

Anti-Nectin-4 Antibody Exatecan conjugates

DESCRIPTION

5 The present invention relates to an antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof wherein the monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-4 and wherein the drug is a topoisomerase I inhibitor, particularly exatecan or deruxtecan.

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BACKGROUND OF THE INVENTION

Nectins are adhesion molecules that help organize epithelial and endothelial junctions and serve as receptors for the entry of the herpes simplex virus, measles virus and poliovirus.

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They belong to the immunoglobulin superfamily and are homologs of the poliovirus receptor (PVR / CD155), and for this, have also been called poliovirus receptor bound proteins (PRR). To date, 5 members have been described PVR / CD155, Nectin-1 / PRR1 / CD111, Nectin-2 / PRR2 / CD112, Nectin-3 / PRR3 and Nectin-4 / PRR4. Their ectodomain is made up of three immunoglobulin-like (Ig) -type V, C, C domains that share between 30 and 55% identity in their amino acid sequences.

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Expression of Nectin / PRR molecules is generally extensive in tissues, including hematopoietic, neuronal, endothelial and epithelial cells, with the exception of Nectin-3 and -4, which exhibit more restricted expression profiles.

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Nectin-4 is a particularly interesting target. It is expressed during fetal development, but its expression decreases and is very restricted in adult tissues in comparison to that of other members of the Nectin family. Nectin-4 is a tumor associated antigen in 83 % of bladder cancers, 78 % of breast

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cancers (mainly triple negative and ERBB2+), 71 % of pancreatic cancers, 55 % of lung cancers, 57% of ovarian cancers, 59% of head and neck cancers, and 55% of esophageal cancers.

5 Expression of Nectin-4 in these pathologies is associated with poor prognosis, probably as a consequence of the ability of Nectin-4 to confer to tumor cells *in vitro*, higher capacities of migration, proliferation and formation of metastases. In normal tissues, Nectin-4 is only detected in skin, salivary glands, urinary bladder and esophagus. The recent approval by health authorities of
10 Enfortumab vedotin for the 2nd line treatment of advanced urothelial cancer has completed the validation of Nectin-4 as a target for the treatment of cancer.

SUMMARY OF THE INVENTION

15 A first aspect of the present invention is an antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof wherein the monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-4 and wherein the drug is a topoisomerase I inhibitor, particularly exatecan or deruxtecan.

20 A further aspect of the present invention is a pharmaceutical composition comprising an active agent, which is an antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof wherein the monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-
25 4 and wherein the drug is a topoisomerase I inhibitor, particularly exatecan, and a pharmaceutically acceptable carrier and/or excipient.

A further aspect of the present invention is the use of an antibody-drug conjugate or a pharmaceutical composition as described above in medicine,
30 particularly in human medicine.

A further aspect of the present invention the use of an antibody-drug conjugate or a pharmaceutical composition as described above in a method for the prevention and/or treatment of a Nectin-4 associated disorder, particularly for the prevention and/or treatment of a Nectin-4 positive cancer.

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A further aspect of the present invention the use of an antibody-drug conjugate or a pharmaceutical composition as described above in a method for the prevention and/or treatment of cancer, particularly for the prevention and/or treatment of a cancer selected from bladder, urothelial, endometrial, cervical, colorectal, liver, thyroid, breast, pancreatic, lung, ovarian head and neck and/or esophagus cancer.

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A further aspect of the present invention is a method for the prevention and/or treatment of a Nectin-4 associated disorder, particularly for the prevention and/or treatment of a Nectin-4 positive cancer comprising administering to a subject in need thereof a therapeutically effective amount of an antibody-drug conjugate or a pharmaceutical composition as described above.

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A further aspect of the present invention is a method for the prevention and/or treatment of cancer, particularly for the prevention and/or treatment of a cancer selected from bladder, urothelial, endometrial, cervical, colorectal, liver, thyroid, breast, pancreatic, lung, ovarian head and neck and/or esophagus cancer comprising administering to a subject in need thereof a therapeutically effective amount of an antibody-drug conjugate or a pharmaceutical composition as described above.

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DETAILED DESCRIPTION OF THE INVENTION

The present inventors have found that conjugates of anti-Nectin-4 antibodies with topoisomerase-I-inhibitors such as exatecan demonstrate improved anti-cancer activity compared to the marketed antibody Enfortumab vedotin, which is a conjugate of an anti-Nectin-4 antibody and monomethyl auristatin E

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(MMAE). Accordingly, the anti-Nectin-4 antibody conjugates of the invention allow an improved treatment of Nectin-4 associated disorders such as Nectin-4 positive cancer.

5 In particular embodiments, the antibody-drug conjugate of the present invention comprises a novel anti-Nectin-4 antibody, which specifically binds to Nectin-4 expressed by tumors with a higher affinity in comparison to Nectin-4 expressed by human keratinocytes, e.g., in vitro differentiated Nectin-4 expressing keratinocytes. Preferred examples are the monoclonal antibodies
10 (mAb) 15A7.5, 9A2.7, 3A1.4 and/or 8F06.

An in particular preferred example is monoclonal antibody (mAb) 15A7.5 or an antibody derived therefrom. *In vitro*, this selectivity provides mAb 15A7.5 with lower binding affinity, internalization and/or cytotoxic activity towards
15 keratinocytes in comparison to tumor cells and in reference to the activity of the HA22 mAb (Enfortumab), in the same assays.

In particular, the conjugate of the present invention comprises a humanized antibody that derives from the monoclonal anti-Nectin-4 antibody 15A7.5 mAb.
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In vivo, this low binding capacity to keratinocytes endows mAb 15A7.5 with a higher half-life due to a lower absorption rate in the skin.

In a xenograft mouse model, treatment with a single dose I.V. of 4 mg/kg of mAb conjugated with exatecan of established tumors led to a rapid and long-lasting regression.
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More particularly, the inventors demonstrate that, in such configuration, an antibody-exatecan conjugate is more efficient than a surrogate of Enfortumab vedotin, i.e., an Enfortumab vedotin product manufactured using non-GMP methods by a third party that is indistinguishable from the commercially available Enfortumab vedotin.
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ANTIBODIES

5 The antibodies of conjugates of the invention are monoclonal antibodies (mAb) or monoclonal antibody fragments characterized by a specific amino acid sequence. If not indicated differently, the term "monoclonal" refers to a single species, i.e., single amino acid composition of antibodies or antibody fragments.

10 The antibodies provided herein show preferably specific binding to Nectin-4 and no essential or no cross-reactivity to other proteins of the human Nectin-family, in particular Nectin-1, and/or rodent Nectin-4, such as Nectin-4 from rat and/or mouse.

15 The antigen-binding site of an inventive antibody comprises heavy chain variable domains/regions (VH) and/or antibody light chain variable domains/regions (VL), or pairs of VH/VL.

20 The term "antigen-binding site" denotes the region(s) of an antibody molecule to which a ligand (e.g., the antigen, i.e., Nectin-4, or antigen fragment of it) actually binds and which is derived from an antibody.

25 The variable domains/region denote each of the pair of light and heavy chains, which is involved directly in binding the antibody to the antigen. The variable domain of a heavy chain is abbreviated as "VH" and the variable domain of a light chain is abbreviated as "VL".

30 An antigen-binding site of an antibody according to the invention can contain six complementarity determining regions (CDRs) which contribute in varying degrees to the affinity of the binding site for the antigen. There are three heavy chain variable domain CDRs (CDR-H1, CDR-H2 and CDR-H3) and three light chain variable domain CDRs (CDR-L1, CDR-L2 and CDR-L3). Also included

within the scope of the invention are functional antigen binding sites comprised of fewer CDRs (i.e., where binding specificity is determined by three, four or five CDRs). For example, less than a complete set of 6 CDRs may be sufficient for binding. In some cases, a VH or a VL domain will be sufficient.

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According to the present invention, a VH region or the CDRs thereof alone may constitute a complete antigen-binding site. In certain embodiments, the antibody comprises a VH region or the CDRs thereof as defined herein alone. In other embodiments, the antibody comprises a VH region or the CDRs thereof as defined herein together with a VL region or the CDRs thereof, particularly with a VL region or the CDRs thereof as defined herein.

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The position of CDRs within a VH or VL region may be defined according to Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991) or the IMGT numbering system, both of which are known to the person skilled in the art. The IMGT numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species (Lefranc M.-P., "Unique database numbering system for immunogenetic analysis" Immunology Today, 18, 509 (1997); Lefranc M.-P., "The IMGT unique numbering for Immunoglobulins, T cell receptors and Ig-like domains" The Immunologist, 7, 132-136 (1999)). If not stated otherwise, the Kabat system is used herein.

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As used herein, the terms "binding" and "specific binding" refer to the binding of the inventive antibody or fragment thereof to an epitope of the Nectin-4 antigen. The measure of the binding strength of an antibody is referred to as affinity. Methods for determining such a binding and/or affinity using in vitro assays are known to the person skilled in the art. According to the present invention, detection with flow cytometry, immuno-histochemistry and/or fluorescence are described and particularly preferred herein.

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The affinity of the binding of an antibody to an antigen is defined by the terms K_a (rate constant for the association of the antibody from the antibody/antigen complex), K_D (dissociation constant), and K_{dis} (K_D/K_a).

5 In certain embodiments, antibody of the conjugate of the invention may be a chimeric antibody, a multispecific antibody, in particular a bispecific antibody, a human antibody, a humanized antibody, or an antigen-binding fragment thereof.

