VARIANT ANTIBODIES THAT BIND GAMMA-DELTA T CELL RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to EP 22179260.9, filed on June 15, 2022, the content of which is incorporated herein by reference in its entirety.

ELECTRONIC VERSION OF THE SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (LVAT_024_01WO_SeqList_ST26.xml; Size: 101,824 bytes; and Date of Creation: June 14, 2023) are herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to novel antibodies capable of binding the Vδ2 chain of a human Vγ9Vδ2 T cell receptor. The invention further relates to pharmaceutical compositions comprising the antibodies of the invention and to uses of the antibodies of the invention for medical treatment.

BACKGROUND OF THE INVENTION

[0004] Gamma-delta (γδ) T cells are T cells that express a T cell receptor (TCR) that is made up of a gamma chain and a delta chain. The majority of γδ T cells express TCRs comprising Vγ9 and Vδ2 regions. Vγ9Vδ2 T cells can react against a wide array of pathogens and tumor cells. This broad reactivity is understood to be conferred by phosphoantigens that are able to specifically activate this T-cell subset in a TCR dependent fashion. The broad antimicrobial and anti-tumor reactivity of Vγ9Vδ2 T-cells suggest a direct involvement in immune control of cancers and infections.

[0005] Agents that can activate Vγ9Vδ2 T cells can be useful in the treatment of infections or cancer as these may promote Vγ9Vδ2 T cell reactivity towards the pathogen or infected cells or cancer cell. WO2015156673 describes antibodies that bind Vγ9Vδ2 TCRs and are capable of activating Vγ9Vδ2 T cells. WO2020060405 describes bispecific antibodies that bind both Vγ9Vδ2 T cells and a tumor cell target and thus have the potential to recruit Vγ9Vδ2 T cells to a tumor and thus stimulate a therapeutic effect.

SUMMARY OF THE INVENTION

[0006] Recombinant production of antibodies in host cells often results in heterogenous products, comprising different forms of the antibody with various types and degrees of post-translational modifications of the polypeptide chain. Such heterogeneity is undesirable for an antibody product for medical use, as post-translational modifications may alter the functional properties of the

antibodies, for example in terms of affinity for the target antigen, in terms of pharmacokinetic properties, product stability, aggregation, etc.

[0007] The present invention provides improved Vγ9Vδ2 TCR binding antibody sequences that result in a more homogeneous product upon production in a host cell, yet retain good functional properties, with respect to target binding and functional effects on target cells, as well as good structural properties such as stability.

[0008] Production of a bispecific antibody comprising Vγ9Vδ2-TCR binding antibody 5C8 (described in WO 2015/156673) in mammalian cells demonstrated that a subpopulation of the antibody molecules are proteolytically cleaved within the CDR3 region of 5C8, resulting in heterogeneity of the antibody product, as determined by mass spectrometry analysis of purified protein. The cleavage occurs in a sequence not predicted to be prone to endoproteolytic degradation. Furthermore, surprisingly, the residue immediately before the proteolytic cleavage site could be mutated without affecting the antigen-binding properties of the antibody, even though this residue is located in the CDR3 region. The CDR3 region is known to be a main determinant of antigen binding specificity in an antigen-binding region of an antibody.

[0009] Furthermore, the inventors have found that antibody 5C8 undergoes a sulfation at a site in the antibody that was not predicted to be subject to this post-translational modification. Sulfation occurred partially in various host cells, also resulting in a heterogenous antibody product. Surprisingly, the tyrosine residue subject to the sulfation could be mutated without affecting the antigen-binding properties of the antibody even though the amino acid is also located in the CDR3 region. The removal of the sulfation site via mutation resulted in a more homogeneous antibody product.

[0010] Accordingly, in a first aspect, the invention provides an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence as set forth in SEQ ID NO:12, a CDR2 sequence as set forth in SEQ ID NO:13 and a CDR3 sequence as set forth in SEQ ID NO:14.

[0011] In a further aspect, the invention provides bispecific antibodies comprising a first binding region capable of binding to human Vδ2 as defined herein and a second antigen-binding region capable of binding a second antigen, wherein the second antigen is selected from human EGFR, CD123, PSMA, CD1d, CD40, and Nectin 4. In further aspects, the invention relates to pharmaceutical compositions comprising the antibodies of the invention, uses of the antibodies of the invention in medical treatment, and to nucleic acid constructs, expression vectors for producing antibodies of the invention and to host cells comprising such nucleic acid constructs or

expression vector. Furthermore, the invention relates to processes for producing antibodies of the invention that yield more homogeneous products.

[0012] Further aspects and embodiments of the invention are described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1: Representative chromatogram of the size exclusion profile of protein-A purified LAVA compound (VHH 5C8 is shown) using a Superdex-75 column. The fractions of the dominant monomeric peak (fractions 1E11-1G2) were pooled and quantified.

[0014] Figure 2: Representative example of labchip polyacrylamide gel electrophoresis of purified VHH 5C8. Left: non-reducing; right: reducing conditions.

[0015] Figure 3: HP-SEC profiles of purified VHH 5C8 (A) and VHH 5C8var1 (B).

[0016] Figure 4: Representative HP-SEC profiles of purified bispecific VHH (bsVHH) 1D12var5-5C8var1. A: bsVHH 1D12var5-5C8var1 batch that was expressed and purified by protein-A affinity chromatography from the supernatant of Pichia pastoris. B: bsVHH 1D12var5-5C8var1 batch that was expressed and purified by both protein-A and size exclusion chromatography from HEK-293 E cells.

[0017] Figure 5: Labchip analysis of purified VHH 5C8var1-Y105F and 5C8var1-Y105S under non-reducing conditions.

[0018] Figure 6: HP-SEC analysis of VHH 5C8var1-Y105F (A) and 5C8var1-Y105S (B).

[0019] Figure 7: Affinity measurement of VHH fragment binding to recombinant Vγ9Vδ2-TCR protein using BLI. The protein mass (response, in nm) is plotted as a function of time. The dotted vertical line separates the association phase (left) from the dissociation phase (right). A: VHH 5C8var1; B: VHH 5C8var1-Y105F; C: VHH 5C8var1-Y105S. Straight black lines represent fitted data to the actual responses measured.

[0020] Figure 8: Both bsVHH 7D12var8-5C8var1-Y105F and bsVHH 7D12-5C8 induce potent Vγ9Vδ2 T cell activation and cause Vγ9Vδ2 T cell-mediated tumour cell lysis. A: 4 hours degranulation assay: the percentage of CD107A (LAMP-1)+ Vγ9Vδ2 T cells is plotted as a function of the antibody concentration used. Left: 7D12-5C8 (non-humanized); right: 7D12var8-5C8var1-Y105F. B: 24 hours cytotoxicity assay showing the percentage of A431 tumour cell kill as a function of the antibody concentration used. Left: 7D12-5C8 (non-humanized); right: 7D12var8-5C8var1-Y105F.

[0021] Figure 9: Binding of 7D12var8-5C8var1(Y105F)-Fc to primary Vγ9Vδ2 T cells isolated from healthy human PBMCs using flow cytometry. The two panels represent two different donors.

[0022] Figure 10: Binding of 7D12var8-5C8var1(Y105F)-Fc to EGFR on tumor cells by cell-based ELISA.

[0023] Figure 11: Degranulation of Vγ9Vδ2 T cells (indicated as γδ T cells) induced by 7D12var8-5C8var1(Y105F)-Fc dependent on the A431 cell line.

[0024] Figure 12: Viability of A-388 cells in co-culture with Vγ9Vδ2 T cells T cells (indicated as GDT cells) and 7D12-5C8.

[0025] Figure 13: Lysed tumor cells after a 4-hour culture of dissociated tumor cell suspensions (primary CRC: n=10, peritoneal CRC metastases: n=5, liver CRC metastases: n=3, primary HNSCC: n=5, and primary NSCLC: n=4) with healthy donor derived Vγ9Vδ2 T cells (1:1 E:T ratio) and 7D12-5C8 (50 nM) or medium control.

[0026] Figure 14: Lysed tumor cells after a 24-hour culture of dissociated tumor cell suspensions (peritoneal CRC metastases: n=4) with healthy donor derived Vγ9Vδ2 T cells (1:1 E:T ratio) and 7D12-5C8var1(Y105S)-Fc (50 nM), gp120-5C8var1(Y105S)-Fc (50 nM) or medium control.

[0027] Figure 15: Structure of construct for non-human primate studies.

[0028] Figure 16: Binding of 7A5-7D12var8-Fc to antigen targets.

[0029] Figure 17: Degranulation and cytotoxicity mediated by 7A5-7D12var8-Fc.

[0030] Figure 18: PK analyses of 7A5-7D12var8-Fc concentrations in the blood of the three treated animals. Concentration-time curves are shown that demonstrate the molecule to have an IgG-like PK.

[0031] Figure 19: Total number of T cells (CD3+, left graph) and number of Vγ9 positive cells (as percentage of the CD3+population) in the blood of treated animals. Arrows indicate the injections with compound. The numbers in the legend are the numbers of the treated monkeys.

[0032] Figure 20: Levels of the IL-6 cytokine in the blood of the treated animals over time. Only low levels of the cytokine were observed and the release was largely limited to after the first injection. Arrowheads indicate the treatment moments.

[0033] Figure 21: SDS-PAGE analysis of purified VHH-Fc 'heavy chain-only' antibody. 1 and 2 microgram of purified protein were loaded for better identification of the (truncated) impurity product.

[0034] Figure 22: Deconvoluted mass spectrum of VHH-Fc single domain antibody. The intensity of the signal is plotted as a function of the molecular weight in Da. Signals at 78,000 to 79,000 Da are due to deconvolution artifacts. There is a significant signal at 68,8kDa.

[0035] Figure 23| Amino acid sequence of the CDR3 of VHH 5C8 (SEQ ID NO:19) and the modifications introduced to avoid sulfation and/or truncation (SEQ ID NO:36). Y105 (sulfated) was changed to F and the 108 and 109 positions were changed to either A (108 and 109) or K (109).

Also, next to the R109A mutation, the sequence LGIR of the CDR3 region was deleted (("LGIR, "Truncated").

[0036] Figure 24| Affinity measurement of VHH fragment binding to recombinant Vγ9Vδ2-TCR protein using BLI. The protein mass (response, in nm) is plotted as a function of time. The dotted vertical line separates the association phase (left) from the dissociation phase (right). A: VHH 5C8var1-Y105F; B: VHH 5C8var1-Y105F/G108A; C: VHH 5C8var1-Y105F/R109A; D: VHH 5C8var1-Y105F/R109K; E: VHH 5C8var1-Y105F/R109A/"LGIR.

[0037] Figure 25| Binding of bispecific CD123xVδ2 binding, Fc-containing molecules containing position 108 and 109 variants of 5C8 to primary, expanded Vγ9Vδ2 T cells as measured by flow cytometry. The strength of the fluorescent signal measured in FACS is plotted as a function of the concentration of antibody used.

[0038] Figure 26| Fc-containing bispecific molecule-induced Vγ9Vδ2 T cell activation as measured by the appearance of the degranulation marker LAMP-1 (i.e. CD107A) on the surface of the Vγ9Vδ2T cells. After a co-culture of 24 hours of Vγ9Vδ2 T cells and tumor target cells (1:1 E:T ratio), the percentage of CD107A expressing Vγ9Vδ2 T cells (degranulating, activated Vγ9Vδ2 T cells) was measured by flowcytometry as a function of the concentration of compound used in the assay. Two target cell lines were used: MOLM-13 and THP-1 (as indicated).

[0039] Fig. 27A – Fig. 27B show Fc-containing anti-CD123 x Vδ2 bispecific molecule-dependent, Vγ9Vδ2 T cell-mediated tumor cell cytotoxicity. The percentage of living (7AAD negative) cells was determined using flow cytometry after a 24 hours co-culture of the Vγ9Vδ2 T cells and tumor target cells (1:1 E:T ratio) and is plotted as a function of the concentration of compound used. Fig. 27A shows results with MOLM-13 cells. Fig. 27B shows results with THP-1 cells.

[0040] Fig. 28A – Fig. 28B shows analysis of purified protein by CE-SDS (capillary electrophoresis). Shown are electropherograms of 1D2-Fc x 5C8var1-Y105F-Fc (Fig. 28A) and 1D2-Fc x 5C8var1-Y105F-R109A-Fc (Fig. 28B).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0041] The term "human Vδ2", when used herein, refers to the rearranged δ2 chain of the Vγ9Vδ2-T cell receptor (TCR). UniProtKB - A0JD36 (A0JD36_HUMAN) gives an example of a variable TRDV2 sequence.

[0042] The term "human Vγ9", when used herein, refers to the rearranged γ9 chain of the Vγ9Vδ2-T cell receptor (TCR). UniProtKB – Q99603_HUMAN gives an example of a variable TRGV9 sequence.

[0043] The term "EGFR", when used herein, refers to the human EGFR protein (UniProtKB - P00533 (EGFR_HUMAN)).

[0044] The term "CD123", when used herein, refers to the human CD123 protein, also termed interleukin-3 receptor alpha chain (GenBank accession number NM_002183.4, NCBI Reference Sequence: NP_002174.1). The IL3 receptor is a heterodimer of CD123 with CD131, a common beta chain (NCBI Reference Sequence: NP_000386.1).

[0045] The term "PSMA", when used herein, refers to the human Prostate-Specific Membrane Antigen protein (UniProtKB - Q04609 (FOLH1_HUMAN)).

[0046] The term "CD40", when used herein, refers to the CD40 protein, also known as tumor necrosis factor receptor superfamily member 5 (UniProtKB - P25942 (TNR5_HUMAN)), Isoform I.

[0047] The term "CD1d", when used herein, refers to the human CD1d protein (UniProtKB - P15813 (CD1D_HUMAN)).

[0048] The term "Nectin 4" when used herein, refers to the human Nectin 4 protein (UniProtKB – Q96NY8 (NECT4_HUMAN)).

[0049] The term "antibody" is intended to refer to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions with a half-life of significant periods of time, such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen and/or time sufficient for the antibody to recruit an effector activity). The antigen-binding region (or antigen-binding domain) which interacts with an antigen may comprise variable regions of both the heavy and light chains of the immunoglobulin molecule or may be a single-domain antigen-binding region, e.g. a heavy chain variable region only. The constant regions of an antibody, if present, may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells and T cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation.

[0050] The Fc region of an immunoglobulin is defined as the fragment of an antibody which would be typically generated after digestion of an antibody with papain which includes the two CH2-CH3 regions of an immunoglobulin and a connecting region, e.g. a hinge region. The constant domain of an antibody heavy chain defines the antibody isotype, e.g. IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, or IgE. The Fc-region mediates the effector functions of antibodies with cell surface receptors called Fc receptors and proteins of the complement system.

[0051] The term “hinge region” as used herein is intended to refer to the hinge region of an immunoglobulin heavy chain. Thus, for example, the hinge region of a human IgG1 antibody corresponds to amino acids 216-230 according to the EU numbering.

[0052] The term “CH2 region” or “CH2 domain” as used herein is intended to refer to the CH2 region of an immunoglobulin heavy chain. Thus, for example the CH2 region of a human IgG1 antibody corresponds to amino acids 231-340 according to the EU numbering. However, the CH2 region may also be any of the other subtypes as described herein.

[0053] The term “CH3 region” or “CH3 domain” as used herein is intended to refer to the CH3 region of an immunoglobulin heavy chain. Thus, for example the CH3 region of a human IgG1 antibody corresponds to amino acids 341-447 according to the EU numbering. However, the CH3 region may also be any of the other subtypes as described herein.

[0054] Reference to amino acid positions in the Fc region/Fc domain in the present invention is according to the EU-numbering (Edelman et al., Proc Natl Acad Sci U S A. 1969 May;63(1):78-85; Kabat et al., Sequences of proteins of immunological interest. 5th Edition - 1991 NIH Publication No. 91-3242).

[0055] As indicated above, the term antibody as used herein, unless otherwise stated or clearly contradicted by context, includes fragments of an antibody that retain the ability to specifically bind to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antibody" include (i) a Fab’ or Fab fragment, i.e. a monovalent fragment consisting of the VL, VH, CL and CH1 domains, or a monovalent antibody as described in WO2007059782; (ii) F(ab')2 fragments, i.e. bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the VH and CH1 domains; and (iv) a Fv fragment consisting essentially of the VL and VH domains of a single arm of an antibody. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for

instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. The term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies and humanized antibodies, and antibody fragments provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques.

[0056] In some embodiments of the antibodies of the invention, the first antigen-binding region or the second antigen-binding region, or both, is a single domain antibody. Single domain antibodies are well known to the skilled person, see e.g. Hamers-Casterman et al. (1993) Nature 363:446, Roovers et al. (2007) Curr Opin Mol Ther 9:327 and Krah et al. (2016) Immunopharmacol Immunotoxicol 38:21. Single domain antibodies comprise a single CDR1, a single CDR2 and a single CDR3. Examples of single domain antibodies are variable fragments of heavy-chain-only antibodies, antibodies that naturally do not comprise light chains, single domain antibodies derived from conventional antibodies, and engineered antibodies. Single domain antibodies may be derived from any species including mouse, human, camel, llama, shark, goat, rabbit, and cow. For example, single domain antibodies can be derived from antibodies raised in Camelidae species, for example in camel, dromedary, llama, alpaca and guanaco. Like a whole antibody, a single domain antibody is able to bind selectively to a specific antigen. Single domain antibodies may contain only the variable domain of an immunoglobulin chain, i.e. CDR1, CDR2 and CDR3 and framework regions. Such antibodies are also called Nanobody® or VHH.

