of IgM μ -chain antigen in a subject.

In one embodiment, there is provided the use of an antigen-binding molecule as defined herein, a chimeric molecule as defined herein or a pharmaceutical composition as defined herein in the manufacture of a medicament for treating a disease or condition associated with an undesired expression of IgM μ -chain antigen in a subject.

Disclosed herein is a method of detecting the likelihood of the presence of a cancer in a subject, the method comprising determining the level of IgM μ -chain antigen in a sample obtained from the subject, wherein an increased level of IgM μ -chain antigen as compared to a reference indicates the likelihood of the presence of a cancer in the subject.

In one embodiment, the sample is a cell, tissue or blood sample.

In one embodiment, the method comprises contacting the sample with an antigen-binding molecule as defined herein or a chimeric molecule as defined herein to determine the level of IgM μ -chain antigen in the sample.

Throughout this specification and the statements which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

EXAMPLE 1

CL-c17A11 is a chimeric (canine constant region genes-mouse variable region genes) IgGb mAb developed to target B cell lymphoma. It was generated by immunizing B cell lymphoma cells, CLBL-1 to immunocompetent mice. A total of 1580 hybridoma clones were screened for specific binding to CLBL-1 but not normal dog cells (Table 1). The lead candidate clone CL-m17A11 (mouse IgG2b) was selected based on highly efficient cytotoxicity to CLBL-1 as an ADC and low reactivity to normal cells and tissues.

It binds to an antigen present on the cell surface of CLBL-1 and xenograft but not to normal canine cells (Figures 2 and 3). Several characterization assays were carried out to determine the identity of the antigen. CL-m17A11 binds predominantly to a protein band of molecular weight ~190 kDa (Figure 4A). Immunoprecipitation (IP) of antigen using Protein G capture of CL-m17A11 was done in duplicate and the samples were reproduced on a parallel SDS-PAGE gel and excised for mass spectrometry (Figure 4B). The identity of antigen was determined to be IgM μ -chain, protein accession number E2RE31 (Table 2). A polyclonal commercial antibody from Biorad, goat anti-dog IgM was used for validation. The cross-probing IP data validated that CL-m17A11 binds to an IgM-like molecule (Figure 5A and 5B) and does not compete and bind to the same epitope as the commercial mAb (Figure 6). From the glycan removal assays, the data suggested that CL-m17A11 binds to an O-linked glycoprotein epitope and the binding is sensitive to reducing conditions (Figure 8).

In cell-based assay, the presence of naked mAb was observed to slow CLBL-1 cells growth. When conjugated to MMAE, cytotoxicity was observed (Figure 10). In vivo animal models were set up to study both efficacy and dose escalation of CL-17A11-MMAE. For efficacy studies, immunocompromised mice were given treatment with CL-m17A11-MMAE upon inoculation with CLBL-1 cells in a chase model. Groups of mice were given a total of 3 treatments, once every 4 days, via intraperitoneal administration route. Mice treated with CL-m17A11-MMAE did not produce tumours

while control groups given buffer were terminated after tumour volume reached 1000mm³ or when ulceration was observed (Figures 11-12). High doses of naked antibody CL-m17A11 exhibited cytostatic activity and delayed the formation of tumour in mice (Figures 11-12).

CL-m17A11 was converted to a chimeric mAb by replacing the Fc region with a canine IgGb equivalent and the translated heavy and light chain protein sequence have been mapped (Figure 9) by DNA sequencing. There was no observed change in the specificity and function between the mouse and chimeric mAb (Figure 10).

In dose escalation experiments, the mice were inoculated with CLBL-1 cells to allow tumour formation prior to treatment. The tumour-bearing mice were randomized and 2 treatment doses of CL-c17A11-MMAE were investigated. Tumour regression was observed for mice treated with CL-c17A11-MMAE (Figures 13-14) for more than 30 days. CL-c17A11-MMAE has potential to be developed as an antibody-drug conjugate (ADC).