10 According to the present invention, a "chimeric" antibody 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.

15 "Multispecific antibodies" bind two or more different epitopes. The epitopes may be on the same or different antigens. A preferred example of a multispecific antibody is a "bispecific antibody" which binds two different epitopes.

20 In preferred embodiments, the antibody of the invention is a humanized antibody.

The term "humanized antibody" or "humanized version of an antibody" refers to antibodies for which both heavy and light chains are humanized as a result of antibody engineering. A humanized chain is typically a chain in which the V-region amino acid sequence has been changed so that, analyzed as a whole, is closer in homology to a human germline sequence than to the germline sequence of the species of origin. For example, a murine CDR may be grafted into the framework region of a human antibody to prepare the "humanized antibody." See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M. S., et al., Nature 314 (1985) 268-270. Other forms of humanized antibodies encompassed by the present invention are those in which the

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constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention. Humanization assessment is based on the resulting amino acid sequence and not on the methodology per se.

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Another preferred embodiment refers to conjugates comprising human antibodies.

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The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M. A., and van de Winkel, J. G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production.

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The antibody of the conjugate of the present invention may be of any suitable class. The term "class" refers to the type of constant domain or constant region possessed by its heavy chain. As used herein, "constant domain" or "constant region" 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 effector functions. The antibody may be of any of the five major classes of antibodies, particularly of the five major classes of human antibodies: IgA, IgD, IgE, IgG, and IgM, or any subclass thereof (isotype), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ and μ , respectively. According to the present invention, an antibody of the class, particularly of the human class IgG, IgA or IgM or a fragment thereof is particularly suitable.

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According to a preferred embodiment, an antibody of the conjugate of the invention is selected from class IgG, particularly from the class of human IgG, e.g., of subclass IgG1, IgG2, IgG3 or IgG4, of class IgM, of class IgA or an antigen-binding fragment thereof.

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In certain embodiments, the antibody comprises a constant domain, particularly a heavy chain constant domain, more particularly a heavy chain constant domain of the class IgG, particularly of the class human IgG, e.g., of subclass IgG1, IgG2, IgG3 or IgG4, of class IgM, of class IgA, which has a reduced effector function compared to a wild-type sequence of the same subclass, e.g., which has a reduced binding to the Fc receptor. Examples of heavy chain constant domains with reduced effector functions may contain at least one of the mutations D265C, L234A, L235A, P331S, L234Q and L235F of human IgG, e.g., IgG1 or IgG4 sequences.

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An "antigen-binding fragment" of an antibody refers to a molecule comprising a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multi-specific antibodies formed from antibody fragments. The term also encompasses a fusion protein, e.g., a fusion protein with a non-immunoglobulin peptide or polypeptide, and a conjugate with a non-proteinaceous structure, e.g., a label or a toxin. The terms "antigen-binding fragment of an antibody (thereof)" and "fragment of an antibody (thereof)" may be used interchangeably herein.

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The antibody or the antigen-binding fragment may be mono- or multivalent, i.e., it may comprise a single antigen-binding site or multiple antigen-binding sites. For example, Fab fragments have single antigen-binding site, antibodies of the IgG class or Fv or scFv fragments have two antigen-binding sites and antibodies of the IgM class have 5 antigen-binding sites. The term "antibody" also encompasses hetero-specific antibodies, e.g., hetero-bispecific

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antibodies, which have different antigen-binding sites, particularly antibodies, which are directed to two different epitopes on the antigen. As used herein, "epitope" is a region of an antigen that is bound by an antibody. The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody.

TUMOR-SELECTIVE ANTI-NECTIN-4 ANTIBODIES

In a particular embodiment, the antibody of the conjugate is a tumor-selective anti-Nectin-4 antibody, which specifically binds to Nectin-4 expressed by tumors with a higher affinity in comparison to Nectin-4 expressed by human keratinocytes, e.g., in vitro differentiated Nectin-4 expressing keratinocytes, e.g., a monoclonal antibody derived from the monoclonal antibody (mAb) 15A7.5, 9A2.7, 3A1.4 and/or 8F06. The mAb 15A7.5 is in particular preferred

For comparison, the binding affinity may be detected by flow cytometry, immuno-histochemistry and/or fluorescence. Such specific binding allows, inter alia, less side effects during a treatment based on the inventive antibodies.

Accordingly, an antibody-drug conjugate comprising antibody 15A7.5, 9A2.7, 3A1.4 and/or 8F06 and in particular a humanized variant derived therefrom, represents a new way to improve therapeutic index of Nectin-4 positive cancer treatment through lower associated skin toxicity and higher anti-tumor selectivity and efficacy.

Tumor-selective anti-Nectin-4 antibodies and antigen-binding fragments thereof preferably show a dissociation constant K_D of at least 40, preferably at least 45, more preferably at least 50 and most preferably at least 55 (nM).

Beside a lower binding affinity, the tumor-selective antibodies and fragments provided herein show also preferably lower internalization and/or cytotoxic activity towards keratinocytes in comparison to tumor cells.

5 According to an especially preferred embodiment, the tumor-selective antibodies and fragments show lower binding affinity, lower internalization, and lower cytotoxic activity towards keratinocytes in comparison to tumor cells.

Of course, a specific binding to Nectin-4 expressed by tumors is preferred.

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According to a further aspect, the present invention also relates to an antibody-drug conjugate comprising a monoclonal antibody or antigen-binding fragment thereof characterized by binding to Nectin-4 expressed by tumors with a higher affinity in comparison to Nectin-4 expressed by human keratinocytes. For
15 comparison, the binding affinity may be detected by flow cytometry, immuno-histochemistry and/or fluorescence.

In certain embodiments, the tumor-selective monoclonal ant-Nectin-4 antibody or antigen-binding fragment thereof comprises

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(a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein

(i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO: 1, 7, 88, 96, or 104,
25 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

(ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO: 2, 8, 89, 97 or 105,
30 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion at least one amino acid, e.g., 1 or 2 amino acids,

(iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO: 3,9, 90, 98 or 106,

or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids, and optionally

(b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein

(i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO: 4,10,92, 100 or 108,

or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

(ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO: 5,11, 93, 101 or 109,

or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

(iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO: 6,12, 94, 102 or 110,

or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids.

SEQ ID NO: 1-6 define the six CDR sequences of the inventive parental antibody 15A7.5 according to the IMGT numbering system. SEQ ID NO: 7-12 define the six CDR sequences of the inventive parental antibody according to Kabat.

Specific amino acid sequences for the SEQ ID NO: used herein are provided in the attached sequence listing.

SEQ ID NO:s 88-95 characterize mAb 9A2.7.

SEQ ID NO:s 96-103 characterize mAb 3A1.4.

SEQ ID NO:s 104-111 characterize mAb 8F06.

5 According to the present invention, a substitution or insertion of at least one amino acid, e.g., 1 or 2 amino acids in these CDR sequences is possible. In particular embodiments, a conservative amino acid substitution is preferable, i.e. a substitution of an amino acid by another amino acid with similar biochemical properties, for example a substitution of an aliphatic amino acid,
10 e.g., Gly, Ala, Val, Leu or Ile, for another aliphatic amino acid, a basic amino acid, e.g., His, Lys or Arg, against another basic amino acid, an acidic amino acid or an amide thereof, e.g., Asp, Glu, Asn or Gln, against another acidic amino acid or an amide thereof, an aromatic amino acid, e.g., Phe, Tyr or Trp, against another aromatic amino acid, or a hydroxy or sulfur containing amino
15 acid, e.g., Ser, Thr, Met or Cys, against another hydroxy or sulfur containing amino acid. In further particular embodiments, at least one amino acid, e.g., 1 or 2 histidine residues are inserted into one or more CDR sequences, e.g., CDR1, CDR2 or CDR3 of the heavy chain or the light chain. This might result in a preferential binding at a low pH, e.g., pH between 5.5 to 6.5 versus a
20 neutral pH, e.g., pH 7.0 to 7.5.

According to a further preferred embodiment, the antibody or antigen-binding fragment thereof comprises according to the IMGT numbering system

- 25 (i) a VH region comprising the CDR-H1 of SEQ ID NO:1, the CDR-H2 of SEQ ID NO:2, and the CDR-H3 of SEQ ID NO:3, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:4, the CDR-L2 of the SEQ ID NO:5 and the CDR-L3 of the SEQ ID NO:6.

30 According to another preferred embodiment, the antibody or antigen-binding fragment thereof comprises according to Kabat

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- (i) a VH region comprising the CDR-H1 of SEQ ID NO:7, the CDR-H2 of SEQ ID NO:8, and the CDR-H3 of SEQ ID NO:9, and optionally
 - (ii) a VL region comprising the CDR-L1 of SEQ ID NO:10, the CDR-L2 of the SEQ ID NO:11 and the CDR-L3 of the SEQ ID NO:12.

According to another preferred embodiment, the antibody or antigen-binding fragment thereof comprises

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- (i) a VH region comprising the CDR-H1 of SEQ ID NO:88, the CDR- of SEQ ID NO:89, and the CDR-H3 of SEQ ID NO:90, and optionally
 - (ii) a VL region comprising the CDR-L1 of SEQ ID NO:92, the CDR-L2 of the SEQ ID NO:93 and the CDR-L3 of the SEQ ID NO:94.