[0057] The term “immunoglobulin” as used herein is intended to refer to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds. The term “immunoglobulin heavy chain”, “heavy chain of an immunoglobulin” or “heavy chain” as used herein is intended to refer to one of the chains of an immunoglobulin. A heavy chain is typically comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH) which defines the isotype of the immunoglobulin. The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chain constant region further comprises a hinge region. Within the structure of the immunoglobulin (e.g. IgG), the two heavy chains are inter-connected via disulfide bonds in the hinge region. Equally to the heavy chains, each light chain is typically comprised of several

regions; a light chain variable region (VL) and a light chain constant region (CL). Furthermore, the VH and VL regions may be subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. CDR sequences may be determined by use of various methods, e.g. the methods provided by Chothia and Lesk (1987) J. Mol. Biol. 196:901 or Kabat et al. (1991) Sequence of protein of immunological interest, fifth edition. NIH publication. Various methods for CDR determination and amino acid numbering can be compared on www.abysis.org (UCL).

[0058] The term “isotype” as used herein, refers to the immunoglobulin (sub)class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) or any allotype thereof, such as IgG1m(za) and IgG1m(f) that is encoded by heavy chain constant region genes. Each heavy chain isotype can be combined with either a kappa (κ) or lambda (λ) light chain. An antibody of the invention can possess any isotype.

[0059] The term “parent antibody”, is to be understood as an antibody which is identical to an antibody according to the invention, but wherein the parent antibody does not have one or more of the specified mutations. A “variant” or “antibody variant” or a “variant of a parent antibody” of the present invention is an antibody molecule which comprises one or more mutations as compared to a “parent antibody”. Amino acid substitutions may exchange a native amino acid for another naturally-occurring amino acid, or for a non-naturally-occurring amino acid derivative. The amino acid substitution may be conservative or non-conservative. In the context of the present invention, conservative substitutions may be defined by substitutions within the classes of amino acids reflected in one or more of the following three tables:

Amino acid residue classes for conservative substitutions

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

Alternative conservative amino acid residue substitution classes

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

Alternative Physical and Functional Classifications of Amino Acid Residues

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

[0060] In the context of the present invention, a substitution in a variant is indicated as: Original amino acid – position – substituted amino acid. The three-letter code, or one letter code, are used, including the codes Xaa and X to indicate amino acid residue. Accordingly, the notation “T366W” means that the variant comprises a substitution of threonine with tryptophan in the variant amino acid position corresponding to the amino acid in position 366 in the parent antibody.

[0061] Furthermore, the term “a substitution” embraces a substitution into any one of the other nineteen natural amino acids, or into other amino acids, such as non-natural amino acids. For example, a substitution of amino acid T in position 366 includes each of the following substitutions: 366A, 366C, 366D, 366G, 366H, 366F, 366I, 366K, 366L, 366M, 366N, 366P, 366Q, 366R, 366S, 366E, 366V, 366W, and 366Y.

[0062] The term “full-length antibody” when used herein, refers to an antibody which contains all heavy and light chain constant and variable domains corresponding to those that are normally found in a wild-type antibody of that isotype.

[0063] The term “chimeric antibody” refers to an antibody wherein the variable region is derived from a non-human species (e.g. derived from rodents) and the constant region is derived from a different species, such as human. Chimeric antibodies may be generated by genetic engineering. Chimeric monoclonal antibodies for therapeutic applications are developed to reduce antibody immunogenicity.

[0064] The term “humanized antibody” refers to a genetically engineered non-human antibody, which contains human antibody constant domains and non-human variable domains modified to contain a high level of sequence homology to human variable domains. This can be achieved by grafting of the six non-human antibody complementarity-determining regions (CDRs), which together form the antigen binding site, onto a homologous human acceptor framework region (FR). In order to fully reconstitute the binding affinity and specificity of the parental antibody, the substitution of framework residues from the parental antibody (i.e. the non-human antibody) into the human framework regions (back-mutations) may be required. Structural homology modeling may help to identify the amino acid residues in the framework regions that are important for the binding properties of the antibody. Thus, a humanized antibody may comprise non-human CDR sequences, primarily human framework regions optionally comprising one or more amino acid back-mutations to the non-human amino acid sequence, and, optionally, fully human constant regions. Optionally, additional amino acid modifications, which are not necessarily back-mutations, may be introduced to obtain a humanized antibody with preferred characteristics, such as affinity and biochemical properties. Humanization of non-human therapeutic antibodies is performed to minimize its immunogenicity in man while such humanized antibodies at the same time maintain the specificity and binding affinity of the antibody of non-human origin.

[0065] The term “multispecific antibody” refers to an antibody having specificities for at least two different, such as at least three, typically non-overlapping, epitopes. Such epitopes may be on the same or on different target antigens. If the epitopes are on different targets, such targets may be on the same cell or different cells or cell types. In some embodiments, a multispecific antibody may comprise one or more single-domain antibodies.

[0066] The term “bispecific antibody” refers to an antibody having specificities for two different, typically non-overlapping, epitopes. Such epitopes may be on the same or different targets. If the epitopes are on different targets, such targets may be on the same cell or different cells or cell types. In some embodiments, a bispecific antibody may comprise one or two single-domain antibodies.

[0067] Examples of different classes of multispecific, such as bispecific, antibodies include but are not limited to (i) IgG-like molecules with complementary CH3 domains to force

heterodimerization; (ii) recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; (iii) IgG fusion molecules, wherein full length IgG antibodies are fused to extra Fab fragment or parts of Fab fragment; (iv) Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant- domains, Fc-regions or parts thereof; (v) Fab fusion molecules, wherein different Fab- fragments are fused together, fused to heavy-chain constant-domains, Fc-regions or parts thereof; and (vi) ScFv-and diabody-based and heavy chain antibodies (e.g., domain antibodies, Nanobodies®) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, Nanobodies®) are fused to each other or to another protein or carrier molecule fused to heavy-chain constant-domains, Fc-regions or parts thereof.

[0068] Examples of IgG-like molecules with complementary CH3 domains molecules include but are not limited to the Triomab® (Trion Pharma/Fresenius Biotech), the Knobs-into-Holes (Genentech), CrossMAbs (Roche) and the electrostatically-matched (Amgen, Chugai, Oncomed), the LUZ-Y (Genentech, Wranik et al. J. Biol. Chem. 2012, 287(52): 43331-9, doi: 10.1074/jbc.M112.397869. Epub 2012 Nov 1), DIG-body and PIG-body (Pharmabcine, WO2010134666, WO2014081202), the Strand Exchange Engineered Domain body (SEEDbody)(EMD Serono), the Biclonics (Merus, WO2013157953), FcΔAdp (Regeneron), bispecific IgG1 and IgG2 (Pfizer/Rinat), Azymetric scaffold (Zymeworks/Merck), mAb-Fv (Xencor), bivalent bispecific antibodies (Roche, WO2009080254) and DuoBody® molecules (Genmab).

[0069] Examples of recombinant IgG-like dual targeting molecules include but are not limited to Dual Targeting (DT)-Ig (GSK/Domantis, WO2009058383), Two-in-one Antibody (Genentech, Bostrom, et al 2009. Science 323, 1610–1614), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star), ZybodiesTM (Zyngenia, LaFleur et al. MAbs. 2013 Mar-Apr;5(2):208-18), approaches with common light chain, κλBodies (NovImmune, WO2012023053) and CovX-body® (CovX/Pfizer, Doppalapudi, V.R., et al 2007. Bioorg. Med. Chem. Lett. 17,501–506).

[0070] Examples of IgG fusion molecules include but are not limited to Dual Variable Domain (DVD)-Ig (Abbott), Dual domain double head antibodies (Unilever; Sanofi Aventis), IgG-like Bispecific (ImClone/Eli Lilly, Lewis et al. Nat Biotechnol. 2014 Feb;32(2):191-8), Ts2Ab (MedImmune/AZ, Dimasi et al. J Mol Biol. 2009 Oct 30;393(3):672-92) and BsAb (Zymogenetics, WO2010111625), HERCULES (Biogen Idec), scFv fusion (Novartis), scFv fusion (Changzhou Adam Biotech Inc) and TvAb (Roche).

[0071] Examples of Fc fusion molecules include but are not limited to ScFv/Fc Fusions (Academic Institution, Pearce et al Biochem Mol Biol Int. 1997 Sep;42(6):1179), SCORPION (Emergent BioSolutions/Trubion, Blankenship JW, et al. AACR 100th Annual meeting 2009 (Abstract #5465); Zymogenetics/BMS, WO2010111625), Dual Affinity Retargeting Technology (Fc-DARTTM) (MacroGenics) and Dual(ScFv)2-Fab (National Research Center for Antibody Medicine – China).

[0072] Examples of Fab fusion bispecific antibodies include but are not limited to F(ab)2 (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock® (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech).

[0073] Examples of ScFv-, diabody-based and domain antibodies include but are not limited to Bispecific T Cell Engager (BiTE®) (Micromet, Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DARTTM) (MacroGenics), Single-chain Diabody (Academic, Lawrence FEBS Lett. 1998 Apr 3;425(3):479-84), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack, WO2010059315) and COMBODY molecules (Epigen Biotech, Zhu et al. Immunol Cell Biol. 2010 Aug;88(6):667-75), dual targeting nanobodies® (Ablynx, Hmila et al., FASEB J. 2010), dual targeting heavy chain only domain antibodies.

[0074] In the context of antibody binding to an antigen, the terms “binds” or “specifically binds” refer to the binding of an antibody to a predetermined antigen or target to which binding typically is with an apparent affinity corresponding to a KD of about 10-6 M or less, e.g. 10-7 M or less, such as about 10-8 M or less, such as about 10-9 M or less, about 10-10 M or less, or about 10-11 M or even less, e.g. when determined using flow cytometry as described in the Examples herein. Alternatively, KD values can be determined using for instance surface plasmon resonance (SPR) technology in a BIAcore T200 or bio-layer interferometry (BLI) in an Octet RED96 instrument using the antigen as the ligand and the binding moiety or binding molecule as the analyte. Specific binding means that the antibody binds to the predetermined antigen with an affinity corresponding to a KD that is at least ten-fold lower, such as at least 100-fold lower, for instance at least 1,000-fold lower, such as at least 10,000-fold lower, for instance at least 100,000-fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The degree with which the affinity is lower is dependent on the KD of the binding moiety or binding molecule, so that when the KD of the binding moiety or binding molecule is very low (that is, the binding moiety or binding molecule is highly specific), then the degree with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000-fold. The term "KD" (M), as used herein, refers to the dissociation equilibrium constant of a particular interaction between the antigen and the binding moiety or binding molecule.

[0075] In the context of the present invention, "competition" or "able to compete" or "competes" refers to any detectably significant reduction in the propensity for a particular binding molecule to bind a particular binding partner in the presence of another molecule that binds the binding partner. Typically, competition means an at least about 25 percent reduction, such as an at least about 50 percent, e.g. an at least about 75 percent, such as an at least 90 percent reduction in binding, caused by the presence of another molecule, such as an antibody, as determined by, e.g., ELISA analysis or flow cytometry using sufficient amounts of the two or more competing molecules, e.g. antibodies. Additional methods for determining binding specificity by competitive inhibition may be found in for instance Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc, and Wiley InterScience N. Y., (1992, 1993), and Muller, Meth. Enzymol. 92, 589-601 (1983)).

[0076] In one embodiment, the antibody of the present invention binds to the same epitope on EGFR as antibody 7D12, to the same epitope on CD123 as antibody 1D2, to the same epitope on CD1d as antibody 1D12, to the same epitope on PSMA as antibody LV1050, to the same epitope on CD40 as antibody v19, to the same epitope on Nectin 4 as antibody LV1184, LV1181, LV1185, LV1186, LV1178, LV1183, or LV1187, or to the same epitope on Vδ2 as antibody 5C8 or a variant thereof. There are several methods available for mapping antibody epitopes on target antigens known in the art, including but not limited to: crosslinking coupled mass spectrometry, allowing identification of peptides that are part of the epitope, and X-ray crystallography identifying individual residues on the antigen that form the epitope. Epitope residues can be determined as being all amino acid residues with at least one atom less than or equal to 5 Å from the antibody. 5 Å was chosen as the epitope cutoff distance to allow for atoms within a van der Waals radius plus a possible water-mediated hydrogen bond. Next, epitope residues can be determined as being all amino acid residues with at least one atom less than or equal to 8 Å. Less than or equal to 8 Å is chosen as the epitope cutoff distance to allow for the length of an extended arginine amino acid. Crosslinking coupled mass spectrometry begins by binding the antibody and the antigen with a mass labeled chemical crosslinker. Next the presence of the complex is confirmed using high mass MALDI detection. Because after crosslinking chemistry the Ab/Ag complex is extremely stable, many various enzymes and digestion conditions can be applied to the complex to provide many different overlapping peptides. Identification of these peptides is performed using high resolution mass spectrometry and MS/MS techniques. Identification of the crosslinked peptides is determined using mass tag linked to the cross-linking reagents. After MS/MS fragmentation and data analysis, peptides that are crosslinked and are derived from the antigen

are part of the epitope, while peptides derived from the antibody are part of the paratope. All residues between the most N- and C-terminal crosslinked residue from the individual crosslinked peptides found are considered to be part of the epitope or paratope. The epitope of antibody 7D12 has been determined by X-ray crystallography, described in Schmitz et al. (2013) Structure 21:1214 and consists of a flat surface on domain III (residues R353, D355, F357, Q384, N420) that corresponds to the domain III ligand-binding site.

[0077] The terms “first” and “second” antigen-binding regions when used herein do not refer to their orientation / position in the antibody, i.e. they have no meaning with regard to the N- or C-terminus. The terms “first” and “second” only serve to correctly and consistently refer to the two different antigen-binding regions in the claims and the description.

[0078] “% sequence identity”, when used herein, refers to the number of identical nucleotide or amino acid positions shared by different sequences (i.e., % identity = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci 4, 11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Further aspects and embodiments of the invention

[0079] As described above, in a first aspect, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence as set forth in SEQ ID NO:12, a CDR2 sequence as set forth in SEQ ID NO:13 and a CDR3 sequence as set forth in SEQ ID NO:14.

[0080] In one embodiment, X1 in SEQ ID NO:12 is S (Ser). In another embodiment, X1 in SEQ ID NO:12 is G (Gly).

[0081] In one embodiment, X2 in SEQ ID NO:14 is selected from the group consisting of: A, C, D, E, G, H, F, I, K, L, M, N, P, Q, R, S, T, V, W and Y. In one embodiment, X2 in SEQ ID NO: 14 is F or S.

[0082] In one embodiment, X2 in SEQ ID NO:14 is selected from the group consisting of: A, C, D, E, G, H, F, I, K, L, M, N, Q, R, S, T, V, W and Y.

[0083] In one embodiment, X2 in SEQ ID NO:14 is F (Phe). In another embodiment, X2 in SEQ ID NO:14 is S (Ser).

[0084] In one embodiment, X1 in SEQ ID NO:12 is S and X2 in SEQ ID NO:3 is F.

[0085] In one embodiment, X1 in SEQ ID NO:12 is S and X2 in SEQ ID NO:3 is S.

[0086] In one embodiment, X1 in SEQ ID NO:12 is G and X2 in SEQ ID NO:3 is F.

[0087] In one embodiment, X1 in SEQ ID NO:12 is G and X2 in SEQ ID NO:3 is S.

[0088] In one embodiment, X3 in SEQ ID NO:14 is selected from the group consisting of: A, C, D, E, G, H, F, I, K, L, M, N, P, Q, S, T, V, W and Y.

[0089] In one embodiment, X3 in SEQ ID NO:14 is selected from the group consisting of: A, C, D, E, G, H, F, I, K, L, M, N, Q, S, T, V, W and Y.

[0090] In one embodiment, X3 in SEQ ID NO:14 is A (Ala). In another embodiment, X3 in SEQ ID NO:14 is K (Lys).

[0091] In one embodiment, X1 in SEQ ID NO:12 is S and X3 in SEQ ID NO:14 is A.

[0092] In one embodiment, X1 in SEQ ID NO:12 is S and X3 in SEQ ID NO:14 is K.

[0093] In one embodiment, X1 in SEQ ID NO:12 is G and X3 in SEQ ID NO:14 is A.

[0094] In one embodiment, X1 in SEQ ID NO:12 is G and X3 in SEQ ID NO:14 is K.

[0095] In one embodiment, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is A.

[0096] In one embodiment, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is K.

[0097] In one embodiment, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is A.

[0098] In one embodiment, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is K.

[0099] In one embodiment:

(a) X1 in SEQ ID NO:12 is S and X2 in SEQ ID NO:14 is F,

(b) X1 in SEQ ID NO:12 is S and X2 in SEQ ID NO:14 is S,

(c) X1 in SEQ ID NO:12 is S and X3 in SEQ ID NO:14 is A, or

(d) X1 in SEQ ID NO:12 is S and X3 in SEQ ID NO:14 is K.

[0100] In one embodiment, X1 in SEQ ID NO:12 is S, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is A.

[0101] In one embodiment, X1 in SEQ ID NO:12 is S, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is K.

[0102] In one embodiment, X1 in SEQ ID NO:12 is S, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is A.

[0103] In one embodiment, X1 in SEQ ID NO:12 is S, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is K.

[0104] In one embodiment, X1 in SEQ ID NO:12 is G, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is A.