Table 1 Screening of clones by Flow Cytometry. A total of 1580 clones were screened and the final list of clones were selected for binding to cancer cells CLBL-1 but not to normal cells MDCK and cf2TH. The lead candidate 17A11 (in bold) was chosen for further characterization and development due to the high binding affinity to CLBL-1 cells.

+++ (>80%)	+++ (40-80%)	++ (20-39%)
Tier1	Tier2	Tier3
2D4	1A12	2F1
2G9	1C4	3C9
2G10	1E3	4A8
2H6	1H4	5E9
3A5	3G1	6C10
3H4	4D10	10C10
4E11	4E2	12E7
4G8	4G10	
4G12	6E1	
6G5	7C5	
7D5	7F7	
7D9	10C9	
7D11	12G7	
8H2	13B11	
9A5	14B8	
9F11		
9H10		
10B8		
12C3		
12E10		
13H7		
15B9		
16D11		
16F4		
15G5		
17A11		

Table 2. Mass spectrometry (MS) identified the protein that CL-m17A11 targeted is IgM μ -chain (protein accession number E2RE31) and was detected in both the gel samples. Band 1 is the predominant band and is likely a dimer of Band 2 ~80kDa band.

Band 1 (~190kDa)

Accession	Description	Score	MW [kDa]	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	calc. pI
E2RE31	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=2 - [E2RE31_CANLF]	108.66	53.8	25.00	2	12	12	38	492	5.29
L7N098	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=1 - [L7N098_CANLF]	54.11	14.7	16.15	2	2	2	16	130	5.24
L7N0P9	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=1 - [L7N0P9_CANLF]	22.86	13.6	33.33	1	3	3	11	126	7.99
F1PQL8	Uncharacterized protein OS=Canis lupus familiaris GN=ACTG1 PE=3 SV=2 - [F1PQL8_CANLF]	10.34	41.4	11.26	4	4	4	4	373	5.96
F2Z4Q6	Serum albumin OS=Canis lupus familiaris GN=ALB PE=3 SV=1 - [F2Z4Q6_CANLF]	9.34	68.6	3.29	2	2	2	4	608	5.69
E2RLS3	Uncharacterized protein OS=Canis lupus familiaris GN=HSP90AB1 PE=3 SV=2 - [E2RLS3_CANLF]	5.89	83.2	3.59	1	2	2	2	724	5.03

Band 2 (~80kDa)

Accession	Description	Score	MW [kDa]	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	calc. pI
E2RE31	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=2 - [E2RE31_CANLF]	87.30	53.8	23.37	2	11	11	28	492	5.29
L7N098	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=1 - [L7N098_CANLF]	37.04	14.7	16.15	2	2	2	8	130	5.24
F1PYU9	Keratin, type I cytoskeletal 10 OS=Canis lupus familiaris GN=KRT10 PE=3 SV=2 - [F1PYU9_CANLF]	31.71	57.7	15.85	2	10	10	10	568	5.15
F1PTY1	Keratin, type II cytoskeletal 1 OS=Canis lupus familiaris GN=KRT1 PE=3 SV=1 - [F1PTY1_CANLF]	26.03	63.7	10.82	2	6	7	8	619	7.84
L7N0P9	Uncharacterized protein OS=Canis lupus familiaris PE=4 SV=1 - [L7N0P9_CANLF]	15.35	13.6	19.05	1	3	3	10	126	7.99
Q6EIZ1	Keratin, type II cytoskeletal 2 epidermal OS=Canis lupus familiaris	14.20	64.5	6.48	2	3	5	5	633	7.74