15 According to another preferred embodiment, the antibody or antigen-binding fragment thereof comprises

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- (i) a VH region comprising the CDR-H1 of SEQ ID NO:96, the CDR- of SEQ ID NO:97, and the CDR-H3 of SEQ ID NO:98, and optionally
 - (ii) a VL region comprising the CDR-L1 of SEQ ID NO:100, the CDR-L2 of the SEQ ID NO:101 and the CDR-L3 of the SEQ ID NO:102.

According to another preferred embodiment, the antibody or antigen-binding fragment thereof comprises

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- (i) a VH region comprising the CDR-H1 of SEQ ID NO:104, the CDR-H2 of SEQ ID NO:105, and the CDR-H3 of SEQ ID NO:106, and optionally
 - (ii) a VL region comprising the CDR-L1 of SEQ ID NO:108, the CDR-L2 of the SEQ ID NO:109 and the CDR-L3 of the SEQ ID NO:110.

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More particularly, the antibody or antigen-binding fragment thereof may comprise

- 5 (a) a VH region comprising an amino acid sequence according to SEQ ID NO:13, 91, 99 or 107 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence, and optionally
- 10 (b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:14, 95, 103 or 111 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

According to a preferred embodiment, the antibody or antigen-binding fragment thereof may comprise

- 15 (a) a VH region comprising an amino acid sequence according to SEQ ID NO:13 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence, and optionally
- 20 (b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:14 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

According to a preferred embodiment, the antibody or antigen-binding fragment thereof may comprise

- 25 (a) a VH region comprising an amino acid sequence according to SEQ ID NO:91 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence, and optionally
- 30 (b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:95 or an amino sequence

having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

5 According to a preferred embodiment, the antibody or antigen-binding fragment thereof may comprise

- (a) a VH region comprising an amino acid sequence according to SEQ ID NO:99 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence,
10 and optionally
- (b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:103 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

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According to a preferred embodiment, the antibody or antigen-binding fragment thereof may comprise

- (a) a VH region comprising an amino acid sequence according to SEQ ID NO:107 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence,
20 and optionally
- (b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:111 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

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"Percent (%) amino acid sequence identity" with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in
30 a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence

identity, Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST.

- 5 In particular embodiments, the inventive humanized or human antibodies are defined by combination of at least 3, preferably 6, complementarity-determining regions (CDRs), i.e., relate to antibodies or antigen-binding fragments thereof comprising
- 10 (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein
- (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO:21, 35, 49 or 63,
- 15 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO:22, 36, 50 or 64
- 20 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO:23, 37, 51 or 65,
- 25 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids, and optionally
- (b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein
- 30 (i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO:24, 38, 52 or 66,

- or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 5 (ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO:25, 39, 53 or 67,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 10 (iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO:26, 40, 54 or 68,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids.
- 15 Specific humanized or human antibodies according to the present invention may comprise
- (a) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23, and optionally
- 20 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26, or
- (b) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37, and optionally
- 25 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40, or
- (c) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51, and optionally
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- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54,
or
- 5 (d) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68,
or
- 10 (e) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40,
or
- 15 (f) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:5, and the CDR-L3 of the SEQ ID NO:54,
or
- 20 (g) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68,
or
- 25 (h) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37,
and optionally
- 30 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26,

- or
- (j) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37, and optionally
- 5 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54, or
- (k) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37, and optionally
- 10 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68, or
- (l) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51, and optionally
- 15 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26, or
- (m) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51, and optionally
- 20 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40, or
- 25 (n) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68, or
- 30 (o) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-

- H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26,
or
- 5 (p) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40,
or
- 10 (q) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54.
- 15

In particular preferred are the humanized antibodies (a), (b), (c), (d), (j), (k), (n) and (q) as defined above.

20

In particular embodiments, the inventive humanized or human antibodies are defined by combination of at least 3, preferably 6, complementarity-determining regions (CDRs), i.e., relate to antibodies or antigen-binding fragments thereof comprising

- 25 (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein
- (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO:21, 35, 49 or 63,
- 30 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

- (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO:22, 36, 50 or 64
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO:23, 37, 51 or 65,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
and optionally
- (b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein
- (i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO:24, 38, 52 or 66,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO:25, 39, 53 or 67,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO:26, 40, 54 or 68,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids

Specific humanized or human antibodies according to the present invention may comprise

- 5 (a) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26, or
- 10 (b) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40, or
- 15 (c) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54, or
- 20 (d) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68, or
- 25 (e) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23, and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40, or
- 30 (f) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-

- H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54,
or
- 5 (g) (i) a VH region comprising the CDR-H1 of SEQ ID NO:21, the CDR-H2 of SEQ ID NO:22, and the CDR-H3 of SEQ ID NO:23,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68,
or
- 10 (h) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26,
or
- 15 (j) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54,
or
- 20 (k) (i) a VH region comprising the CDR-H1 of SEQ ID NO:35, the CDR-H2 of SEQ ID NO:36, and the CDR-H3 of SEQ ID NO:37,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68,
or
- 25 (l) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51,
and optionally
- 30

- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26,
or
- 5 (m) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40,
or
- 10 (n) (i) a VH region comprising the CDR-H1 of SEQ ID NO:49, the CDR-H2 of SEQ ID NO:50, and the CDR-H3 of SEQ ID NO:51,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:66, the CDR-L2 of the SEQ ID NO:67, and the CDR-L3 of the SEQ ID NO:68,
or
- 15 (o) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- (ii) a VL region comprising the CDR-L1 of SEQ ID NO:24, the CDR-L2 of the SEQ ID NO:25, and the CDR-L3 of the SEQ ID NO:26,
or
- 20 (p) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- 25 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:38, the CDR-L2 of the SEQ ID NO:39, and the CDR-L3 of the SEQ ID NO:40,
or
- (q) (i) a VH region comprising the CDR-H1 of SEQ ID NO:63, the CDR-H2 of SEQ ID NO:64, and the CDR-H3 of SEQ ID NO:65,
and optionally
- 30 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:52, the CDR-L2 of the SEQ ID NO:53, and the CDR-L3 of the SEQ ID NO:54.

Particularly preferred are the humanized antibodies (a), (b), (c), (d), (j), (k), (n) and (q) as defined above.

5 Of course, the humanized or human antibodies according to the invention may be also defined by their VH and/or VL regions.

Such antibodies may comprise

- 10 (i) a VH region comprising an amino acid sequence selected from SEQ ID NO:27, 41, 55 or 69 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
- (ii) a VL region, particularly a VL region comprising an amino acid
15 sequence selected from SEQ ID NO:28, 42, 56, 70 or 71 or an amino
sequence having an identity thereof of at least 85%, at least 90%, at
least 95% or at least 99%.

Specific humanized or human antibodies according to the invention may be defined by their VH and/or VL regions, wherein such antibodies comprise

- 20 (a) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:27 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
- (ii) a VL region, particularly a VL region comprising an amino acid
25 sequence according to SEQ ID NO:28 or an amino sequence having an
identity thereof of at least 85%, at least 90%, at least 95% or at least
99%,
or
- (b) (i) a VH region comprising an amino acid sequence according to
30 SEQ ID NO:41 or an amino acid sequence having an identity thereof of
at least 85%, at least 90%, at least 95% or at least 99%,
and optionally

- (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:42 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
5 or
- (c) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:55 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
10 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:56 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 15 (d) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:69 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
20 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:70 or 71 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 25 (e) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:27 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
30 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:42 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or

- (f) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:27 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 5 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:56 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 10 (g) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:27 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 15 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:70 or 71 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 20 (h) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:41 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 25 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:28 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 30 (j) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:41 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally

- (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:56 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
5 or
- (k) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:41 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
10 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:70 or 71 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 15 (l) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:55 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
20 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:28 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 25 (m) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:55 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
and optionally
30 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:42 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or

- (n) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:55 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 5 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:70 or 71 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 10 (o) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:69 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 15 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:28 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 20 (p) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:69 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- 25 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:42 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
or
- 30 (q) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:69 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally

- (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:56 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%.

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In particular preferred are the humanized antibodies (a), (b), (c), (d), (j), (k), (n) and (q) as defined above.

FURTHER ANTI-NECTIN-4 ANTIBODIES

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In a further embodiment, the antibody of the conjugate of the invention is the antibody 5A12.2 or an antigen-binding fragment thereof.

SEQ ID NO:s 80-87 characterize mAb 5A12.2.

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According to a preferred embodiment, the 5A12.2 antibody or antigen-binding fragment thereof comprises

- (i) a VH region comprising the CDR-H1 of SEQ ID NO:80, the CDR-
of SEQ ID NO:81, and the CDR-H3 of SEQ ID NO:82,
and optionally
(ii) a VL region comprising the CDR-L1 of SEQ ID NO:84, the CDR-
L2 of the SEQ ID NO:85 and the CDR-L3 of the SEQ ID NO:86.

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According to another preferred embodiment, the 5A12.2 antibody or antigen-binding fragment thereof may comprise

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- (a) a VH region comprising an amino acid sequence according to
SEQ ID NO:83 or an amino acid sequence having an identity
thereof of at least 85%, at least 90%, at least 95% or at least 99%
over the whole length of the sequence,
and optionally
(b) a VL region, particularly a VL region comprising an amino acid
sequence according to SEQ ID NO:87 or an amino sequence

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having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the sequence.