[0105] In one embodiment, X1 in SEQ ID NO:12 is G, X2 in SEQ ID NO:14 is F and X3 in SEQ ID NO:14 is K.

[0106] In one embodiment, X1 in SEQ ID NO:12 is G, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is A.

[0107] In one embodiment, X1 in SEQ ID NO:12 is G, X2 in SEQ ID NO:14 is S and X3 in SEQ ID NO:14 is K.

[0108] In one embodiment:

(a) X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is F (Phe) and X3 in SEQ ID NO:14 is A (Ala), or

(b) X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is F (Phe) and X3 in SEQ ID NO:14 is K (Lys).

[0109] In one embodiment:

(a) X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is S (Ser) and X3 in SEQ ID NO:14 is A (Ala), or

(b) X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is S (Ser) and X3 in SEQ ID NO:14 is K (Lys).

[0110] In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:17. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:21. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 29.

[0111] In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:18. In some embodiments, the antibody comprises a first antigen binding

region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 26. In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:22. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 30.

[0112] In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:19. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 27. In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:23. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 31.

[0113] In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:20. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 28. In some embodiments, the invention relates to an antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:24. In some embodiments, the antibody comprises a first antigen binding region capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 32.

[0114] In a preferred embodiment, the antibody is able to activate human Vγ9Vδ2 T cells. Activation of Vγ9Vδ2 T cells may be measured through measuring alterations in gene-expression and/or (surface) marker expression (e.g., activation markers, such as CD25, CD69, or CD107a) and/or secretory protein (e.g., cytokines or chemokines) profiles. In a preferred embodiment, the antibody is able to induce activation (e.g. upregulation of CD69 and/or CD25 expression) resulting

in degranulation marked by an increase in CD107a expression and/or cytokine production (e.g. TNF, IFNγ) by Vγ9Vδ2 T cells.

[0115] In a further preferred embodiment, the antibody is able to increase the number of cells positive for CD107a at least 2-fold, such as at least 5-fold, when tested as described in Example 9 herein, e.g. at a concentration of 1nM, preferably 100pM, preferably 10pM, preferably 1pM, even more preferably 100fM. In another preferred embodiment, the antibody of the invention has an EC50 value for increasing the percentage of CD107a positive cells of 100 pM or less, such as 50 pM or less, e.g. 25 pM or less, such as 20 pM or less, e.g. 15 pM or less when tested using Vγ9Vδ2 T cells and A431 target cells as described herein in Example 9.

[0116] In one embodiment, the first antigen-binding region is a single-domain antibody. Thus, in one embodiment, the antibody of the invention comprises a single-domain antibody capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence as set forth in SEQ ID NO:12, a CDR2 sequence as set forth in SEQ ID NO:13 and a CDR3 sequence as set forth in SEQ ID NO:14.

[0117] In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:17. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 25. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:21. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 29.

[0118] In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:18. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:22. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 30.

[0119] In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:19. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 27. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:23. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 31.

[0120] In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:20. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence of SEQ ID NO:24. In some embodiments, the antibody comprises a single-domain antibody capable of binding to human Vδ2 and comprising or consisting of the amino acid sequence of SEQ ID NO: 32.

[0121] In another embodiment, the first antigen-binding region is humanized, wherein preferably the antigen-binding region comprises or consists of: (a) the sequence set forth in SEQ ID NO:16, or (b) a sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to a sequence as set forth in SEQ ID NO:16, wherein X1 is S (Ser), X2 is F (Phe) and X3 is A (Ala). In another embodiment, the first antigen-binding region is humanized and comprises or consists of SEQ ID NO: 25, 26, 27, or 28. In another embodiment, the first antigen-binding region is humanized and comprises or consists of SEQ ID NO: 29, 30, 31, or 32.

[0122] In some embodiments, the antibody of the invention is a multispecific antibody, such as a bispecific antibody. Thus, in one embodiment, the antibody further comprises a second antigen-binding region. In one embodiment, the second antigen-binding region is a single-domain antibody.

[0123] In a further embodiment, the antibody is a bispecific antibody wherein both the first antigen-binding region and the second antigen-binding region are single-domain antibodies. In a further embodiment, the multispecific antibody is a bispecific antibody, wherein the first antigen-binding region is a single-domain antibody and the second antigen-binding region is a single-domain antibody. In one embodiment, the second antigen-binding region is humanized.

[0124] In some embodiments, the antibody comprises a second antigen-binding region capable of binding human EGFR. Bispecific antibodies targeting both Vγ9Vδ2-T cells and EGFR have been shown to induce potent Vγ9Vδ2 T cell activation and tumor cell lysis both in vitro and in an in vivo mouse xenograft model (de Bruin et al. (2018) Oncoimmunology 1, e1375641).

[0125] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:33, the CDR2 sequence set forth in SEQ ID NO:34, and the CDR3 sequence set forth in SEQ ID NO:35. In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting SEQ ID NO:36, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO:36.

[0126] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human EGFR with an antibody having the sequence set forth in SEQ ID NO:36, preferably the second antigen-binding region binds the same epitope on human EGFR as an antibody having the sequence set forth in SEQ ID NO:36.

[0127] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 33, a CDR2 sequence comprising SEQ ID NO: 34, and a CDR3 sequence comprising SEQ ID NO: 35. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 33, a CDR2 sequence comprising SEQ ID NO: 34, and a CDR3 sequence comprising SEQ ID NO: 35.

[0128] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 36. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from

SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 36.

[0129] In a further embodiment, the antibody is capable of mediating killing of human EGFR-expressing cells. In a preferred embodiment, the antibody is able to increase Vγ9Vδ2 T cell mediated killing of EGFR expressing cells, such as A431 cell at least 25%, such as at least 50%, e.g. at least 2-fold, when tested as described in Example 9 herein.

[0130] In a further embodiment, the antibody is not capable of mediating killing of EGFR-negative cells, such as EGFR negative human cells.

[0131] In one embodiment, the antibody of the invention comprises a second antigen-binding region capable of binding human CD123.

[0132] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:37, the CDR2 sequence set forth in SEQ ID NO:38, and the CDR3 sequence set forth in SEQ ID NO:39. In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting SEQ ID NO:40, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO:40.

[0133] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human CD123 with an antibody having the sequence set forth in SEQ ID NO:40, preferably the second antigen-binding region binds the same epitope on human CD123 as an antibody having the sequence set forth in SEQ ID NO:40.

[0134] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 37, a CDR2 sequence comprising SEQ ID NO: 38, and a CDR3 sequence comprising SEQ ID NO: 39. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 37, a CDR2 sequence comprising SEQ ID NO: 38, and a CDR3 sequence comprising SEQ ID NO: 39.

[0135] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 40. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from

SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 40.

[0136] In some embodiments, the antibody comprises a second antigen-binding region capable of binding human PSMA. Multispecific antibodies having antigen-binding regions capable of binding a human PSMA suitable for use in the present invention have for example been described in WO 2022/008646 (herein incorporated by reference).

[0137] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:41, the CDR2 sequence set forth in SEQ ID NO:42, and the CDR3 sequence set forth in SEQ ID NO:43. In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting SEQ ID NO:44, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO:44.

[0138] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human PSMA with an antibody having the sequence set forth in SEQ ID NO:44, preferably the second antigen-binding region binds the same epitope on human PSMA as an antibody having the sequence set forth in SEQ ID NO:44.

[0139] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 41, a CDR2 sequence comprising SEQ ID NO: 42, and a CDR3 sequence comprising SEQ ID NO: 43. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 41, a CDR2 sequence comprising SEQ ID NO: 42, and a CDR3 sequence comprising SEQ ID NO: 43.

[0140] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 44. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 44.

[0141] In some embodiments, the antibody comprises a second antigen-binding region capable of binding human CD1d. Antibodies having antigen-binding regions capable of binding a human

CD1d suitable for incorporation into a multispecific antibody to be used in the invention have for example been described in WO 2016/122320 and WO 2020/060405 (both herein incorporated by reference).

[0142] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:45, the CDR2 sequence set forth in SEQ ID NO:46, and the CDR3 sequence set forth in SEQ ID NO:47. In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting SEQ ID NO:48, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO:48.

[0143] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human CD1d with an antibody having the sequence set forth in SEQ ID NO:48, preferably the second antigen-binding region binds the same epitope on human CD1d as an antibody having the sequence set forth in SEQ ID NO:48.

[0144] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 45, a CDR2 sequence comprising SEQ ID NO: 46, and a CDR3 sequence comprising SEQ ID NO: 47. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 45, a CDR2 sequence comprising SEQ ID NO: 46, and a CDR3 sequence comprising SEQ ID NO: 47.

[0145] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 48. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 48.

[0146] In some embodiments, the antibody comprises a second antigen-binding region capable of binding human CD40. Antibodies having antigen-binding regions capable of binding a human CD40 suitable for incorporation into a multispecific antibody to be used in the invention have for example been described in WO 2020/159368 (herein incorporated by reference).

[0147] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:49, the CDR2 sequence set forth in SEQ ID NO:50, and the CDR3 sequence set forth in SEQ ID NO:51. In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting SEQ ID NO:52, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO:52.

[0148] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human CD40 with an antibody having the sequence set forth in SEQ ID NO:52, preferably the second antigen-binding region binds the same epitope on human CD40 as an antibody having the sequence set forth in SEQ ID NO:52.

[0149] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 49 a CDR2 sequence comprising SEQ ID NO: 50, and a CDR3 sequence comprising SEQ ID NO: 51. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 49, a CDR2 sequence comprising SEQ ID NO: 50, and a CDR3 sequence comprising SEQ ID NO: 51.

[0150] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 52. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 52.

[0151] In some embodiments, the antibody comprises a second antigen-binding region capable of binding human Nectin 4.

[0152] In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:53, the CDR2 sequence set forth in SEQ ID NO:54, and the CDR3 sequence set forth in SEQ ID NO:55. In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:57, the CDR2 sequence set forth in SEQ ID NO:58, and the CDR3 sequence set forth in SEQ ID NO:59. In some embodiments, the antibody comprises a second antigen-binding region

comprising the CDR1 sequence set forth in SEQ ID NO:61, the CDR2 sequence set forth in SEQ ID NO:62, and the CDR3 sequence set forth in SEQ ID NO:63. In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:65, the CDR2 sequence set forth in SEQ ID NO:66, and the CDR3 sequence set forth in SEQ ID NO:67. In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:69, the CDR2 sequence set forth in SEQ ID NO:70, and the CDR3 sequence set forth in SEQ ID NO:71. In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:73, the CDR2 sequence set forth in SEQ ID NO:74, and the CDR3 sequence set forth in SEQ ID NO:75. In some embodiments, the antibody comprises a second antigen-binding region comprising the CDR1 sequence set forth in SEQ ID NO:77, the CDR2 sequence set forth in SEQ ID NO:78, and the CDR3 sequence set forth in SEQ ID NO:79.

[0153] In some embodiments, the antibody comprises a second antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO:56, 60, 64, 68, 72, 76, and 80, or comprising an amino acid sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80.

[0154] In some embodiments, the second antigen-binding region competes (i.e. is able to compete) for binding to human Nectin 4 with an antibody having the sequence set forth in SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80, preferably the second antigen-binding region binds the same epitope on human Nectin 4 as an antibody having the sequence set forth in SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80.

[0155] In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 17, 18, 19, and 20; and (b) a second antigen-binding region comprising a CDR1 sequence selected from SEQ ID NO: 49, 53, 57, 61, 65, 69, 73, and 77, a CDR2 sequence comprising SEQ ID NO: 50, 54, 58, 62, 66, 70, 74, and 78, and a CDR3 sequence comprising SEQ ID NO: 51, 55, 59, 63, 67, 71, 75, and 79. In some embodiments, the antibody comprises (a) a first antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 8, a CDR2 sequence comprising SEQ ID NO: 13, and a CDR3 sequence selected from SEQ ID NO: 21, 22, 23, and 24; and (b) a second antigen-binding region comprising a CDR1 sequence comprising SEQ ID NO: 49, 53, 57, 61, 65, 69, 73, and 77, a CDR2 sequence comprising SEQ ID NO: 50, 54, 58, 62, 66, 70, 74, and 78, and a CDR3 sequence comprising SEQ ID NO: 51, 55, 59, 63, 67, 71, 75, and 79.

[0156] In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 25, 26, 27, and 28 and a second antigen-binding region comprising or consisting of SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80. In some embodiments, the antibody comprises a first antigen-binding region comprising or consisting of a sequence selected from SEQ ID NO: 29, 30, 31, and 32 and a second antigen-binding region comprising or consisting of SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80.

[0157] Exemplary combinations of binding domains are provided in Table A below.

Table A: Exemplary binding domain combinations

First antigen-binding domain					Second antigen-binding domain				
Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)	Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)
Vδ2	8	13	17	25	EGFR	33	34	35	36
Vδ2	8	13	18	26	EGFR	33	34	35	36
Vδ2	8	13	19	27	EGFR	33	34	35	36
Vδ2	8	13	20	28	EGFR	33	34	35	36
Vδ2	8	13	21	29	EGFR	33	34	35	36
Vδ2	8	13	22	30	EGFR	33	34	35	36
Vδ2	8	13	23	31	EGFR	33	34	35	36
Vδ2	8	13	24	32	EGFR	33	34	35	36
Vδ2	8	13	17	25	CD123	37	38	39	40
Vδ2	8	13	18	26	CD123	37	38	39	40
Vδ2	8	13	19	27	CD123	37	38	39	40
Vδ2	8	13	20	28	CD123	37	38	39	40
Vδ2	8	13	21	29	CD123	37	38	39	40
Vδ2	8	13	22	30	CD123	37	38	39	40
Vδ2	8	13	23	31	CD123	37	38	39	40
Vδ2	8	13	24	32	CD123	37	38	39	40
Vδ2	8	13	17	25	PSMA	41	42	43	44
Vδ2	8	13	18	26	PSMA	41	42	43	44
Vδ2	8	13	19	27	PSMA	41	42	43	44
Vδ2	8	13	20	28	PSMA	41	42	43	44
Vδ2	8	13	21	29	PSMA	41	42	43	44
Vδ2	8	13	22	30	PSMA	41	42	43	44
Vδ2	8	13	23	31	PSMA	41	42	43	44
Vδ2	8	13	24	32	PSMA	41	42	43	44
Vδ2	8	13	17	25	CD1d	45	46	47	48
Vδ2	8	13	18	26	CD1d	45	46	47	48
Vδ2	8	13	19	27	CD1d	45	46	47	48
Vδ2	8	13	20	28	CD1d	45	46	47	48
Vδ2	8	13	21	29	CD1d	45	46	47	48
Vδ2	8	13	22	30	CD1d	45	46	47	48

First antigen-binding domain					Second antigen-binding domain				
Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)	Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)
Vδ2	8	13	23	31	CD1d	45	46	47	48
Vδ2	8	13	24	32	CD1d	45	46	47	48
Vδ2	8	13	17	25	CD40	49	50	51	52
Vδ2	8	13	18	26	CD40	49	50	51	52
Vδ2	8	13	19	27	CD40	49	50	51	52
Vδ2	8	13	20	28	CD40	49	50	51	52
Vδ2	8	13	21	29	CD40	49	50	51	52
Vδ2	8	13	22	30	CD40	49	50	51	52
Vδ2	8	13	23	31	CD40	49	50	51	52
Vδ2	8	13	24	32	CD40	49	50	51	52
Vδ2	8	13	17	25	Nectin 4	53	54	55	56
Vδ2	8	13	18	26	Nectin 4	53	54	55	56
Vδ2	8	13	19	27	Nectin 4	53	54	55	56
Vδ2	8	13	20	28	Nectin 4	53	54	55	56
Vδ2	8	13	21	29	Nectin 4	53	54	55	56
Vδ2	8	13	22	30	Nectin 4	53	54	55	56
Vδ2	8	13	23	31	Nectin 4	53	54	55	56
Vδ2	8	13	24	32	Nectin 4	53	54	55	56
Vδ2	8	13	17	25	Nectin 4	57	58	59	60
Vδ2	8	13	18	26	Nectin 4	57	58	59	60
Vδ2	8	13	19	27	Nectin 4	57	58	59	60
Vδ2	8	13	20	28	Nectin 4	57	58	59	60
Vδ2	8	13	21	29	Nectin 4	57	58	59	60
Vδ2	8	13	22	30	Nectin 4	57	58	59	60
Vδ2	8	13	23	31	Nectin 4	57	58	59	60
Vδ2	8	13	24	32	Nectin 4	57	58	59	60
Vδ2	8	13	17	25	Nectin 4	61	62	63	64
Vδ2	8	13	18	26	Nectin 4	61	62	63	64
Vδ2	8	13	19	27	Nectin 4	61	62	63	64
Vδ2	8	13	20	28	Nectin 4	61	62	63	64
Vδ2	8	13	21	29	Nectin 4	61	62	63	64
Vδ2	8	13	22	30	Nectin 4	61	62	63	64

First antigen-binding domain					Second antigen-binding domain				
Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)	Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)
Vδ2	8	13	23	31	Nectin 4	61	62	63	64
Vδ2	8	13	24	32	Nectin 4	61	62	63	64
Vδ2	8	13	17	25	Nectin 4	65	66	67	68
Vδ2	8	13	18	26	Nectin 4	65	66	67	68
Vδ2	8	13	19	27	Nectin 4	65	66	67	68
Vδ2	8	13	20	28	Nectin 4	65	66	67	68
Vδ2	8	13	21	29	Nectin 4	65	66	67	68
Vδ2	8	13	22	30	Nectin 4	65	66	67	68
Vδ2	8	13	23	31	Nectin 4	65	66	67	68
Vδ2	8	13	24	32	Nectin 4	65	66	67	68
Vδ2	8	13	17	25	Nectin 4	69	70	71	72
Vδ2	8	13	18	26	Nectin 4	69	70	71	72
Vδ2	8	13	19	27	Nectin 4	69	70	71	72
Vδ2	8	13	20	28	Nectin 4	69	70	71	72
Vδ2	8	13	21	29	Nectin 4	69	70	71	72
Vδ2	8	13	22	30	Nectin 4	69	70	71	72
Vδ2	8	13	23	31	Nectin 4	69	70	71	72
Vδ2	8	13	24	32	Nectin 4	69	70	71	72
Vδ2	8	13	17	25	Nectin 4	73	74	75	76
Vδ2	8	13	18	26	Nectin 4	73	74	75	76
Vδ2	8	13	19	27	Nectin 4	73	74	75	76
Vδ2	8	13	20	28	Nectin 4	73	74	75	76
Vδ2	8	13	21	29	Nectin 4	73	74	75	76
Vδ2	8	13	22	30	Nectin 4	73	74	75	76
Vδ2	8	13	23	31	Nectin 4	73	74	75	76
Vδ2	8	13	24	32	Nectin 4	73	74	75	76
Vδ2	8	13	17	25	Nectin 4	77	78	79	80
Vδ2	8	13	18	26	Nectin 4	77	78	79	80
Vδ2	8	13	19	27	Nectin 4	77	78	79	80
Vδ2	8	13	20	28	Nectin 4	77	78	79	80
Vδ2	8	13	21	29	Nectin 4	77	78	79	80
Vδ2	8	13	22	30	Nectin 4	77	78	79	80

First antigen-binding domain					Second antigen-binding domain				
Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)	Target antigen	CDR1 (SEQ ID)	CDR2 (SEQ ID)	CDR3 (SEQ ID)	VHH (SEQ ID)
Vδ2	8	13	23	31	Nectin 4	77	78	79	80
Vδ2	8	13	24	32	Nectin 4	77	78	79	80

[0158] In one embodiment, the antibody comprises a first antigen-binding region and a second antigen-binding region wherein the first antigen-binding region and second antigen-binding region are covalently linked via a peptide linker, e.g. a linker having a length of from 1 to 20 amino acids, e.g. from 1 to 10 amino acids, such as 2, 3, 4, 5, 6, 7, 8 or 10 amino acids. In one embodiment, the peptide linker comprises or consists of the sequence GGGGS, set forth in SEQ ID NO:81.