	GN=KRT2 PE=2 SV=1 - [K22E_CANLF]									
F1PTS8	Uncharacterized protein OS=Canis lupus familiaris GN=LOC486523 PE=3 SV=1 - [F1PTS8_CANLF]	12.53	60.6	5.08	2	2	3	4	571	8.16
A0A077KFB1	Heat shock protein 90 kDa beta member 1 (Fragment) OS=Canis lupus familiaris GN=HSP90 beta PE=2 SV=1 - [A0A077KFB1_CANLF]	7.14	76.1	3.92	3	2	2	2	663	5.10
F1PQL8	Uncharacterized protein OS=Canis lupus familiaris GN=AC1G1 PE=3 SV=2 - [F1PQL8_CANLF]	4.83	41.4	5.63	7	2	2	2	373	5.96

Table 3. *In vivo* tumour-chase animal model using CB-17 SCIDs mice. Groups of CB-17 SCIDs mice were inoculated subcutaneously with 2×10^6 CLBL-1 cells on the day of first dose administration. Each group of 5 mice were given a total of 3 doses, 1 dose every 4 days via intraperitoneal route. Mice were culled when tumour volume exceeds 1000 mm^3 or ulceration occurs.

Group	Description
1	PBS buffer (control)
2	m11A12 (isotype control)
3	m11A12*MMAE (isotype control)
4	m17A11 (L) 1.875mg/kg
5	m17A11 (H) 7.5 mg/kg
6	m17A11*MMAE 1.875mg/kg

Table 4. *In vivo* animal model using CB-17 SCIDs mice with established tumours ($\sim 100 \text{ mm}^3$). Groups of mice were inoculated subcutaneously with 2×10^6 CLBL-1 cells. Each group of 5 mice were given a total of 3 doses, 1 dose per week (1Q3W) via intravenous route. Mice were culled when tumour volume exceeded 1000 mm^3 or when ulceration occurred.

Group	Description
1	PBS buffer
2	MMAE
3	c17A11*MMAE (8mg/kg)
4	c17A11*MMAE (15mg/kg)
5	c17A11 (15mg/kg)

CLAIMS

1. An antigen-binding molecule that specifically binds to IgM μ -chain antigen.
2. The antigen-binding molecule of claim 1, wherein the IgM μ -chain antigen is a canine IgM μ -chain antigen.
3. The antigen-binding molecule of claim 1 or 2, wherein the antigen-binding molecule comprises:
 - a) a heavy chain variable (VH) region comprising the VHCDR1 amino acid sequence K A S G Y S F T D Y N I Y (SEQ ID NO: 1), the VHCDR2 amino acid sequence F F D P H N G N T T Y N Q K F K (SEQ ID NO: 2) and the VHCDR3 amino acid sequence E G Y L Y A M D Y (SEQ ID NO: 3); and
 - b) a light chain variable (VL) region comprising the VLCDR1 amino acid sequence R S S Q S I E H S N G N T Y L E (SEQ ID: 4), the VLCDR2 amino acid sequence F K V S N R F S (SEQ ID NO: 5) and the VLCDR3 amino acid sequence F Q G S H V P P T (SEQ ID NO: 6).
4. The antigen-binding molecule of any one of claims 1 to 3, wherein the antigen-binding molecule comprises:
 - a) a VH region comprising an amino acid sequence having at least 70% sequence identity to:

Q I Q L Q Q S G P E L V K P G A S V K V S C K A S G Y S F T D Y N I Y
W V K Q S H G K S L E W I G F F D P H N G N T T Y N Q K F K G K A T
L T V D K S S T A F M H L N S L T S E D S A V Y Y C A R E G Y L Y A
M D Y W G Q G T S V T V S S (SEQ ID NO: 7); and
 - b) a VL region comprising an amino acid sequence having at least 70% sequence identity to:

D V L M T Q T P L S L P V S L G D Q A S I S C R S S Q S I E H S N G N T
Y L E W Y L Q K P G Q A P K L L I F K V S N R F S G V P D R F S G S G S
G T D F T L K I S R M E A E D L G V Y Y C F Q G S H V P P T F G G G T
K L E I K R A D A A P T V S (SEQ ID NO: 8).