5 In a further embodiment, the antibody of the conjugate of the invention is the antibody HA22, which is the antibody of the marketed antibody-drug conjugate Enfortumab vedotin, or a HA22-derived antibody.

10 HA22 is a human monoclonal anti-Nectin-4 antibody described in WO2012/047724, the content of which is herein incorporated by reference. A HA22 antibody or a HA22-derived antibody particularly refers to an IgG1 antibody comprising a heavy chain G1m17 or G1m3 haplotype sequence optionally with a constant region having a reduced effector function as described above, e.g., the TM mutation: P331S, L234Q and L235F, or the LALA mutation: L234A and L235A and a kappa light chain sequence. In particular, the HA22 VH sequence and its human IgG1 constant region is shown in Fig. 3A, and the HA22 VL sequence and its human kappa constant region is shown in Fig. 3B of WO 2012/047724.

20 In certain embodiments, a HA22-derived monoclonal anti-Nectin-4 antibody or antigen-binding fragment thereof is defined by its CDR sequences and comprises

- (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein
 - (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO:72, or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
 - (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO:73,

- or an amino acid sequence comprising a substitution or insertion,
particularly a conservative substitution and/or histidine insertion
of at least one amino acid, e.g., 1 or 2 amino acids,
- 5 (iii) the CDR-H3 comprises an amino acid sequence
according to SEQ ID NO:74,
or an amino acid sequence comprising a substitution or insertion,
particularly a conservative substitution and/or histidine insertion
of at least one amino acid, e.g., 1 or 2 amino acids,
and optionally
- 10 (b) a variable light chain (VL) region, particularly a VL region
comprising complementarity-determining regions (CDRs) CDR-L1,
CDR-L2 and CDR-L3, wherein
- (i) the CDR-L1 comprises an amino acid sequence according
to SEQ ID NO:75,
- 15 or an amino acid sequence comprising a substitution or insertion,
particularly a conservative substitution and/or histidine insertion
of at least one amino acid, e.g., 1 or 2 amino acids,
- (ii) the CDR-L2 comprises an amino acid sequence according
to SEQ ID NO:76,
- 20 or an amino acid sequence comprising a substitution or insertion,
particularly a conservative substitution and/or histidine insertion
of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-L3 comprises an amino acid sequence according
to SEQ ID NO:77,
- 25 or an amino acid sequence comprising a substitution or insertion,
particularly a conservative substitution and/or histidine insertion
of at least one amino acid, e.g., 1 or 2 amino acids.

30 SEQ ID NO: 72-77 define the six CDR sequences of the parental antibody
HA22 according to the IMGT numbering system.

In particular embodiments, a HA22 derived antibody may be defined by its VH and/or VL regions, wherein such antibody comprises

- (a) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:78 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%, and optionally
- (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:79 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%.

In even more particular embodiments, a HA22 derived antibody comprises

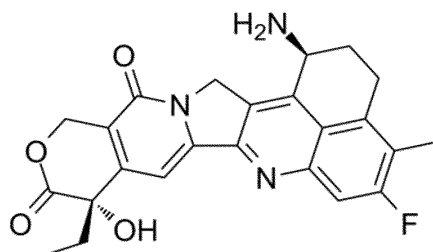
- (a) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:78,
- and
- (ii) a VL region comprising an amino acid sequence according to SEQ ID NO:79.

TOPOISOMERASE I INHIBITORS

The drug of the antibody conjugate of the invention is a topoisomerase I inhibitor. A topoisomerase I inhibitor is a compound, which is capable of forming a ternary complex with topoisomerase I and DNA, thereby preventing DNA re-ligation and introducing DNA strand breaks in the cellular genome. The topoisomerase I inhibitor may be e.g., selected from camptothecin or analogs thereof, indenoisoquinolines and indolocarbazoles.

In a particular embodiment, the topoisomerase-I-inhibitor is camptothecin or an analog thereof, i.e. a compound comprising the pentacyclic basic structure of camptothecin and modified substituents optionally resulting in the presence of a further ring. Specific examples are camptothecin, topotecan, irinotecan, SN-38, belotecan, exatecan including derivatives thereof such as deruxtecan, lurtotecan or atiratecan.

In a particular embodiment, the topoisomerase I inhibitor is exatecan:



Exatecan is typically conjugated to an antibody via its NH₂ group.

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CONJUGATION

The topoisomerase-I-inhibitor, particularly exatecan, is covalently conjugated to the antibody or antigen-binding fragment thereof.

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In principle, the topoisomerase-I-inhibitor, e.g., exatecan, may be conjugated to any suitable position of the monoclonal antibody or antigen-binding fragment thereof, particularly to any position, which does not abolish the binding of the antibody to Nectin-4. For example, the topoisomerase-I-inhibitor, e.g., exatecan, may be conjugated to a reactive amino acid residue on the antibody, e.g., an amino acid residue having a side chain comprising an amino, hydroxy or thiol group, or a reactive group in the antibody glycan structure. In particular embodiments, the topoisomerase-I-inhibitor, e.g., exatecan, is conjugated to a reactive thiol group in the side chain of an accessible cysteine residue on the antibody.

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20

In certain embodiments, the antibody drug conjugate comprises has a molar drug-antibody/antibody fragment ratio (DAR) of greater than 1, i.e., more than one drug molecule is attached to an antibody/antibody fragment. Typically, the conjugate has a DAR of about 2:1 to about 16:1, particularly of about 4:1 to

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about 10:1 and more particularly of about 6:1 to about 8:1. The DAR may be calculated from a statistical distribution according to known methods.

5 In certain embodiments, the topoisomerase-I-inhibitor is conjugated to the antibody or antigen-binding fragment via a linker. In certain embodiments, the linker is a cleavable linker, i.e., a linker cleavable under physiological conditions, e.g., by physiological enzymes. Specific examples of cleavable linkers are peptide-based linkers, which may be subject to cleavage by a protease, or glycoside-based linkers, which may be subject to cleavage by a
10 glycosidase.

In certain embodiments, the linker is a hydrophilic polysarcosine linker e.g., as described by Conilh et al. "Exatecan antibody drug conjugates based on a hydrophilic polysarcosine drug-linker platform" (Pharmaceuticals 14 (2021),
15 247). Further preferred linkers include linkers comprising at least one ethylene glycol unit, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more ethylene glycol units, e.g., the linker of the antibody-drug conjugate MEDI7247, an mcc-triazole spacer-PEG7-x-Lys-PABC glycol linker from Trodelvy®, Further preferred linkers include oligopeptide, particularly di- to decapeptide, e.g., tetrapeptide
20 sequences such as glycine-glycine-phenylalanine-glycine from Enhertu®. Further preferred linkers include highly polar spacers such as an acyl group, carbamoyl group and/or sulfamide group added to at least at least one ethylene glycol unit, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more ethylene glycol units, e.g., the linker technology called Hydraspace™.

25 In certain embodiments, the linker is a hydrophilic polysarcosine linker comprising, e.g., up to 15 sarcosine units, and at least one ethylene glycol unit, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more ethylene glycol units, wherein the linker is subject to cleavage by a glycosidase, and particularly subject to
30 cleavage by a glucuronidase.

5 In certain embodiments, the linker is a hydrophilic polysarcosine linker comprising, e.g., about 8-12 sarcosine units, and at least one ethylene glycol unit, e.g., up to 10 ethylene glycol units, and wherein the linker is subject to cleavage by a glycosidase, and particularly subject to cleavage by a glucuronidase.

10 In certain embodiments, the linker is a hydrophilic polysarcosine linker comprising 10 sarcosine units, and 2 ethylene glycol units, and wherein the linker is subject to cleavage by a glucuronidase.

10 The antibody drug conjugate of the present invention may be prepared by known methods.

15 The antibody or the antigen-binding fragment thereof may be produced in a suitable host cell comprising a nucleic acid molecule, e.g., a DNA molecule, encoding an antibody VH region, or an antibody VL region, or encoding a complete antibody or an antibody fragment, or a vector or vector system, i.e. a plurality of vectors, comprising said nucleic acid molecule(s), preferably in operative linkage with an expression control sequence, particularly with a heterologous expression control sequence. The host cell may be any known

20 host cell for producing antibodies or antibody fragments, e.g., a prokaryotic cell such as an E. coli cell, a yeast cell, an insect cell, or a mammalian cell, e.g., a CHO cell or a hybridoma cell.

25 The antibody or antigen-binding fragment thereof may be reacted with a linker-drug conjugate to obtain the antibody-drug conjugate. The linker-drug conjugate comprises a drug molecule, e.g., having attached thereto a suitable linker, wherein the linker comprises a reactive group capable of reacting with desired attachment positions on the antibody or antigen-binding fragment thereof. For attachment to cysteine residues, the linker-drug conjugate

30 comprises a thiol-reactive group, e.g., a maleimide group.

A further aspect of the invention relates to a linker-drug conjugate comprising:

- 5 (i) a hydrophilic polysarcosine linker comprising, e.g., about 8-12 sarcosine units, and at least one ethylene glycol unit, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more ethylene glycol units, wherein the linker is subject to cleavage by a glycosidase, and particularly subject to cleavage by a glucuronidase, and wherein the linker comprises a thiol-reactive group, e.g., a maleimide group capable of reacting with cysteine residues on an antibody or antigen-binding fragment thereof, and
- 10 (ii) a topoisomerase I inhibitor, particularly exatecan, covalently attached to the linker.