[0159] In another embodiment, the antibody comprises a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region capable of binding human Vδ2 is located C-terminally of the second antigen-binding region capable of binding a human EGFR, CD123, PSMA, CD1d, CD40, or Nectin 4. In another embodiment, the antibody comprises a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region capable of binding human Vδ2 is located N-terminally of the second antigen-binding region capable of binding a human EGFR, CD123, PSMA, CD1d, CD40, or Nectin 4

[0160] In one embodiment of the invention, the antibody further comprises a half-life extension domain. In one embodiment, the antibody has a terminal half-life that is longer than about 168 hours when administered to a human subject. Most preferably the terminal half-life is 336 hours or longer. The “terminal half-life” of an antibody, when used herein refers to the time taken for the serum concentration of the polypeptide to be reduced by 50%, in vivo in the final phase of elimination.

[0161] In one embodiment, the antibody further comprises a half-life extension domain and the half-life extension domain is an Fc region. In a further embodiment, the antibody is a multispecific antibody, such as a bispecific antibody comprising an Fc region. Various methods for making bispecific antibodies have been described in the art, e.g. reviewed by Brinkmann and Kontermann (2017) MAbs 9:182. In one embodiment of the present invention, the Fc region is a heterodimer comprising two Fc polypeptides, wherein the first antigen-binding region is fused to the first Fc polypeptide and the second antigen-binding region is fused to the second Fc polypeptide and wherein the first and second Fc polypeptides comprise asymmetric amino acid mutations that favor the formation of heterodimers over the formation of homodimers. (see e.g. Ridgway et al. (1996) 'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng 9:617). In a further embodiment hereof, the CH3 regions of the Fc polypeptides comprise said asymmetric amino acid mutations, preferably the first Fc polypeptide comprises a T366W substitution and the second Fc polypeptide comprises T366S, L368A and Y407V substitutions, or vice versa, wherein the amino acid positions correspond to

human IgG1 according to the EU numbering system. In a further embodiment, the cysteine residues at position 220 in the first and second Fc polypeptides have been deleted or substituted, wherein the amino acid position corresponds to human IgG1 according to the EU numbering system. In a further embodiment, the region comprises the hinge sequence set forth in SEQ ID NO:82.

[0162] In some embodiments, the first and/or second Fc polypeptides contain mutations that render the antibody inert, i.e. unable to, or having reduced ability to, mediate effector functions. In one embodiment, the inert Fc region is in addition not able to bind C1q. In one embodiment, the first and second Fc polypeptides comprise a mutation at position 234 and/or 235, preferably the first and second Fc polypeptide comprise an L234F and an L235E substitution, wherein the amino acid positions correspond to human IgG1 according to the EU numbering system. In another embodiment, the antibody contains a L234A mutation, a L235A mutation and a P329G mutation. In another embodiment, the antibody contains a L234F mutation, a L235E mutation and a D265A mutation.

[0163] In a preferred embodiment, the first antigen-binding region comprises the sequence set forth in SEQ ID NO:16, the second antigen-binding region comprises a sequence selected from SEQ ID NO: 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76 and 80 and (a) the first Fc polypeptide comprises the sequence set forth in SEQ ID NO:83 and the second Fc polypeptide comprises the sequence set forth in SEQ ID NO:84, or (b) the first Fc polypeptide comprises the sequence set forth in SEQ ID NO:84 and the second Fc polypeptide comprises the sequence set forth in SEQ ID NO:83.

[0164] In one embodiment, X7 in SEQ ID NO:83 is not present. In another embodiment, X7 in SEQ ID NO: 83 is present and is a K (lysine).

[0165] In one embodiment, X7 in SEQ ID NO:84 is not present. In another embodiment, X7 in SEQ ID NO:87 is present and is a K (lysine).

[0166] In a further preferred embodiment, the antibody comprises or consists of: (a) the sequence set forth in SEQ ID NO:85, and (b) a sequence selected from the group of sequences set forth in: SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89 and SEQ ID NO:90.

[0167] In a further preferred embodiment, the antibody comprises or consists of: (a) the sequence set forth in SEQ ID NO:86, and (b) a sequence selected from the group of sequences set forth in: SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89 and SEQ ID NO:90.

[0168] In one embodiment, X7 in SEQ ID NO:85 is not present. In another embodiment, X7 in SEQ ID NO:85 is present and is a K (lysine).

[0169] In one embodiment, X7 in SEQ ID NO:86 is not present. In another embodiment, X7 in SEQ ID NO:86 is present and is a K (lysine).

[0170] In one embodiment, X7 in SEQ ID NO:87 is not present. In another embodiment, X7 in SEQ ID NO:22 is present and is a K (lysine).

[0171] In one embodiment, X7 in SEQ ID NO:87 is not present. In another embodiment, X7 in SEQ ID NO:23 is present and is a K (lysine).

[0172] In one embodiment, X7 in SEQ ID NO:88 is not present. In another embodiment, X7 in SEQ ID NO:24 is present and is a K (lysine).

[0173] In one embodiment, X7 in SEQ ID NO:88 is not present. In another embodiment, X7 in SEQ ID NO:25 is present and is a K (lysine).

[0174] In a further main aspect, the invention relates to a pharmaceutical composition comprising an antibody according to the invention as described herein and a pharmaceutically-acceptable excipient.

[0175] Antibodies may be formulated with pharmaceutically-acceptable excipients in accordance with conventional techniques such as those disclosed in Rowe et al. 2012 Handbook of Pharmaceutical Excipients, ISBN 9780857110275). The pharmaceutically-acceptable excipient as well as any other carriers, diluents or adjuvants should be suitable for the antibodies and the chosen mode of administration. Suitability for excipients and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen antibody or pharmaceutical composition of the present invention (e.g., less than a substantial impact (10% or less relative inhibition, 5% or less relative inhibition, etc.) upon antigen binding).

[0176] A pharmaceutical composition may include diluents, fillers, salts, buffers, detergents (e.g., a nonionic detergent, such as Tween-20 or Tween-80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition. Further pharmaceutically-acceptable excipients include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption-delaying agents and the like that are physiologically compatible with an antibody of the present invention.

[0177] In a further main aspect, the invention relates to the antibody according to the invention as described herein for use as a medicament.

[0178] An antibody according to the invention enables creating a microenvironment that is beneficial for killing of tumor cells by Vγ9Vδ2 T cells. Accordingly, in a preferred embodiment, the antibody is for use in the treatment of cancer.

[0179] In one embodiment, the antibody targets EGFR and is for use in the treatment of primary or metastatic colon or rectal cancer. In another embodiment, the antibody is for use in the treatment of cancer of the peritoneum. In another embodiment, the antibody is for use in the treatment of liver cancer. In another embodiment, the antibody is for use in the treatment of head and neck squamous cell carcinoma (HNSCC). In another embodiment, the antibody is for use in the treatment of non-small cell lung carcinoma (NSCLC). In another embodiment, the antibody is for use in the treatment of squamous cell carcinoma of the skin.

[0180] In another embodiment, the antibody targets CD123 and is for use in the treatment of acute myeloid leukemia, B-cell acute lymphoblastic leukemia, hairy cell leukemia, Hodgkin lymphoma, blastic plasmacytoid dendritic cell neoplasm, chronic myeloid leukemia, chronic lymphocytic leukemia, B-cell chronic lymphoproliferative disorders or myelodysplastic syndrome.

[0181] In another embodiment, the antibody targets CD1d and is for use in the treatment of hematological malignancies such as T cell lymphoma, multiple myeloma, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, mantle cell lymphoma, B cell lymphoma, smoldering myeloma, Hodgkin lymphoma, myelomonocytic leukemias, lymphoplasmacytic lymphoma, hairy cell leukemia, or splenic marginal zone lymphoma, or solid tumors, such as renal cell carcinoma, melanoma, colorectal carcinoma, head and neck cancer, breast cancer, prostate cancer, lung cancer, pancreatic cancer, gastro-esophageal cancer, small bowel carcinoma, central nervous system tumors, medulloblastomas, hepatocellular carcinoma, ovarian cancer, glioma, neuroblastoma, urothelial carcinomas, bladder cancer, sarcoma, penile cancer, basal cell carcinoma, merkel cell carcinoma, neuroendocrine carcinoma, neuroendocrine tumors, carcinoma of unknown primary (CUP), thymoma, vulvar cancer, cervical carcinoma, testicular cancer, cholangiocarcinoma, appendicular carcinoma, mesothelioma, ampullary carcinoma, anal cancer or choriocarcinoma.

[0182] n another embodiment, the antibody targets PSMA and is for use in the treatment of prostate cancer, such as non-metastatic or metastatic prostate cancer, for example metastatic castration resistant prostate cancer, such as therapy refractory metastatic castration resistant prostate cancer. For example, the antibody may comprise a first antigen-binding region capable of binding PSMA and be used for the treatment of prostate cancer in patients that have failed at least one line of taxane-based chemotherapy and optionally have received a 2nd generation or later androgen receptor targeted therapy/androgen biosynthesis inhibitor (e.g. abiraterone, enzalutamide, and/or apalutamide).

[0183] Furthermore, the antibody capable of binding PSMA may be for use in the treatment of cancers in which PSMA is expressed on the tumor neo-vasculature or tumor-associated

endothelial cells of primary or metastatic tumors including from colorectal cancer, lung cancer, breast cancer, endometrial and ovarian cancer, gastric cancer, renal cell cancer, urothelial cancer, hepatocellular cancer, oral squamous cancer, thyroid tumors and glioblastomas. Moreover, the antibody capable of binding PSMA may be for use in the treatment of adenoid cystic carcinoma of the head and neck.

[0184] Similarly, the invention relates to a method of treating a disease comprising administration of a multispecific antibody according to the invention as described herein to a human subject in need thereof. In one embodiment, the disease is cancer.

[0185] In some embodiments, the antibody is administered as monotherapy. However, antibodies of the present invention may also be administered in combination therapy, i.e., combined with other therapeutic agents relevant for the disease or condition to be treated.

[0186] “Treatment” or “treating” refers to the administration of an effective amount of an antibody according to the present invention with the purpose of easing, ameliorating, arresting, eradicating (curing) or preventing symptoms or disease states. An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. An effective amount of a polypeptide, such as an antibody, may vary according to factors such as the disease stage, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. Administration may be carried out by any suitable route, but will typically be parenteral, such as intravenous, intramuscular or subcutaneous.

[0187] Multispecific antibodies of the invention are typically produced recombinantly, i.e. by expression of nucleic acid constructs encoding the antibodies in suitable host cells, followed by purification of the produced recombinant antibody from the cell culture. Nucleic acid constructs can be produced by standard molecular biological techniques well-known in the art. The constructs are typically introduced into the host cell using an expression vector. Suitable nucleic acid constructs and expression vectors are known in the art. Host cells suitable for the recombinant expression of antibodies are well-known in the art, and include CHO, HEK-293, Expi293F, PER-C6, NS/0 and Sp2/0 cells.

[0188] Accordingly, in a further aspect, the invention relates to a nucleic acid construct encoding an antibody according to the invention. In one embodiment, the construct is a DNA construct. In another embodiment, the construct is an RNA construct.

[0189] In a further aspect, the invention relates to an expression vector comprising a nucleic acid construct encoding an antibody according to the invention.

[0190] In a further aspect, the invention relates to a host cell comprising one or more nucleic acid constructs encoding an antibody according to the invention or an expression vector comprising a nucleic acid construct encoding an antibody according to the invention.

[0191] In a further aspect, the invention relates to a process for manufacturing an antibody of the invention, comprising expressing one or more nucleic acids encoding the antibody according to the invention in a host cell.

[0192] In a further aspect, the invention the relates to a process for manufacturing a clinical batch of antibody of the invention, comprising expressing one or more nucleic acids encoding the antibody according to the invention in a host cell. A "clinical batch" when used herein refers to a product composition that is suitable for use in humans.

[0193] In a further aspect, the invention relates to a process for manufacturing an antibody free of proteolysis and/or tyrosine sulfation, comprising expressing one or more nucleic acids encoding the antibody according to the invention in a host cell.

[0194] In a further aspect, the invention relates to a process for avoiding proteolysis and/or tyrosine sulfation of an antibody capable of activating human Vγ9Vδ2 T cells, said process comprising constructing a nucleic acid encoding an antibody of the invention and producing said antibody by expression said nucleic acid in a host cell.

[0195] In a further aspect, the invention relates to a process for producing a homogeneous antibody preparation of an antibody capable of activating human Vγ9Vδ2 T cells, said process comprising constructing a nucleic acid encoding an antibody of the invention and producing said antibody by expression said nucleic acid in a host cell.

[0196] In one embodiment, the host cell in the above manufacturing process is a mammalian cell, such as a CHO cell or a HEK cell, or a yeast cell, such as a Pichia pastoris cell.