5. The antigen-binding molecule of any one of claims 1 to 4, wherein the antigen-binding fragment is an antibody or antigen-binding fragment thereof.
6. The antigen-binding molecule of claim 5, wherein the antibody or antigen-binding molecule therefore is caninized or chimerized.
7. The antigen-binding molecule of claim 5 or 6, wherein the antibody or antigen binding fragment thereof is a full-length antibody, a substantially intact antibody, a Fab fragment, a scFab, a Fab', a single chain variable fragment (scFv) or a one-armed antibody.
8. The antigen-binding molecule of claim 7, wherein the antibody or antigen binding fragment thereof is a full-length antibody and comprises a canine constant region.
9. A chimeric molecule comprising an antigen-binding molecule according to any one of the preceding claims and a heterologous moiety.
10. The chimeric molecule of claim 9, wherein the heterologous moiety is a detectable moiety, a half-life extending moiety or a therapeutic moiety.
11. The chimeric molecule of claim 10, wherein the therapeutic moiety is a toxin.
12. The chimeric molecule of claim 11, wherein the toxin is auristatin, saporin, Mertansine (DM1).
13. The chimeric molecule of claim 12, wherein the auristatin is Monomethyl auristatin E (MMAE).
14. An isolated polynucleotide comprising a nucleic acid sequence encoding the antigen-binding molecule according to any one of claims 1 to 8, or the chimeric molecule of any one of claims 9 to 13.

15. A construct comprising a polynucleotide of claim 14 in operable connection with one or more control sequences.
16. A host cell that contains the construct of claim 15.
17. A pharmaceutical composition comprising an antigen-binding molecule according to any one of claims 1 to 8 or a chimeric molecule according to any one of claims 9 to 13.
18. An antigen-binding molecule according to any one of claims 1 to 8, a chimeric molecule according to any one of claims 9 to 13 or a pharmaceutical composition according to claim 17 for use as a medicament.
19. A method for reducing or inhibiting proliferation and/or viability of a cancer cell, the method comprising contacting the cancer cell with a therapeutically effective amount of an antigen-binding molecule according to any one of claims 1 to 8, a chimeric molecule according to any one of claims 9 to 13 or a pharmaceutical composition according to claim 17.
20. The method of claim 19, wherein the cancer cell is a lymphoma cell.
21. The method of claim 20, wherein the lymphoma cell is a B lymphoma cell.
22. A method of reducing or inhibiting proliferation, survival and/or viability of a cancer cell in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule according to any one of claims 1 to 8, a chimeric molecule according to any one of claims 9 to 13 or a pharmaceutical composition according to claim 17 to the subject.
23. A method of treating a cancer in a subject, the method comprising administering a therapeutically effective amount of an antigen-binding molecule according to any one of claims 1 to 8, a chimeric molecule according to any one of claims 9 to 13 or a pharmaceutical composition according to claim 17 to the subject.

24. A method of treating a disease or condition associated with an undesired expression of IgM μ -chain antigen in a subject, wherein the method comprises administering a therapeutically effective amount of an antigen-binding molecule according to any one of claims 1 to 8, a chimeric molecule according to any one of claims 9 to 13 or a pharmaceutical composition according to claim 17 to the subject.
25. A method of detecting the likelihood of the presence of a cancer in a subject, the method comprising determining the level of IgM μ -chain antigen in a sample obtained from the subject, wherein an increased level of IgM μ -chain antigen as compared to a reference indicates the likelihood of the presence of a cancer in the subject.
26. The method of claim 25, wherein the sample is a cell, tissue or blood sample.
27. The method of claim 25 or 26, wherein the method comprises contacting the sample with an antigen-binding molecule according to any one of claims 1 to 8 or a chimeric molecule according to any one of claims 9 to 13 to determine the level of IgM μ -chain antigen in the sample.