In particular embodiments, the linker-drug conjugate comprises:

- 15 (i) a hydrophilic polysarcosine linker comprising 10 sarcosine units, and 2 ethylene glycol units, wherein the linker is subject to cleavage by a glycosidase, and particularly subject to cleavage by a glucuronidase, and wherein the linker comprises a maleimide group capable of reacting with cysteine residues on an antibody or antigen-binding fragment thereof, and
- 20 (ii) exatecan covalently attached to the linker.

25 The linker-drug conjugate is particularly suitable for attachment to an anti-Nectin-4 antibody as describes herein. It should be noted, however, that the linker-drug conjugate is also suitable for attachment to any antibody or antigen-binding fragment thereof, e.g., an antibody binding to a tumor antigen or an antigen-binding fragment thereof.

PHARMACEUTICAL COMPOSITIONS

30 A further aspect of the present invention is a pharmaceutical composition comprising an active agent, which is an antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof wherein the

monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-4 and wherein the drug is a topoisomerase I inhibitor, particularly exatecan, and a pharmaceutically acceptable carrier and/or excipient.

5 Examples of suitable carriers and excipients for formulating antibody drug
conjugates include saline and aqueous buffer solutions and are well known in
the art. Typically, the pharmaceutical composition is adapted for parenteral
administration, e.g., for subcutaneous, intramuscular, or intravenous injection
or by infusion. In further embodiments, the pharmaceutical composition is
10 adapted for local administration, e.g., for intravesical instillation into the
bladder.

Depending on the stage and the severity of the disorder, the pharmaceutical composition may be administered once or several times in a therapeutically effective dose to a subject in need thereof, particularly to a human subject. For example, it may be administered once or several times daily, each second day, two times weekly or weekly for a suitable period, e.g., of at least one week, or at least one month.

20 MEDICAL APPLICATIONS

According to a further aspect of the invention, the antibody-drug conjugate or pharmaceutical composition as described above is used in medicine, including human and veterinary medicine, particularly in human medicine.

25 In a particular embodiment, the antibody-drug conjugate or pharmaceutical composition as described above is used in a method for the prevention and/or treatment of a Nectin-4 associated disorder, particularly for the prevention and/or treatment of a Nectin-4 positive cancer, more particularly for the prevention and/or treatment of a cancer associated with Nectin-4 overexpression.

5 The cancer to be prevented and/or treated may be any kind of cancer, wherein the term "cancer" is used herein to refer to proliferative diseases. The cancer to be prevented and/or treated according to the present invention is preferably selected from the group consisting of bladder, urothelial, endometrial, cervical, colorectal, liver, thyroid, breast, pancreatic, lung, ovarian head and neck and/or esophagus cancer.

10 In therapeutic applications, the active agent is administered in an effective amount to a subject in need thereof, particularly to a human subject. The dose will depend on the specific type of agent, e.g., type of antibody or antibody fragment, the type of disease, and the mode of administration, e.g., locally or systemically.

15 In the prevention and/or treatment of cancer, a therapeutic dose of an antibody drug conjugate is typically from about 0.3 mg/kg to about 10 mg/kg.

20 The antibody drug conjugate may be administered alone or together with a further active agent, which may be selected from chemotherapeutic agents, e.g., anti-metabolites, alkylating agents, intercalating agents, or anti-mitotic agents), inhibitors of specific kinases e.g., tyrosine kinase inhibitors, serine/threonine kinase inhibitors or phosphoinositide kinase inhibitors, immunotherapeutic compounds, e.g., immune checkpoint inhibitors, CAR-T cells, therapeutic vaccines or oncolytic viruses.

25 Further, the present invention is explained in more detail by the following Tables, Figures and Examples.

FIGURES

FIG. 1: Ch-15A7.5 and Ch-5A12.2 recognize human Nectin-4. Detection by flow cytometry.

5 Flow cytometry analysis of MDA-MB231 transfected with the N-terminal Flag-tagged epitope of Nectin-4 using a dose range (0.3 ng/mL – 5 µg/mL) of Ch-15A7.5 or Ch-5A12.2 antibodies. Parental MDA-MB231 cells are included as controls. Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody. The normalized mean fluorescence intensity is shown. Inlay: staining of parental MDA-MB231 cells with anti Nectin-1, -2 and -3 mAbs (5 µg/mL). Mean fluorescence intensity is represented.

15 **FIG. 2:** Ch-15A7.5 recognizes IgV-like domain of human Nectin-4. Detection by ELISA.

Ninety-six-wells plates were coated, as indicated, with 5 µg/mL Nectin-1 extracellular domain Fc fusion recombinant protein (Nec1-VCC), or Nectin-4 IgV-like domain Fc fusion recombinant protein (Nec4-V), or Nectin-4 extracellular domain Fc fusion recombinant protein (Nec4-VCC) overnight at 4°C. Ch15A7.5 recognizes the Nectin-4 extracellular domain, more precisely the Nectin-4 IgV-like domain and not the Nectin-1 extracellular domain.

25 **FIG. 3:** Characterization of Ch-15A7.5 epitope. Competition assay was performed by ELISA.

Ninety-six-wells plates were coated with 5 µg/mL of Nectin-4 extracellular domain Fc fusion recombinant protein (Nec4-VCC) overnight at 4°C. Binding of peroxidase-conjugated Ch-15A7.5 mAb (5 µg/mL) was measured in presence of increasing concentrations (2.75 ng/mL - 6 µg/mL) of Rituximab, Ch-15A7 mAb, Enfortumab (HA22), Ch-N41 mAb and Ch-14A5 mAb.

HA22, Ch-N41 and CH-14A5 recognize the IgV domain of Nectin-4.

FIG. 4: Competition of Ch-15A7.5 and 5A12.2 mAbs with Nectin-1 for Nectin-4 binding. Competition was assessed by flow cytometry. Ninety-six-wells plates were seeded with 50,000 CHO cells transfected with human Nectin-4 cDNA. Cells were incubated 45 minutes with increasing concentrations of isotypic control, Ch-15A7.5 or Ch-5A12.2 mAb (8 ng/mL – 5 µg/mL). After washing, plates were incubated with 20 µg/mL of Nectin-1 extracellular domain Fc fusion recombinant protein (Nec1-VCC). Nectin-1 binding was revealed after incubation with goat anti human Fc antibody coupled to phycoerythrin.

FIG. 5: Cross-reactivity with Nectin-4 from cynomolgus monkey, rat and mouse. Detection by flow cytometry. Flow cytometry analysis of CHO cells transfected with cynomolgus monkey, rat or mouse Nectin-4 using a dose range of Ch-15A7 (A), Ch-3A1.4 (B), Ch-5A12.2 (C), Ch-9A2.7 (D) or Enfortumab (HA22, B) antibodies (0.05 ng/mL – 5 µg/mL). Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody. Normalized mean fluorescence intensity is shown. Tables report the estimated EC₅₀ values determined by GraphPad Prism 9 software using non-linear curve fitting (4 parameters). ~ symbol indicates that the reported value is ambiguous due to poor curve fitting.

FIG.6: Differential binding to Nectin-4 expressed by tumor cell line and normal differentiated human keratinocytes. Detection by flow cytometry. Flow cytometry analysis of TNBC cell line (SUM190, plain symbols) and normal differentiated (0.1 mM CaCl₂) human

keratinocytes (NHEK, open symbols) using a dose range (1.2 ng/mL – 5 µg/mL) of Enfortumab (HA22, squares), Ch-15A7.5 (circles), Ch-3A1.4 (triangles) and Ch-9A2.7 (diamonds). Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody. Normalized mean fluorescence intensities are shown. Non-linear curve fitting (4 parameters) was done with GraphPad Prism 9 software. Horizontal bar is placed at 50% of maximum staining intensity obtained with HA22 antibody on SUM190 cells.

FIG. 7: Differential binding to Nectin-4 expressed by tumor cell line and normal differentiated human keratinocytes. Detection by flow cytometry.

Flow cytometry analysis of TNBC cell line (SUM190, A) and normal differentiated (0.1 mM CaCl₂) human keratinocytes (NHEK, B) using 5 µg/mL of HA22 (Enfortumab), Ch-5A12.2, Ch-15A7.5, and Ch-8F06. Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody. Normalized mean fluorescence intensities are shown.

FIG. 8: Differential binding to Nectin-4 expressed by tumor cell line and human keratinocytes. Detection by immuno-histochemistry.

Cryo-preserved OCT-embedded blocks of high expressing levels of Nectin-4 tumor cell line (SUM190), low expressing levels of Nectin-4 tumor cell line (SUM149), human and cynomolgus skin were processed for staining with 15A7.5 mAb (A) and 9A2.7 mAb (B). Quick score reported for each mAb are shown (A, B). C, ratio of quick score SUM190 over human skin.

FIG. 9: Differential internalization in tumor cell line and human keratinocytes. Detection by fluorescence

Enfortumab (HA22), Ch-15A7.5 and Isotypic control mAbs were coupled to pHAB thiol reactive dye to reach dye antibody ratio comprised between 4.58 and 5.55. A dose range of each of these antibody dye conjugates (1.6 ng/mL – 5 µg/mL) was incubated in duplicates with SUM190PT Nectin-4-expressing cell line (A) normal human differentiated (0.1 mM CaCl₂) keratinocytes (B). Intracellular fluorescence was recorded after 24 hours with fluorescence microplate reader (ClarioStar). Reported are the fluorescence intensities in function of dye antibody conjugate concentration.