Table 1: Sequence listing

SEQ ID	code	Description	Sequence
1	5C8	CDR1	NYAMG
2	5C8	CDR2	AISWSGGSTSYADSVKG
3	5C8	CDR3	QFSGADYGFGRLGIRGYEYDY
4	5C8	VHH	EVQLVESGGGLVQAGGSLRLSCAASGRPFSNYA MGWFRQAPGKEREFVAAISWSGGSTSYADSVKG RFTISRDNAKNTVYLQMNSPKPEDTAIYYCAAQ FSGADYGFGRLGIRGYEYDYWGQGTQVTVSS
5	5C8 var	CDR1	NYAMX_1, wherein X_1 is S or G
6	5C8 var	CDR2	AISWSGGSTSYADSVKG
7	5C8 var	CDR3	QFSGADYGFGRLGIRGYEYDY
8	5C8 var1	CDR1	NYAMS

SEQ ID	code	Description	Sequence
9	5C8 var1	CDR2	AISWSGGSTSYADSVKG
10	5C8 var1	CDR3	QFSGADYGFGRLGIRGYEYDY
11	5C8var1	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADYGFGRLGIRGYEYDYWGQGTQVTVSS
12	5C8 var (Y105+R109)	CDR1	NYAMX$_1$, wherein X$_1$ is S or G
13	5C8 var (Y105+R109)	CDR2	AISWSGGSTSYADSVKG
14	5C8 var (Y105+R109)	CDR3	QFSGADX$_2$GFGX$_3$LGIRGYEYDY, wherein X$_2$ can be any amino acid and wherein X$_3$ is not R
15	5C8 var (Y105+R109)ΔLGIR	CDR3	QFSGADX$_2$GFGX$_3$GYEYDY, wherein X$_2$ can be any amino acid and wherein X$_3$ is not R
16	5C8 var (Y105+R109)	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MX$_1$WFRQAPGKEREFVSAISWSGGSTSYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA QFSGADX$_2$GFGX$_3$LGIRGYEYDYWGQGTQVTVS S, wherein X$_1$ is S or G, wherein X$_2$ can be any amino acid and wherein X$_3$ is not R
17	5C8 var1 (Y105F+R109A)	CDR3	QFSGADFGFGALGIRGYEYDY
18	5C8 var1 (Y105S+R109A)	CDR3	QFSGADSGFGALGIRGYEYDY
19	5C8 var1 (Y105F+R109K)	CDR3	QFSGADFGFGKLGIRGYEYDY
20	5C8 var1 (Y105S+R109K)	CDR3	QFSGADSGFGKLGIRGYEYDY
21	5C8 var1 (Y105F+R109A) ΔLGIR	CDR3	QFSGADFGFGAGYEYDY
22	5C8 var1 (Y105S+R109A) ΔLGIR	CDR3	QFSGADSGFGAGYEYDY
23	5C8 var1 (Y105F+R109K) ΔLGIR	CDR3	QFSGADFGFGKGYEYDY
24	5C8 var1 (Y105S+R109K) ΔLGIR	CDR3	QFSGADSGFGKGYEYDY
25	5C8 var1 (Y105F+R109A)	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGALGIRGYEYDYWGQGTQVTVSS
26	5C8 var1 (Y105S+R109A)	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGALGIRGYEYDYWGQGTQVTVSS
27	5C8 var1 (Y105F+R109K)	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG

SEQ ID	code	Description	Sequence
			RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGKLGIRGYEYDYWGQGTQVTVSS
28	5C8 var1 (Y105S+R109K)	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGKLGIRGYEYDYWGQGTQVTVSS
29	5C8 var1 (Y105F+R109A) ΔLGIR	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGAGYEYDYWGQGTQVTVSS
30	5C8 var1 (Y105S+R109A) ΔLGIR	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGAGYEYDYWGQGTQVTVSS
31	5C8 var1 (Y105F+R109K) ΔLGIR	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGKGYEYDYWGQGTQVTVSS
32	5C8 var1 (Y105S+R109K) ΔLGIR	VHH	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGKGYEYDYWGQGTQVTVSS
33	7D12 (EGFR)	CDR1	SYGMG
34	7D12 (EGFR)	CDR2	GISWRGDSTGYADSVKG
35	7D12 (EGFR)	CDR3	AAGSAWYGTLYEYDY
36	7D12var8 (EGFR)	VHH	EVQLVESGGGSVQPGGSLRLSCAASGRTSRSYG MGWFRQAPGKEREFVSGISWRGDSTGYADSVKG RFTISRDNAKNTVDLQMNSLRAEDTAVYYCAAA AGSAWYGTLYEYDYWGQGTLVTVSS
37	1D2 (CD123)	CDR1	GRTASSYVMX$_4$, wherein X$_4$ is G or S
38	1D2 (CD123)	CDR2	VINWNGDSTYYX$_5$DSVKG, wherein X$_5$ is A or T
39	1D2 (CD123)	CDR3	DTRREWYRDGX$_6$WGPPARYEYDY, wherein X$_6$ is Y or F
40	1D2 (CD123)	VHH	EVQLVESGGGLVQAGGSLRLSCAASGRTASSYV MGWFRQAPGKEREFVAVINWNGDSTYYTDSVKG RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTQVTVSS
41	LV1050 (PSMA)	CDR1	RFMISEYSMH
42	LV1050 (PSMA)	CDR2	TINPAGTTDYADSVKG
43	LV1050 (PSMA)	CDR3	DGYGY
44	LV1050 (PSMA)	VHH	EVQLVESGGGLVQPGGSLRLSCAASRFMISEYS MHWVRQAPGKGLEWVSTINPAGTTDYADSVKGR FTISRDNAKNTLYLQMNSLRAEDTAVYYCDGYG YRGQGTQVTVSS
45	1D12 (CD1d)	CDR1	DNVMG

SEQ ID	code	Description	Sequence
46	1D12 (CD1d)	CDR2	TIRTGGSTNYADSVKG
47	1D12 (CD1d)	CDR3	TIPVPSTPYDY
48	1D12 (CD1d)	VHH	QVQLVESGGGLVQAGGSLRLSCAASGSMFSDNV MGWYRQAPGKQRELVATIRTGGSTNYADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCRHTI PVPSTPYDYWGQGTQVTVSS
49	v19 (CD40)	CDR1	RSAMG
50	v19 (CD40)	CDR2	AIGTRGGSTKYADSVKG
51	v19 (CD40)	CDR3	RGPGYPSAAIFQDEYHY
52	v19 (CD40)	VHH	QVQLQESGGGLVQAGGSLRLSCAASGRTFGRSA MGWFRQAPGKEREFVAAIGTRGGSTKYADSVKG RFTISTDNASNTVYLQMDSLKPEDTAVYRCAVR GPGYPSAAIFQDEYHYWGQGTQVTVSS
53	LV1184 (Nectin 4)	CDR1	INLMG
54	LV1184 (Nectin 4)	CDR2	SISPGGSVRYADSVKG
55	LV1184 (Nectin 4)	CDR3	ESERTYYFDS
56	LV1184 (Nectin 4)	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSISSINL MGWFRQAPAKQRELVTSISPGGSVRYADSVKGR FTISRDVAKNTVDLQMNSLKPEDTAVYYCAAES ERTYYFDSWGQGTQVTVSS
57	LV1181 (Nectin 4)	CDR1	TITRGGSTNYADSVKG
58	LV1181 (Nectin 4)	CDR2	VEPSGMGWRDY
59	LV1181 (Nectin 4)	CDR3	EVQLVESGGGLVQAGGSLRLSCAASGSISS
60	LV1181 (Nectin 4)	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSISSINL MGWHRQAPGKQRELVATITRGGSTNYADSVKGR FTISRDNGKNTVYLQMNSLKPEDTAAYYCVDVE PSGMGWRDYWGQGTQVTVSS
61	LV1185 (Nectin 4)	CDR1	LNIMG
62	LV1185 (Nectin 4)	CDR2	TITTGGSTNYADSVRG
63	LV1185 (Nectin 4)	CDR3	ELVRRGPTTY
64	LV1185 (Nectin 4)	VHH	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNI MGWYRQAPGKQREYVATITTGGSTNYADSVRGR FTISRDNAEDTVYLQMNSLKPEDTAVYYCTAEL VRRGPTTYWGRGTQVTVSS
65	LV1186 (Nectin 4)	CDR1	LNIMG
66	LV1186 (Nectin 4)	CDR2	TITSGGSTNYADSVRG
67	LV1186 (Nectin 4)	CDR3	ELVRRGPTTY
68	LV1186 (Nectin 4)	VHH	EVQLVESGGGLVQAGGSLRLSCAASRSTFSLNI MGWYRQAPGKQREYVATITSGGSTNYADSVRGR FTISRDNAENTVYLQMDSLKPEDTAVYYCTAEL VRRGPTTYWGRGTQVTVSS
69	LV1178 (Nectin 4)	CDR1	RLTMG
70	LV1178 (Nectin 4)	CDR2	RVYASGGLTDYADSVKG
71	LV1178 (Nectin 4)	CDR3	GLWADMRTMTSTRGY

SEQ ID	code	Description	Sequence
72	LV1178 (Nectin 4)	VHH	EVQLVESGGALVQAGGSLRLSCSVSGLTASRLT MGWFRQAPGKEREFVARVYASGGLTDYADSVKG RFTISRDNAKNTVYLQMNSLEPEDTAVYYCVAG LWADMRTMTSTRGYWGQGTQVTVSS
73	LV1183 (Nectin 4)	CDR1	INLMG
74	LV1183 (Nectin 4)	CDR2	SITRGGSTWYVDSVKG
75	LV1183 (Nectin 4)	CDR3	ESIGSYTFDR
76	LV1183 (Nectin 4)	VHH	EVQLVESGGGLVQAGGSLRLSCAASGSIFDINL MGWYRQAPGKLRELVASITRGGSTWYVDSVKGR FIISRDSAKNTVYLQMNSLKPGDTAVYYCAAES IGSYTFDRWGQGTQVTVSS
77	LV1187 (Nectin 4)	CDR1	INVMG
78	LV1187 (Nectin 4)	CDR2	GITSGGSRRLADSVKG
79	LV1187 (Nectin 4)	CDR3	EAVVGDEPY
80	LV1187 (Nectin 4)	VHH	EVQLVESGGGLVQAGGFLRLSCAASRSVFRINV MGWYRQAPGKQRELVAGITSGGSRRLADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCFAEA VVGDEPYWGQGTQVTVSS
81	linker		GGGGS
82		Modified hinge	AAASDKTHTCPPCP
83	IgG1 L234F L235E T366W	Heavy chain constant region variant	AAASDKTHTCPPCPAPEFEGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX $_{7}$, wherein X_{7} is a K that may or may not be present
84	IgG1 L234F L235E T366S L368A Y407V	Heavy chain constant region variant	AAASDKTHTCPPCPAPEFEGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX $_{7}$, wherein X_{7} is a K that may or may not be present
85	7D12var8-Fc	VHH-Fc	EVQLVESGGGSVQPGGSLRLSCAASGRTSRSYG MGWFRQAPGKEREFVSGISWRGDSTGYADSVKG RFTISRDNAKNTVDLQMNSLRAEDTAVYYCAAA AGSAWYGTLYEYDYWGQGTLVTVSSAAASDKTH TCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNV

SEQ ID	code	Description	Sequence
			FSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
86	1D2-Fc	VHH-Fc	EVQLVESGGGLVQAGGSLRLSCAASGRTASSYV MGWFRQAPGKEREFVAVINWNGDSTYYTDSVKG RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTQVTVSSA AASDKTHTCPPCPAPEFEGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
87	5C8var1 (Y105F R109A)-Fc	VHH-Fc	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGALGIRGYEYDYWGQGTQVTVSSAA ASDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
88	5C8var1 (Y105S R109A)-Fc	VHH-Fc	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGALGIRGYEYDYWGQGTQVTVSSAA ASDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
89	5C8var1 (Y105F R109K)-Fc	VHH-Fc	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADFGFGKLGIRGYEYDYWGQGTQVTVSSAA ASDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK

SEQ ID	code	Description	Sequence
			EYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
90	5C8var1 (Y105S R091K)-Fc	VHH-Fc	EVQLLESGGGSVQPGGSLRLSCAASGRPFSNYA MSWFRQAPGKEREFVSAISWSGGSTSYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAQ FSGADSGFGKLGIRGYEYDYWGQGTQVTVSSAA ASDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGX$_7$, wherein X$_7$ is a K that may or may not be present
91	1D2 var1	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MGWFRQAPGKEREFVSVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTQVTVSS
92	1D2 var2	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MGWVRQAPGKEREWVSVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
93	1D2 var3	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MSWFRQAPGKEREWVAVINWNGDSTYYADSVKG RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
94	1D2 var4	VHH	EVQLVESGGGVVQPGGSLRLSCAASGRTASSYV MSWFRQAPGKEREWVAVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTTVTVSS
95	1D2 var5	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MSWFRQAPGKEREFVAVINWNGDSTYYADSVKG RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
96	1D2 var6	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MGWVRQAPGKGLEWVSVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
97	1D2 var7	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MSWVRQAPGKGLEWVSVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
98	1D2 var8	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MSWFRQAPGKGLEWVAVINWNGDSTYYADSVKG

SEQ ID	code	Description	Sequence
			RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTLVTVSS
99	1D2 var9	VHH	EVQLVESGGGLVQAGGSLRLSCAASGRTASSYV MGWFRQAPGKEREWVAVINWNGDSTYYTDSVKG RFAISRDNAKNTVYLQMNSLRAEDTAVYYCAAD TRREWYRDGYWGPPARYEYDYRGQGTQVTVSS
100	1D2 var10	VHH	EVQLVESGGGLVQPGGSLRLSCAASGRTASSYV MGWFRQAPGKEREFVSVINWNGDSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAD TRREWYRDGFWGPPARYEYDYRGQGTQVTVSS

[0197] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application herein is not, and should not be, taken as acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

EXAMPLES

Example 1 | Production and purification of VHH compounds

[0198] VHH compounds were mostly produced by transient transfection of the encoding plasmids in HEK293-E 253 cells and purification of the proteins from the conditioned medium (after a week of production) by protein-A affinity chromatography, followed by preparative gel filtration. The dominant monomeric peak (fractions 1E11-1G2) observed in preparative size exclusion using a Superdex-75 column was purified: Figure 1.

[0199] Purified proteins were shown to migrate as a single band under reducing- and non-reducing conditions in polyacrylamide gel electrophoresis: Figure 2 shows a representative example.

Example 2 | HP-SEC analysis of purified VHH compounds

[0200] The Waters Acquity ARC-bio system was used for HP-SEC analysis of purified VHH compounds. 10 µg of antibody (10µL of antibody with a concentration of 1 mg/mL) was injected on a Waters BEH200 SEC column (bead size 2.5 µm, column dimensions 7.8 x 300 mm). The mobile phase consisted of 50 mM Sodium Phosphate, 0.2 M Sodium Chloride buffer pH 7.0 and

a buffer velocity of 0.8 mL/min was used for the run. Protein was detected by measuring absorption at a wavelength of 214 nm. The total analysis time was 15 minutes per injection. VHH compounds were produced and purified as described in Example 1. Surprisingly, when VHH 5C8 (SEQ ID NO:13) (previously described in WO2015156673) and 5C8var1 (SEQ ID NO:14) (previously described in WO2020060405) were tested in HP-SEC analysis for integrity and monomericity, two peaks were observed: Figure 3.

Example 3 | Mass spec analysis of 5C8 reveals an additional mass of 80Da

[0201] To determine whether the two isoforms of 5C8 differed in mass, the protein preparation of 5C8 was analyzed by LC-ESI-MS Mass spectrometry. The main species found in this analysis was 5C8 without post translational modifications or signal peptide. A second species was a protein with a mass difference of + 80.3 Dalton (Da), which indicated possible sulfation or phosphorylation. This was further investigated by treatment with phosphatase or sulfatase and subsequent LC-ESI-MS mass spec analysis of peptides after proteolytic digestion. It was shown that sulfatase treatment reduced the mass of the peptides containing Y105, the 7th residue of the CDR3 (SEQ ID NO:15) by 80Da, whereas phosphatase treatment had no effect. This proves the Y105 in 5C8 to be post-translationally modified by sulfation. This sulfation was present in roughly 30% of the protein preparation.

Example 4 | 1D12var5-5C8var1 containing the same anti-Vδ2 VHH shows the same heterogeneity in HP-SEC and the same extra mass of 80Da

[0202] 1D12var5-5C8var1 is a bispecific VHH compound composed of an anti-CD1d VHH, coupled via a flexible linker to 5C8var1 (described in SEQ ID NO:87 in WO2020060405). This protein was expressed in HEK 293E cells as described above. In addition, protein preparations were obtained from different expression systems: the bispecific VHH was also expressed in Pichia pastoris and in Chinese hamster ovarian (CHO) cells. When different protein preparations were tested in HP-SEC analysis, a pre-peak was systematically observed: Figure 4.

[0203] The pre-peak observed is indicative of a significant percentage of the protein being a different isoform again. As the 5C8 VHH was shown to be sulphated and the 5C8var1 contains the exact same CDR3 sequence, the 1D12var5-5C8var1 batches were also analysed by mass spectrometry for their molecular weight. Dependent on the protein batch, between 15% and 40% was found to contain an additional mass of 80Da. This is consistent with a sulfation, as observed for VHH 5C8.

Example 5 | In silico analysis of VHH 5C8 and 5C8var1

[0204] A homology model of 5C8 and 5C8var1 was built using Maestro (Schrödinger) based on PDB ID 5M2W. CDR1 and CDR3 required refinement by de novo loop prediction using Prime (Schrödinger). The generated models demonstrated that CDR3 residue Y105 shows a high solvent-accessibility surface area of 205.1 Å2 in the model of 5C8var1 and 122.2 Å2 in the model of 5C8 and is therefore expected to contribute to antigen binding. Subsequently, the models were analyzed for reactive residues, indicating residues that are prone to post-translational modifications (PTM). Next, the protein sequences were analyzed using ModPred, a sequence based PTM prediction tool. Modifications that were predicted in both structure and sequence are listed in table 2. The individual predicted PTMs could not explain the mass difference observed in HP-SEC analyses.

Table 2 | Predicted PTMs in Maestro and ModPred for 5C8 and 5C8var1.

Residue number	Residue	Type
13*	Q	Deamidation
32	Y	Oxidation
39	Q	Deamidation
60	Y	Oxidation
62	D	Proteolysis (ASP)
73	D	Proteolysis (ASP)
74	N	Deamidation
80	Y	Oxidation
84	N	Deamidation
90	D	Proteolysis (ASP)
94	Y	Oxidation
95	Y	Oxidation
104	D	Proteolysis (ASP)
105	Y	Oxidation
115	Y	Oxidation
117	Y	Oxidation
118	D	Proteolysis (ASP)
119	Y	Oxidation
122	Q	Deamidation

**Q13 deamidation was only predicted for 5C8. Type describes the PTM as predicted by Maestro.*

Example 6 | Design and production of 5C8var1 VHH CDR3 mutants Y105F and Y105S

[0205] The homology models, as described in Example 5, were used to introduce mutations that would prevent sulfation of Y105 in 5C8 and 5C8var1. Two different mutants were designed on the basis of a model structure of the VHH: Y105S (retaining the alcohol function) and Y105F (retaining the aromatic ring). Residue Y105 is the 7th residue of CDR3; by introducing mutations, an effect on binding was expected. Both mutations were designed in the humanized VHH sequence 5C8var1 and both proteins were produced in HEK293E cells and purified as described above. The CDR3 amino acid sequence of the humanized VHH was kept identical to the one of the non-humanized.

[0206] Both 5C8var1-Y105F and 5C8var1-Y105S were well produced and appeared as monomeric proteins in preparative size exclusion (data not shown). Both proteins were highly pure (Figure 5) and migrated as a single species in polyacrylamide gel electrophoresis.

Example 7 | HP-SEC analysis of purified VHH containing designed CDR3 mutations

[0207] HP-SEC analysis was performed as described for 5C8. Both 5C8var1-Y105F as well as 5C8var1-Y105S were analyzed (Figure 6).

[0208] As can be concluded from the HP-SEC analysis of the purified VHH molecules containing the designed CDR3 mutations, no heterogeneity was observed anymore for either mutant. This indicated that the observed Y105 post translational modification was absent and the proteins were homogeneous.

Example 8 | Affinity measurement of 5C8var1, 5C8var1-Y105F and 5C8va1-Y105S using Biolayer Interferometry (BLI) shows no difference in affinity

[0209] Binding of the 5C8var1 VHH antibody fragment and variants 5C8var1-Y105F and 5C8var1-Y105S to the Vy9Vδ2 TCR was measured by biolayer interferometry using an Octet RED96 instrument (ForteBio). Recombinant human Vy9Vδ2-Fc fusion protein (20 µg/ml) was captured as ligand on anti-human Fc capture biosensors. Sensorgrams were recorded when ligand captured biosensors were incubated with a dilution series of VHH antibody fragments (40 to 0.63 nM) in 10X kinetics buffer (ForteBio). Global data fitting to a 1:1 binding model was used to estimate values for the kon (association rate constant) and koff (dissociation rate constant). These values were used to calculate the KD (equilibrium dissociation constant) using KD = koff/kon.

[0210] As can be concluded from figure 7 and from Table 3, the KD values found for the two different Y105 VHH mutants did not differ substantially from the value found for 5C8var1. Especially the Y105F mutant had a comparable affinity to that found for 5C8var1.

Table 3 | Affinity values found in BLI measurements of 5C8var1, 5C8var1-Y105F and 5C8var1-Y105S VHH binding to recombinant Vγ9Vδ2 TCR protein.

VHH compound	KD (nM) +/- SD
5C8var1	0.81 +/- 0.02
5C8var1-Y105F	0.78 +/- 0.23
5C8var1-Y105S	1.59 +/- 0.31

The values depicted are the means of at least three independent measurements +/- standard deviation.

Example 9 | The functionality of an anti-(EGFR x Vδ2 TCR) bispecific VHH containing the Y105F mutation is fully retained

[0211] To determine whether the equal affinity of the VHH 5C8var1-Y105F compared to that of 5C8var1 could be translated into a comparable functionality, the bispecific VHH 7D12var8-5C8var1-Y105F was designed: a humanized anti-EGFR VHH 7D12var8 (based on the VHH described in Gainkam et al. (2008) J Nucl Med 49(5):788) was coupled via a G4S linker to the 5C8var1-Y105F VHH, forming 7D12var8-5C8var1-Y105F. The two VHH molecules were separated by a flexible G4S linker sequence. This molecule was produced and purified as described above and then tested for its ability to induce Vγ9Vδ2 T cell activation dependent on an EGFR positive tumor cell line (A431) and to cause Vγ9Vδ2 T cell-mediated tumor cell lysis. Briefly, Vγ9Vδ2 T cells were isolated from the blood of healthy donors using magnetic activated cell sorting (MACS) in combination with an anti-Vδ2 antibody according to standardized procedures. These cells were then expanded for a week using a mix of cytokines and irradiated feeder cells: a mix of the JY cell line and PBMC from a different donor. Vγ9Vδ2 T cells were always >90% pure (stained double positive for Vγ9 and for Vδ2 in FACS) when used in an assay. The A431 cell line (ATCC, cat nr. CRL-1555) was cultured according to the supplier's recommendation. For an activation or cytotoxicity assay, 50,000 tumor target cells were plated in 96 wells tissue culture plates the day before the assay. The next day, 50,000 expanded, purified Vγ9Vδ2 T cells were added in medium together with a concentration range of bispecific VHH compound. In the activation assay, Vγ9Vδ2-T cell degranulation was assessed using a mix of a labeled anti-CD3 and anti-CD107A antibodies that was added to the mix. After 4 hours, cells were harvested, washed and analyzed by FACS for expression of the degranulation marker CD107A.

For the cytotoxicity assay, the supernatants of the co-cultures were examined the day after for the presence of protease (indicative of cell death) using the CytoTox-Glo cytotoxicity assay kit: (Promega G9290). Cell lysis using detergent was used to set 100% killing at the end of the assay. Figure 8 shows the data.

[0212] Figure 8 shows that 7D12var8-5C8var1-Y105F, as well as the non-humanized 7D12-5C8 induced potent Vγ9Vδ2 T cell activation and tumor cell lysis. These results are in line with the potency of the non-humanized 'precursor' molecule without the Y105 mutation 7D12wt-5C8. Table 4 shows the EC50 values that were obtained after curve fitting. 7D12var8-5C8var1-Y105F had a slightly lower EC50 in the cytotoxicity assay as compared to 7D12-5C8.

Table 4 | EC50 values found after curve fitting of the data represented in Figure 8 using GraphPad software.

Antibody used	EC50 degranulation (pM)	EC50 cytotoxicity (pM)
7D12-5C8	10	9
7D12var8-5C8var1-Y105F	11	2.5

[0213] The maximal level of tumor cell kill was slightly lower in case of 7D12var8-5C8var1-Y105F, compared to the level of tumor cell kill observed for 7D12-5C8. However, these were two different measurements using two different Vγ9Vδ2 T cell donors and this maximal level of cytotoxicity may be particularly donor dependent.

Example 10 | The temperature stability of VHH 5C8var1 containing the Y105 mutations was not changed

[0214] To determine whether the mutation introduced in the different variants affected the thermostability of the VHH folding, the melting temperatures of the mutants were measured using NanoDSF (Differential Scanning Fluorimetry). Antibody samples were diluted using PBS until they were equal to the sample with the lowest concentration. The antibody samples were subsequently filled in nanoDSF grade capillaries and measured with the Prometheus NT.48. During the experiment, temperature was ramped from 20 to 95°C. The intrinsic fluorescence of the protein was detected at 350 and 330 nm and was recorded together with the amount of reflected light. From these measurements, the apparent melting temperature (Tm) and aggregation onset (Tagg) were determined. For all three antibody fragments, the onset melting temperature (Ton) and melting temperature (Tm) at which the VHH were completely unfolded were reported (Table 5). Melting temperatures measured for 5C8var1-Y105F and 5C8var1-Y105S were in line with that of 5C8var1: Table 5.

Table 5 | 5C8var1-Y105F and 5C8var1-Y105S show similar melting temperatures as 5C8var1, indicating stability is not impaired by the introduced mutations.

	T_{on} (°C)	Tm (°C)
5C8var1	61.8	86.7
5C8var1-Y105F	64.5	85.5
5C8var1-Y105S	63.9	86.6

Example 11 | Half-life extended (Fc containing) bispecific constructs

[0215] In order to obtain a molecule with a longer in vivo plasma half-life, the 7D12var8-5C8var1-Y105F bispecific VHH was re-formatted into a therapeutic antibody format containing a human Fc. Both VHH domains were coupled to a human IgG1 Fc (i.e. CH2 and CH3) domain with the following characteristics: the VHH was coupled to a modified hinge (AAA, followed by SDKTHTCPPCP, cysteine 220 was deleted) and human CH2 and CH3 domains. The CH2 domain was Fc-silenced by the LFLE mutational pair (L234F, L235E) and the CH3 domains were mutated with the 'knobs-into-holes' mutations (knob: T366W and hole: T366S, L368A and Y407V) that enforce hetero-dimerization, upon co-expression of the two chains in the same cell. This mutational pair has been described in the scientific literature (Ridgway et al. (1996) Protein Eng 9:617). The sequences of the construct are set forth in SEQ ID NO:16 and SEQ ID NO:17. The resulting antibody construct 7D12var8-5C8var1(Y105F) with Fc region was termed 7D12var8-5C8var1(Y105F)-Fc. Similarly, a construct was prepared wherein Y at position 105 was replaced with S (7D12var8-5C8var1(Y105S)-Fc). The sequences of that construct are set forth in SEQ ID NO:16 and SEQ ID NO:18.

[0216] Protein was made via co-transfection of the encoding two expression vectors in HEK293E cells and purification from the culture supernatant by means of protein-A affinity chromatography, followed by preparative size exclusion chromatography, as described in Example 1. This yielded a highly monomeric protein preparation.

Example 12 | Binding of 7D12var8-5C8var1(Y105F)-Fc to primary Vγ9Vδ2 T cells isolated from healthy human PBMCs

[0217] To demonstrate the binding of 7D12var8-5C8var1(Y105F)-Fc to the Vγ9Vδ2 T cell receptor (TCR), human Vγ9Vδ2 T cells were isolated from healthy peripheral blood mononuclear cells (PBMCs) by magnetic activated cell sorting (MACS) and subsequently expanded as described (de Bruin et al., Clin. Immunology 169 (2016), 128-138; de Bruin et al., J. Immunology 198(1) (2017), 308-317). Expanded polyclonal and pure (>95%) Vγ9Vδ2T cells were then seeded at a concentration of 50000 cells/well and incubated with either the 7D12var8-5C8var1(Y105F)-

Fc antibodies or GP120-5C8var1(Y105F)-Fc antibodies as a positive control in a half-log titration starting at 100 nM for one hour at 4°C. Binding of the antibodies to the Vγ9Vδ2 TCR was visualized by flow cytometry using a fluorescently labelled secondary anti-IgG1 antibody. Figure 9 shows the mean fluorescent intensity (MFI) signal of the anti-IgG1 antibody staining as measured by flow cytometry for two different PBMC donors (D336 and D339). The sigmoidal curves underline a significant binding of the 7D12var8-5C8var1(Y105F)-Fc to Vγ9Vδ2 T cells with a half maximal effective concentration (EC50) in the low nanomolar range (~3 nM).

Example 13 | Binding of 7D12var8-5C8var1(Y105F)-Fc to EGFR positive tumor cells by cell-based ELISA

[0218] The binding of 7D12var8-5C8var1(Y105F)-Fc to the epidermal growth factor receptor (EGFR) was tested in a cell-based enzyme-linked immunosorbent assay (ELISA) using EGFR-expressing tumor cell lines A-431, HCT-116 and HT-29. To this end, tumor cells were first seeded at different concentrations on day -1 to reach a concentration of approximately 50,000 cells/well on day 0. On day 0, a half-log titration of 7D12var8-5C8var1(Y105F)-Fc antibodies or GP120-5C8var1(Y105F)-Fc antibodies as a negative control was added to the tumor cells starting at 100 nM for one hour at 37°C. Bound antibodies were then labelled in a secondary incubation step with anti-IgG1-HRP for one hour at 37°C. The secondary antibody binding was then resolved by the addition of 3,3′,5,5′-Tetramethylbenzidine and the colorimetric change induced by the HRP, followed by the addition of H_2SO_4 to stop the reaction. The optical density (OD) was then measured in a UV spectrometer at a wavelength of 450 nm. Figure 10 shows that 7D12var8-5C8var1(Y105F)-Fc strongly binds to A-431, HCT-116 and HT-29 tumor cells with an EC50 of ~7 nM, whereas the non-targeting control antibody did not measurably bind any of the cell lines tested.

Example 14 | Degranulation of Vγ9Vδ2 T cells dependent on A-431 cells induced by 7D12var8-5C8var1(Y105F)-Fc

[0219] To investigate the potential of 7D12var8-5C8var1(Y105F)-Fc to activate Vγ9Vδ2 T cells, Vγ9Vδ2 T cells were first isolated and expanded as described before. Next, Vγ9Vδ2 T cells were then cultured together with A-431 tumor cells in a 1:1 E:T ratio in the presence of different concentrations of 7D12var8-5C8var1(Y105F)-Fc antibody and a PE-labelled anti-CD107a fluorescent antibody. After 24h, cells were harvested and stained with fluorescently labelled anti-Vγ9 and anti-CD3 antibodies to differentiate Vγ9Vδ2 T cells from tumor cells. Using flow cytometry, the degree of CD107a expression on Vγ9Vδ2 T cells, reflecting target-dependent degranulation, was investigated. Figure 11 shows that with increasing concentrations of

7D12var8-5C8var1(Y105F)-Fc, Vγ9Vδ2 T cells were efficiently induced to degranulate dependent on A-431 cells. The EC50 for Vγ9Vδ2 T cell degranulation induced by 7D12var8-5C8var1(Y105F)-Fc lies in the picomolar range (~40-90 pM).

Example 15 | Antibody 7D12-5C8 induces T cell-mediated target cell cytotoxicity

[0220] To investigate whether the bispecific VHH 7D12-5C8 was efficient in inducing Vγ9Vδ2 T cell-mediated cytotoxicity against target cells, the viability of the A-388 epidermoid tumor cell line (ATCC, CRL-7905) was assessed in a co-culture setting with Vγ9Vδ2 T cells and the bsVHH antibody fragment. In this assay, Vγ9Vδ2 T cells were used that were isolated from healthy PBMCs as described previously but subsequently frozen and stored at -150 °C. Frozen Vγ9Vδ2 T cells were thawed and rested overnight in IL-2 supplemented medium. A-388 tumor cells were seeded either alone or with rested Vγ9Vδ2 T cells in a 1:1 or 1:0.1 target to effector ratio, with or without 7D12-5C8 (10 nM). As an additional control, Vγ9Vδ2 T cells were seeded alone with or without antibody 7D12-5C8 (10 nM). After 72h, the viability of cells was determined by the addition of ATP Lite (Perkin Elmer, 6016731) and readout of the luminescent signal with a microplate reader. Figure 12 shows the ATP-derived fluorescence signal, representing the metabolically active cells and thereby number of viable cells. At a 1:1 E:T ratio it can be observed that the antibody induces a reduction of viable cells of ca. 50% whereas untreated co-cultures of A-388 and Vγ9Vδ2 T cells are unaffected, underlining its potential to induce T cell-mediated cytotoxicity.

Example 16 | Tumor cell killing by 7D12-5C8 and 7D12-5C8var1(Y105S)-Fc activated Vγ9Vδ2 T cells

[0221] To investigate whether bsVHH 7D12-5C8 and antibody 7D12-5C8var1(Y105S)-Fc were able to induce Vγ9Vδ2 T cell-mediated cytotoxicity against patient-derived tumor cells, the viability of such tumor cells was assessed in a co-culture setting with Vγ9Vδ2 T cells and the antibodies. Various different types of tumor cells were tested.

[0222] Tissue samples (i.e. primary and metastatic colorectal cancer, head and neck squamous cell carcinoma (HNSCC) and non-small cell lung carcinoma (NSCLC) samples) were collected from cancer patients at the Amsterdam UMC (location VUmc) after written informed consent. Tissue was cut into small pieces with a surgical blade (size no. 22; Swann Morton Ltd), resuspended in dissociation medium composed of IMDM supplemented with 0.1% DNAse I, 0.14% Collagenase A, 5% FCS, 100 IU/ml sodium penicillin, 100 µg/ml streptomycin sulfate and 2.0 mM L-glutamine, transferred to a sterile flask with stir bar and incubated on a magnetic stirrer for 45 minutes in a 37°C water bath. After incubation, cell suspensions were run through 100 µM

cell strainers. Tumor was dissociated three times after which cells were washed for viable cell count with trypan blue exclusion.

[0223] Dissociated patient-derived tumor cells were incubated with healthy donor derived Vγ9Vδ2 T cells (1:1 E:T ratio) in the presence or absence of 50 nM 7D12-5C8 for 4 hours or 7D12-5C8var1(Y105S)-Fc or gp120-5C8var1(Y105S)-Fc for 24 hours.

[0224] Adhered cells were, if needed, detached after the culture period using trypsin-EDTA and resuspended in FACS buffer (PBS supplemented with 0.5% bovine serum albumin and 20 µg/ml NaN3), incubated with fluorescent dye labeled antibodies for 30 minutes at 4 degrees after which staining was measured by flow cytometry using an LSR Fortessa XL-20 (BD).

[0225] Living cells were identified using the life/death marker 7AAD in combination with 123counting eBeadsTM according to manufacturer's instructions. Flow cytometry data were analyzed using Kaluza Analysis Version 1.3 (Beckman Coulter) and FlowJo Version 10.6.1 and 10.7.2 (Becton Dickinson).

[0226] 7D12-5C8 and 7D12-5C8var1(Y105S)-Fc induced Vγ9Vδ2 T cell-mediated cytotoxicity of tumor cells was assessed by incubating expanded healthy donor derived Vγ9Vδ2 T cells with single cell suspensions of various malignant tumors (primary CRC, CRC metastases in peritoneum and liver, head and neck squamous cell carcinoma and non-small cell lung carcinoma).

[0227] As illustrated in Figure 13, 7D12-5C8 induced substantial lysis of patient tumor cells by Vγ9Vδ2 T cells (mean % of lysis induced by 7D12-5C8: CRC primary 52.3% and p-value 0.0003, CRC peritoneum 46.0% and p-value 0.0052, CRC liver 31.8% and p-value 0.0360, head and neck squamous cell carcinoma 46.1% and p-value 0.0187 and non-small cell lung carcinoma 64.1% and p-value 0.0153).

[0228] Furthermore, as shown in Figure 14, 7D12-5C8var1(Y105S)-Fc induced significant amount of lysis of patient tumor cells by Vγ9Vδ2 T cells (mean % of lysis induced by 7D12-5C8var1(Y105S)-Fc: 71.2% and p <0.0001 and 0.0012). The control compound gp120-5C8var1(Y105S)-Fc did not induce any measurable tumor cell lysis.