FIG. 10: Differential *in vitro* cytotoxic activity to tumor cells and normal differentiated human keratinocytes. Cell survival measured by MTT assay.

Enfortumab (HA22, diamonds), Ch-15A7.5 (triangles) and Ch-9A2.7 (squares) mAbs were coupled to α-amanitin to generate antibody drug conjugates which cytotoxic activity of a dose range (7.7 pg/mL – 15 µg/mL) was comparatively evaluated on SUM190PT Nectin-4-expressing cell line (A, D) or normal human differentiated (0.1 mM CaCl₂) keratinocytes (B, E). Viability (MTT assay) after a 5-days incubation period is reported. EC₅₀ values were determined by GraphPad Prism 9 software using non-linear curve fitting (4 parameters). For each condition, the ratio of EC₅₀ of Ch-15A7.5 ADC (C) or Ch-9A2.7 ADC (F) to that of HA22-ADC was calculated and reported. Data shown is representative of 2 different Nectin-4 expressing tumor cell lines (SUM190PT and MDA-MB468) and 3 independent donors for human keratinocytes.

FIG. 11: Treatment of SUM190 grafted NSG mice with Ch-15A7.5-MA-PS-βGlu-Exatecan ADC induces a long-lasting tumor regression period.

NSG mice (n=5/group) were orthotopically xenografted bilaterally with the SUM190PT cells embedded in Matrigel. Three different ADC were tested: Isotypic Control, ICT- and Ch-15A7.5-MA-PS- β Glu-Exatecan and Enfortumab vedotin surrogate, HA22-MC-vc-PABC-MMAE. Treatment of mice (single intravenous injection) started when tumors reached approximately 150 mm³. Ch-15A7.5-MA-PS- β Glu-Exatecan (Ch-15A7.5-Ex) and Ch-5A12.2-MA-PS- β Glu-Exatecan (Ch-5A12.2-Ex) were evaluated at 2 doses (4 or 8 mg/kg), ICT-MA-PS- β Glu-Exatecan (ICT-Ex) was given at 8 mg/kg and Enfortumab vedotin (EV) surrogate was given at 4 mg/kg. Tumor sizes (n=10/group) were monitored with a caliper twice a week thereafter and sizes were reported with the following formula ($L \times l \times h \times \pi / 6$).

FIG. 12: Apparent affinity of humanized variants to tumor cells. Detection by flow cytometry.

T47D human tumor cells expressing Nectin-4 were numbered and incubated with a dose range (169 pg/mL – 30 μ g/mL) of Ch-15A7.5 or indicated humanized variants. Numbers associated with H and L refer to the number of back mutations introduced. Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody and analyzed by flow cytometry. Mean fluorescence intensities are reported. GraphPad Prism 9 software was used non-linear curve fitting (4 parameters).

FIG. 13: Treatment of SUM190 grafted NSG mice with HA22-MA-PS- β Glu-Exatecan ADC induces a long-lasting tumor regression period.

NSG mice (n=5/group) were orthotopically xenografted bilaterally with the SUM190PT cells embedded in Matrigel. Three different ADC were tested: Isotypic Control, ICT- and HA22-MA-PS- β Glu-Exatecan and Enfortumab vedotin surrogate, HA22-MC-vc-

PABC-MMAE. Treatment of mice (single intravenous injection) started when tumors reached approximately 150 mm³. HA22MA-PS-βGlu-Exatecan (HA22-Ex) was evaluated at 2 doses (4 or 8 mg/kg), ICT- MA-PS-βGlu-Exatecan (ICT-Ex) was given at 8 mg/kg and Enfortumab vedotin (EV) surrogate was given at 4 mg/kg. Tumor sizes (n=10/group) were monitored with a caliper twice a week thereafter and sizes were reported with the following formula ($L \times l \times h \times \pi / 6$).

FIG. 14: Treatment of SUM190 grafted NSG mice with HA22-MA-PS-βGlu-Exatecan and HA22-MC-GGFG-DX8951 ADC induces a long-lasting tumor regression period.

NSG mice (n=5/group) were orthotopically xenografted bilaterally with the SUM190PT cells embedded in Matrigel. Five different ADC were tested: Isotypic Control, ICT- and HA22 coupled either with MA-PS-βGlu-Exatecan (ICT-Ex and HA22-Ex) or MC-GGFG-DX8951 (ICT- and HA22-Dxd) and Enfortumab vedotin surrogate, HA22-MC-vc-PABC-MMAE (EV). Treatment of mice (single intravenous injection) started when tumors reached approximately 150 mm³. HA22-Ex and HA22-Dxd were evaluated at 2 doses (4 or 8 mg/kg), ICT-EX and ICT-Dxd were given at 8 mg/kg and Enfortumab vedotin (EV) surrogate was given at 4 mg/kg. Tumor sizes (n=10/group) were monitored with a caliper twice a week thereafter and sizes were reported with the following formula ($L \times l \times h \times \pi / 6$).

FIG. 15: Differential binding of Ch-15A7.5 and its humanized variants to Nectin-4 expressed by tumor cell line and normal differentiated human keratinocytes. Detection by flow cytometry.

Flow cytometry analysis of TNBC cell line (SUM190, upper panels) and normal differentiated (0.1 mM CaCl₂) human keratinocytes (NHEK, lower panels) using a dose range (8 pM –

33 nM) of Ch-5A12.2, Ch-15A7.5 and its humanized variants 15A7.5-H1L2, -H1L3, -H2L2, -H2L3, -H3L0, -H3L2, -H3L3. Cells were then stained with phycoerythrin conjugate goat anti human Fc antibody. Normalized mean fluorescence intensities are shown. Non-linear curve fitting (4 parameters) was done with GraphPad Prism 9 software. Note the much lower apparent EC₅₀ of Ch-15A7.5 and its humanized variants towards keratinocytes (lower panels) in comparison to that of Ch-5A12.2.

- 5
- 10 **FIG. 16:** Amino acid sequence of the variable heavy chain (VH) and variable light chain (VK) of the parental antibody clone 5A12.2. The H-CDR and L-CDR sequences according to the IMTG nomenclature are underlined.
- 15 **FIG 17:** Amino acid sequences of the variable heavy chains (VH) and variable light chains (VK) of humanized variants of 9A2.7. The H-CDR and L-CDR sequences according to the IMTG nomenclature are underlined.
- 20 **FIG 18:** Amino acid sequences of the variable heavy chains (VH) and variable light chains (VK) of humanized variants of 3A1.4. The H-CDR and L-CDR sequences according to the IMTG nomenclature are underlined.
- 25 **FIG 19:** Amino acid sequences of the variable heavy chains (VH) and variable light chains (VK) of humanized variants of 8F06. The H-CDR and L-CDR sequences according to the IMTG nomenclature are underlined.
- 30 **FIG 20:** Amino acid sequences of the variable heavy chains (VH) and variable light chains (VL) of humanized variants of 15A7.5. The H-

CDR and L-CDR sequences according to the IMTG nomenclature are gray-shaded

FIG. 21: Affinity values of humanized 15A7.5 variants.

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FIG. 22: Amino acid sequences of the parental antibody clone 15A7.5 variable heavy chains (VH) and variable light chains (VK). The H-CDR and L-CDR sequences according to the IMTG nomenclature are gray-shaded.

10

FIG. 23: Amino acid sequence of the variable heavy chain (VH) and variable light kappa chain (VK) of the antibody HA22. The H-CDR and L-CDR sequences according to the IMTG nomenclature are gray-shaded.

15

EXAMPLES

Materials and Methods

20

Cell lines:

Human breast carcinoma cell line MDA-MB231 (ATCC, Manassas, VA) was cultured in DMEM supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, 50 µg/mL streptomycin and 2 mM glutamine. The cells were transfected with expression vector p3XFLR4.C1 containing a PVRL4 cDNA.

25

Human triple negative breast cancer cell line SUM190PT (BioIVT, Westbury, NY) was cultured in Ham's F12 medium with 5% fetal bovine serum, 1% non-essential amino acids, 1% Hepes, 1% insulin, 1 µg/mL hydrocortisone, 6.8 ng/mL Triiodo L-tyrosine, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM glutamine. Chinese hamster ovary CHO cell line was cultured in DMEM

30

supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM glutamine. Human breast carcinoma MDA-MB-468 cell

line and T47D cell line were cultured in RPMI supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin.

ELISA:

5 A sandwich enzyme-linked immunosorbent assay was used to control specificity of Ch-15A7.5 antibody and to perform competition assays between different mAbs. Ninety-six-wells plates were coated with 10 nM of Nectin-4-VCC-Fc, Nectin-1-VCC-Fc (entire extracellular part) or Nectin-4-V-Fc (comprising only the IgV domain) overnight at +4°C. After washes and
10 saturation with PBS 1% BSA, cells were incubated for 2 hours at 25°C with 10 nM of peroxidase-conjugated Ch-15A7.5 mAb. In the case of competition, binding of 0.5 nM of peroxidase conjugated Ch-15A7.5 mAb was measured in the presence of variable concentration (0.018 nM to 40 nM) of "cold" mAb. One hundred µL of peroxidase substrate was added (One Step ABST, Pierce), and
15 OD was read at 405 nm.