Example 17 | Design, production and purification of construct 7A5-7D12var8-Fc for use in non-human primate studies

[0229] For in vivo studies in non-human primates, a construct with a binding domain that cross-reacts with the cynomolgus Vγ9 TCR chain was generated (Figure 15). This binding domain was based on antibody 7A5, a TCR Vγ9-specific antibody (Janssen et al., J. Immunology 146(1) (1991), 35-39). Antibodies based on 7A5 have been found to bind cynomolgus V□9V□2 T cells

(see Example 1 of WO2021052995). A bispecific Fc-containing antibody comprising 7A5 and anti-EGFR VHH 7D12var8 was constructed. The molecule contained a human IgG1 Fc tail that was engineered for hetero-dimerization using the knob-in-hole technology (KiH; Carter et al., 2001 Imm. Meth. 2001: 248, 7; Knob: T366W; Hole: T366S, L368A and Y407V). The Vγ9 binding scFv of the 7A5 antibody was coupled to the 'knob' chain; the EGFR binding VHH 7D12var8 was cloned in-frame with the 'hole' chain of the KiH Fc pair. In addition, the upper hinge was engineered to 'AAASDKTHTCPPCP' to remove the cysteine (C220) that normally bridges to the CL and to introduce more flexibility by changing 'EPK' to 'AAA'. The N-terminal part of CH2 was engineered to abrogate Fc receptor (CD16, -32 and -64) interaction (silencing mutations L234F, L235E), while maintaining the FcRn binding. The resulting construct was termed 7A5-7D12var8-Fc.

[0230] The molecule was produced by transient co-transfection of two plasmids encoding the two different chains in HEK293E cells and purified from the culture supernatant by protein-A affinity chromatography, followed by preparative size exclusion chromatography (Example 1). The molecule was shown to bind with roughly 3 nanomolar (nM) apparent affinity to either target using ELISA and recombinant forms of both antigens (Figure 16). The functionality of the molecule was demonstrated by showing that it caused target-dependent activation (CD107a expression) of in vitro expanded Vγ9Vδ2 T cells and subsequent T-cell mediated tumor cell lysis (Figure 17).

Example 18 | Bispecific antibody 7A5-7D12var8-Fc was well tolerated in an exploratory multiple-dose non-human primate (NHP: Cynomolgus monkey) study

[0231] In a multiple-dose exploratory NHP study, 7A5-7D12var8-Fc was administered to three female cynomolgus monkeys at 1 mg/kg, 5 mg/kg and 23 mg/kg doses respectively. The antibody was given in half an hour infusions at 5 mL/kg; 4 weekly infusions were administered. The first two dose groups (1 animal per dose) of 1 and 5mg/kg were dosed simultaneously and after three (weekly) doses, the third dose group (23 mg/kg) received the first dose. Blood was regularly drawn from the animals for PK analyses, analyses of clinical chemistry parameters, measurements of cytokine levels and for analyses of blood cell subsets by flow cytometry. One day after the last dose was given, animals were euthanized and tissues were harvested and prepared for histopathological examination and for immunohistochemistry (IHC).

[0232] Pharmacokinetic analysis of 7A5-7D12var8-Fc concentrations in the blood of treated animals (measured in ELISA, Figure 18) revealed that the antibody displayed an IgG-like PK with a half-life that ranged between 84 and 127 hours. In the animal that was dosed at 1 mg/kg, the antibody showed a shorter half-life after the third injection, which could be due to a possible anti-drug antibody (ADA) response in that animal.

[0233] Clearance values found were between 0.36 and 0.72 mL/h/kg and the volume of distribution was between 58.5 and 115.2 mL/kg. The systemic exposure increased dose-proportionally between 1 and 23 mg/kg. However, no accumulation was observed after repeated dosing.

[0234] The compound could be detected by IHC in different tissues (lymph node, muscle, skin and colon); as expected, there was a dose-proportional intensity of compound staining in these tissues (data not shown). Flow cytometric analysis of blood cells showed several transient decreases in lymphocytes (Figure 19) that are often observed in these kinds of multiple-dose studies and that are procedure-related. Figure 19 shows transient decreases in T cell counts at every time point 2 hours after dosing. However, the number of T cells returned to baseline levels two days after the injection.

[0235] In contrast, Vγ9 positive T-cells decreased in numbers in peripheral blood and did not regain their former frequency. These cells stayed almost absent over the course of the study, demonstrating a specific pharmacodynamic effect of the compound. Measurements of cytokines in the blood of treated animals showed that the treatment caused very little cytokine release and that this was almost exclusively restricted to the first injection with compound. Figure 20 shows the levels of IL-6 measured as an example.

[0236] As a general conclusion, treatment of NHP with 7A5-7D12var8-Fc was very well tolerated and showed no clinical signs of toxicity. In addition, no macroscopic, nor microscopic aberrations of any of the examined organs were noted in histopathology (data now shown). In comparison: an anti-EGFR x CD3 BiTE was lethal for NHP at a dose of 31 µg/kg/day of continuous infusion (Lutterbuese et al., Proc Natl Acad Sci U S A 2010: 107(28), 12605).

Example 19 | VHH 5C8var1 is partially cleaved at position 109 in productions of Fc containing bispecific antibody format, irrespective of the residue at position Y105

[0237] As described above, the Fc bearing format comprises two VHH molecules fused to a modified human IgG1 Fc, forming a humanized 'heavy chain-only' antibody molecule. During cell line development for large scale production of therapeutic Fc-containing bispecific molecules comprising the Vδ2 binding VHH 5C8var1 in CHO cells, a small percentage of the product was found truncated in SDS-PAGE analysis (Figure 21).

[0238] LC-ESI-MS mass spec analysis (Figure 22 and Table 6) showed that, surprisingly, the truncated species seen in SDS-PAGE could be attributed to a small percentage of the 5C8var1 VHH being cleaved after position 109, located in the CDR3 of the VHH. This phenomenon, which was not predicted on the basis of the in silico analysis (Example 5), was observed for different

Fc-containing bispecific molecules, irrespective of the (other) targeting VHH arm on the second chain and irrespective of the amino acid at position 105 in VHH 5C8var1.

[0239] Because truncation beyond position 109 causes the majority of the VHH to be absent, this leads to the generation of a non-functional protein. The percentage of N-terminally truncated protein was low (1-3%) and constant over time, suggesting cleavage occurred during intracellular production and processing of the protein.

Table 6 | Mass annotation of LC-ESI-MS measurement of the VHH-Fc heavy chain-only antibody.

Compound	Theoretical mass (Da)	Measured mass (Da)	Mass error (Da)	Relative intensity (MS signal)
Hetero-dimer, 2x C-term truncation of lysine, 2x G0F glycosylation, N-terminal truncation (1-109)	68805.1	68806.7	+1.6	3.4%
Hetero-dimer, 2x C-term truncation of lysine, G0F glycosylation	79111.6	79115.0	+3.4	<0.1%
Hetero-dimer, 2x C-term truncation of lysine, 2x G0F glycosylation	80556.9	80558.7	+1.8	96.5%

Example 20 | Design of sequence variants of 5C8var1 to increase product homogeneity

[0240] The sequence of the CDR3 of 5C8 was changed at two positions to avoid the proteolysis that was observed in the different Fc-containing bispecific molecules. The G at position 108 (G108) was changed to A and the R at position 109 (R109) was changed to either K, to keep the same net charge, or to A: Figure 23. In addition, a variant (R109AΔGIR) was produced in which the R109 position was changed to 'A' and four residues were deleted from the CDR3 region (ΔGIR).

[0241] These four different sequence variants (G108A, R109A, R109K and R109AΔLGIR) were then expressed and purified as single VHH and as bispecific Fc-containing molecules with a CD123-binding targeting VHH as second binding arm (SEQ ID NO:12) (previously described in PCT/EP2022/054993 (incorporated by reference)). The protein chain encoding the (mutated) VHH directed to the TCR fused to Fc was extended with a C tag for purification purposes. Proteins were expressed by transient transfection of the encoding plasmids in HEK293E cells and secreted proteins were purified from the conditioned cell culture supernatant by C-tag affinity chromatography, followed by preparative size exclusion chromatography. Intact mass analysis

(LC-ESI-MS) of the proteins showed they were all >98% pure and that no truncation was observed in any of the protein batches produced in these small-scale productions.

Example 21 | Affinity measurement by BLI shows position 109 to be permissive.

[0242] To determine the possible effects of the mutations at positions 108 and 109 on the binding characteristics of the different VHH molecules, single VHH proteins were tested for binding to a recombinant form of the Vγ9Vδ2 T cell receptor by biolayer interferometry (BLI) using an Octet system. Figure 24 and Table 7 show that the original humanized 5C8 VHH (5C8var1) with the Y105F mutation has an affinity of 0.74 nM and that the VHH molecules additionally containing a R109A or a R109K mutation retained binding (the affinity not being significantly changed). However, the 5C8var1-Y105F containing an additional mutation at position 108 had a 10-fold reduced affinity. The R109AΔLGIR variant did not measurably bind to the Vγ9Vδ2 TCR anymore. These data demonstrate that, although all (mutated) VHH molecules were well expressed, only the R109 position was permissive for Vγ9Vδ2 T cell receptor binding and permitted a change in sequence. The absence of binding observed for the R109AΔLGIR variant showed that the CDR3 is required for specific binding.

Table 7 | Affinity values found in BLI measurements of 5C8var1-Y105F, 5C8var1-Y105F/G108A, 5C8var1-Y105F/R109A and 5C8var1-Y105F/R109K VHH binding to recombinant Vγ9Vδ2 TCR protein.

VHH clone	Affinity as measured in BLI (nM)
5C8var1-Y105F	0.74
5C8var1-Y105F/G108A	9.2
5C8var1-Y105F/R109A	1.2
5C8var1-Y105F/R109K	0.82
5C8var1-Y105F-R109AΔLGIR	ND

ND = not determined due to lack of binding.

Example 22 | Apparent affinity determination of position 108 and 109 VHH variants using flow cytometry

[0243] To test whether the mutations at positions 108 and 109 of the 5C8var1 VHH affected the binding to cell-expressed Vγ9Vδ2 T cell receptor, the Fc-containing forms of the mutated VHH molecules were tested for binding to primary, expanded Vγ9Vδ2 T cells in flow cytometry. Primary Vγ9Vδ2 T cells were isolated from the PBMC fraction of blood from a healthy volunteer and expanded using a mixed lymphocyte reaction using irradiated feeder cells and a mix of cytokines. Aliquots of 50,000 Vγ9Vδ2 T cells were then incubated with a concentration range of Fc-

containing bispecific molecules and binding was detected with a PE-labeled anti-human IgG detection antibody. In line with the results obtained in BLI, the molecules containing the R109 mutations were indistinguishable from the original molecule in apparent affinity (i.e. binding to Vγ9Vδ2 T cells) (Figure 25); EC50 values for binding were very similar. The bispecific molecule containing the R109AΔLGIR CDR3 did not significantly bind to the cells (not shown) and the G108 mutant showed a very weak binding.

Example 23 | Bispecific, Fc-containing R109 mutant molecules are equally potent in inducing Vγ9Vδ2 T cell activation and target-dependent Vγ9Vδ2 T cell mediated cytotoxicity.

[0244] Bispecific, Fc-containing anti-CD123 x TCR bispecific molecules containing position 108 and 109 variants of 5C8var1 were tested for their ability to induce target-dependent Vγ9Vδ2 T cell activation (determined by analysis of CD107a expression) and Vγ9Vδ2 T cell-mediated target cell cytotoxicity, as described in Examples 14 and 15. Two target cell lines that both express the CD123 molecule were used: MOLM-13 and THP-1. Figures show a representative result using Vγ9Vδ2 T cells from one healthy donor; two different Vγ9Vδ2T cell donors were tested, which both yielded comparable results.

[0245] Figures 26 and 27 show that all bispecific molecules were able to induce Vγ9Vδ2 T cell activation and Vγ9Vδ2 T cell-mediated target cell cytotoxicity. The variants containing the R109 mutations (to either A or K) were as potent as the original molecule that only had the Y105F mutation. The EC50 values (Table 8) for the R109A variant were slightly lower than those for the other two variants.

Table 8 | EC50 values of Fc-containing bispecific molecule-induced Vγ9Vδ2 T cell activation and tumor cell toxicity.

Compound	Molm13 EC50 degranulation (pM)	Molm13 EC50 cytotox (pM)	Thp-1 EC50 degranulation (pM)	Thp-1 EC50 cytotox (pM)
anti-CD123 x 5C8var1-Y105F	2.6	1.4	1.0	0.5
+ R109A	2.6	1.2	0.8	0.2
+ R109K	2.6	1.2	1.0	0.5

Example 24 | Stability of the antibodies

[0246] To determine whether the introduction of the changes at position 109 in the Vδ2 binding VHH had any influence on the thermal and colloidal stability of the antibody, the unfolding of the Fc-containing bispecific antibodies was tested using nano-DSF (differential scanning fluorimetry).

Table 9 gives the temperatures at which unfolding was observed and the onset of turbidity (protein aggregation). The R109 mutants had almost identical thermal stability compared with the parental molecule.

Table 9 | Thermal stability parameters of the different Fc-containing bispecific antibodies. IP: infliction point.

Compound	IP #1 (°C)	IP #2 (°C)	Turbidity on (°C)
Anti-CD123 x 5C8var1-Y105F	65.65	71.61	67.75
+ R109A	64.53	70.79	68.69
+ R109K	65.17	70.75	67.15

[0247] In addition, the antibodies were tested in accelerated stress tests: proteins were incubated at elevated temperature and in acidic or basic conditions, or under oxidative stress conditions.

[0248] For elevated temperature, protein samples were adjusted with phosphate buffer, pH 7.4 to 1 g/L and were incubated at 40°C for 1 week in an incubator, after which samples were aliquoted and stored at 2-8°C or at -80°C until analysis.

[0249] For low pH conditions, protein samples were rebuffered by ultrafiltration (molecular weight cut-off (MWCO) 30 kDa) against 50 mM acetate buffer, pH 5.0, to 1 g/L and incubated at 40°C and low pH conditions for 1 week in an incubator. For high pH conditions, the protein samples were rebuffered by ultrafiltration (MWCO 30 kDa) to 100 mM phosphate buffer, pH 8.5, to 1 g/L and incubated at 40°C and high pH conditions for 1 week in an incubator.

[0250] For oxidative conditions, protein samples were rebuffered by ultrafiltration (MWCO 30 kDa) against phosphate buffer, pH 7.4 and 0.05% H2O2, to 1 g/L and incubated at RT for 24 hours.

[0251] After incubation in acidic, basic or oxidative conditions, the samples were rebuffered by ultrafiltration (MWCO 10 kDa) against phosphate buffer, pH 7.4, aliquoted and stored at 2-8°C or at -80°C until analysis.

[0252] Analysis of aggregates and fragments was performed by SEC-UV (Table 10), in which no or only minor changes in the percentage of high molecular weight species (HMWS) and/or low molecular weight species (LMWS) were observed after incubation under the indicated accelerated stress conditions. Importantly, the changes observed for the R109 mutants were almost similar to those for the parental molecule.

Table 10| Summary of HMWS, main peak and LMWS determined by SEC-UV of the different Fc-containing bispecific antibodies before and after stress treatment.

Compound	Quality Attribute	non-stressed samples	elevated temperature	acidic conditions	basic conditions	oxidative conditions
Anti-CD123 x 5C8var1-Y105F	HMWS [%]	1.23	1.90	0.88	2.06	4.71
	Main peak [%]	98.77	97.66	99.12	97.18	95.07
	LMWS [%]	n.d.	0.44	n.d.	0.75	0.23
+ R109A	HMWS [%]	3.36	3.54	2.30	3.49	4.66
	Main peak [%]	96.64	96.36	97.55	95.99	95.34
	LMWS [%]	n.d.	0.10	0.15	0.51	n.d.
+ R109K	HMWS [%]	3.09	2.78	2.00	3.44	4.48
	Main peak [%]	96.90	97.07	97.87	96.07	95.51
	LMWS [%]	n.d.	0.15	0.13	0.49	n.d.

[0253] Additional analysis of fragments was performed by CGE/CE-SDS after reduction (Table 11). No or minor changes in the percentages of LMWS were observed, and changes observed for the R109 mutants were very similar to those found for the parental molecule.

Table 11| Summary of HMWS, main peak and LMWS determined by SEC-UV of the different Fc-containing bispecific antibodies before and after stress treatment.

Compound	Quality Attribute	non-stressed samples	elevated temperature	acidic conditions	basic conditions	oxidative conditions
Anti-CD123 x 5C8var1-Y105F	LMWS (%)	1.00	1.14	0.96	4.52	1.02
	Main peak (%)	99.00	98.86	99.04	95.48	98.98
+ R109A	LMWS (%)	6.71	7.13	6.57	9.36	7.14
	Main peak (%)	93.29	92.87	93.43	90.64	92.86
+R109K	LMWS (%)	5.19	6.35	5.31	7.63	6.40
	Main peak (%)	94.81	93.65	94.69	92.37	93.60

Example 24 | Humanization of anti-CD123 VHH 1D2 using CDR grafting

[0254] The 1D2 VHH antibody fragment was humanized using CDR-grafting technology (see e.g. U.S. Patent No. 5,225,539 and Williams, D.G. et al., 2010, Antibody Engineering, volume 1, Chapter 21). First, human germline sequences were identified using IgBLAST (Ye J. et al., 2013, Nucleic Acids Res. 41:W34-40). As closest human germline sequence, V-gene IGVH3-23*04 was identified (78.4% identity). This germline sequence was used to directly graft the llama CDRs (91.8 % identity with human germline IGVH3-23*04), resulting in the construct of SEQ ID NO: 32.