Flow Cytometry:

Cells or cell lines (10,000 – 50,000) expressing Nectin-4 (naturally or transfected) were incubated with dose range of the indicated antibodies. After
20 washing, cells were then stained with phycoerythrin-conjugated goat anti human antibody (5 µg/mL) Jackson Immuno Research). After fixation, cells were stained with a viability dye (e780, Invitrogen) before flow cytometry acquisition.

Immuno-histochemistry:

25 Frozen samples stored at -80°C were kept on dry ice and placed in plastic cryomolds in a way that would allow to maximize the number of subsequent sections and were embedded in Optimal Cutting Temperature (OCT) medium. Frozen blocks of OCT were mounted on disks with OCT and cryosection were
30 performed at -20°C on a NX70 cryostat (Thermo Scientific). Cryosections (7 µm) were mounted on superfrost + slides (VWR). Only the requested number of slides for each series of tests were prepared and stored at -80°C until use.

Remaining blocks were stored at -80°C until further use. Sections were arranged on the slide and fixed in Acetone at -20°C for 10 minutes. Endogenous peroxidases were inhibited by immersing the slides in hydrogen peroxide (H₂O₂) as part of Roche DAB kit protocol. Several dilution of each mAb were tested to optimize noise to signal ratio and 0.5 µg/mL of mouse 15A7.5 mAb and 10 µg/mL of mouse 9A2.7 mAb were determined as optimal. In order to mitigate the non-specific binding of the secondary antibody to the mouse tissue, a Rabbit anti-mouse IgG (4 µg/mL, Abcam) was added following primary Ab incubation. The omni-map anti-rabbit HRP (Roche) was then used to perform the DAB staining according to manufacturer's instruction (Ventana automat). An assessment of both the staining intensity and the proportion of stained cells was performed. Two trained technicians observed the same microscope field independently and sequentially. Both the stained cells proportion and the staining intensity were evaluated for each field. For each staining, 10 fields were chosen randomly at the magnification which allowed the best visualization of tissues. Scoring procedure was as follows: (i) The proportion of cells stained positively was estimated and a score from 0 to 4 was be assigned for each field (0= 0-5%; 1= 5- 25%; 2= 25-50%; 3= 50-75%; and 4= 75-100%); and (ii) The staining intensity was scored as 0, 1, 2, or 3 corresponding to the presence of negative, weak, intermediate, and strong brown staining, respectively. The final Score for each field and for each observer was the multiplication of the two values (0 = No staining in the tissue; 12= tissue contains strongly stained cells). For each tissue type, on each of the 5 slides of each set of slides, 10 independent observation fields were scored in parallel for both staining intensity and percentage of labeled cells. Technicians simultaneously observed a given field (virtual slide, on computer screen) and independently scored, blind of the other observer results. For each slide, the 10 scores of each technician were averaged to set the final score for each observer. Final scores of the two observers were averaged yielding the tissue score for the given slide. For the human skin, only results from the first set of the repeatability are presented.

Internalization assay

Ten thousand SUM190PT or human differentiated keratinocytes (0.1 mM CaCl₂) were seeded in 96-wells plates and subsequently incubated for 24 hours at 37°C, with a dose range (1.6 ng/mL – 1 µg/mL) of anti Nectin-4 mAbs and isotypic control conjugated with pHAB thiol reactive dye. Upon internalization and endo-lysosomal processing, the acidic environment causes the dye to fluoresce. Fluorescence intensity was monitored on a plate reader with an excitation at 532 nm and an emission at 560 nm.

In vitro cytotoxic assay

To analyze the in vitro cytotoxic activity of ADC, cell viability was assessed using the AlamarBlue staining protocol as recommended by the manufacturer (Biosource, CA, USA). The test incorporates a fluorescent oxidation-reduction indicator. Fluorescence intensity is proportional to cellular metabolic reduction. Experiments were done by incubating 3000 cells/well (SUM190PT or differentiated NHEK) in triplicate with serial dilutions of ADC at Day 0 in 96-wells plates. AlamarBlue was measured at Day 5 by incubating 1/10 volume of alamarBlue solution for 2 h at 37° C and read at 595 nm (FLUOstar Optima, BMG Labtech).

Mouse experiments

NOD/SCID (nonobese diabetic/severe combined immunodeficient)/gc null mice (NSG) were obtained from Charles River Laboratory (Margate, UK). Six to seven-weeks old females (n=5/group) were orthotopically xenografted bilaterally with the SUM190PT (0.5 x 10⁶) cells embedded in Matrigel. Treatment with ADC was performed as mentioned in the respective experiments. Tumor sizes (n=10/group) were monitored with a caliper twice a week thereafter and sizes were reported with the following formula (LxIxhxPi/6).

Hybridoma sequencing

For hybridoma sequencing, RNA was first extracted from hybridoma cell pellets. cDNA was generated by reverse transcription and VH and VL domains were amplified by polymerase chain reaction using Prime STARMax DNA Polymerase (Takara). PCR products were subsequently cloned into dedicated heavy and light chain expression vector and then sequenced.

Chimeric antibodies generation, production, purification, and control

Light chain expression vector is coding for a V kappa chain. Depending on the payload used, 2 different heavy chain expression vectors were used, one coding for a Fc fragment with D265C (ThiomAb) L234A and L235A mutations, one coding for a Fc fragment with P331S, L234Q and L235F mutations. Both Fc fragments are "Fc-silent". The sequences of anti-Nectin-4 Enfortumab (HA22) were also cloned in the same vectors.

Light and heavy chain vectors were transfected in HEK293 seeded with a 1.2/1 ratio. After 6 days of production, culture supernatants were clarified and mAbs were purified using MabSelect Prisma resin (GE Healthcare) according to the manufacturer's instructions. 0.5 M Glycine, 3 M NaCl, pH8.9 was used as binding buffer, and 0.1 M Citrate pH 3 was used for elution. Instant neutralization was done with 10% (V/V) 1 M Tris-HCl pH 9. Monoclonal chimeric antibodies were then dialyzed against PBS 1X pH 7.4 (Mini dialysis devices, 2 mL-10k, Thermo Scientific) followed by filtration on 0.22 µm filter (Milelex GV hydrophilic PVDF, Millipore). Concentration was determined with a Nanodrop 2000 Spectrophotometer (Thermo Scientific) taking into account the specific extinction coefficient ($E^{1\%}_{280nm}$) of each monoclonal antibody. Purity was determined by UPLC-SEC using an Acquity UPLC-HClass Bio (Waters) using a Protein-BEH 200A column equilibrated in 0.2 M NaPO₄, 0.3 M NaCl pH 6.9 supplemented with 10% isopropanol. The mass of the antibodies was determined in a Xevo G2-S Q-ToF mass spectrophotometer (Waters) using a reversed-phase column (PLRP-S 4000A, Agilent technologies). All samples were analyzed after deglycosylation with PNGase F glycosidase (New England Biolabs) at 37°C, according to the manufacturer's

instructions. Fragmentation and/or aggregation of the final material was evaluated by SDS-PAGE. Endotoxin load was determined using a chromogenic LAL-kinetic assay (Charles River Endosafe).

5 Antibody conjugation

The thiol reactive dye pHAB (Promega) was conjugated to cysteine of selected anti-Nectin-4 ThiomAb (D265C, L234A, L235A) antibodies using maleimide chemistry according to manufacturer's instructions. Briefly, antibodies were dialyzed against 0.1 M Phosphate buffer pH 7.0 before being reduced with 2.5 mM for 1 h at room temperature under mild agitation. Subsequently, DTT was removed by washing/centrifugation twice on Zeba spin desalting columns (7 MWCO). 1.2 L of pHdye reagent (10 µg/mL DMSO) was added to 100 µg of antibody and incubated 1 hour at room temperature protected from light. After removal of excess dye with Zeba desalting columns, Dye antibody ratio were calculated to verify equivalent conjugation between different antibodies.

The cysteine reactive linker-exatecan compound Maleimide-Gly-PSAR10-glucuronide-exatecan (MabLink) was conjugated to cysteine residues of selected anti-Nectin-4 mAbs (P331S, L234Q and L235F). In brief, mAbs in PBS 1X, 1 mM EDTA were reduced with 14 molar equivalents of TCEP for 2 hours at 37°C, after which the buffer was exchanged (Amicon ultra 30 kDa) to 100 mM KPO₄, 1 mM EDTA pH 7.4. Twelve molar equivalents of the cysteine reactive linker-exatecan compound were used for conjugation with reactive cysteines for 35 min at room temperature. Buffer was then exchanged to 100 mM KPO₄ pH 8.0, before incubation at 37°C for 24 hours in absence of oxygen to allow the maleimide to self-hydrolyze. The final exchange buffer was performed in 20 mM His pH 6.0 before filtration 0.22 µm filter. The drug-antibody ratio (DAR) according to LC-MS analysis was comprised between 7.77 and 7.82 toxins per conjugated mAb. As determined by SEC-HPLC, less than 8% material was aggregated.

Claims

1. An antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof and a drug, wherein the monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-4, and the drug is a topoisomerase I inhibitor.
2. The antibody-drug conjugate of claim 1 comprising an antibody selected from a chimeric antibody, a multispecific antibody, in particular a bispecific antibody, a human antibody, a humanized antibody, or an antigen-binding fragment thereof.
3. The antibody-drug conjugate of claim 1 or 2 comprising an antibody selected from an antibody of class IgG, e.g., of subclass IgG1, IgG2, IgG3 or IgG4, of class IgM, of class IgA or an antigen-binding fragment thereof, or a single-chain antibody, or an antibody Fv fragment, wherein the antibody optionally has a heavy chain constant domain having a reduced effector function, e.g., which has a reduced binding to the Fc receptor.
4. The antibody-drug conjugate of any one of claims 1-3 comprising an antibody, which specifically binds to Nectin-4 expressed by tumors with a higher affinity in comparison to Nectin-4 expressed by human differentiated keratinocytes, or an antigen-binding fragment thereof.
5. The antibody-drug conjugate of any one of claims 1-4 comprising an antibody or antigen-binding fragment thereof comprising:
- (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein
- (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO: 1 or 7, 88, 96 or 104,

- or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 5 (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO: 2 or 8, 89, 97 or 105, or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 10 (iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO: 3 or 9, 90, 98 or 106, or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids, and optionally
- 15 (b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein
- (i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO: 4 or 10, 92, 100 or 108,
- 20 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO: 5 or 11, 93, 101 or 109,
- 25 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO: 6 or 12, 94, 102 or 110,
- 30 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids.

6. The antibody-drug conjugate of any one of claims 1-5 comprising an antibody or antigen-binding fragment thereof comprising:

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(a) (i) a VH region comprising the CDR-H1 of SEQ ID NO:1, the CDR-H2 of SEQ ID NO:2, and the CDR-H3 of SEQ ID NO:3, and optionally

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(ii) a VL region comprising the CDR-L1 of SEQ ID NO:4, the CDR-L2 of the SEQ ID NO:5, and the CDR-L3 of the SEQ ID NO:6, or

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(b) (i) a VH region comprising the CDR-H1 of SEQ ID NO:7, the CDR-H2 of SEQ ID NO:8, and the CDR-H3 of SEQ ID NO:9, and optionally

(ii) a VL region comprising the CDR-L1 of SEQ ID NO:10, the CDR-L2 of the SEQ ID NO:11, and the CDR-L3 of the SEQ ID NO:12, or

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(c) (i) a VH region comprising the CDR-H1 of SEQ ID NO:88, the CDR-H2 of SEQ ID NO:89, and the CDR-H3 of SEQ ID NO:90, and optionally

(ii) a VL region comprising the CDR-L1 of SEQ ID NO:92, the CDR-L2 of the SEQ ID NO:93, and the CDR-L3 of the SEQ ID NO:94, or

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(d) (i) a VH region comprising the CDR-H1 of SEQ ID NO:96, the CDR-H2 of SEQ ID NO:97, and the CDR-H3 of SEQ ID NO:98, and optionally

30

(ii) a VL region comprising the CDR-L1 of SEQ ID NO:100, the CDR-L2 of the SEQ ID NO:101, and the CDR-L3 of the SEQ ID NO:102,

or

(e) (i) a VH region comprising the CDR-H1 of SEQ ID NO:104, the CDR-H2 of SEQ ID NO:105, and the CDR-H3 of SEQ ID NO:106, and optionally

5 (ii) a VL region comprising the CDR-L1 of SEQ ID NO:108, the CDR-L2 of the SEQ ID NO:109, and the CDR-L3 of the SEQ ID NO:110.

10 7. The antibody-drug conjugate of any one of claims 1-6 comprising an antibody or antigen-binding fragment thereof comprising:

(a) a VH region comprising an amino acid sequence according to SEQ ID NO:13, 91, 99 or 107 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 15 99%,
and optionally

(b) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:14, 95, 103 or 111 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 20 95% or at least 99%.

8. The antibody-drug conjugate of any one of claims 1-7 comprising an antibody or antigen-binding fragment thereof comprising:

(a) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:13 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,
25 and optionally

(ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:14 or an amino sequence having an

identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

or

- 5 (b) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:91 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

and optionally

- 10 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:95 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

or

- 15 (c) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:99 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

and optionally

- 20 (ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:103 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

or

- (d) (i) a VH region comprising an amino acid sequence according to SEQ ID NO:107 or an amino acid sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%,

25 and optionally

(ii) a VL region, particularly a VL region comprising an amino acid sequence according to SEQ ID NO:111 or an amino sequence having an identity thereof of at least 85%, at least 90%, at least 95% or at least 99%.

5 9. The an antibody-drug conjugate of any one of the preceding claims comprising an antibody or antigen-binding fragment thereof derived, comprising:

10 (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein

 (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO:21, 35, 49 or 63, or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

15 (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO:22, 36, 50 or 64 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,

20 (iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO:23, 37, 51 or 65, or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids, and optionally

25 (b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein

30 (i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO:24, 38, 52 or 66,

- or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 5 (ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO:25, 39, 53 or 67,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 10 (iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO:26, 40, 54 or 68,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids.
- 15 10. The antibody-drug conjugate of any one of claims 1-3 comprising an antibody or antigen-binding fragment thereof derived, comprising:
- (a) a variable heavy chain (VH) region comprising complementarity-determining regions (CDRs) CDR-H1, CDR-H2 and CDR-H3, wherein
- 20 (i) the CDR-H1 comprises an amino acid sequence according to SEQ ID NO: 72 or 80,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 25 (ii) the CDR-H2 comprises an amino acid sequence according to SEQ ID NO: 73 or 81,
or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- 30 (iii) the CDR-H3 comprises an amino acid sequence according to SEQ ID NO: 74 or 82,

- or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids, and optionally
- 5 (b) a variable light chain (VL) region, particularly a VL region comprising complementarity-determining regions (CDRs) CDR-L1, CDR-L2 and CDR-L3, wherein
- (i) the CDR-L1 comprises an amino acid sequence according to SEQ ID NO: 75 or 84,
- 10 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (ii) the CDR-L2 comprises an amino acid sequence according to SEQ ID NO: 76 or 85,
- 15 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids,
- (iii) the CDR-L3 comprises an amino acid sequence according to SEQ ID NO: 77 or 86,
- 20 or an amino acid sequence comprising a substitution or insertion, particularly a conservative substitution and/or histidine insertion of at least one amino acid, e.g., 1 or 2 amino acids.

11. The antibody-drug conjugate of any one of claims 1-10, wherein the
25 topoisomerase I inhibitor is a camptothecin or analog thereof, and wherein the topoisomerase I inhibitor is particularly exatecan or deruxtecan.

12. The antibody-drug conjugate of any one of claims 1-11, which has a
30 molar drug-antibody/antibody fragment ratio (DAR) of about 2:1 to about 16:1, particularly of about 4:1 to about 10:1 and more particularly of about 6:1 to about 8:1.

13. The antibody-drug conjugate of any one of claims 1-12, wherein the drug is conjugated to a reactive amino acid residue on the antibody, e.g., an amino acid residue having a side chain comprising an amino, hydroxy or thiol group, or a reactive group in the antibody glycan structure and
5 wherein the drug is particularly conjugated to a reactive thiol group in the side chain of a cysteine residue on the antibody or antigen-binding fragment thereof.

14. The antibody-drug conjugate of any one of claims 1-13, wherein the
10 drug is conjugated to the antibody or antigen-binding fragment thereof via a linker, particularly wherein the linker is particularly a cleavable linker, and/or wherein the linker is particularly a hydrophilic polysarcosine linker, a hydrophilic linker comprising at least one ethylene glycol unit, a linker comprising a highly polar spacer, or an oligopeptide linker.

15. The antibody-drug conjugate of claim 14, wherein the linker is a hydrophilic polysarcosine linker and at least one ethylene glycol unit, wherein the linker is subject to cleavage by a glycosidase, and particularly subject to cleavage by a glucuronidase.

20 16. The antibody-drug conjugate of claim 14 or 15, wherein the linker is a hydrophilic polysarcosine linker comprising, e.g., about 8-12 sarcosine units, and at least one ethylene glycol unit, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more ethylene glycol units, wherein the linker is subject to cleavage by a
25 glycosidase, and particularly subject to cleavage by a glucuronidase.

17. The antibody-drug conjugate of any one of claims 14-16, wherein the linker is a hydrophilic polysarcosine linker comprising 10 sarcosine units, and 2 ethylene glycol units,

30 18. The antibody-drug conjugate of any one of claims 14-17, wherein the linker is subject to cleavage by a glucuronidase.

19. The antibody-drug conjugate of any one of claims 14-18, wherein the linker is attached to a cysteine residue on the antibody or antigen-binding fragment thereof, via a thiol-reactive group, particularly a maleimide group.

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20. A pharmaceutical composition comprising an active agent, which is an antibody-drug conjugate comprising a monoclonal antibody or an antigen-binding fragment thereof wherein the monoclonal antibody or the antigen-binding fragment thereof binds to Nectin-4 and wherein the drug is a topoisomerase I inhibitor, and a pharmaceutically acceptable carrier and/or excipient.

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21. Use of an antibody-drug conjugate of any one of claims 1-19 or a pharmaceutical composition of claim 19 in medicine, particularly in human medicine.

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22. Use of an antibody-drug conjugate of any one of claims 1-19 or a pharmaceutical composition of claim 19 in a method for the prevention and/or treatment of a Nectin-4 associated disorder, particularly for the prevention and/or the treatment of a Nectin-4 positive cancer, particularly for the prevention and/or the treatment of a cancer selected from bladder, urothelial, endometrial, cervical, colorectal, liver, thyroid, breast, pancreatic, lung, ovarian, head and neck and/or esophagus cancer.

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