Next, the NCBI NR database (downloaded Sept 27 2020) was queried using BLASTP (version 2.10.0+) to identify human template sequences that demonstrated the highest identity to the 1D2 sequence. Two VH sequences were identified that demonstrated a similarity score of 70% or higher and that displayed similar CDR lengths, preferably identical to those in 1D2 CDR1, CDR2, CDR3, respectively. The frameworks encoded by GenBank (Benson, D.A. et al., 2013, Nucleic Acids Res. 41(D1):D36-42) accession # CAD60357.1, and AKU38567.1 were selected as templates for grafting of the 1D2 CDRs, resulting in the following cDNA constructs: SEQ ID NO: 29 and 33, respectively. Framework and CDR definition were those as determined by Kabat et al. ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)). To understand the effect of humanized framework residues on the structure of the VHH, a homology model of 1D2 VHH was made using the 'Antibody Prediction'-tool (default parameters) within BioLuminate 4.2.156 (Schrödinger). The homology model was built on basis of PDB ID 6GKU. The CDRs were grafted in silico to study the effect of the human residues for features as loop conformation of the CDRs, the hydrophobicity of the surface, and structural integrity (e.g. increased rigidity). The resulting constructs were checked for these features, resulting in the design of additional constructs: SEQ ID NO: 26, 27, 28, 30, 31, 34 and 35. The sequences of these humanized 1D2- VHHs were then re-formatted to bispecific VHH with a Vδ2 specific VHH (5C8var1; SEQ ID NO:18) in the orientation: N-term-humanized-anti-1D2 VHH-linker-anti-Vδ2 VHH-C-tag. The cDNAs encoding these molecules were then synthesized and cloned into an expression vector for the expression in HEK293E cells. Protein was made by transient transfection of the cells and was purified from the culture supernatant using C-tag affinity chromatography, followed by preparative size exclusion chromatography.

Example 25 | Affinity determination of humanized 1D2-5C8var1(Y105F) variants for binding CD123 using biolayer interferometry (BLI)

[0255] To determine the kinetics of binding of humanized 1D2-5C8var1 variants to CD123, recombinant purified CD123-Fc fusion protein (Bio-Techne/R&D Systems) was loaded to a density of 1nm onto anti-human IgG Fc Capture sensors (using a concentration of 5µg/ml) for an Octet Red96e (Sartorius) instrument. Different sensors were then dipped in different concentrations of humanized 1D2-5C8var1 variants, starting at 50 nM and two-fold dilutions thereof; dilutions were made in 10x kinetic buffer (10xKB) provided by the supplier. From the obtained sensorgrams, the kinetic association- and dissociation rate constants were determined by curve fitting. The association- and dissociation rate constants, when fitting was possible, were used to calculate the affinity of the humanized 1D2-5C8var1 variants for binding to CD123.

Measurements were performed twice and the affinity of the different humanized 1D2-5C8var1 variants for CD123 ranged from 2.6 nM, similar compared to parental 1D2, to non-binding variants as shown in Table 12. For reference, the non-humanized (parental) 1D2 was included in these experiments.

Table 12| affinity of humanized variants of 1D2 to recombinant CD123

SEQ ID No:	KD (nM)	SD
1	3.1	0.7
26	7.2	0.4
27	24.5*	
28	14.9	4.3
29	20.0	7.5
30	7.7	1.5
31	29.1*	
32	n.b.	
33	44.1*	
34	2.6	0.5
35	10.4	7.5

**low binding (>20 nM), one measurement only, n.b.: no binding observed*

Example 26 | Development of cell pools stably expressing bispecific antibodies.

[0256] cDNA's encoding all protein chains (1D2-Fc (SEQ ID NO:21), 5C8var1-Y105F-Fc (SEQ ID NO:37) and 5C8var1-Y105F-R109A-Fc (SEQ ID NO:22)) were designed by reverse-translation and then codon-optimized for expression in Chinese Hamster Ovary (CHO)-CD390 cells at a contract research organization (CRO). cDNA's were generated by synthetic gene synthesis and cloned into a proprietary expression vector harboring an antibiotic resistance (selection) gene (giving either puromycin, or methotrexate resistance). Two different plasmids encoding the two different chains (in the combinations 1D2-Fc x 5C8var1-Y105F-Fc and 1D2-Fc x 5C8var1-Y105F-R109A-Fc) were then co-transfected to CHO-CD390 cells at different plasmid ratios. After 48 hours, selection on both puromycin and methotrexate was started and after 13 days of culture, clone pools resistant to both antibiotics were obtained and cryopreserved.

Example 27 | Purification of bispecific antibody from conditioned cell culture supernatant

[0257] Production of soluble antibody by the selected cell pools was initiated using a generic fed-batch protocol in an Infors HT multitron cell incubation shaker (12ml scale bioreactor tubes, 14 days of culture). After the production, antibody was purified from the conditioned cell supernatant using protein-A affinity chromatography. Briefly, protein was captured onto a Protein A HP SpinTrap (protein A Sepharose) column (GE Healthcare) in 200mM Sodium phosphate (pH7.0).

After binding and washing, the antibody was eluted using a pH shock (100mM sodium citrate pH3.6) and the solution was immediately neutralized by the addition of 1M Tris-HCl, pH9.0. The amount of purified antibody was quantified by biolayer interferometry (BLI) using an Octet Red96 system (Sartorius) equipped with protein A sensors and human IgG as standard.

Example 28 | Analysis of purified protein by CE-SDS (capillary electrophoresis)

[0258] Capillary electrophoresis is capable of separating proteins by their apparent molecular weight and quantifying all proteinaceous components by UV spectrophotometry. Sample preparation involved heat denaturation of protein in the presence of the denaturing agent sodium dodecyl sulfate (SDS). Upon denaturation, sample components were separated based on electro-mobility differences in a capillary containing a replaceable SDS polymer matrix. In Figure 28, the electropherograms of non-reduced analyses of 1D2-Fc x 5C8var1-Y105F-Fc (Fig. 28A) and 1D2-Fc x 5C8var1-Y105F-R109A-Fc (Fig. 28B) are depicted. Analyses were performed using a PA800 Plus system (SCIEX) equipped with a PDA detector for protein detection at a wavelength of 220 nm. Due to protein denaturation under non-reducing conditions, the main component of both samples is the heterodimer with a retention time of 24-25 minutes. In case of 1D2-Fc x 5C8var1-Y105F-Fc, a truncated version of 5C8var1-Y105F-Fc denoted as 'Peak 2' is detected, whereas that peak is absent in the electropherogram of 5C8var1-Y105F-R109A-Fc, demonstrating that 5C8var1-Y105F-R109A-Fc is not prone to fragmentation as observed for 5C8var1-Y105F-Fc. Table 13 summarizes the quantification of the various species. The main peak at 25min corresponds to the intact hetero-dimer; 'Peak 1' represents a slight amount of half-body present in the preparation of 1D2-Fc x 5C8var1-Y105F-Fc. 'Peak 2' is the truncation observed at position 108 in 5C8var1-Y105F-Fc. 'Peak 3' corresponds to a de-glycosylated variant of the protein.

Table 13| quantification of the different species found in the electropherograms shown in Figure 28.

		Peak 1	Peak 2	Peak 3	Main peak
Pool ID	**Molecule**	**Half-body**	**Truncation**	**Glyc variant**	**Dimer**
DGC8-T1	1D2-FC x 5C8var1-Y105F-Fc	5.6	1.4	2.1	90.9
DGC8-T2	1D2-FC x 5C8var1-Y105F-Fc	1.4	1.4	2.3	94.9
DGC8-T3	1D2-FC x 5C8var1-Y105F-Fc	0.7	1.0	1.6	96.7
DGC8-T4	1D2-FC x 5C8var1-Y105F-Fc	0.0	0.9	1.5	97.7
DGC8-T5	1D2-FC x 5C8var1-Y105F-R109A-Fc	4.4	0.0	1.5	94.1
DGC8-T6	1D2-FC x 5C8var1-Y105F-R109A-Fc	1.1	0.0	1.5	97.4
DGC8-T7	1D2-FC x 5C8var1-Y105F-R109A-Fc	1.1	0.0	1.6	97.3

Example 29 | Analysis of purified 1D2-Fc x 5C8var1-Y105F-R109A-Fc protein by mass spectrometry (MS)

[0259] Protein-A purified protein of 1D2-Fc x 5C8var1-Y105F-R109A-Fc was sent to a CRO for MS analysis. Proteins were N-deglycosylated using N-glycosidase F (Roche) in an optimized enzyme:protein ratio overnight at 37°C. The samples were diluted in 0.1% formic acid (FA) and separated on a UPLC-system (Waters Acquity Premier H-class) using a reversed phase column (MAbPac RP 4µm 2.1 x 50mm, Thermo Scientific). Eluents were 0.1% FA in water and 0.1% FA in acetonitrile. The mass spectrometric analysis was performed with a Compact QTOF mass spectrometer (Bruker Daltonik). The recorded LC-ESI-MS spectra were summed, deconvoluted and smoothed using the software DataAnalysis (Bruker Daltonik). For annotation, completely oxidized/reduced cysteines were assumed (depending on the sample preparation). 4 different batches of 1D2-Fc x 5C8var1-Y105F-R109A-Fc were analyzed. Despite some heterogeneity with regard to C-terminal lysine clipping and some heterogeneity in glycosylation, no truncation product of 5C8var1-Y105F-R109A-Fc was identified, proving again that the R109A mutation prohibited the formation of the truncation (data not shown).

CLAIMS

1. An antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence as set forth in SEQ ID NO:12, a CDR2 sequence as set forth in SEQ ID NO:13 and a CDR3 sequence as set forth in SEQ ID NO:14.

2. The antibody according to claim 1, wherein

X1 in SEQ ID NO:12 is S (Ser) and X2 in SEQ ID NO:14 is F (Phe),

X1 in SEQ ID NO:12 is S (Ser) and X2 in SEQ ID NO:14 is S (Ser),

X1 in SEQ ID NO:12 is S (Ser) and X3 in SEQ ID NO:14 is A (Ala), or

X1 in SEQ ID NO:12 is S (Ser) and X3 in SEQ ID NO:14 is K (Lys).

3. The antibody according to claim 1 or 2, wherein

X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is F (Phe) and X3 in SEQ ID NO:14 is A (Ala), or

X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is F (Phe) and X3 in SEQ ID NO:14 is K (Lys).

4. The antibody according to claim 1 or 2, wherein

X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is S (Ser) and X3 in SEQ ID NO:14 is A (Ala), or

X1 in SEQ ID NO:12 is S (Ser), X2 in SEQ ID NO:14 is S (Ser) and X3 in SEQ ID NO:14 is K (Lys).

5. The antibody according to any one of the preceding claims, wherein the first antigen-binding region is a single-domain antibody.

6. The antibody according to any one of the preceding claims, wherein the first antigen-binding region comprises or consists of:

a sequence as set forth in SEQ ID NO:16, or

a sequence having at least 90%, such as at least 92%, e.g. at least 94%, such as at least 96%, e.g. at least 98% sequence identity to a sequence as set forth in SEQ ID NO:16, wherein X1 is S (Ser), X2 is F (Phe) and X3 is A (Ala).

7. An antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence selected from SEQ ID NO:17, 18, 19, and 20.

8. The antibody of claim 7, comprising:

(a) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO:17;

(b) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 18;

(c) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 19; or

(d) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 20.

9. The antibody of claim 7 or claim 8, wherein the first antigen-binding region comprises or consists of a sequence selected from SEQ ID NO: 25, 26, 27, and 28.

10. An antibody comprising a first antigen-binding region capable of binding to human Vδ2, wherein said first antigen-binding region comprises a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 sequence selected from SEQ ID NO:21, 22, 23, and 24.

11. The antibody of claim 10, comprising:

(a) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO:21;

(b) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 22;

(c) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 23; or

(d) a CDR1 sequence of SEQ ID NO:8, a CDR2 sequence of SEQ ID NO:13 and a CDR3 of SEQ ID NO: 24.

12. The antibody of claim 10 or claim 11, wherein the first antigen-binding region comprises or consists of a sequence selected from SEQ ID NO: 29, 30, 31, and 32.

13. The antibody according to any one of the preceding claims, wherein the antibody further comprises a second antigen-binding region and wherein the second antigen-binding region preferably is a single-domain antibody.

14. The antibody according to claim 13, wherein the antibody is a bispecific antibody.

15. The antibody of claim 13 or 14, wherein the second antigen-binding region is capable of binding to an antigen selected from CD123, PSMA, CD1d, CD40, and Nectin 4.

16. The antibody of claim 15, wherein:

(a) the second antigen-binding region is capable of binding CD123 and comprises the CDR1 sequence of SEQ ID NO: 37, the CDR2 sequence of SEQ ID NO: 38, and the CDR3 sequence of SEQ ID NO: 39;

(b) the second antigen-binding region is capable of binding PSMA and comprises the CDR1 sequence of SEQ ID NO: 41, the CDR2 sequence of SEQ ID NO: 42, and the CDR3 sequence of SEQ ID NO: 43;

(c) the second antigen-binding region is capable of binding CD1d and comprises the CDR1 sequence of SEQ ID NO: 45, the CDR2 sequence of SEQ ID NO: 46, and the CDR3 sequence of SEQ ID NO: 47;

(d) the second antigen-binding region is capable of binding CD40 and comprises the CDR1 sequence of SEQ ID NO: 49, the CDR2 sequence of SEQ ID NO: 50, and the CDR3 sequence of SEQ ID NO: 51; or

(e) the second antigen-binding region is capable of binding Nectin 4 and comprises the CDR1 sequence selected from SEQ ID NO: 53, 57, 61, 65, 69, 73, and 77, the CDR2 sequence selected from SEQ ID NO: 54, 58, 62, 66, 70, 74, and 78, and the CDR3 sequence selected from SEQ ID NO: 55, 59, 63, 67, 71, 75, and 79.

17. The antibody of claim 15 or 16, wherein:

(a) the second antigen-binding region is capable of binding CD123 and comprises or consists of SEQ ID NO: 40;

(b) the second antigen-binding region is capable of binding PSMA and comprises or consists of SEQ ID NO: 44;

(c) the second antigen-binding region is capable of binding CD1d and comprises or consists of SEQ ID NO: 48;

(d) the second antigen-binding region is capable of binding CD40 and comprises or consists of SEQ ID NO: 52; or

(e) the second antigen-binding region is capable of binding Nectin 4 and comprises or consists of a sequence selected from SEQ ID NO: 56, 60, 64, 68, 72, 76, and 80.

18. The antibody of any one of claims 13-17, wherein the first antigen-binding region and second antigen-binding region are covalently linked via a peptide linker.

19. The antibody according to any one of the preceding claims, wherein the antibody further comprises a half-life extension domain, such as an Fc region.

20. The antibody according to any one of the preceding claims, wherein the antibody comprises an Fc region, wherein the Fc region is a heterodimer comprising two Fc polypeptides, wherein the first antigen-binding region is fused to the first Fc polypeptide and the second antigen-binding region is fused to the second Fc polypeptide and wherein the first and second Fc polypeptides comprise asymmetric amino acid mutations that favor the formation of heterodimers over the formation of homodimers.

21. The antibody according to claim 20, wherein the CH3 regions of the Fc polypeptides comprise said asymmetric amino acid mutations, preferably the first Fc polypeptide comprises a T366W substitution and the second Fc polypeptide comprises T366S, L368A and Y407V substitutions, or vice versa, wherein the amino acid positions correspond to human IgG1 according to the EU numbering system.

22. The antibody according to claim 20 or 21, wherein the first and second Fc polypeptides comprise a mutation at position 234 and/or 235, preferably the first and second Fc polypeptide comprise an L234F and an L235E substitution, wherein the amino acid positions correspond to human IgG1 according to the EU numbering system.

23. The antibody according to any one of claims 19 to 22, wherein

the first Fc polypeptide comprises the sequence set forth in SEQ ID NO:83 and the second Fc polypeptide comprises the sequence set forth in SEQ ID NO:84, or

the first Fc polypeptide comprises the sequence set forth in SEQ ID NO:84 and the second Fc polypeptide comprises the sequence set forth in SEQ ID NO:83.

24. The antibody according to any one of claims 19 to 23 wherein the antibody comprises or consists of:

the sequence set forth in SEQ ID NO:86, and

a sequence selected from the group of sequences set forth in: SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89 and SEQ ID NO:90.

25. A pharmaceutical composition comprising an antibody according to any one of the preceding claims and a pharmaceutically-acceptable excipient.

26. The antibody according to any one of claims 1 to 24 for use as a medicament, preferably for use in the treatment of cancer.

27. A method of treating a disease comprising administration of an antibody according to any one of claims 1 to 24 to a human subject in need thereof.

28. A nucleic acid construct encoding the antibody according to any one of claims 1 to 24.

29. An expression vector comprising the nucleic acid construct of claim 28.

30. A host cell comprising one or more nucleic acid constructs encoding the antibody according to any one of claims 1 to 25.

31. A process for manufacturing an antibody, comprising expressing one or more nucleic acids encoding the antibody according to any one of claims 1 to 24 in a host cell, wherein the host cell preferably is a Chinese Hamster Ovary cell, a Human Embryonic Kidney cell or a Pichia pastoris cell.